Screening plant extracts for anticancer potential; Characterization of active fractions from *Melia dubia* Cav. and elucidation of their effects on cancer cell lines

Thesis submitted to
University of Calicut in partial fulfilment of
the requirement for the award of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

by

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UNIVERSITY OF CALICUT
2024

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I also certify that the corrections/suggestions recommended by adjudicators have been incorporated into the thesis

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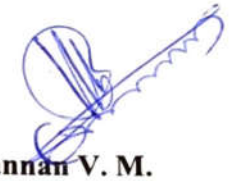
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Declaration

I hereby declare that the work presented in the thesis entitled **"Screening plant extracts for anticancer potential; Characterization of active fractions from *Melia dubia* Cav. and elucidation of their effects on cancer cell lines."** is based on the original work done by me under the guidance of **Prof. (Dr.) Kannan V. M.**, Biochemistry and Molecular Biology division, Department of Zoology, University of Calicut and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI-generated contents.



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Abbreviations

ACLA	:	*Anamirta cocculus* leaf acetone extract
ACLC	:	*Anamirta cocculus* leaf chloroform extract
ACLE	:	*Anamirta cocculus* leaf ethyl acetate extract
ACLM	:	*Anamirta cocculus* leaf methanol extract
ACLP	:	*Anamirta cocculus* leaf petroleum ether extract
ACLW	:	*Anamirta cocculus* leaf water extract
ANOVA	:	Analysis of variance
AO	:	Acridine orange
Bax	:	Bcl-2 associated X
Bcl2	:	B cell lymphoma 2
BSA	:	Bovine serum albumin
CDK	:	Cyclin-dependent kinase
cDNA	:	Complementary DNA
c-Myc	:	Cellular myelocytomatosis
COX-2	:	Cyclooxygenase 2
C_T	:	Cycle threshold
DAPI	:	4′,6-diamidino-2-phenylindole
DLS	:	Dynamic Light Scattering
DMEM	:	Dulbecco's Modified Eagle's Medium
DMSO	:	Dimethyl sulfoxide
EB	:	Ethidium bromide
EDTA	:	Ethylenediaminetetraacetic acid
EMT	:	Epithelial-mesenchymal transition
FACS	:	Fluorescence activated cell sorting
FBS	:	Foetal bovine serum
FESEM	:	Field-emission scanning electron microscopy
FITC	:	Fluorescein isothiocyanate
GAPDH	:	Glyceraldehyde 3-phosphate dehydrogenase
GC-MS	:	Gas chromatography-mass spectrometry
HPTLC	:	High performance thin layer chromatography
HRP	:	Horse radish peroxidase
IgG	:	Immunoglobulin G
JC1	:	5,5,6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazoylcarbocyanine iodide
LC-MS	:	Liquid chromatography-mass spectrometry
MAPK	:	Mitogen activated protein kinase
MEM	:	Minimum essential medium
MMP	:	Mitochondrial membrane potential
MMP-2	:	Matrix metallopeptidase 2
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	:	Nuclear factor kappa B
PBS	:	Phosphate buffer saline

PDB	:	Protein data bank
PI	:	Propidium iodide
PTEN	:	Phosphate and tensin
RIPA	:	Radioimmunoprecipitation assay
ROS	:	Reactive oxygen species
rpm	:	rotations per minute
RTPCR	:	Real time PCR
SC	:	Silica gel column chromatography
SEM	:	Standard error mean
SNAI	:	Snail family transcriptional repressor
TBST	:	Tris-buffered saline with Tween 20
TLC	:	Thin layer chromatography
TRI reagent	:	Total RNA Isolation Reagent
VEGF	:	Vascular endothelial growth factor
w/v	:	weight/volume

Abstract

Cancer is a disease which still needs better therapeutic intervention in spite of the advances in the treatment modalities currently available, as some of the cancers are hard to cure and may cancers develop resistance against the currently used drugs. Plants are a repository of molecules with anticancer activities. To explore the anticancer activity of molecules in the plant extracts, we screened plant extracts and identified extracts with potential anticancer compounds and purified enriched fractions with anticancer molecules.

In this study 426 extracts from 65 plants were screened for their cytotoxic effects on cancer cell lines. Among the 10 cancer cell lines tested many extracts showed high toxicity against many of the cell lines tested with minimum toxicity to normal cell line HaCaT. Notably, 28 extracts showed over 50% mortality in HT29 cells with minimal toxicity to HaCaT cells, demonstrating differential cytotoxicity. Similar results were obtained for other cancer lines, with 18 extracts selective for HCT116, 24 for A431, 21 for A498. This is the first report of anticancer activity in plants, such as *Pancratium triflorum* and *Anamirta cocculus*, *Chassalia curviflora, Antigonon leptopus, Otacanthus caeruleus, Hewittia malabarica* and *Melia dubia.*

The chloroform extract of *Hewittia malabarica* aerial parts exhibited notable cytotoxicity against the human colorectal carcinoma cell line HT29, with an IC_{50} of 31.97±3.07 µg/mL. GC-MS analysis revealed 17 compounds, with tritetracontane and neodene being the most abundant. *Pancratium triflorum* extracts also displayed selective cytotoxicity with chloroform extract showing 92.36±0.81% mortality in HeLa cells. *Conyza canadensis* petroleum ether extract exhibited nearly complete mortality against PANC1 and HT29 cancer cell lines. *Anamirta cocculus* extract was most potent on the PA1 ovarian cancer cell line, achieving 98.64±0.40% mortality. *Melia dubia* extracts also demonstrated selective cytotoxicity with petroleum ether extract showed 97.48±1.77% mortality in PANC1 cancer cell lines. GC-MS analysis identified a variety of bioactive compounds, including tetratetracontane, (E)-Phytol, and 3-eicosene, which could contribute to their anticancer properties.

The ethyl acetate extract of *Anamirta cocculus* exhibited the potent anticancer activity, showing high cytotoxicity against ovarian cancer (PA1) and colorectal adenocarcinoma (HT29) cell lines, with IC_{50} values of 8.30±0.38 µg/mL and

17.97±0.63 µg/mL, respectively. The ethyl acetate extract further inhibited colony formation in HT29 and PA1 cells, induced apoptosis, which was confirmed by characteristic markers such as membrane blebbing, chromatin condensation, and DNA fragmentation.

The chloroform extract of *Melia dubia* leaves was subjected to fractionation through silica gel column chromatography to enrich fractions with anticancer activity. The crude extract showed an IC_{50} of 61.26±2.55 µg/mL, while the more purified fractions SC1-F8, SC2-F8, SC3-F8, and TLC-B3 exhibited IC_{50} values of 31.07±4.46, 17.86±1.29, 10.27±0.79, and 7.87±1.32 µg/mL, respectively. The fraction, TLC-B3, was subjected to GC-MS analysis, revealing ten compounds that are likely responsible for its potent anticancer effects.

The cytotoxicity analysis of *Melia dubia* purified extracts revealed significant mortality in HT29, MCF7, PA1, and UM-SCC-83B cancer cell lines. The purified fraction, SC3-F8, effectively inhibited colony formation and cell migration and apoptosis in these lines. The purified fraction SC3-F8 demonstrated reduction in mitochondrial membrane potential ($\Delta\Psi M$) after 48 hours. Annexin-V/propidium iodide co-staining showed an increase in apoptotic cells in a dose-dependent manner. DNA damage was evident as longer comet tail lengths were observed in all treated cancer cell lines. Additionally, SC3-F8 induced G1 phase cell cycle arrest and triggered the up regulation of pro-apoptotic genes while down regulating anti-apoptotic genes. The fraction also inhibited cancer cell migration and suppressed components of the epithelial-mesenchymal transition (EMT) pathway, potentially disrupting angiogenesis.

SC1-F8 when incorporated into liposome resulted in decrease in IC_{50} from 31.07±4.46 µg/mL to 16.73±0.77 µg/mL indicating its usefulness for the delivery of anticancer molecules enriched from *M. dubia. In silico* molecular docking studies on the ligands identified by GC-MS from the TLC-B3 fraction of *M.dubia* on key apoptotic proteins, including caspase-3, caspase-9, and P53,resulted in identification of Petroselinic acid and 9-Octadecenoic acid as probable modulators of apoptosis in *M.dubia*. It is worth investigating the potential of these compounds as anticancer agents.

സംഗ്രഹം

നിലവിൽ ലഭ്യമായ ചികിത്സാരീതികളിൽ പുരോഗമനമുണ്ടായിട്ടും മെച്ചപ്പെട്ട ചികിത്സാ ഇടപെടലുകൾ ആവശ്യമായ ഒരു രോഗമാണ് അർബുദം/കാൻസർ. എന്തുകൊണ്ടെന്നാൽ ,മാത്രമല്ല .ചില അർബുദങ്ങൾ ഭേദമാകാൻ പ്രയാസമാണ് , .അർബുദങ്ങൾ നിലവിൽ ഉപയോഗിക്കുന്ന മരുന്നുകളോട് പ്രതിരോധം വളർത്തിയേക്കാം രുദ്ധശേഷിഅർബുദവി/ആന്റികാൻസർ ശേഷിയുള്ള തൻമാത്രകളുടെ ഒരു ശേഖരമാണ് സസ്യങ്ങൾസസ്യസത്തുകളുടെ അർബുദവിരുദ്ധശേഷി ഗവേഷണം ചെയ്യ .ാൻ ഞങ്ങൾ വിവിധ സസ്യസത്തുകൾ/പ്ലാന്റ് എക്സ്ട്രാറ്റുകൾ പരിശോധിച്ചുആന്റികാൻസർ . ഷ്ടമായ സംയുക്തങ്ങളുള്ള സത്തുകളും ശുദ്ധീകരിച്ച ആന്റികാൻസർ തന്മാത്രകളുടെ സമ്പു .ഫ്രാക്ഷനുകളും ഞങ്ങൾ കണ്ടെത്തി

ഈ പഠനത്തിൽ സസ്യങ്ങളിൽ നിന്നുമായി 65426 സത്തുകൾ ഉപയോഗിച്ച് കാൻസർ സെൽലൈനുകളിൽ അവയുടെ കോശനശീകരണശേഷി പരിശോധിച്ചു . അർബുദരഹിത സാധാരണ ,പരീക്ഷിച്ച പത്തു കാൻസർ സെൽലൈനുകളിലും സെൽലൈനായHaCaT കോശങ്ങളിലുള്ള ഏറ്റവും കുറഞ്ഞ വിഷാംശത്തിൽ പല സസ്യസത്തകളും ഉയർന്ന കോശനശീകരണശേഷി പ്രകടിപ്പിച്ചു 28 ശ്രദ്ധേയമായി . സത്തുകൾHaCaT കോശങ്ങളിൽ ഏറ്റവും കുറഞ്ഞ വിഷാംശനിരക്കിൽ പ്രകടമാക്കിക്കൊണ്ട് ,HT29 അർബുദ കോശങ്ങളിൽ 50% മരണനിരക്ക് കാണിച്ചുമറ്റ . .അർബുദകോശങ്ങളിലും സമാനമായ ഫലമാണ് കിട്ടിയത്HCT 116കോശങ്ങളിൽ 18 എക്സ്ട്രാക്ടുകളുംA431 കോശങ്ങളിൽ എക്സ്ട്രാക്ടുകളും 24A498 കോശങ്ങളിൽ 21 എക്സ്ട്രാക്ടുകളും ഉയർന്ന കോശനശീകരണശേഷി പ്രകടമാക്കി .*പാൻക്രേഷ്യം ടൈഫ്ലോറംആന്റിഗൺ ,ചസാലിയ കർവിഫ്ലോറ ,അനാമിർട്ട കോക്യുലസ് , മീലിയ ഡൂബിയ ,ഹെവിറ്റിയ മലബാറിക്ക ,ഒട്ടാകാന്തസ് സെറൂലിയസ് ,ലെപ്റ്റോപസ്* തുടങ്ങിയ സസ്യങ്ങളുടെ അർബുദവിരുദ്ധശേഷിയുടെ ആദ്യ പഠനമാണിത് .*ഹെവിറ്റിയ മലബാറിക്ക*യുടെ ഭൗമോപരിതല ഭാഗങ്ങളുടെ ക്ലോറോഫോം എക്സ്ട്രാക്ട് മനുഷ്യ വൻകുടൽ അർബുദകോശമായ HT29 ന് എതിരെ 31.97±3.07 µg/ml IC_{50} മൂല്യത്തോടുകൂടി ശ്രദ്ധേയമായ കോശനശീകരണശേഷി പ്രദർശിപ്പിച്ചു .GC-MS വിശകലനം 17 .സംയുക്തങ്ങളുടെ സാന്നിധ്യം എക്സ്ട്രാക്ടിൽ വെളിപ്പെടുത്തി അതിൽ ടൈടെട്രാകോണ്ടെയ്നും നിയോസിനും സമൃദ്ധമായി കാണപ്പെട്ടു. *പാൻക്രേഷ്യം ടൈഫ്ലോറം* എക്സ്ട്രാക്ടുകൾ സിലക്ടീവ് സൈറ്റോടോക്സിസിറ്റി കാണിച്ചു .HeLa കോശങ്ങളിൽ *പാൻക്രേഷ്യം ടൈഫ്ലോറം* ക്ലോറോഫോം എക്സ്ട്രാക്ട് 92.36±0.81% മരണനിരക്ക് കാണിച്ചു .PANC1, HT29 അർബുദ കോശങ്ങൾക്കെതിരെ *കൊനൈസ കാനഡൻസിസ്* പെട്രോളിയം ഈഥർ എക്സ്ട്രാക്ട് ഏതാണ്ട് സമ്പൂർണ്ണ മരണനിരക്കാണ് പ്രകടിപ്പിച്ചത് . PAI അണ്ഡാശയാർബുദകോശങ്ങളിൽ വളരെ വീര്യമാണ് *അനാമിർട്ട കോക്യുലസ്* ഈഥൈൽ അസറ്റേറ്റ് എക്സ്ട്രാക്ട് കാണിച്ചത് മരണനിരക്ക്)=64.00 ±0.40%(. *മീലിയ ഡൂബിയയുടെ* പെട്രോളിയം ഈഥർ എക്സ്ട്രാക്ടിനാൽ PANC1 അർബുദകോശങ്ങളിൽ 97.48±01.77% മരണനിരക്കോടുകൂടി സെലക്ടീവ് സൈറ്റോടോക്സിസിറ്റി പ്രകടമാക്കി .GC-MS വിശകലനത്തിൽ ടെട്രാടെട്രാകോൺഡെയ്ൻ) ,E)-ഫൈറ്റോൾഇക്കോസീൻ -3 , എന്നിവയുൾപ്പെടുന്ന ബയോ ആക്ടീവായ അർബുദകോശനശീകരണ ഗുണങ്ങൾക്ക് കാരണമാകുന്ന സംയുക്തങ്ങളെ തിരിച്ചറിഞ്ഞു .

PAI അണ്ഡാശയാർബുദകോശങ്ങളിലും വൻകുടൽ അർബുദകോശങ്ങളിലും *അനാമിർട്ട കോക്യുലസി*ന്റെ ഈഥൈൽ അസറ്റേറ്റ് എക്സ്ട്രാക്ടിന്റെ പ്രവർത്തനം പ്രശംസനീയമായിരുന്നു ,രണ്ടു അർബുദകോശങ്ങളിലും ഉയർന്ന സൈറ്റോടോക്സിസിറ്റി .IC_{50} മൂല്യം യഥാക്രമം 8.30±0.38 µg/ml ഉം 17.97±0.63 µg/ml ഉം ആയിരുന്നുഈഥൈൽ . അസറ്റേറ്റ് എക്സ്ട്രാക്ടുHT29 കോശങ്ങളിലും PA1 കോശങ്ങളിലും കോളനി രൂപീകരണം തടയുകയും കോശമരണത്തിലേക്ക് നയിക്കുകയും ചെയ്തുകോശങ്ങളിൽ മുകുള രൂപീകരണം . എ നശീകരണം എന്നീ സവിശേഷതകളാൽ .എൻ .ഡി ,ക്രൊമാറ്റിൻ കണ്ടൻസേഷൻ .കോശമരണം സ്ഥിരീകരിച്ചു

മീലിയ വൂബിയ ഇലകളുടെ ക്ലോറോഫോം എക്സ്ട്രാക്ട് സിലിക്കാജെൽ കോളം ക്രൊമറ്റോഗ്രാഫിക്ക് വിധേയമാക്കിആന്റികാൻസർ ശേഷിയുള്ള ഫ്രാക്ഷൻ , കണ്ടെത്തി . ശുദ്ധീകരിക്കാത്ത ക്രൂഡ് എക്സ്ട്രാക്ടിന്റെIC_{50} മൂല്യം 61.26±2.56 µg/ml ആയിരുന്നു . അതേ സമയം കൂടുതൽ ശുദ്ധീകരിച്ച ഫ്രാക്ഷനുകളായSC1-F8, SC2-F8, SC3-F8, TLC-B3 എന്നിവ IC_{50} മൂല്യങ്ങൾ യഥാക്രമം 31.07±4.46, 17.86±1.29, 10.27±0.7 ,9 7.87±1.32 µg/ml എന്ന രീതിയിൽ പ്രദർശിപ്പിച്ചു .TLC-B3 ഫ്രാക്ഷൻ GC-MS വിശകലനത്തിനു വിധേയമാക്കിഅതിൽ അതിശക്തമായ . .ആന്റികാൻസർ ശേഷിക്ക് കാരണമായേക്കാവുന്ന പത്തു സംയുക്തങ്ങൾ തിരിച്ചറിഞ്ഞു

*മീലിയ വൂബിയ*യുടെ ശുദ്ധീകരിച്ച എക്സ്ട്രാക്ടിന്റെ സൈറ്റോടോക്സിസിറ്റി വിശകലനത്തിലൂടെ HT29, MCF7, PA1, UM-SCC-83B കോശങ്ങളിലെ കാര്യമായ മരണ നിരക്ക് കണ്ടെത്തി ശുദ്ധീകരിക്കപ്പെട്ട .SC3-F8 ഫ്രാക്ഷൻ ഈ കോശങ്ങളിൽ കോളനി രൂപീകരണവും (അപൊപ്ടോസിസ്) സെൽ മൈഗ്രേഷനും കോശമരണവും , ഫലപ്രദമായ ി പ്രതിരോധിച്ചു 48 മൈറ്റോകോൺഡ്രിയൽ മെമ്പ്രേയ്ൻ പൊട്ടൻഷ്യൽ . അനക്സിൻ .മണിക്കൂറിനു ശേഷം കുറവു കാണിച്ചു-V/പ്രൊപിഡിയം അയൊഡൈഡ് സ്റ്റെയിനിംഗ് അപൊപ്റ്റോട്ടിക് കോശങ്ങളുടെ എണ്ണം വർദ്ധിക്കുന്നതായി കാണിച്ചു . പരീക്ഷിച്ച അർബുദകോശങ്ങളിൽ എല്ലാം നീളം കൂടിയ വാലുകളോടുകൂടിയ ധൂമകേതുക്കൾ കാണപ്പെട്ടുഎയ്ക്ക് കേടുപാടുകൾ സംഭവിച്ചിരിക്കുന്നത് .എൻ .ഇതിൽ നിന്നും ഡി . കൂടാതെ .പ്രകടമായി SC3-F8, കോശചക്രത്തിലെ G1 ഘട്ടത്തെ പ്രതിരോധിക്കുകയും കോശമരണത്തെ നിയന്ത്രിക്കുന്ന ജീനുകളെ നിയന്ത്രിക്കുകയും കോശമരണത്തിലേക്ക് നയിക്കുന്ന ജീനുകളെ ഉത്തേജിപ്പിക്കുകയും ചെയ്യുന്നതായി കണ്ടുഈ ഫ്രാക്ഷൻ . എപിതീലിയൽ മീസൻകൈമൽ ട്രാൻസിഷൻ ,അർബുദകോശങ്ങളുടെ മൈഗ്രേഷനും)EMTപാതയിലെ ഘടകങ്ങളായ മാംസ്യങ്ങളുടെ രൂപീകരണവും നിയന്ത്രിക്കുന്നതായും (കൂടാതെ അർബുദത്തിന്റെ മറ്റൊരു സവിശേഷതയായ പുതിയ .കാണാൻ കഴിഞ്ഞു രക്തക്കുഴലുകളുടെരൂപീകരണ ആന്റിജിയോ) ജെനസിസ്തെ ഈ ഫ്രാക്ഷൻ (.നിയന്ത്രിക്കുന്നതായും വ്യക്തമായി

SC1-F8 എന്ന ഫ്രാക്ഷൻ ലിപോസോമുകളിൽ സംയോജിപ്പിച്ചപ്പോൾ ഇതിന്റെ IC_{50} മൂല്യം കുറയുകയും .തന്മാത്രകളുടെ വിതരണത്തെ പരിപോഷിപ്പിക്കുകയും ചെയ്തു , ഇൻസിലിക്കോ പഠനത്തിലൂടെTLC-B3 ലെ പെട്രൊസെലിനിക് ആസിഡും -9 ഒക്ടാഡെക്കനോയിക് ആസിഡും കാസ്പേസ് ,9-കാസ്പേസ് ,3-P53 എന്നീ അപൊപ്ടോടിക് മാംസ്യങ്ങളുമായുള്ള സമ്പർക്കം കോശമരണത്തിലേക്ക് നയിച്ചേക്കാം എന്നു തിരിച്ചറിഞ്ഞുഈ സംയുക്തങ്ങളുടെ ആന്റികാൻസർ സാധ്യതകൾ . .അന്വേഷിക്കുന്നത് മൂല്യവത്താണ്

Introduction

0.1.1. Cancer

Cancer is a highly complex and multifactorial condition characterized by the unregulated proliferation of cells. It arises due to multiple genetic mutations that disrupt the regulatory mechanisms controlling cell growth, division, and death. These aberrant cells form tumours that can invade neighbouring tissues and metastasize to distant organs, significantly impacting the body's normal functions (Cooper, 2000). If untreated, cancer can lead to severe morbidity and mortality.

Cancers are classified based on the origin of the affected tissue and cell type. The major categories include carcinomas, which originate from epithelial cells; sarcomas, from mesenchymal cells; leukaemia, lymphomas, and myelomas, from hematopoietic cells; and cancers of the brain and spinal cord, from cells of the central nervous system (Wani and Malik, 2021). The development of cancer, or carcinogenesis, is a multifactorial process involving both genetic predispositions and environmental exposures (Parsa, 2012).

Understanding cancer involves studying its biology, risk factors, progression, and the complex interplay between genes and the environment. This knowledge is crucial for developing effective diagnostic tools, treatments, and preventive strategies. Despite significant advancements in cancer research and treatment, challenges remain in managing this disease due to its heterogeneity and the potential for resistance to therapies. Consequently, ongoing research and innovation are essential to improve patient outcomes and reduce the global burden of cancer.

0.1.2. Global cancer statistics

Cancer remains one of the leading causes of death globally. In 2022 alone, there were approximately 20 million new cases and 9.7 million deaths worldwide. By 2050, these figures are expected to surge to 35 million new cases and 15.3 million deaths annually (Figure 0.1.1). Typically, cancer rates are highest in countries with high life expectancy, education levels, and living standards. However, for certain

cancers like cervical cancer, the highest incidence rates are found in countries with lower rankings in these areas.

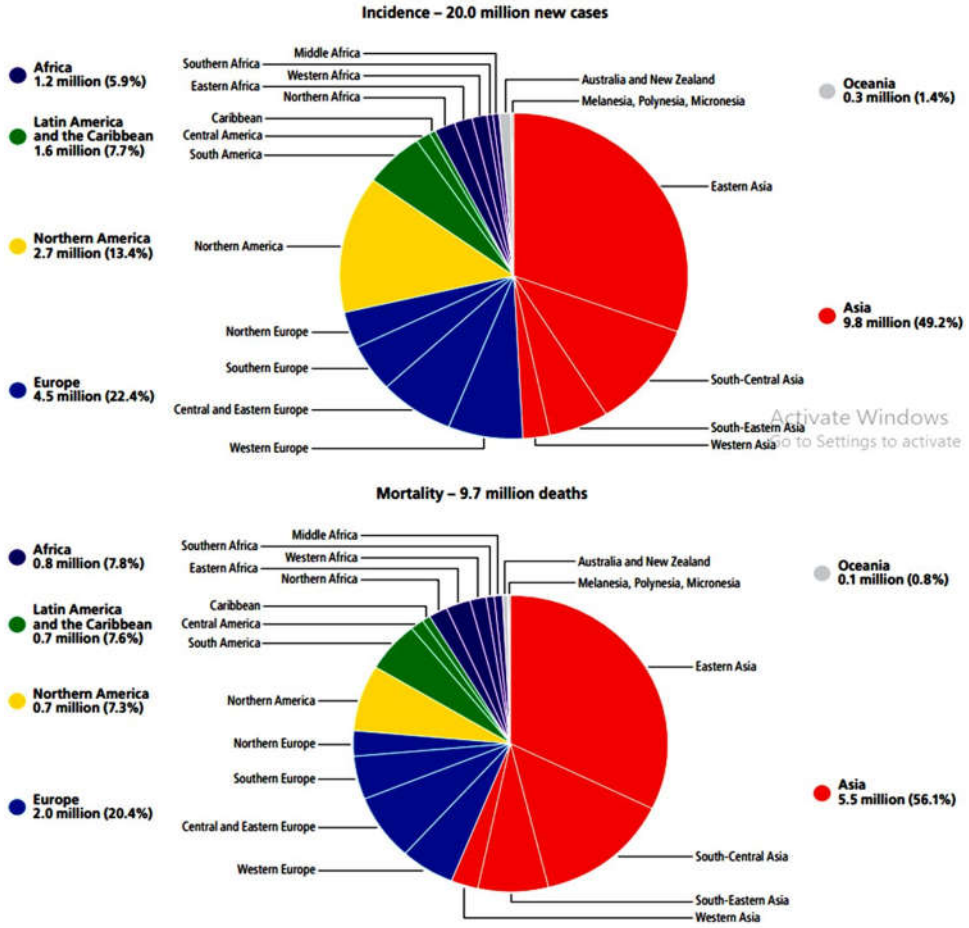


Figure 0.1.1. Estimated number of new cancer cases and cancer mortality in million by World region, 2022 (Source: GLOBOCAN 2022-gco.iarc.fr/today).

The most prevalent cancers, in descending order of estimated new cases, include lung cancer (24,80,675) breast cancer (22,96,840), colorectal cancer (19,26,425), prostate cancer (14,67,854), stomach cancer (9,68,784), liver cancer (8,66,136), thyroid cancer (8,21,214), cervix uteri cancer (6,62,301), bladder cancer (6,14,298), non-Hodgkin lymphoma (5,53,389), oesophagus cancer (5,11,054), pancreatic cancer (5,10,992), leukemia (4,87,294), kidney and renal pelvis cancer (4,34,840), and corpus uteri cancer (4,20,368) (Figure 0.1.2 A). Lung, prostate, and colorectal cancers are projected to make up 48% of all cancer diagnoses in men (Figure 0.1.2 B), while breast, lung, and colorectal cancers are expected to account for 51% of diagnoses in women (Figure 0.1.2 C).

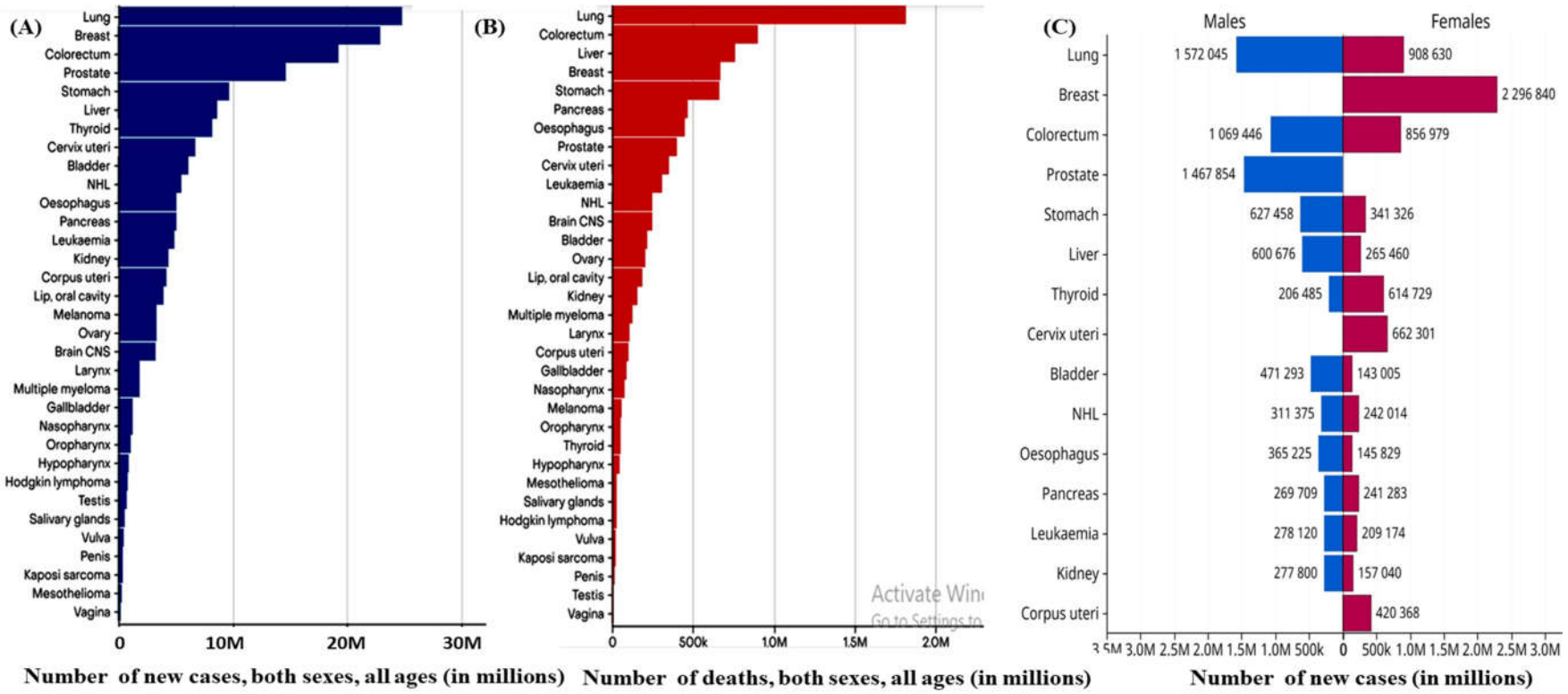


Figure 0.1.2. Global cancer site ranking in 2022 (A) Incidence – Number of new cases in both sexes in all age groups (in million), (B) Incidence – Number of deaths in both sexes in all age groups (in million), (C) Incidence –Number of new cases with absolute numbers in females and males separately. (Source: Cancer TODAY | IARC - https://gco.iarc.who.int **Data version**: Globocan 2022 (version 1.1) - 08.02.2024)

Worldwide, the incidence of cancer stands at 213 cases per 100,000 men and 186 cases per 100,000 women, with significant regional variation ranging from 4 to 5 times different (Figure 0.1.3). This disparity is primarily due to variations in significant cancer risk factors and the accessibility of screening and diagnostic services. In males, Eastern Europe has the highest mortality rate at 160 per 100,000, with lung and colorectal cancers being the most common. For females, the highest rates are observed in Melanesia, with an incidence rate of 116 per 100,000 and Southern Africa, at 108 per 100,000, experience a substantial burden of breast and cervical cancers. In the US, the cancer incidence rate is 440.5 per 100,000 annually (based on 2017-2021 data), with a mortality rate of 146.0 per 100,000 per year (based on 2018–2022 data). Among racial and ethnic groups, non-Hispanic Black men have the highest mortality rate at 208.3 per 100,000, while non-Hispanic Asian/Pacific Islander women have the lowest at 82.6 per 100,000 (GLOBOCAN, 2022).

Social factors such as human development indices (HDI), age, and sex also influence cancer incidence. Cancer was the primary cause of premature death (before the age of 70) in 57 out of 183 countries in 2019, primarily those with very high HDI, and the second-leading cause in 55 countries, mostly high HDI. In 71 countries with medium or low HDI, cancer ranked lower due to higher deaths from infectious diseases like malaria, HIV/AIDS, and tuberculosis. However, cancer is becoming an increasing concern due to longer life expectancy, population growth, and the adoption of unhealthy Western lifestyles. In 2022, 47% of new cancer cases were in very high-HDI countries, which house only 21% of the global population. Cancer deaths are less common in very high-HDI countries (37%), indicating better survival rates. The largest rise in cancer cases is anticipated in low-Human Development Index (HDI) countries, where the incidence is expected to double by 2050. These nations are the least prepared to manage the growing cancer burden due to limited healthcare infrastructure and resources. Cancer rates rise with HDI for incidence but not for mortality, particularly among women, due to variations in cancer distribution, detection rates, and treatment access. Disparities are most pronounced among women in low-HDI countries, where cancer is less common but mortality rates are highest (ACS, 5th edition).

As of January 2022, the US had an estimated 18.1 million cancer survivors, and this number is expected to rise to 22.5 million by 2032. Approximately 40.5% of people will be diagnosed with cancer at some point in their lives, according to 2017–2019 data. In 2024, about 14,910 children and adolescents (ages 0 to 19) are expected to be diagnosed with cancer, with 1,590 expected deaths (Gallicchio *et al.,* 2022).

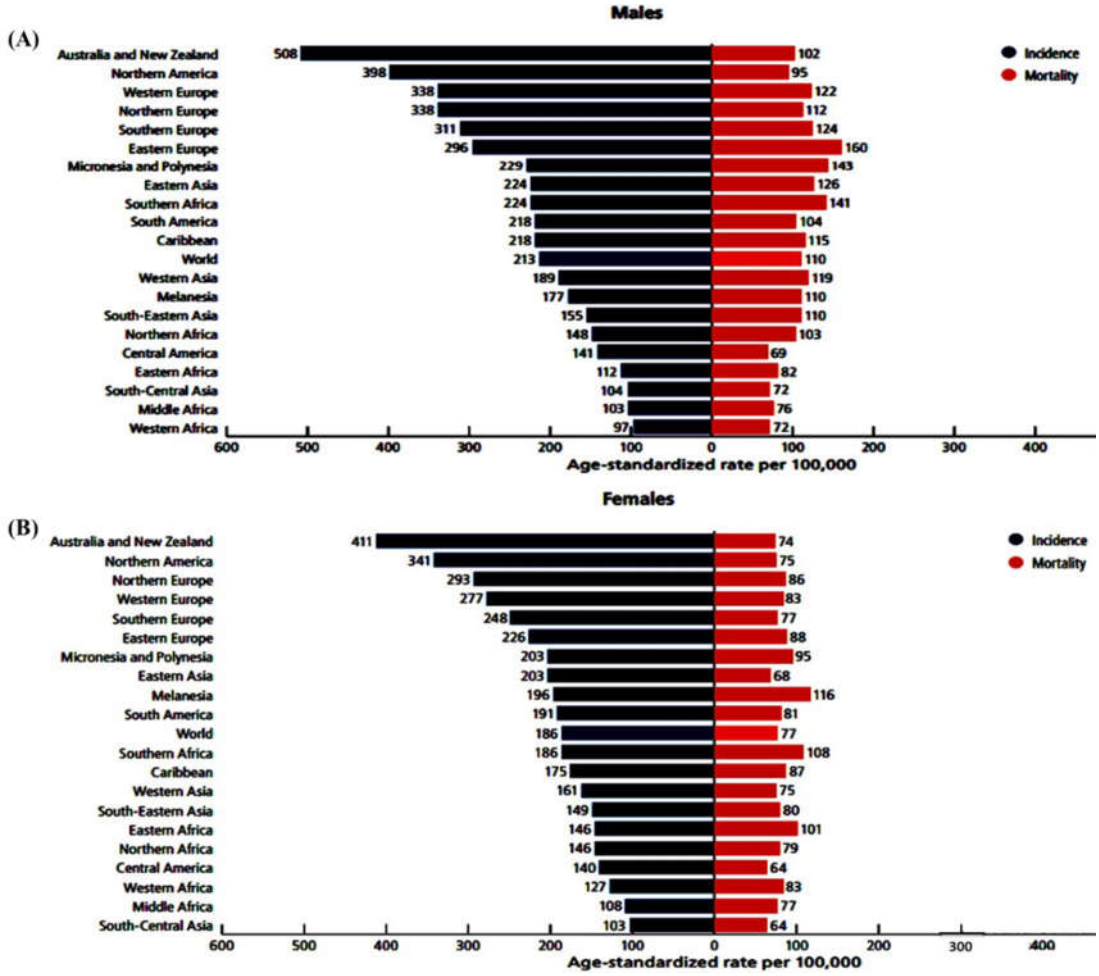


Figure 0.1.3. Global cancer incidence and mortality rates by sex for 20 world regions, 2022 in millions. (A) Incidence and mortality rates in males in million. (B) Incidence and mortality in females in millions. Source: GLOBOCAN 2022 (gco.iarc.fr/today/).

Global cancer statistics provide essential insights that inform research, diagnosis, treatment, and outcomes. Ongoing surveillance, research, and adherence to screening guidelines are critical in the global fight against cancer. International collaborations and data sharing are vital for advancing our understanding of cancer and developing effective strategies to reduce its impact on individuals and healthcare systems worldwide.

0.1.3. Cancer in India

Cancer poses a major public health challenge in India, with numerous studies highlighting its prevalence, management, and impact on the healthcare system. These studies emphasize the complex nature of cancer care and the need for a comprehensive strategy to address this growing issue.

In India, cancer types vary across regions and demographic groups. According to the American Cancer Society, there were 1,413,316 new cancer cases in India in 2022, with an incidence rate of 98.5 per 100,000. Cancer-related deaths are also significant, with 916,827 fatalities and a mortality rate of 64.4 per 100,000. The top three cancers in terms of both incidence and mortality are breast cancer, lip and oral cavity cancers, and cervical cancer. Among women, breast, cervical, and ovarian cancers are the most common, while in men, lip and oral cavity cancers, lung cancer, and esophageal cancer are leading causes of death (Figure 0.1.4). Breast cancer, in particular, is a growing concern in India, mirroring global trends, with a steady increase in cases reported (GLOBOCAN, 2022).

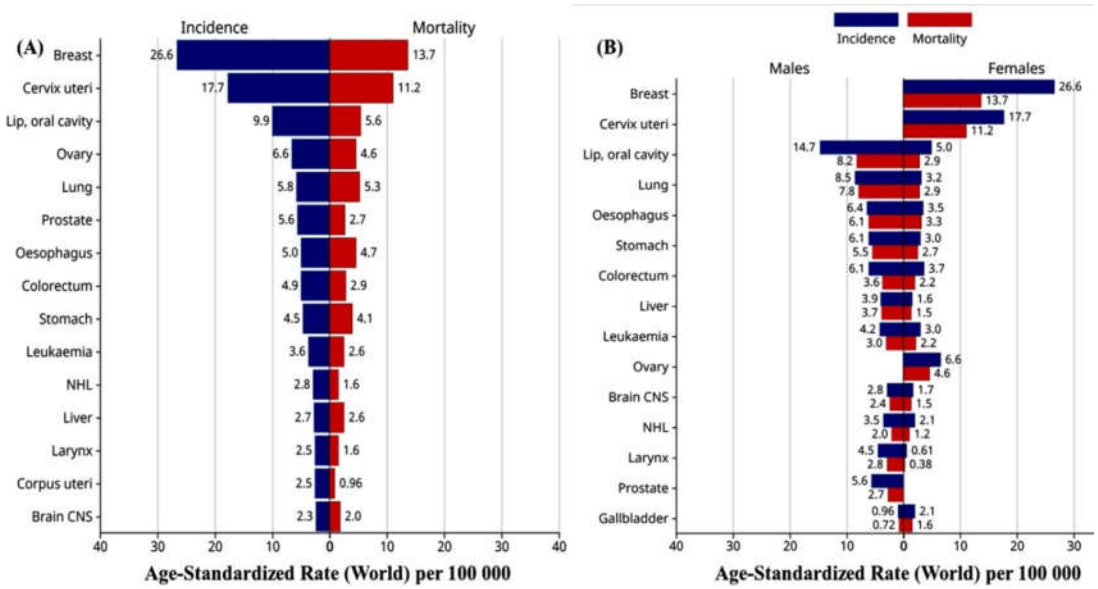


Figure 0.1.4. Incidence and mortality of cancer in India in 2022. (A) Age standardised rate (world) per 100000, incidence and mortality in both sexes in millions. (B) Age standardised rate (world) per 100000, incidence and mortality in males and females in millions. Source: GLOBOCAN 2022 (gco.iarc.fr/today/).

The impact of the COVID-19 pandemic on cancer care worldwide, including in India, has been profound (Ranganathan *et al.,* 2021). Singh *et al.* and Mitra *et al.* studied the pandemic's impact on cancer treatment in India. Singh *et al.,* assessed the preparedness of cancer hospitals and the changes in oncosurgical practices, while Mitra *et al.,* examined the challenges faced by cancer patients in accessing healthcare during the crisis. These studies highlighted the need for robust healthcare systems and adaptive strategies to ensure continuity of cancer care during global health emergencies (Singh *et al.,* 2021; Mitra *et al.,* 2022).

The body of research on cancer in India encompasses a wide range of topics, including cancer types, cervical cancer screening, radiotherapy advancements, cancer incidence and distribution, biomarkers for ovarian cancer, oral cancer diagnosis, the impact of COVID-19 on cancer care, and awareness of cervical cancer prevention among healthcare professionals (Prinja *et al.,* 2023). These studies collectively provide valuable insights into the current status of cancer management in India, highlighting both the challenges and opportunities for improving cancer care. Continued research and innovation are essential to address the complex and evolving landscape of cancer treatment and prevention in the country.

0.1.4. Plants as traditional medicine

For thousands of years, nature has been an invaluable source of medicinal products. According to the World Health Organization (WHO) defines traditional medicine as encompassing the full spectrum of skills, knowledge, and practices derived from the beliefs, and theories of different cultures. These are used to maintain health, prevent, diagnose, improve, or treat physical and mental illnesses. In ancient medicinal systems like those in India, China, and Africa, natural products have always been central. The WHO reports that approximately 80% of the world's population, mainly in developing countries, relies on plant-based traditional medicines for their primary healthcare needs (WHO, 2023).

Traditional medicine systems like Ayurveda, traditional Chinese medicine (TCM), and various African medicinal practices have utilized natural products extensively.

These systems have evolved over centuries, leveraging the medicinal properties of plants to treat a wide range of ailments. This reliance on nature's pharmacy is not just a relic of the past but continues to influence modern drug discovery and development processes (WHO, 2023).

The ethnomedical approach, highlighted by Fiorenza *et al.,* (1997), is a testament to the potential of traditional knowledge in discovering new drugs. This approach, which involves ethnobotany and traditional medicine, seeks to explore and validate the therapeutic uses of plants as practiced by indigenous cultures. It provides an alternative pathway to drug discovery, especially for diseases like non-insulin-dependent diabetes mellitus (NIDDM) (Fiorenza *et al.,* 1997).

Fabricant and Farnsworth discuss various methods for selecting higher plants as candidates for drug development. They emphasize the role of ethnomedicine, which uses information from traditional medicine systems to identify plants with high potential for therapeutic success (Fabricant and Farnsworth, 2001). This method has been particularly effective in regions such as China and the Himalayan countries, where ethnobotanical studies have been instrumental in drug production and the development of new drugs. Sheng-Ji underscores the importance of conserving traditional medical knowledge and medicinal plant resources, which are critical for ongoing and future drug discovery efforts (Sheng-Ji, 2001).

Natural products have been central to drug discovery, especially for agents targeting cancer and infectious diseases. For example, three plant materials endemic to Peru-Cat's claw (*Uncaria tomentosa*), Maca (*Lepidium meyenii*), and Dragon's blood (*Croton lechleri*)-have been extensively studied for their wide range of therapeutic uses. Gonzales *et al.* present an evidence-based analysis of the chemistry, biological properties, and anti-tumour activities of these plants, showcasing their potential as specific anti-cancer agents derived from traditional usage (Gonzales *et al.,* 2006).

Despite the proven efficacy of many medicinal plants, drug discovery from these sources is often constrained by the availability of adequate plant material, the capacity to perform high-throughput screening bioassays, and the ability to produce

bioactive compounds in large volumes. Ramawat *et al.* reviewed the use of herbs in traditional systems and the bioactive therapeutic molecules responsible for their medicinal activities (Ramawat *et al.,* 2009).

The discovery of drugs based on natural products depends significantly on advancements in the fields of biology and chemistry. Chantarasriwong *et al.* summarize explorations of the caged *Garcinia xanthones*, a family of plant metabolites with unique chemical structures, potent bioactivities, and promising pharmacology for drug design and development (Chantarasriwong *et al.,* 2010). Traditional medicine systems like Ayurveda, traditional Chinese medicine, and typically posit that the combined effects of all plant components enhance therapeutic efficacy to the greatest extent. Ulrich-Merzenich *et al.* provide examples of such synergistic effects among plant constituents (Ulrich-Merzenich *et al.,* 2010).

Saussurea lappa, known for its significant pharmacological activities, presents considerable potential for new drug discovery. Madhuri *et al.* explore the different phytoconstituents and pharmacological activities of *Saussurea lappa*, highlighting its promise as a source of new therapeutic agents (Madhuri *et al.,* 2012). There is, however, ongoing controversy about the efficacy of traditional medicine in guiding drug discovery. Saslis-Lagoudakis *et al.* employ phylogenetic methods from community ecology to reveal significant clustering of 1,500 traditionally used species, demonstrating shared phylogenetic patterns across different medicinal floras and their therapeutic uses (Saslis-Lagoudakis *et al.,* (2012).

Shikov *et al.* aimed to summarize and critically appraise data concerning plants used in Russian medicine. This knowledge can inform future research on the safe, evidence-based application of traditional Russian medicinal plants in European and global phytopharmacotherapy, as well as aid in the discovery of new drug leads. In certain regions, people strongly believe in the positive impact of plants on respiratory disorders, and they have used medicinal plants for these conditions since ancient times (Shikov *et al.,* 2014). Asadbeigi *et al.* highlight the importance of

herbal medicines and traditional medicine as resources for future drug discovery (Asadbeigi *et al.,* 2014).

Traditional medicine systems, including traditional Ayurveda, Chinese medicine, Unani, traditional Korean medicine, and Kampo, have developed into well-regulated medical practices. Yuan *et al.,* review the literature on the relationship among natural products, traditional medicines, and modern medicine, exploring concepts and methodologies from traditional medicines to further develop drug discovery (Yuan *et al.,* 2016). Indigenous cultures around the world have used herbal medicine to treat numerous ailments for centuries. Li and Weng provide an evolutionary and historical perspective on the significance of plants as medicines for humans (Li and Weng, 2017).

India, with its rich flora of indigenous medicinal plants, has used these resources in traditional Indian medicine for centuries. Mohanraj *et al.* present IMPPAT, a comprehensive online database of 1,742 Indian medicinal plants, 9,596 phytochemicals, and 1,124 therapeutic uses, facilitating computational approaches to natural-product-based drug discovery (Mohanraj *et al.,* 2018). Recently, attention has increased on the pharmacognostical, phytochemical, and pharmacological studies of traditional medicinal plants. Süntar emphasizes ethnopharmacological aspects of herbal medicine and the plant-based drug discovery process, discussing key issues in their use as complementary medicine (Süntar, 2019).

Other influential works in this field include contributions from Kinghorn *et al.,* Cos *et al.,* Pieters *et al.,* and Itokawa *et al.* each of which has significantly advanced the understanding and potential of natural products in drug discovery (Kinghorn *et al.,* 1993; Cos *et al.,* 2008; Pieters *et al.,* 2005; Itokawa *et al.,* 2008).

The successful identification and development of new drugs from natural products depend on advanced biological and chemical research. As highlighted by Batova *et al.*, Chantarasriwong *et al.,* and Ulrich-Merzenich *et al.* understanding the chemical properties and synergistic effects of plant constituents is crucial. This comprehensive approach not only honours traditional knowledge but also applies

rigorous scientific methods to validate and expand upon it (Batova *et al.,* 2010; Chantarasriwong *et al.,* 2010; Ulrich-Merzenich *et al.,* 2010).

The on-going research and development efforts in this field, including those by Madhuri *et al.* and Saslis-Lagoudakis *et al.* continue to reveal the vast potential of natural products in modern medicine. By bridging the gap between traditional practices and contemporary scientific research, these studies contribute to the discovery of new, effective therapeutic agents that can address a wide range of health issues, from cancer and infectious diseases to respiratory and neurological disorders (Madhuri *et al.,* 2011; Saslis-Lagoudakis *et al.,* 2012).

Ultimately, the integration of traditional knowledge with modern scientific techniques offers a promising pathway for future drug discovery and development. The collaborative efforts of researchers globally, as demonstrated by the works cited, underscore the importance of preserving traditional medical knowledge while advancing our understanding of natural products' therapeutic potential. This dual approach not only enriches the field of pharmacology but also enhances global health outcomes by providing new and effective treatments derived from nature.

0.1.5. Importance of natural products in cancer treatment

Natural products have long played a crucial role in cancer treatment, offering a wealth of bioactive compounds with unique mechanisms of action that can target various pathways involved in cancer development and progression. Historically, many cancer treatments have been derived from natural sources. For instance, the discovery of paclitaxel (Taxol) from the Pacific yew tree (Suffness, 1993) and the vinca alkaloids from the Madagascar periwinkle plant (Moudi *et al.,* 2013) are landmark examples. These compounds have become cornerstone treatments for various cancers, demonstrating the profound potential of natural products in oncology. Natural products provide an unparalleled diversity of chemical structures that are often more complex than synthetic compounds. This diversity allows for the discovery of unique molecules that can interact with biological targets in ways that synthetic compounds may not. This complexity can lead to the development of

novel drugs with unique mechanisms of action, enhancing the arsenal of available cancer therapies.

Many natural compounds exhibit multi-targeted actions, meaning they can simultaneously affect multiple pathways involved in cancer cell survival, proliferation, and metastasis. For example, curcumin, derived from turmeric, has been shown to influence several signaling pathways, including those related to inflammation, apoptosis, and cell cycle regulation (Liczbiński *et al.,* 2020). This multi-targeted approach can be particularly beneficial in treating complex diseases like cancer, where multiple pathways are often dysregulated. Natural products often exhibit lower toxicity compared to conventional chemotherapeutic agents. This is particularly important in cancer treatment, where the therapeutic window is narrow, and minimizing adverse effects can significantly improve patient quality of life. Compounds like epigallocatechin gallate (EGCG) from green tea (Wang and Bachrach, 2002) and resveratrol from grapes (Rauf *et al.,* 2018) have shown potential anti-cancer properties with minimal toxicity. They can enhance the efficacy of conventional cancer treatments and may act synergistically with chemotherapy and radiation, potentially allowing for lower doses of these drugs while maintaining or even improving therapeutic outcomes. For example, studies have shown that compounds like quercetin and genistein can enhance the sensitivity of cancer cells to chemotherapy (To and Cho, 2021).

They often work through novel mechanisms that are distinct from those of traditional chemotherapeutic agents. For instance, certain natural compounds can modulate the immune system to enhance anti-tumour immunity or inhibit angiogenesis, the process by which tumours develop new blood vessels to sustain their growth (Wu *et al.,* 2021). This can provide new avenues for treatment, particularly for cancers that are resistant to existing therapies.

0.1.6. Present treatment modalities and their shortcomings

Cancer treatment has witnessed significant progress in recent decades, offering a variety of options to combat different types and stages of the disease. These methods

encompass a range of approaches, including local treatments like surgery and radiation therapy, as well as systemic treatments such as chemotherapy, immunotherapy, targeted therapy, stem cell transplantation, and hormone therapy (Baykara, 2015). While these treatments have demonstrated effectiveness in many cases, they are not without limitations and drawbacks, prompting the ongoing search for improved therapeutic strategies.

Surgery continues to be a fundamental approach in cancer treatment, especially for localized tumours that can be effectively removed through resection. The primary objective of surgery is to remove the tumour along with surrounding healthy tissue to ensure complete eradication of cancer cells. However, surgery has several limitations. It is invasive and carries inherent risks such as infection, bleeding, and damage to adjacent structures. Furthermore, surgery may not be feasible for metastatic cancers, and achieving complete tumour removal is not always possible, leading to the potential for recurrence (Tringale *et al.,* 2018).

Chemotherapy involves the use of drugs to target and destroy rapidly dividing cancer cells throughout the body. While chemotherapy is effective against various cancers, it lacks specificity and can also damage healthy cells that undergo rapid proliferation. Consequently, chemotherapy is associated with notable side effects, including fatigue, immunosuppression, hair loss, nausea and vomiting. Moreover, the toxic nature of chemotherapy limits the doses that can be administered, potentially compromising its efficacy. Additionally, cancer cells can develop resistance to chemotherapy over time, rendering treatment less effective (Zeimet *et al.,* 2009).

Radiation therapy utilizes high-energy particles or waves to target and damage cancer cells. It is particularly effective for localized tumours and is often used in conjunction with surgery or chemotherapy. However, radiation therapy can cause collateral damage to surrounding healthy tissues, leading to side effects such as skin burns, fatigue, and long-term organ damage. Furthermore, there is a risk of developing secondary cancers due to exposure to radiation, and the efficacy of

radiation therapy may be limited by tumour location and type (Jaffray and Gospodarowicz, 2015).

Targeted therapy involves drugs or substances designed to specifically target cancer cells by interfering with specific molecules involved in cancer growth and progression. While targeted therapy is often more precise and less toxic than traditional chemotherapy, it is typically effective only against cancers with specific genetic mutations or markers. Moreover, cancer cells can develop resistance to targeted therapies, necessitating the development of alternative treatment strategies (Tsimberidou, 2015).

Immunotherapy harnesses the body's immune system to fight cancer by enhancing the immune response against cancer cells. While immunotherapy has shown promise in treating certain types of cancer, it can cause immune-related side effects such as inflammation and autoimmune reactions. Additionally, the response to immunotherapy can vary widely among patients, with some experiencing significant benefits while others see limited or no improvement (Kennedy and Salama, 2020).

Bone marrow or Stem cell transplantation involves the replacement of damaged or destroyed bone marrow with healthy stem cells, allowing normal blood cell production to be restored. Although this approach can be curative for some certain blood cancers, it carries substantial risks, such as, graft-versus-host disease, infection and organ damage. Furthermore, finding a compatible donor can be challenging, and patients must undergo intensive chemotherapy or radiation therapy before transplantation, which can have severe side effects (Pavletic and Armitage, 1996).

Hormone therapy is used to treat hormone-dependent cancers, like breast and prostate cancers, by either inhibiting the body's hormone production or interfering with the hormones' effects on cancer cells. This therapy is primarily effective for hormone-sensitive cancers and can cause side effects including fatigue, hot flashes, and a higher risk of other health issues. Moreover, not all patients respond to

hormone therapy, and resistance can develop over time (Abraham and Staffurth, 2016).

Despite the advantages in cancer treatment, several shortcomings persist (Chakraborty and Rahman, 2012). A major concern is the severe side effects that accompany many therapies, often diminishing patients' quality of life and hindering adherence to treatment plans. Over time, cancer cells may develop resistance to these treatments, leading to disease progression and eventual failure of therapy. Furthermore, some treatments are limited in their efficacy, only working for specific cancer types or a stage, which leaves patients with advanced or metastatic conditions with fewer viable options. Additionally, many treatments cause toxicity not only to cancer cells but also to healthy cells, resulting in systemic harm and potential long-term health issues. Even advanced therapies like targeted therapy and immunotherapy, while promising, are often prohibitively expensive, making them inaccessible to many patients.

While current cancer treatments have significantly improved patient outcomes, they are not without limitations and drawbacks. Addressing these shortcomings will require continued research and innovation to develop more effective, targeted, and less toxic therapies. Additionally, efforts to improve treatment accessibility and affordability are essential to ensure that all patients have access to the care they need. By addressing these challenges, we can move closer to our goal of effectively managing and ultimately curing cancer.

0.1.7. Need for alternative cancer treatments

The need for alternative cancer treatments arises from the limitations and significant side effects associated with conventional therapies, alongside the potential benefits and promising efficacy of plant-derived compounds. Conventional cancer treatments, including chemotherapy, radiation therapy, and surgery, often come with a host of severe side effects and limitations. Chemotherapy, for example, targets rapidly dividing cells but lacks specificity, leading to the destruction of healthy cells, including those in the hair follicles, bone marrow, and gastrointestinal tract. This

results in side effects like nausea, vomiting, hair loss, and immunosuppression. Radiation therapy, while more localized, can still cause damage to surrounding healthy tissues, leading to skin burns, fatigue, and long-term damage to organs. Surgical interventions, though necessary in many cases, can be invasive and may not always guarantee the complete removal of cancerous cells, potentially leading to recurrence.

Plant-derived compounds offer several potential benefits that address some of the shortcomings of conventional cancer treatments. These natural products often exhibit a range of biological activities that can target cancer cells more selectively, reducing harm to normal cells. Many plant compounds, such as curcumin from turmeric, have been shown to affect multiple cellular pathways, including those involved in inflammation, apoptosis (programmed cell death), and cell cycle regulation (Moon, 2024). This multi-targeted approach can be particularly beneficial in treating complex diseases like cancer, where multiple pathways are often dysregulated.

They generally exhibit lower toxicity compared to traditional chemotherapeutic agents. This lower toxicity profile can lead to fewer side effects and better patient quality of life. For instance, resveratrol from grapes and epigallocatechin gallate (EGCG) from green tea have demonstrated anti-cancer properties with minimal adverse effects (Amin *et al.,* 2021). Natural compounds can enhance the efficacy of conventional cancer therapies. They may act synergistically with chemotherapy and radiation, potentially allowing for lower doses of these treatments while maintaining or improving therapeutic outcomes. Studies have shown that compounds like quercetin and genistein can increase the sensitivity of cancer cells to chemotherapy.

Natural products often work through novel mechanisms that are distinct from those of traditional chemotherapeutic agents. For instance, some natural compounds can modulate the immune system to enhance anti-tumour immunity or block angiogenesis, the process by which tumours form new blood vessels to support their growth. Several plant-based drugs have been developed and approved for use in

cancer treatment, highlighting the therapeutic potential of natural products (Nan *et al.,* 2023).

Derived from the Pacific yew tree (*Taxus brevifolia*), paclitaxel (Taxol) is a widely used chemotherapeutic agent that stabilizes microtubules and inhibits cell division, effectively treating various cancers, including ovarian, breast, and lung cancers (Weaver, 2014). Vinblastine and vincristine, obtained from the Madagascar periwinkle (*Catharanthus roseus*), are used to treat cancers, including Hodgkin's lymphoma, non-Hodgkin's lymphoma, and leukemia. They work by inhibiting microtubule formation, thus preventing cell division (Sharma *et al.,* 2022). Camptothecin, originally derived from the Chinese tree *Camptotheca acuminata*, and its derivatives, such as irinotecan and topotecan, inhibit the enzyme topoisomerase I, which is essential for DNA replication (Martino *et al.*, 2017; Basili and Moro, 2009). These drugs are used to treat colorectal and ovarian cancers. Etoposide, derived from the mayapple plant (*Podophyllum peltatum*) (Canel *et al.,* 2000), is used to treat lung cancer, testicular cancer, and lymphoma. It works by inhibiting topoisomerase II, leading to DNA damage and cell death (Hande, 1998).

The need for alternative cancer treatments is driven by the desire to find more effective, less toxic therapies that can complement or replace conventional options. Plant-derived compounds offer significant promise in this regard, providing unique mechanisms of action, multi-targeted effects, and potential synergy with existing treatments. Continued research into these natural products is essential to fully realize their potential in cancer therapy and improve patient outcomes.

Objectives

1. Screening of plant extracts to identify anticancer activity.

2. Study of the differential cytotoxicity of selected extracts on cancer cell lines and analysis of its composition by GC-MS.

3. Bioactivity guided enrichment of anticancer molecules from the leaf extract of *Melia dubia* Cav.; Characterisation and *in silico* studies.

4. Study of anticancer activity of leaf extract from *Anamirta cocculus* and active fraction from *Melia dubia*.

5. Liposome incorporation of active fraction from *Melia dubia*: Characterization and cytotoxicity analysis.

0.3.1. Overview of cancer and classification

Cancer, a complex and multifaceted disease, represents a significant challenge to global health due to its diverse manifestations and profound impact on affected individual and society at large. The aberrant proliferating nature in cancer condition disrupts normal cellular functions, leading to the formation of tumours and compromising the function of vital organs.

The understanding of cancer has evolved significantly over the years, driven by extensive research efforts aimed at unraveling its molecular underpinnings, elucidating its pathogenesis, and developing effective treatment modalities. Key studies, such as those by Lu *et al.*, Koizumi *et al.* and Li *et al.* have shed light on various aspects of cancer biology, including the role of genetic predisposition, tumour microenvironment, and cancer stem cells in disease progression (Lu *et al.,* 2005; Koizumi *et al.,* 2007; Li *et al.,* 2015).

Classification of cancer is crucial for effective diagnosis, prognosis, and treatment planning. Studies by Chen *et al.* and Bray *et al.* have provided valuable insights into the epidemiology and burden of cancer, highlighting the prevalence of specific cancer types and their associated mortality rates. Common cancer types such as lung cancer, stomach cancer, colorectal cancer, and breast cancer, continue to pose significant challenges to healthcare systems worldwide, understanding the importance of targeted interventions and preventive measures (Chen *et al.,* 2012; Chen *et al.,* 2017; Bray *et al.,* 2018).

Furthermore, advancements in cancer research, as exemplified by studies on cancer metastasis, cancer-associated thrombosis (Razak *et al.,* 2018), and biomarker detection (Khanmohammadi *et al.,* 2019), have paved the way for innovative diagnostic and therapeutic approaches. These developments hold promise for

improving patient outcomes and enhancing our ability to combat this formidable disease.

Thus, cancer represents a complex interplay of genetic, environmental, and lifestyle factors, leading to heterogeneous manifestations and treatment responses. By integrating insights from diverse disciplines and leveraging cutting-edge technologies, researchers continue to unravel the mysteries of cancer, paving the way for more targeted, personalized, and effective approaches to prevention, diagnosis, and treatment.

0.3.2. Cancer

According to American Cancer Society (ACS), cancer is a collection of diseases marked by the uncontrolled growth and spread of abnormal cells. This definition encapsulates the fundamental essence of cancer pathology, emphasizing the dysregulated cellular proliferation and dissemination that underpin its malignant behavior. The ACS definition underscores the diverse and multifaceted nature of cancer, encompassing a broad spectrum of malignancies with varying histological origins and clinical presentations.

0.3.2.1. Characteristics of cancer

0.3.2.1.1. Cellular abnormality

At the heart of cancer lies the aberrant behavior of cells, deviating from their normal physiological functions to proliferate uncontrollably. While cells are the fundamental units of life, facilitating essential processes such as tissue repair and growth, cancerous cells undergo malignant transformation, losing their specialized functions and proliferating indiscriminately. This loss of cellular identity and function underscores the pathological basis of cancer, wherein normal regulatory mechanisms governing cell growth and division are subverted, paving the way for tumour formation (Swanton *et al.*, 2024).

0.3.2.1.2. Uncontrollable proliferation

Fundamental to cancer progression is the uncontrolled proliferation of malignant cells, driven by dysregulated cell cycle progression. Unlike normal cells, which undergo regulated division to maintain tissue homeostasis, cancer cells circumvent growth control mechanisms, perpetuating their unbridled proliferation. This unchecked cellular replication results in the accumulation of abnormal cells, culminating in the formation of disorganized masses or tumours, a hallmark feature of cancer pathology (Tufail *et al.,* 2024).

0.3.2.1.3. Invasive behaviour

A distinguishing feature of cancer is its invasive propensity, wherein malignant tumours infiltrate and destroy surrounding tissues, compromising organ function and integrity. Unlike benign growths, which remain localized and encapsulated, malignant tumours exhibit invasive behavior, penetrating adjacent tissues and facilitating metastatic spread to distant sites (Welch, D. R., and Hurst, 2019). This invasive capacity not only exacerbates tissue damage but also complicates treatment strategies, necessitating comprehensive approaches to mitigate disease progression and metastatic dissemination.

0.3.2.2. Classification of cancer

Cancer is a diverse group of diseases categorized by their cell type and primary site of origin. Histologically, cancers fall into six main categories (Visvader, 2011):

Carcinoma: The most common type, comprising about 90% of human cancers, arises from epithelial tissues lining internal and external body surfaces. Subtypes include adenocarcinoma (from glandular tissues) and squamous cell carcinoma (from squamous epithelium), found in organs like the skin, lungs, and gastrointestinal tract.

Sarcoma: These uncommon cancers arise from connective tissues such as fat, muscles and bones. Examples include chondrosarcoma, osteosarcoma and liposarcoma, each reflecting the characteristics of its tissue of origin.

Myeloma: Originates from plasma cells in the bone marrow, leading to abnormal cell proliferation, bone destruction, and immune dysfunction. Multiple myeloma is the most common form, disrupting normal hematopoiesis.

Leukemia: Known as "liquid cancer," leukemia arises from the bone marrow, causing the overproduction of abnormal white blood cells. It is classified into subtypes like AML, CML, ALL, and CLL, each with distinct features.

Lymphoma: Develops from lymphoid tissues and is divided into Hodgkin lymphoma, characterised by the presence of Reed-Sternberg cells, and non-Hodgkin lymphoma, which includes various malignancies with different prognoses.

Mixed Types: These cancers exhibit mixed histological features from multiple cell lineages, such as adenosquamous carcinoma and carcinosarcoma, posing diagnostic and therapeutic challenges.

Cancers are also classified by their primary site:

Head and Neck Cancer: Begins in squamous cells in the mouth, throat, or less commonly, in salivary glands, sinuses, or nearby muscles.

Skin Cancer: Includes squamous cell carcinoma, basal cell carcinoma and melanoma, with UV radiation serving as a primary cause, particularly for aggressive melanomas.

Lung Cancer: Often detected late, leading to high mortality.

Breast Cancer: Common in women over 50, with risks including family history and lifestyle factors.

Prostate Cancer: Affects older men and shares symptoms with benign conditions.

Colon and Rectal Cancer: Among the most common cancers, with symptoms like blood in the stool and changes in bowel habits.

Uterine Cancer: The most common gynecologic cancer, mainly affecting older women, typically presents with abnormal bleeding.

Metastatic Cancer develops when cancer cells spreads from their initial site to other areas of the body, while still being referred to by the name of the the original cancer. Common metastatic sites include bones, liver, and lungs. Understanding metastasis is vital for effective treatment (https://training.seer.cancer .gov/disease/categories/site.html).

0.3.2.3. Development of cancer and causes

Cancer is characterized by the clonal origin of tumours, which develop from single cells that begin to proliferate abnormally. This concept has been illustrated by examining X chromosome inactivation (Wang *et al.,* 2020). In female cells, one of the two X chromosomes is randomly silenced during embryonic development, resulting in different cells having different inactive X chromosomes. Consequently, normal tissues in heterozygous females express both alleles of an X chromosome gene. Tumour tissues, however, generally express only one allele, indicating that all tumour cells are derived from a single progenitor cell with a fixed X inactivation pattern.

At the cellular level, cancer development involves mutations and the selection of cells with enhanced abilities for proliferation, survival, invasion, and metastasis (Cooper, 2000). The first step, tumour initiation, results from a genetic alteration that leads to the abnormal proliferation of a single cell. This proliferation results in a population of clonally derived tumour cells. Tumour progression advances as further mutations arise within this cell population (Nowell, 1976). Certain mutations may confer a selective advantage, such as faster growth, leading to clonal selection where the descendants of these mutated cells dominate the tumour population. This process of clonal selection continues throughout tumour development, resulting in tumours that grow more rapidly and become increasingly malignant.

Carcinogens, substances that cause cancer, have been identified through research involving experimental animals and epidemiological studies analysing cancer prevalence in humans. For example, the high incidence of lung cancer among cigarette smokers highlights a clear link. The complexity of cancer development,

being a multistep process, means that many factors can affect the probability of developing cancer. It is overly simplistic to attribute most cancers to single causes. However, numerous agents, including chemicals, radiation and viruses, are known to induce cancer in both animals and humans.

Radiation and many chemical carcinogens act by damaging DNA and inducing mutations. These carcinogens are often referred to as initiating agents, as the induction of mutations in key target genes is the initial event leading to cancer development. Examples include solar ultraviolet radiation, which is a leading cause of skin cancer, and carcinogenic chemicals present in tobacco smoke (Cooper, 2000). Tobacco smoke contains compounds such as benzo(a)pyrene and dimethylnitrosamine, which are major factors that contribute to the development of cancer in humans (Hecht and Hatsukami, 2022). Smoking is the leading cause of 80 to 90% of lung cancers and is also linked in cancers of the oral cavity, larynx, pharynx esophagus, and other sites (Cooper, 2000). Overall, smoking is estimated to be responsible for nearly one-third of all cancer deaths, highlighting its significant impact as a carcinogenic agent.

Certain carcinogenic agents promote cancer progression by enhancing cell proliferation rather than directly inducing genetic mutations. These substances, known as tumour promoters, facilitate increased cell division, which is essential for expanding the cell population during the early stages of tumourigenesis. A notable example is phorbol esters, which activate protein kinase C to drive cell proliferation. This mechanism was elucidated in studies on chemically induced skin tumours in murine models, where tumour formation occurred only when a tumour promoter was used to stimulate the growth of pre-existing mutated cells (Evans and Farrar, 1998).

Hormones, especially oestrogens, act as tumour promoters in some human cancers (Cooper, 2000, Henderson and Feigelson, 2000). Oestrogens stimulate the proliferation of uterine endometrial cells, and excess oestrogen exposure increases the likelihood of developing endometrial cancer (Yang, *et al.,* 2019). Extended use of high-dose oestrogen alone for postmenopausal hormone replacement therapy

markedly elevates this risk. The risk is minimized by administering progesterone to counteract oestrogen's stimulatory effect on proliferation of endometrial cells (Furness, *et al.,* 2012). However, long-term therapy with oestrogen and progesterone combinations may increase the risk of breast cancer.

In addition to chemicals and radiation, some viruses induce cancer in both experimental animals and humans. Human cancers caused by viruses include liver cancer and cervical carcinoma, which together account for 10 to 20% of worldwide cancer incidence. These viruses are significant not only as causes of cancer but also because studies of tumour viruses have been crucial in understanding the molecular events that lead to cancer induced by both viral and nonviral carcinogens (McLaughlin-Drubin and Munger, 2008).

0.3.2.4. Mechanism of malignant transformation

While significant strides have been made in elucidating the biological and molecular mechanisms underlying primary tumour formation, understanding the subsequent processes leading to invasion and metastasis has remained somewhat enigmatic until recent years. Over the past five years, various avenues of research have begun to outline the conceptual framework explaining the emergence of high-grade malignancies. These discussions often revolve around the widely acknowledged sequence termed the "invasion-metastasis cascade," which describes the sequential steps through which primary tumour cells invade adjacent tissues, enter the bloodstream, migrate to distant sites, and establish secondary tumours (Valastyan and Weinberg, 2011).

Empirical observations are crucial for resolving debates regarding the timing of metastatic trait acquisition, particularly in the context of early versus late mutations. The complexity of the invasion-metastasis cascade parallels that of the initial steps leading to primary tumour formation, prompting questions about whether acquiring metastatic traits necessitates accumulating mutations comparable to those driving primary tumour initiation.

Mounting evidence suggests that metastatic dissemination may not depend on additional late-acquired genetic mutations beyond those present in primary tumours. Discussions center on "driver" mutations that confer selective advantages rather than "passenger" mutations representing background genetic noise (Bozic, *et al.,* 2010). Evidence against the necessity of metastasis-specific mutations comes from diverse sources.

Firstly, gene expression profiles of primary tumours can predict their propensity for metastatic relapse, reflecting the differentiation program of the normal cell of origin perturbed by subsequent mutations. For instance, gene expression arrays have enabled the prediction of metastatic potential in primary breast carcinomas, suggesting that many neoplastic cells within a tumour express genes influencing metastatic behaviour early in tumour progression (Weigelt, *et al.,* 2003).

Secondly, research involving the transformation of normal human cells into tumourigenic states has demonstrated that the differentiation program of the normal cell of origin strongly influences tumour behaviour. For instance, transformed mammary epithelial cells yielded tumours with distinct histopathological characteristics and metastatic tendencies depending on their culture conditions. Similarly, transformed melanocytes generated primary tumours with vastly different metastatic potentials, underscoring the role of the normal cell of origin in determining metastatic behaviour (Ince, *et al.,* 2007).

Thirdly, studies comparing the genomes of primary tumour cells with their derived metastases have generally found the same genetic lesions in both, suggesting that metastatic spread does not necessarily require late-acquired mutations. Together, these lines of evidence suggest that metastatic dissemination may occur as an inadvertent byproduct of primary tumour formation rather than as an actively selected trait (Yates, *et al.,* 2017).

The epithelial – mesenchymal transition (EMT) emerges as a key process in cancer pathogenesis, facilitating the acquisition of invasive and metastatic traits by carcinoma cells. EMT, which occurs in various embryonic developmental stages,

enables epithelial cells to acquire mesenchymal traits, promoting their motility and invasiveness. Transcription factors (TFs) regulate EMT transiently during embryogenesis in response to contextual signals from the surrounding microenvironment (Ribatti, *et al.*, 2020).

These TFs, including Slug, Snail, and Twist, are expressed in human tumour cells, correlating with aggressive traits like invasion and resistance to apoptosis (Yu, *et al.*, 2010). Their expression may be regulated by signals from neighbouring cells, mirroring the contextual regulation observed in embryogenesis. The relevance of EMT to cancer pathogenesis is underscored by the resemblance between aggressive carcinoma cells and embryonic cell phenotypes (Nakamura, *et al.*, 2018).

Metastatic dissemination in cancer appears to be influenced significantly by the differentiation program of the normal cell of origin and may not necessarily require additional late-acquired genetic mutations. EMT and its regulatory TFs play crucial roles in facilitating invasive and metastatic behaviours in carcinoma cells, drawing parallels with mechanisms observed in normal embryonic development (Lu and Kang, 2019). This understanding shifts the focus from late-acquired genetic mutations to the intrinsic properties of tumour cells shaped early in their development.

Using an in vitro model of keratinocyte transformation, studies have shown that certain genetic modifications significantly increase the frequency of spontaneous transformation. For example, primary keratinocytes with specific genetic alterations display higher transformation rates than control genotypes, and the introduction of exogenous factors can suppress malignant transformation (Thépot, *et al.*, 2011).

Further investigations into the mechanisms of action of certain apoptotic pathways have found that they are independent of traditional regulatory proteins like bcl-2 and bax. These pathways are active only in malignant cells, not in normal or activated lymphocytes (Qian, *et al.*, 2022). Additionally, studies on malignant hypertension have demonstrated that certain molecular changes, such as reduced ADAMTS-13 levels and endothelial dysfunction, play critical roles in the disease's pathogenesis

(van den Born, *et al.*, 2008). Superoxide anion, for instance, contributes to various forms of hypertension through mechanisms dependent on nitric oxide.

Research has also explored the role of microRNAs in the malignant transformation of cells exposed to carcinogens like arsenic. Findings indicate that depletion of specific microRNAs is causally linked to malignant transformation and tumour formation following arsenic exposure (Nail, *et al.*, 2023). This highlights the complex interplay of genetic and environmental factors in cancer development.

Malignant multiple sclerosis (MS), a severe form of the disease, is defined by rapid progression to significant disability within a short time from symptom onset. Studies have examined the incidence and predictors of malignant MS, revealing insights into the demographic and clinical characteristics of affected patients (Gholipour, *et al.*, 2011). Other research has focused on the potential biases in epidemiological studies, such as the inverse association between smoking and melanoma, suggesting that smoking-related competing risks might skew the observed relationships (Thompson, *et al.*, 2013).

The mechanisms of DNA damage in cadmium-induced malignant transformation have been elucidated, showing that cadmium can cause significant genomic instability, leading to cancer. Similarly, research on malignant progression in certain tumour types has revealed that some tumours can evolve to higher grades, underscoring the need for long-term follow-up in patients with initially lower-grade tumours.

The mechanisms of DNA damage in cadmium-induced malignant transformation have been elucidated, showing that cadmium can cause significant genomic instability, leading to cancer (Zhou, *et al.*, 2014). Similarly, research on malignant progression in certain tumour types has revealed that some tumours can evolve to higher grades, underscoring the need for long-term follow-up in patients with initially lower-grade tumours.

Investigations into the therapeutic potential of compounds like apigenin have shown promising effects on malignant mesothelioma cell lines, suggesting possible new treatment avenues (Masuelli, *et al.*, 2017). The carcinogenic mechanisms of malignant melanoma have been reviewed, considering factors such as ultraviolet radiation, mechanical stress, and trauma.

In the context of gastric cancer, research has focused on the regulatory mechanisms underlying malignant transformation induced by specific nitrosamine compounds (Toh, *et al.*, 2020). Models of gastric epithelial cell transformation have been established to study these processes. Additionally, studies on the biological characteristics and functions of circular RNAs (circRNAs) in paediatric malignant solid tumours have advanced our understanding of their roles in cancer development (Galardi, *et al.*, 2022; Shen, *et al.*, 2022).

Recent research has explored the role of exosomes in gastric cancer progression, revealing that certain long non-coding RNAs (lncRNAs) from cancer cells can enhance malignant transformation of normal cells. This highlights the importance of intercellular communication in tumourigenesis (Hu, *et al.*, 2021 and Sun, *et al.*, 2023).

Endometriosis – associated ovarian cancer (EAOC) is another area of focus, with studies summarizing the latest advancements in understanding the biological characteristics and early diagnosis of malignant transformation in endometriosis (Kobayashi, *et al.*, 2023 and Steinbuch, *et al.*, 2024). This research aims to improve diagnostic strategies and patient outcomes.

0.3.2.5. Cancer progression and metastasis

The process of cancer progression and metastasis is a multifaceted journey marked by a series of interconnected events, each playing a crucial role in determining the fate of malignant cells. Metastasis, the dissemination of cancer cells from the primary tumour to distant locations in the body, represents a major challenge in cancer treatment and is a leading cause of cancer-related deaths (Arvelo, *et al.*,

2016). Despite significant advancements in our understanding of cancer biology and treatment modalities, metastasis remains a formidable adversary, often leading to therapy failure and poor patient outcomes.

Metastasis begins with the dissemination of cancer cells from the primary tumour (Figure 0.2.1), followed by their infiltration into nearby tissues and eventual entry into the bloodstream or lymphatic system (Lugassy and Escande, 1997). Once in circulation, these circulating tumour cells (CTCs) face numerous challenges, including immune surveillance and harsh environmental conditions, before successfully extravasating into secondary sites and establishing metastatic colonies. This intricate process, often referred to as the "invasion-metastasis cascade," involves several discrete steps, each presenting unique opportunities for therapeutic intervention (Martin, *et al.*, 2013).

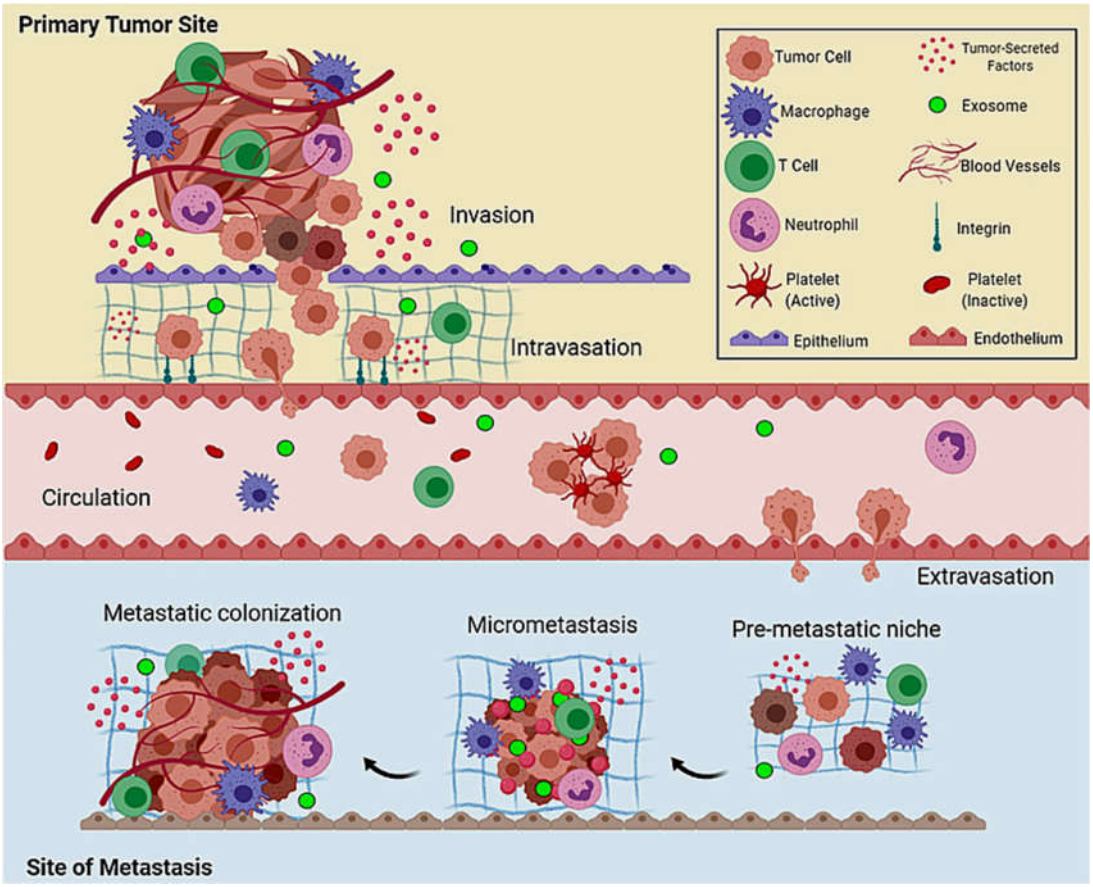


Figure 0.2.1. Overview of the metastatic cascade: The five key steps of metastasis include invasion, intravasation, circulation, extravasation, and colonization (Fares *et al.,* 2020).

Genetic and epigenetic alterations drive the acquisition of metastatic properties by cancer cells, leading to the selection and expansion of subpopulations with enhanced invasive and migratory capabilities (Jones and Laird, 1999; Sharma *et al.*, 2010). Despite the vast number of cancer cells entering the bloodstream, only a minute fraction successfully survives and establishes metastatic colonies, highlighting the inefficiency of the metastatic process. However, this small subset of metastatic cells possesses remarkable plasticity and adaptability, allowing them to overcome the myriad challenges encountered during metastatic dissemination.

Epithelial – mesenchymal transition (EMT) stands as a critical process in cancer progression and metastasis, enabling epithelial cells to acquire mesenchymal traits associated with increased motility and invasiveness. EMT is driven by complex signaling networks involving various growth factors, cytokines, and transcription factors, orchestrating the downregulation of epithelial markers and the upregulation of mesenchymal markers (Ribatti *et al.,* 2020). This phenotypic switch not only enhances the migratory capacity of cancer cells but also promotes resistance to conventional therapies, posing significant challenges in clinical management.

The tumour microenvironment (TME) plays a pivotal role in modulating cancer progression and metastasis, providing a supportive niche for the survival and growth of metastatic cells. Inflammatory mediators, immune cells, and stromal components within the TME create a pro-tumourigenic milieu, fostering immune evasion, angiogenesis, and tissue remodelling. Chronic inflammation fuels the production of cytokines and chemokines that promote tumour growth and metastasis, highlighting the intricate interplay between inflammation and cancer progression (Wang *et al.*, 2023).

Angiogenesis, the process of new blood vessel formation, is essential for supplying nutrients and oxygen to growing tumours and facilitating metastatic spread. Angiogenic factors like vascular endothelial growth factor (VEGF) promote the formation of new blood vessels, while antiangiogenic factors help maintain vascular quiescence (Saman *et al.*, 2020). Dysregulated angiogenesis contributes to tumour

progression and metastasis, underscoring the therapeutic potential of targeting angiogenic pathways in cancer treatment.

The establishment of pre-metastatic niches, orchestrated by primary tumours, primes distant organs for subsequent metastatic colonization. Extracellular vesicles, secreted factors, and interactions between cancer cells and the stromal components contribute to the formation of these supportive niches, providing a fertile soil for incoming metastatic cells (Ghoroghi *et al.*, 2021). Age-related changes in the extracellular matrix (ECM) and immune responses influence metastasis patterns, with older patients exhibiting altered ECM complexity and increased lymphatic permeability.

Cancer cell plasticity, characterized by dynamic transitions between differentiated and stem-like states, facilitates therapy resistance and malignant progression. This phenotypic plasticity allows cancer cells to adapt to changing microenvironmental cues and evade therapeutic interventions, posing significant challenges in clinical management (Bhat *et al.*, 2024). Targeting key regulators of cancer cell plasticity holds promise for disrupting metastatic progression and overcoming therapy resistance in cancer patients.

Despite significant progress in our understanding of cancer progression and metastasis, numerous challenges remain in translating these insights into effective therapeutic strategies. The heterogeneity of cancer cells, dynamic nature of the tumour microenvironment, and complex interplay between metastatic cells and host tissues underscore the need for innovative approaches in cancer treatment. By unravelling the intricacies of cancer metastasis and developing targeted therapies that disrupt key molecular pathways, we can hope to improve patient outcomes and ultimately conquer this formidable foe.

0.3.2.6. Biology of cancer

0.3.2.6.1. Carcinogenesis

Carcinogenesis is the process by which normal cells transform into cancerous cells, characterized by uncontrolled growth and the ability to invade tissues (Parodi and

Mancuso, 1996). This transformation is driven by genetic mutations, such as point mutations, chromosomal imbalances, and epigenetic modifications, like DNA methylation, which can silence tumour suppressor genes. While external factors like chemical damage can trigger these changes, most mutations arise during DNA replication and become heritable. The proliferation of mutated cells allows further genetic alterations, leading to the evasion of regulatory mechanisms. Key mutations occur in oncogenes, which drive cell proliferation, and tumour suppressor genes, which inhibit growth. As mutations accumulate, cancer cells gain the ability to grow autonomously and resist apoptosis, highlighting the genetic basis of cancer development.

External agents like chemicals and radiation can cause DNA damage, leaving molecular signatures that indicate exposure to carcinogens. DNA replication errors, spontaneous damage (like depurination and cytidine deamination), and oxidative by-products of metabolism are common sources of mutations. If these mutations persist due to improper DNA repair, they can lead to cancer. Additionally, ionizing radiation and UV light can directly damage DNA, causing breaks and thymine dimers, respectively, which distort normal base pairing and lead to mutations.

0.3.2.6.2. Tumour microenvironment and metastasis

Cancer begins with premalignant lesions caused by genetic alterations or environmental factors, such as viral infections. These changes eventually convert premalignant cells into malignant ones, resulting in primary tumours. The tumour microenvironment (TME), comprising the extracellular matrix (ECM), stromal cells, and a network of cytokines, chemokines, and growth factors, play a critical role in cancer development. These components facilitate communication between tumour and non-tumour cells, supporting tumour growth (Neophytou *et al.,* 2021).

Tumour angiogenesis, the formation of new blood vessels, is driven by hypoxia and nutrient deprivation in the TME. Inflammatory signals recruit vascular cells to form the tumour's blood vessel network, enabling tumour growth. As tumours acquire more mutations, cancer cells develop the ability to migrate, leading to metastasis.

This process relies on the creation of a pre-metastatic niche, allowing tumour survival at distant sites (Neophytou *et al.,* 2021).

0.3.2.6.3. Cancer progression

Cancer is a progressive condition that initially manifests as a small, benign lesion restricted to its original tissue (Shomar *et al.,* 2022). If detected early, it can be surgically removed, preventing patient death. However, if undetected, cancer can grow and spread within the organ, eventually invading nearby tissues and the lymphatic system. From there, cancer cells spread to distant organs, forming metastases in areas like bones, lungs, liver, or brain. This dissemination is often supported by angiogenesis, which supplies the tumour with oxygen and nutrients, promoting further growth (Gerstberger *et al.,* 2023).

0.3.2.6.4. DNA damage and repair in cancer

DNA instability, caused by replication errors and environmental factors, is a major contributor to cancer. Cells with unrepaired DNA damage accumulate mutations, leading to uncontrolled cell proliferation. Environmental carcinogens, like radiation and chemical agents, also cause DNA damage, creating mutations that can trigger cancer. Ionizing radiation, for instance, causes DNA strand breaks, while UV light induces thymine dimers, both of which distort DNA and increase the risk of mutations (Turgeon *et al.,* 2018).

Cancer treatments, such as chemotherapy and radiation, often target cancer cells by inducing DNA damage. However, these therapies also raise the risk of secondary cancers, as they can damage healthy DNA. Alkylating agents and antibiotics like Doxorubicin are examples of treatments that increase cancer risk by altering DNA. This highlights the need for a delicate balance between effectively treating cancer and maintaining the integrity of healthy DNA (Turgeon *et al*., 2018).

Carcinogenesis is a complex process driven by genetic mutations and environmental factors. The tumour microenvironment and angiogenesis play critical roles in

supporting tumour growth and metastasis. Early detection is crucial, as cancer is most treatable in its benign stages before spreading. Understanding the molecular mechanisms of cancer development is essential for developing effective therapies, and advances in genomic research and personalized medicine offer promising avenues for targeted treatments that disrupt the molecular drivers of cancer. By continuing to explore the biology of cancer, we can improve patient outcomes and develop more effective, less toxic therapies (Turgeon *et al.,* 2018).

0.3.2.6.5. Mechanisms of carcinogenesis

Recent studies have outlined key steps in transforming normal cells into cancerous ones by violating three fundamental rules. The first rule mandates that cells divide only in response to appropriate signals. Cancer cells bypass this by permanently activating cell division pathways, usually triggered by hormones or growth factors. The second rule states that cells should undergo self-destruction under stressful or unsuitable conditions for DNA replication. In cancer cells, this pathway is evaded through mutations in the *RB1* gene and the *TP53* gene, which disable the P53 protein's ability to halt cell division under stress. The third rule restricts normal cells to a finite number of divisions, regulated by telomeres (Martin *et al.,* 2013 and Sever and Brugge, 2015). Cancer cells bypass this limitation by activating telomerase, which maintains telomere length and enables indefinite cell division, effectively granting them "permanent cell youth."

At the molecular level, these transformations disrupt normal cell functions and often lead to apoptosis, a process that eliminates abnormal cells. For cancer cells to thrive, they must simultaneously coordinate these changes, influenced by genetic predisposition and environmental factors. Genetic factors can enhance the likelihood of multiple simultaneous mutations, while environmental factors act as selective forces, allowing more fit abnormal cells to survive and proliferate.

Despite their diversity, cancer cells share common regulatory mechanisms in cell proliferation and death. Oncogenes and tumour suppressor genes are frequently altered across various cancers, forming a network of factors that control cell behaviour. This network, or "cancer box," includes three main signalling processes: two growth-promoting and one growth-suppressive (Baker, 2020). The growth-promoting processes involve beta-catenin, which, when mutated, stimulates cell proliferation, and cell-surface receptors like EGFR, which activate intracellular signalling cascades. The anti-proliferative process is controlled by the *TP53* gene, which encodes the P53 protein, a stress sensor that halts cell cycle progression, induces differentiation, or triggers apoptosis (He and Gan, 2023).

A unique chromosomal locus at the end of chromosome 9's short arm contains the *CDKN2a* gene, which produces p16 and p14ARF proteins. P16 inhibits cyclin-dependent kinases (CDKs), blocking cell proliferation, while p14ARF regulates P53 activation (Wagner and Wagner, 2022). This locus integrates various components of the cancer box, making it a frequent target for alterations in cancer. Overall, the representation of cell life cycles—including division, differentiation, and programmed death—highlights the interconnected roles of these genes and their mutations, with p53 serving as a universal "brake" on cell division.

0.3.2.7. The hallmarks of cancer

Tumours are complex tissues composed not only of proliferating cancer cells but also of various recruited normal cells within the tumour-associated stroma. These normal cells actively participate in tumourigenesis, contributing significantly to the development and expression of hallmark capabilities. Over the past decade, advancements in cancer research have refined our understanding of these capabilities and prompted consideration of new additions to the existing roster, necessitating a broader perspective on tumour biology that includes the dynamic influence of the tumour microenvironment.

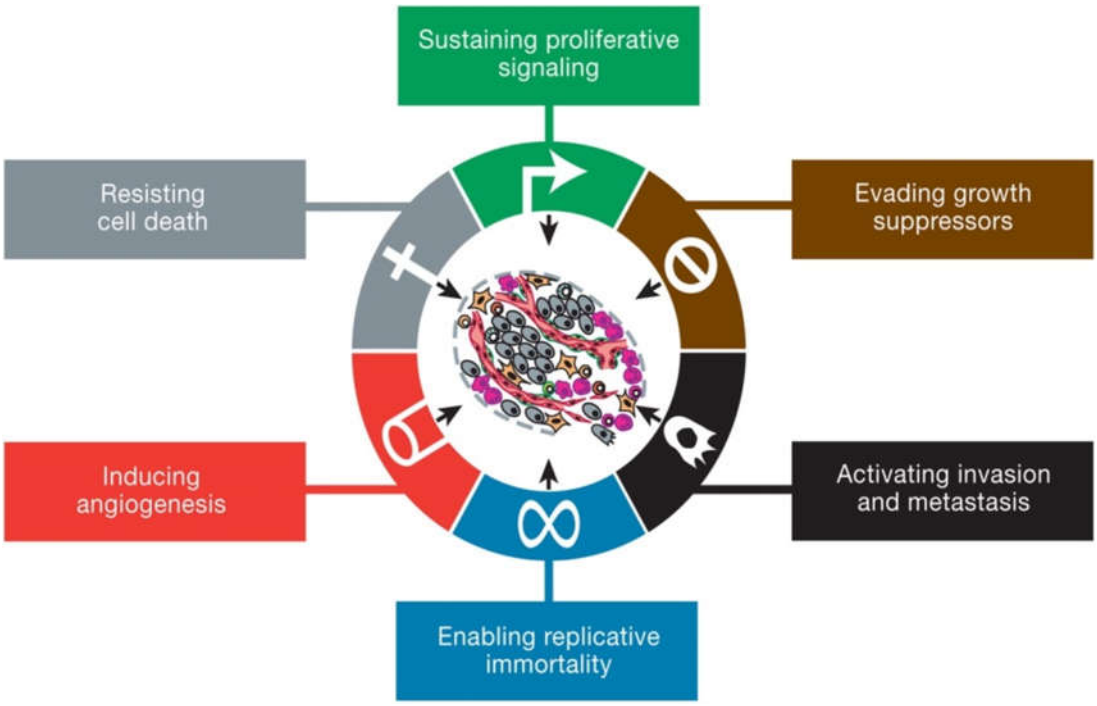


Figure 0.2.2. The Hallmarks of Cancer. This graphic outlines the six hallmark characteristics first introduced in our 2000 overview. Over the last ten years, significant advancements have been made in comprehending the underlying mechanisms associated with each hallmark. (Hanahan and Weinberg, 2011).

Conceptually, the hallmarks of cancer—fundamental capabilities enabling tumour growth and metastasis—continue to provide a foundational framework. The main hallmarks (Figure 0.2.2) are:

0.3.2.7.1. Sustaining proliferative signalling

Cancer cells autonomously maintain continuous proliferation through various mechanisms, such as producing their own growth factors, influencing stromal cells to supply growth factors, enhancing receptor expression, or activating downstream signalling pathways independent of ligand-mediated activation. These strategies subvert normal growth controls and contribute to uncontrolled cell division characteristic of cancer (Hanahan and Weinberg, 2011).

0.3.2.7.2. Evading growth suppressors

Cancer cells evade stringent regulatory mechanisms, including those mediated by tumour suppressor genes like *RB* and *TP53*. Loss of these regulators allows

unchecked cell proliferation by disabling critical checkpoints that inhibit cell division in response to growth-inhibitory signals, and compromising contact inhibition mechanisms essential for tissue architecture maintenance (Hanahan and Weinberg, 2011).

0.3.2.7.3. Resisting cell death

Cancer cells evade programmed cell death, particularly apoptosis, through mechanisms such as inactivating TP53, overexpressing antiapoptotic proteins, or downregulating proapoptotic factors. Additionally, autophagy and necrosis play dual roles in cancer progression, either promoting survival under stress conditions or inadvertently supporting tumour growth through inflammatory responses (Hanahan and Weinberg, 2011).

0.3.2.7.4. Enabling replicative immortality

Cancer cells achieve unlimited replicative potential by overcoming senescence and crisis barriers through telomere maintenance mechanisms, notably activating telomerase to sustain telomere length and avoid cellular aging-associated growth arrest. The intricate regulation of telomeres and alternative mechanisms of telomere maintenance underscore their role in promoting cancer cell proliferation (Hanahan and Weinberg, 2011).

0.3.2.7.5. Inducing angiogenesis

Tumour growth and survival depend on inducing angiogenesis, the process of forming new blood vessels. Cancer cells sustain an "angiogenic switch" by upregulating proangiogenic factors like VEGF-A and FGFs, thereby ensuring adequate nutrient and oxygen supply for continued growth. Disrupted vascular structure and interactions with inhibitory factors like TSP-1 highlight the complexity of tumour angiogenesis regulation (Hanahan and Weinberg, 2011).

0.3.2.7.6. Activating invasion and metastasis

Cancer cells acquire invasive properties through processes like epithelial-mesenchymal transition (EMT), allowing them to invade surrounding tissues and

metastasize to distant sites. Interactions with stromal cells further enhance invasive capabilities by promoting extracellular matrix degradation and altering cell adhesion properties, facilitating metastatic spread (Hanahan and Weinberg, 2011).

The hallmarks of cancer not only define the fundamental capabilities of cancer cells but also reflect the intricate interactions between cancer cells and their microenvironment. Integrating these hallmarks with genomic insights continues to advance our understanding of cancer biology and informs the development of targeted therapies aimed at disrupting critical pathways driving tumour progression. This comprehensive approach holds promise for improving treatment outcomes and addressing the challenges posed by tumour heterogeneity and therapeutic resistance.

0.3.2.8. Traditional treatments of cancer

Cancer is a complex series of pathological conditions characterized by a gradual loss of regulatory control over growth. For many decades, the primary treatment options for cancer were limited to surgery, radiation therapy, and chemotherapy, either as standalone treatments or in combination. However, recent advancements have dramatically improved our understanding of the pathways involved in cancer progression, leading to the development of combinatorial strategies that involve multiple targeted therapies or traditional chemotherapeutics, such as taxanes and platinum compounds, which have shown synergistic effects (Mokhtari *et al.*, 2017; Debela *et al.,* 2021). New approaches, including drugs, biological molecules, and immune-mediated therapies, are being explored, even though they have not yet fully met the desired therapeutic levels to significantly reduce mortality rates and extend survival times for metastatic cancer patients.

The development of new cancer therapies or the effectiveness of targeted drugs relies on the specific pathways and features of various tumour types. Chemotherapy continues to be one of the most effective and commonly utilized treatments for cancer, either as a standalone therapy or in conjugation with radiotherapy. Chemotherapy drugs primarily target tumour cells through genotoxicity, generates reactive oxygen species that destroy tumour cells. Hormonal treatments are also extensively used for specific types of cancer and are classified as cytostatic. They inhibit tumour progression by restricting the availability of hormonal growth factors

through the hypothalamic – pituitary – gonadal axis (HPGA), blocking hormone receptors, and reducing adrenal steroid production (Debela *et al.*, 2021).

0.3.2.9. Cancer treatment modalities

Cancer treatment approaches can be categorized into conventional (traditional) and advanced (novel or modern) types. Worldwide, more than half of all active medical treatment trials are dedicated to cancer therapies. The choice of treatment is influenced by factors such as the type of cancer, its location, and severity guide the selection of treatment options and their progress. Traditional methods commonly used include surgery, radiotherapy, and chemotherapy. In contrast, modern treatments encompass hormone therapy, anti-angiogenic therapies, stem cell therapies, immunotherapy, and dendritic cell – based immunotherapy (Arruebo *et al.*, 2011; Debela *et al.*, 2021).

0.3.2.9.1. Conventional cancer therapies

The most commonly recommended conventional cancer treatments include surgical tumour removal, followed by radiotherapy using X-rays and/or chemotherapy. Surgery is most effective in the early stages of cancer. Radiation therapy, however, can also harm healthy cells, organs, and tissues. Although chemotherapy has significantly decreased morbidity and mortality, it often damages healthy, rapidly dividing cells. Drug resistance is a major challenge, occurring when initially responsive cancer cells become resistant to the treatment, largely due to decreased drug absorption and increased drug expulsion. Conventional chemotherapy faces several limitations, including difficulties in dosage selection, lack of specificity, rapid drug metabolism, and adverse side effects.

Chemotherapy remains the most effective and widely used cancer treatment.it works mainly through genotoxicity, producing reactive oxygen species that destroy tumour cells (El-Hussein *et al.*, 2021). Hormonal treatments are also prevalent, especially for cancers influenced by hormonal growth factors. These treatments work blocking hormone receptors or limiting hormone production, thus restricting cancer growth.

0.3.2.9.2. Advanced and innovative cancer therapies

Modern cancer treatments include hormone therapy, stem cell therapy, anti-angiogenic therapy, immunotherapy, and dendritic cell-based immunotherapy (Charmsaz *et al.*, 2019). These therapies aim to address the limitations of traditional treatments, such as drug resistance and non-specific targeting of cancer cells.

0.3.2.9.2.1. Stem cell therapy

Stem cell therapy involves using undifferentiated cells from bone marrow or other sources to treat cancer (Debela *et al.,* 2021). These therapies are still largely experimental but show promise in regenerating damaged tissues and targeting tumours. Different types of stem cells used in cancer treatment include:

Pluripotent stem cells, thatvderived from embryos, these cells can become any cell type except placental cells. They are used to induce effector T cells and natural killer cells (Li *et al.*, 2018), as well as for anti-tumour vaccine preparation (Ouyang *et al.*, 2019).

Adult stem cells include mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and neural stem cells (NSCs). HSCs are utilised in FDA-approved treatments for leukemia and multiple myeloma (Trujillo-Alonso *et al.*, 2019), while MSCs and NSCs are explored for their roles in tissue repair and regeneration in cancer therapy (Lin *et al.*, 2019; Chu *et al.*, 2020).

Cancer stem cells (CSCs): These cells arise from normal stem cells through epigenetic mutations and are crucial for cancer growth, metastasis, and recurrence. Targeting CSCs could provide a promising approach for treating solid tumours (Chang, 2016).

0.3.2.9.2.2. Targeted drug therapy

Targeted cancer therapies interfere with specific molecules involved in cancer growth and progression. These therapies aim to be more effective and less harmful than conventional treatments by focusing on cellular mechanisms like cell cycle arrest, apoptosis induction, and metabolic interference (Valter *et al.*, 2017). The types of targeted agents include monoclonal antibodies and small molecules inhibitors.

Monoclonal antibodies: Man – made immune proteins that attack specific targets on cancer cells. They can recruit host immune functions, block essential cancer cell processes, or deliver lethal payloads to target cells (Adams *et al.*, 2005).

Small molecules inhibitors: Small molecules inhibitors: These drugs interfere with intracellular signalling pathways, such as tyrosine kinases, to inhibit cancer cell growth and proliferation. Examples include gefitinib, erlotinib, lapatinib, and sorafenib (Yap *et al.*, 2012; Debela *et al.*, 2021).

0.3.2.9.2.3. Ablation therapy

Ablation therapies destroy tumours without removing them, often using extreme temperatures (Debela *et al.*, 2021). Techniques such as radiofrequency ablation (RFA) that uses high-frequency electrical currents to generate heat and eliminate cancer cells, guided by imaging techniques like ultrasound or MRI, and cryoablation which utilizes extreme cold to freeze and destroy cancer cells. This technique has shown potential in eliciting immune responses that may aid in fighting cancer.

0.3.2.9.2.4. Gene therapy

Gene therapy involves introducing a functional copy of a defective gene into the genome to treat or cure certain genetic disorders. This approach is used to deliver proapoptotic genes, tumour suppressor genes, or genes that induce anti-tumour immune responses. Notable examples include the use of ONYX-015 for NSCLC and Gendicine for head and neck cancer (Ahrendt *et al.*, 2003).

0.3.2.9.2.5. RNA interference (RNAi)

This technology targets gene silencing through mechanisms like small interfering RNAs (siRNAs). siRNAs can be designed to block specific genes involved in cancer proliferation and metastasis, providing a highly targeted treatment approach (Debela *et al.,* 2021).

0.3.2.9.2.6. Natural antioxidants

Natural antioxidants, including polyphenols, vitamins and plant-derived compounds, are being researched for their anti-inflammatory and anti-cancer properties. Examples include curcumin, berberine, and quercetin, which have shown potential

in various cancer types (Chikara *et al.*, 2018; Farooqi *et al.*, 2019). However, their clinical application is limited by challenges related to bioavailability and toxicity.

0.3.2.9.3. Current clinical trials

Recent advancements in cancer treatment have led to numerous clinical trials focusing on innovative therapies. These trials aim to develop safe and effective cancer treatments, improving survival rates and quality of life for patients. Current research is particularly focused on stem cell therapy, targeted drug therapy, immunotherapy, and gene therapy (Debela *et al.*, 2021).

0.3.2.9.4. Key challenges in cancer treatment

Cancer treatment faces several key challenges across different therapeutic strategies, each with its own set of advantages and limitations (Table 0.1). One of the most significant challenges is the recurrence of cancer after initial treatment. Despite advancements in medical science, preventing cancer from returning remains a critical hurdle.

Traditional cancer treatments often focus on eradicating the tumour itself, potentially overlooking the underlying causes and risk factors. This can lead to a "one-size-fits-all" approach that may not address the unique conditions that contributed to the cancer or prevent future occurrences (Krzyszczyk *et al.*, 2018). Additionally, these treatments can inadvertently weaken the immune system, the body's natural defence mechanism, which can hinder the ability to fight cancer effectively and increase susceptibility to infections and other health complications. While chemotherapy and radiation can be life-saving, they often come with severe side effects such as nausea, vomiting, fatigue, and long-term bone marrow depression, significantly impacting quality of life (Amjad *et al.*, 2023). Furthermore, conventional treatments may not be equally effective against all types and stages of cancer, with some cancer cells developing resistance, rendering these therapies less effective.

Stem cell therapy is considered a promising approach due to its safety and effectiveness. It can significantly reduce tumour volumes and extend patient survival, especially when combined with other treatment strategies. However, the

benefits of stem cell therapy are not durable, meaning the effects may not last long-term. Additionally, there is a potential risk of tumourigenesis, where the stem cells themselves could contribute to the formation of new tumours, leading to recurrence.

Targeted therapy stands out for its high specificity, targeting cancer cells more precisely than traditional treatments like chemotherapy, thereby reducing adverse reactions. Despite this, the long-term side effects of targeted therapies are still in question, and there is always the risk of cancer cells developing resistance to these treatments, leading to recurrence. As cancer cells mutate, they may find ways to bypass the mechanisms of targeted therapy, making it less effective over time (Targeted therapy for cancer, 2022).

Ablation therapy offers precise treatment, often used to destroy tumours with minimal damage to surrounding tissues. It can be performed alongside MRI imaging, utilizing magnetic hyperthermia to increase effectiveness. However, its efficiency is mainly limited to localized areas, and it has low penetration power, which means it may not be effective for treating deeper or more widespread cancers (Debela *et al.*, 2021). If any cancer cells remain post-treatment, there is a risk of recurrence, necessitating ongoing monitoring and potential repeat procedures. Additionally, the procedure requires a highly skilled operator, limiting its accessibility and increasing the risk of procedural errors that might leave residual cancer cells behind.

Gene therapy holds great potential by enabling the activation of proapoptotic and chemosensitizing genes, wild-type tumour suppressor genes, and genes that can induce targeted anti-tumour immune responses (Debela *et al.*, 2021). It also offers the possibility of targeted silencing of oncogenes using RNA interference (RNAi). Despite these benefits, gene therapy faces significant challenges, such as the risk of genome integration, which can lead to unintended genetic alterations. Its efficacy is often limited to specific subsets of patients, and there is a high chance of the therapy being counteracted by the immune system. Off-target effects and inflammation are additional concerns, particularly with RNAi-based approaches. Furthermore, effective delivery systems and controlled release mechanisms are required, necessitating extensive research and development. Even if initial treatments are

successful, there is a risk that not all cancerous cells are eradicated, leading to recurrence.

Natural antioxidants are appealing due to their availability in large quantities and the potential to exploit their intrinsic anti-cancer properties. However, they suffer from limited bioavailability, meaning that their absorption and utilization in the body are not always efficient (Aqil *et al.*, 2013). Moreover, there is a possibility of toxicity at higher doses, which can limit their therapeutic use (Debela *et al.*, 2021). The inconsistent effectiveness of natural antioxidants means that while they might help in initial treatments, they may not be reliable in preventing recurrence.

Chemotherapy and radiation therapy, while not mentioned in the initial points, are traditional mainstays of cancer treatment. These therapies are often effective in killing cancer cells but come with severe side effects, including damage to healthy cells, which can lead to complications such as immune suppression, hair loss, and gastrointestinal issues (Baskar *et al.*, 2012). The development of resistance to chemotherapy is also a significant challenge, making it less effective over time. Cancer cells that survive initial treatments can adapt and regrow, leading to recurrence.

Immunotherapy has emerged as a transformative strategy, leveraging the body's immune system to combat cancer. While it has shown remarkable results in some cases, it can also lead to severe immune-related side effects and is not effective for all types of cancer (Kciuk *et al.*, 2023). The cost of immunotherapy is also a major barrier, making it inaccessible to many patients. There is also a risk that the immune system may not completely eradicate the cancer, leading to potential recurrence.

Personalized medicine, an emerging field, customizes treatments based on the genetic profile of a patient's tumour (Krzyszczyk *et al.*, 2018). This approach aims to enhance treatment effectiveness and reduce side effects, but it involves costly and time-consuming genetic testing. However, even personalized therapies cannot fully guarantee the prevention of cancer recurrence, as tumours may evolve and adapt unpredictably.

Table 0.1. Advantages and disadvantages of conventional cancer treatment modalities.

Sl. No.	Treatment approach	Advantage	Disadvantage
1	Stem cell therapy	Safe and efficacious Can be integrated with other treatment strategies Decreases tumour size and improves survival rates	The treatment provides onlytemporary effectiveness Possible induction of tumour formation
2	Targeted therapy	High specificity Decreased side effects	Concerns about long-term side effects remain
3	Ablation therapy	Precise treatment The potential to combine with magnetic resonance imaging (MRI) for magnetic hyperthermia therapy exists.	Primarily effective in localized regions. Low penetration power Requires highly skilled operator
4	Gene therapy	Activation of pro-apototic and chemo-sensitizing genes. Restoration of wild-type tumour suppressor gene expression. Expression of genes that can elicit targeted anti-tumour immune responses. Targeted silencing of oncogenes and ensuring safety through RNA interference (RNAi)	Genome integration Limited effectiveness in certain patient subsets. High chances of being neutralized by the immune system Off-target effects and inflammation (RNAi) Need for ad hoc delivery systems (RNAi) Establishment of dosing regimens and apotimal conditions for controlled release (RNAi)
5	Natural antioxidants	Readily available in large quantities Utilizing their inherent properties	Limited bioavailability potential toxicity

Source: Debela *et al.,* New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021

Cancer recurrence remains a significant challenge across all treatments. While each therapy has its strengths, they all face limitations in preventing the return of cancer. Continued research, technological advancements, and a deeper understanding of cancer biology are crucial to improving long-term outcomes for patients.

0.3.2.10. Natural products in cancer research

The exploration and utilization of natural products have profoundly influenced modern medicine, particularly through the use of plant and microbial secondary metabolites. These compounds have been pivotal in extending human life expectancy by mitigating pain, reducing suffering, and enabling advanced medical procedures such as organ transplantation. The diverse chemical structures of these metabolites are intricately tied to their biological and geographical origins, prompting a global effort in bioprospecting to discover novel pharmaceuticals. Traditionally, terrestrial environments have been the primary source for these bioactive compounds, contributing significantly to pharmaceutical success. However, the vast and largely unexplored marine ecosystem presents a new frontier rich in potential therapeutic agents. The recent focus on marine bioprospecting aims to tap into this underutilized resource, with innovative techniques being developed to cultivate previously unculturable microbes from both terrestrial and marine environments. These secondary metabolites often possess complex structures, and while some can be used directly, others require chemical or biological modifications to become effective medicines. The advent of combinatorial biosynthesis has revolutionized the field, enabling the creation of thousands of new derivatives through biological methods, complementing traditional combinatorial chemistry (Demain and Vaishnav, 2011).

The evolution of secondary metabolism in nature is a testament to the sophisticated natural combinatorial chemistry that has been ongoing for billions of years. Bacteria, having inhabited the Earth for over three billion years, have evolved to produce secondary metabolites that offer survival advantages in their local environments. This evolutionary process has led to the retention and refinement of biosynthetic

genes that produce these useful metabolites. Nature's version of combinatorial chemistry generates exotic molecular structures with rich stereochemistry, complex ring systems, and reactive functional groups. This natural diversity has been harnessed in drug discovery through high-throughput screening, genome mining to uncover cryptic pathways, and combinatorial biosynthesis to create novel secondary metabolites with pharmaceutical potential. The pharmaceutical industry depends significantly on a range of integrated technologies including natural product discovery, high-throughput screening, genomics, proteomics, metabolomics, and combinatorial biosynthesis to identify and develop new drugs (Demain and Vaishnav, 2011). Currently, around one million natural products are known, with plants contributing between 500,000 to 600,000 of these (Mendelsohn and Balick, 1995). By the late 1990s, 160,000 natural product structures had been elucidated, a number that continues to grow annually. Microbial secondary metabolites account for over 20,000 known compounds, with approximately 200,000 to 250,000 biologically active products identified. These compounds have successfully translated into clinical trials, with many becoming integral to modern medicine. More than 60% of approved and pre-NDA drug candidates are natural products or their derivatives, excluding biological such as vaccines and monoclonal antibodies (Newman *et al.,* 2003). In early 2008, 225 natural product-based drugs were undergoing various stages of clinical testing, underscoring their critical role in therapeutic development. Natural products are particularly significant in oncology, with three-quarters of anti-tumour agents derived from natural sources. Since, 1940, over 60% of the 140 approved anticancer agents have originated from natural products. This includes a broad array of structural alsses such as anthracyclines, enediynes, indolocarbazoles, isoprenoids, polyketide macrolides, and non-ribosomal peptides, which often exhibit potent anti-tumour activity through mechanisms such as apoptosis induction, enzyme inhibition, ant anti-angiogenesis. The continuous exploration and development of natural products remain essential for advancing medical science and therapeutic innovation (Demain and Vaishnav, 2011).

0.3.2.10.1. Microbial-anti-tumour products

Microbial anti-tumour products have to be a significant source of anticancer compounds, particularly those produced by microorganisms like actinomycetes. These organisms are renowned for generating a diverse array of natural products with both antimicrobial and anti-tumour properties (Demain and Vaishnav, 2011). In the 1980s, a comprehensive screening of antibiotic compounds for their antagonistic effects on non-microbial organisms has been proposed, leading to the identification and repurposing of failed antibiotics for anti-tumour applications. This initiative resulted in the development of numerous *in vitro* laboratory tests, including enzyme inhibition screens, are used to identify, isolate, and purify potential compounds (Umezawa, 1982). Consequently, microbial metabolites began to be utilized for treating diseases traditionally managed by synthetic drugs, marking a significant advancement in medical treatment. This approach has been particularly successful in the field of anti-tumour agents, where microbial-derived compounds have shown considerable therapeutic efficacy.

In their comprehensive review, Newman and Shapiro (2008) emphasized the pivotal role of microorganisms in identifying effective anti-tumour compounds (Newman and Shapiro, 2008). Many of the key drugs used in chemotherapy today are either microbial antibiotics or their derivatives. Historical examples include the application of actinomycin D in treating Wilm's tumour in children, which has achieved a 90% survival rate for stages I and II of the disease (Jenkin *et al.*, 1976).). The structural complexity and diversity of microbial anti-tumour agents, such as those depicted in Salas and Mendez, highlight their potential in cancer therapy (Salas and Mendez, 1998). Another noteworthy microbial product used in anti-tumour therapy is the enzyme L-asparaginase, which further illustrates the therapeutic versatility of microbial metabolites (Alrumman *et al.*, 2019). These findings underscore the invaluable contributions of microbial products to modern oncology, demonstrating their potential to yield novel and effective treatments for cancer.

Angiogenesis is essential for tumours to acquire oxygen and nutrients, a process stimulated by growth factors secreted by the tumours. Folkman proposed targeting angiogenesis to inhibit tumour growth, focusing on vascular endothelial growth factor (VEGF) as akey target (Folkman, 1971). Early anti-angiogenetic agents include fumagillin, produced by *Aspergillus fumigatus*, and its derivatives like TNP470, which inhibit essential endothelial cell progresses (Ingber *et al.*, 1990). Avastin (bevacizumab), a monoclonal antibody that targets VEGF, is used to treat metastatic colorectal cancer (Ferrara *et al.*, 2005 and Kazazi-Hyseni *et al.*, 2010). Other FDA approved agents, such as Ranibizumab (Lucentis) and Pegaptanib (Mucagen), also inhibit VEGF (Nagpal *et al.*, 2007). By 2008, ten anti-angiogenesis drugs had been approved, eight of which were for cancer and two for age-related macular degeneration. This highlights the therapy's importance in cancer treatment, making it one of the four major modalities in oncology (Demain and Vaishnav, 2011).

0.3.2.10.2. Marine anti-tumour products

The ocean, a hub of biological diversity, houses 34 of the 37 known phyla of life, compared to only 17 on land. Despite this diversity, marine bioprospecting for biotechnological and drug discovery purposes is relatively recent. Marine microorganisms are an especially complex and diverse group, with only about 1% having been cultured or identified (Demain and Vaishnav 2011). Bioprospecting efforts often focus on coral reefs, mangroves, and seagrass beds due to their rich biodiversity and intense interspecies competition, which drives the production of bioactive compounds. Marine sponges, for example, are known for producing numerous compounds exhibiting promising pharmaceutical potential. Notably, cytarabine (Cytostar), a drug used to treat non-Hodgkin's lymphoma, was originally derived from a marine sponge (Jimenez *et al.*, 2018). Many of these compounds are believed to be produced by bacterial symbionts rather than the sponges themselves, highlighting the potential of marine microorganisms.

Marine-derived compounds have made significant contributions to oncology. Compounds like cytarabine, pederin, theopederins, annamides, trabectedin (Yondelis), aplidine, and ecteinascidin 743 (ET743) exhibit potent anti-tumour activity. Approximately 68% of pharmaceutically useful marine natural products target cancer, with the remainder addressing inflammation, pain, asthma, and Alzheimer's disease (Demain and Vaishnav 2011).

0.3.2.10.3. Plant derived anti-tumour products

Plants have significantly contributed to the development of anticancer agents, providing a rich source of compounds that have approved for use in cancer therapy. Since 1961, the USA has approved nine anticancer drugs derived from plant sources, highlighting the longstanding importance of botanical sources in modern medicine (Demain and Vaishnav 2011). These include vincristine, paclitaxel (Taxol), etoposide, vinblastine, topotecan, teniposide, vinorelbine (Navelbine), Camptothecin, docetaxel (Taxotere), and irinotecan. The discovery of these compounds often involved extensive ethnobotanical studies and pharmacological screenings, leveraging traditional knowledge of medicinal plants. For instance, vinblastine and vincristine were isolated from the Madagascar periwinkle, a plant traditionally used in folk medicine for treating diabetes (Deorukhkar *et al.*, 2007). The approval of these drugs underscored the potential of natural products in offering novel therapeutic options for cancer treatment. In addition to their efficacy, many of these plant-derived compounds provided a foundation for the development of synthetic analogues and derivatives, further expanding the arsenal of anticancer agents. The significant commercial success of these drugs, with combined sales reaching billions of dollars annually, reflects their critical role in oncology. Moreover, ongoing research into plant secondary metabolites continues to reveal new compounds with potential anticancer activity, emphasizing the enduring relevance of plants in drug discovery.

0.3.2.10.3.1. Vinca alkaloids

Vinblastine and vincristine, which originate from the Madagascar periwinkle (*Catharanthus roseus*), are critical in treating cancers such as Hodgkin’s lymphoma.

Despite their therapeutic importance, these alkaloids present challenges in synthesis due to their complex structures. Advances in metabolic engineering aim to optimize the biosynthesis of these alkaloids by manipulating pathway enzymes (Demain and Vaishnav 2011). Strictosidine synthase, an enzyme with a narrow substrate range, has been engineered to accept tryptamine analogues, leading to the production of novel β-carboline analogues with potential therapeutic applications (Bernhardt *et al.*, 2007). Additionally, the Madagascar periwinkle produces serpentines, compounds with anticancer potential (Almargo *et al*., 2015). However, due to their toxicity, clinical use has been limited. Researchers have expanded the range of drug candidates by producing halogenated alkaloids, offering new avenues for cancer treatment.

0.3.2.10.3.2. Camptothecin and its derivatives

Camptothecin, a modified monoterpene indole alkaloid synthesized by certain angiosperm species (Wall and Wani, 1996) and the endophytic fungus *Entrophospora infrequens.* This compound has gained recognition as a potent anticancer agent. Although its natural abundance in plant roots is minimal and chemical synthesis yields are inefficient, fungal fermentation has emerged as a promising alternative for large-scale production. Clinically, Camptothecin is formulated as Camptosar and Campto for the treatment of recurrent colorectal and has also demonstrated therapeutic efficacy in combating uterine, ovarian and lung cancers (Amna *et al.*, 2006). It targets type I DNA topoisomerase, an enzyme essential for DNA replication (Lorence and Nessler, 2004). Combining camptothecin derivatives like irinotecan with monoclonal antibodies such as Erbitux (cetuximab) enhances its efficacy in treating metastatic colorectal cancer (Demain and Vaishnav, 2011). This combination impedes the infiltration of normal tissues by vasion of normal tissues by tumour cells and restricts the metastasis of tumours to new sites, offering significant therapeutic benefits.

0.3.2.10.3.3. Taxanes

Taxol (paclitaxel), a diterpene alkaloid initially isolated from the bark of the *Taxus brevifolia* (Pacific yew tree), has been a groundbreaking anti-tumour agent (Dejong

et al., 2006). Taxol is now produced using plant cell cultures or semisynthesis from taxoids generated by various Taxus species, which generate over 350 known taxoid compounds (Baloglu and Kingston, 1999). Advances in genetic engineering have optimized the biosynthesis of taxol, with engineered *Saccharomyces cerevisiae* strains producing significant quantities of taxadiene, a taxol precursor (Dejong *et al.*, 2006 and Demain and Vaishnav 2011). The industrial production of taxol involves cultivating cells of *Taxus chinensis*, with the addition of methyl jasmonate and temperature optimization significantly enhancing yield (Yukimune *et al.*, 2000). Taxol, approved for treating breast and ovarian cancers, works by stabilizing microtubules and inhibiting the division of cancer cells (Manifredi and Horwitz, 1984). The global market for taxanes and camptothecins was substantial, with taxol alone achieving sales of $1.6 billion in 2005 and its analogue, docetaxel (Taxotere), reaching $3 billion in sales by 2009 (Demain and Vaishnav 2011).

0.3.2.10.3.4. Etoposide and Teniposide

Etoposide and teniposide, semisynthetic derivatives of podophyllotoxin from the mayapple plant (*Podophyllum peltatum*), are critical in cancer treatment (Clark and Slevin, 1987; Deorukhkar *et al.,* 2007). Etoposide, a topoisomerase II inhibitor (Baldwin *et al.,* 2005), is approved for various cancers, including choriocarcinoma, lung cancer, lymphoma, ovarian and testicular cancer, and acute myeloid leukemia (Sinkule *et al.,* 1984). Teniposide is used for tumours of the central nervous system, malignant lymphoma, and bladder cancer (Demain and Vaishnav 2011; Wang *et al.,* 2020).

0.3.2.10.3.5. Other plant-derived compounds

Other notable plant-derived compounds include shikonin, a naphthoquinone pigment from *Lithospermum erythrorhizon*, which has shown efficacy in inhibiting tumour growth in mice (Demain and Vaishnav 2011; Long *et al.,* 2012; Zhao *et al.,* 2018). Additionally, compounds like curcumin, genistein, epigallocatechin-3-gallate (EGCG), indole-3-carbinol (I3C), and lycopene and resveratrol have demonstrated potential in combating cancer by disrupting cellular signaling pathways and

inducing apoptosis in cancer cells without harming normal cells (Sarkar *et al.,* 2009; Demain and Vaishnav 2011; Gano *et al.,* 2023). These natural products, acting through various mechanisms, have expanded the scope of cancer therapy, underscoring the importance of plant-derived compounds in modern medicine.

The impact of these plant-derived compounds on cancer treatment has been profound. For example, taxol promotes tubulin polymerization and inhibits rapidly dividing mammalian cancer cells, making it effective against a range of cancers (Schiff and Horwitz, 1981). Similarly, camptothecin and its derivatives have revolutionized the treatment of colon cancer (Krishnan *et al.,* 2017; Li *et al.*, 2022; Li *et al.*, 2023), with commercial success reflected in their sales figures. The combination of metabolic engineering, genetic engineering, and biotechnological advancements has enabled the sustainable production of these compounds, ensuring their availability for clinical use. As research continues, the potential for discovering new plant-derived anti-cancer agents remains high, promising further advancements in cancer therapy.

In summary, the discovery and development of plant-derived anti-cancer agents have played a crucial role in advancing cancer treatment. From alkaloids to taxanes and beyond, these natural products have provided effective therapies for various cancers, demonstrating the immense value of plants in pharmaceutical development. Continued research and innovation in this field hold the promise of even greater breakthroughs in the future.

Chapter 1

Screening plant extracts to identify extracts containing anticancer molecules

- *Extraction of compounds from plant powder using solvents of different polarity indices.*
- *Identification of plant extracts containing anticancer activity against human cancer cell lines.*

REVIEW OF LITERATURE

1.1.1. Inspiration from nature in scientific solutions

Nature has long been a source of inspiration for scientific solutions to various problems, including cancer. The vast and complex interplay of natural processes has often guided scientific inquiry and innovation across disciplines. In the realm of cancer research, the influence of natural principles and the potential of nature-based interventions are increasingly being recognized. There has been a growing interest in utilizing nature-based interventions for cancer survivors, emphasizing the holistic benefits of integrating natural elements into cancer care (Timko *et al.,* 2023). These interventions may include activities such as horticultural therapy, forest bathing, and exposure to natural environments, which have been shown to improve the well-being and quality of life of cancer survivors.

1.1.2. Turning to nature for solutions: The rich biodiversity of plants

Plants produce a diverse range of secondary metabolites, referred to as phytochemicals, which have evolved to protect them against pests, diseases and environmental stresses. These phytochemicals include alkaloids, flavonoids, terpenoids, and polyphenols, each with distinct and potent biological activities. Many of these phytochemicals have shown promise in reducing tumour growth and minimizing adverse reactions associated with traditional cancer treatments (Rudzińska *et al.,* 2023). By harnessing these natural compounds, researchers hope to develop new, more effective, and less toxic anticancer therapies.

1.1.3. Phytochemicals: Nature's anticancer compounds

Plants produce a wide variety of secondary metabolites, known as phytochemicals, which have evolved to protect them against pests, diseases, and environmental stresses. These phytochemicals include alkaloids, flavonoids, terpenoids, and polyphenols, each with distinct and potent biological activities. Many of these phytochemicals have shown promise in reducing tumour growth and minimizing adverse reactions associated with traditional cancer treatments (Kumar *et al.,* 2023).

By harnessing these natural compounds, researchers hope to develop new, more effective, and less toxic anticancer therapies.

1.1.3.1. Screening and isolation of active compounds

Developing anticancer treatments from phytochemicals typically involves a multi-step process. Initially, the plant extracts are screened for biological activity using various *in vitro* assays. These assays help identify extracts with potential anticancer properties. Following this, bioassay-guided fractionation is used to isolate the active compounds from the extracts. This method involves separating the extract into different fractions and testing each fraction for biological activity. The fractions showing the highest activity are further purified to identify specific molecules responsible for the observed effects.

1.1.3.2. Emerging technologies and natural solutions

The intersection of nature and technology is also yielding innovative solutions for cancer detection and treatment. Next-generation liquid biopsy technologies targeting cell-free DNA fragments have shown promise in early cancer detection, offering a minimally invasive method to identify cancer at its earliest stages (Chen and Zhao, 2019). These technologies leverage natural biological processes to provide critical insights into the presence and progression of cancer, potentially improving early diagnosis and treatment outcomes.

Additionally, potential solutions for manufacturing chimeric antigen receptor T cells (CAR T cells) in cancer therapy have been explored, including the use of non-T immune effector cells like natural killer cells. This approach draws on the body's natural immune mechanisms to enhance the efficacy and accessibility of CAR T cell therapies, which have shown remarkable success in treating certain types of cancer (Salmikangas *et al.,* 2018).

1.1.3.3. Advanced techniques and natural insights

In the quest for improved accuracy in cancer tissue decomposition, deep learning techniques such as data augmentation have been employed. These advanced

computational methods mimic natural learning processes to enhance the precision of cancer diagnostics and research. By integrating natural patterns and structures into artificial intelligence algorithms, researches are making significant strides in understanding and combating cancer.

Furthermore, advancements in therapeutic cancer vaccines have shown potential in targeting abnormal activation of suppressors involved in dampening natural immune responses. These vaccines aim to harness and amplify the body's natural defences against cancer, providing a targeted and personalized approach to treatment.

1.1.3.4. Drug development: Lessons from nature

Natural products derived from plants, animals, and microorganisms are pivotal to the pharmaceutical and biotechnological industries due to their unique and structurally diverse chemical components. Plants, in particular, have been the most prolific source of these compounds, owing to their extensive chemical and structural variety (Mathur *et al.,* 2017). Notable drugs like aspirin from willow bark (Desborough and Keeling, 2017), digoxin from *Digitalis lanata* (Mbaveng *et al.,* 2014), and morphine from opium (Norn *et al.,* 2005) highlight the significance of plant-derived compounds. In industrialized nations, over 60% of pharmaceuticals are either natural products or derivatives thereof (Eddershaw *et al.,* 2000). Despite new drug discovery methodologies, natural products continue to provide novel clinical candidates. Key examples include anti-infective agents such as azithromycin (Venditto *et al.,* 2021), amphotericin B (Lemke *et al.,* 2005), antiviral drugs like acyclovir (Gnann, 1983), and anticancer therapies including romidepsin (Frye *et al.,* 2012). These compounds have successfully advanced to clinical trials and commercialization.

Technological advancements in drug discovery, such as high-throughput screening, automated separation methods, and combinatorial chemistry, have enhanced the exploration of natural products. Semi-synthetic therapeutic agents, which combine natural and synthetic sources, include the antibiotic penicillin and the anticancer agent paclitaxel from the Pacific yew tree, *Taxus brevifolia* (Fatima *et al.,* 2017). Natural products introduce novel, chemically diverse entities serving as templates for synthetic modifications, addressing a wide spectrum of diseases. They tend to

exhibit better physiological and pharmacological effects within living cells compared to synthetic drugs, which often have adverse side effects due to their small, non-natural chemical building blocks.

Despite the reduced focus on natural product research within the pharmaceutical industry due to advanced techniques like metagenomics and combinatorial chemistry, the complexity and high cost of synthesizing many natural products remain challenges. However, renewed interest driven by therapeutic needs for conditions like cancer, obesity, and multi-drug resistant infections is revitalizing the exploration of natural products. Plants, microorganisms, fungi, and bacteria continue to be crucial sources for drug development, with vast potential for discovering new therapeutic agents.

1.1.4. Plant biodiversity in India

India’s rich biodiversity and traditional medicinal systems position it as a major player in the global medicinal plant market. Despite covering only 2.4% of the world's land area, India houses 8% of global biodiversity, making it a significant potential exporter of medicinal plants. It is estimated that 90% of the country's medicinal plant diversity is found in its forests, with approximately one-fifth of all plant species in India used for medicinal purposes (Gowthami *et al.,* 2021).

The Western Ghats, a biodiversity hotspot, are particularly notable for their wealth of medicinal plants, hosting around 4,000 species of higher plants. Traditional medicinal practices among indigenous communities offer invaluable insights into the use and conservation of these plants. For instance, the folk medicinal knowledge in regions like North Tripura district is essential for understanding the diverse applications of medicinal plants (Debbarma *et al.,* 2017). Preserving this knowledge is crucial for both cultural heritage and the development of new medicinal products.

India’s diverse agro-climatic conditions and extensive plant biodiversity make it a treasure trove of botanical resources. Approximately 70% of India's medicinal plants are found in the tropical forests of the Western Ghats (Vijayan *et al.,* 2017). The use of plant growth-promoting rhizobacteria (PGPR) in medicinal plants is gaining importance as an alternative to chemical fertilizers and pesticides, promoting sustainable agricultural practices (Egamberdieva *et al.,* 2015).

Globally, the demand for herbal products is on the rise, with over 80% of the world's population relying on herbal medicines. This growing trend underscores the need for sustainable practices in the cultivation and harvesting of medicinal plants to meet global healthcare demands while preserving biodiversity.

1.1.5. Plant as a source of anticancer compounds

Plants and other natural sources have been recognized for their potential in producing anticancer compounds. Over 70% of anticancer compounds used in chemotherapy are either natural products or derivatives thereof, showcasing the significant role of natural substances in cancer treatment (Karikas, 2010). The vast structural diversity of these natural compounds provides a rich reservoir of lead compounds that can be modified for therapeutic improvement. However, despite these contributions, less than 15% of higher plants have been systematically studied for their anticancer properties, highlighting the need for further research and multi-disciplinary collaboration in this field.

Plants remain a crucial source of anticancer drugs, contributing significantly to the treatment of various cancers. Notable plant-derived compounds include taxol, vinca alkaloids, camptothecin, and podophyllotoxins.

1.1.6. Chemoprevention and phytochemicals

Plants represent an important source of agents that can prevent cancer. Chemoprevention is the strategy of using either natural or synthetic compounds to block, delay or reverse the development of cancer. Research has identified over 1000 phytochemicals with such preventive capabilities. These compounds, including flavonoids, terpenoids, alkaloids and polyphenols, can interfere with the cellular pathways that cause genetic damage and stress (Akbari *et al.,* 2022).

1.1.6.1. Classification and Functions of Phytochemicals

1.1.6.1.1. Primary and Secondary Metabolites

Phytochemicals can be broadly classified into primary and secondary metabolites based on their roles and pathways in plants (Nwafor and Orabueze, 2018). Primary metabolites are essential for the basic life processes of plants. These include

carbohydrates, amino acids, and proteins, which are crucial for growth, development, and cellular functions. On the other hand, secondary metabolites are produced through secondary metabolic pathways and serve protective roles for the plant. These compounds, such as alkaloids, flavonoids, and terpenoids, are not directly involved in growth but provide defence against herbivores, pathogens, and environmental stresses.

1.1.6.1.2. Types of secondary metabolites

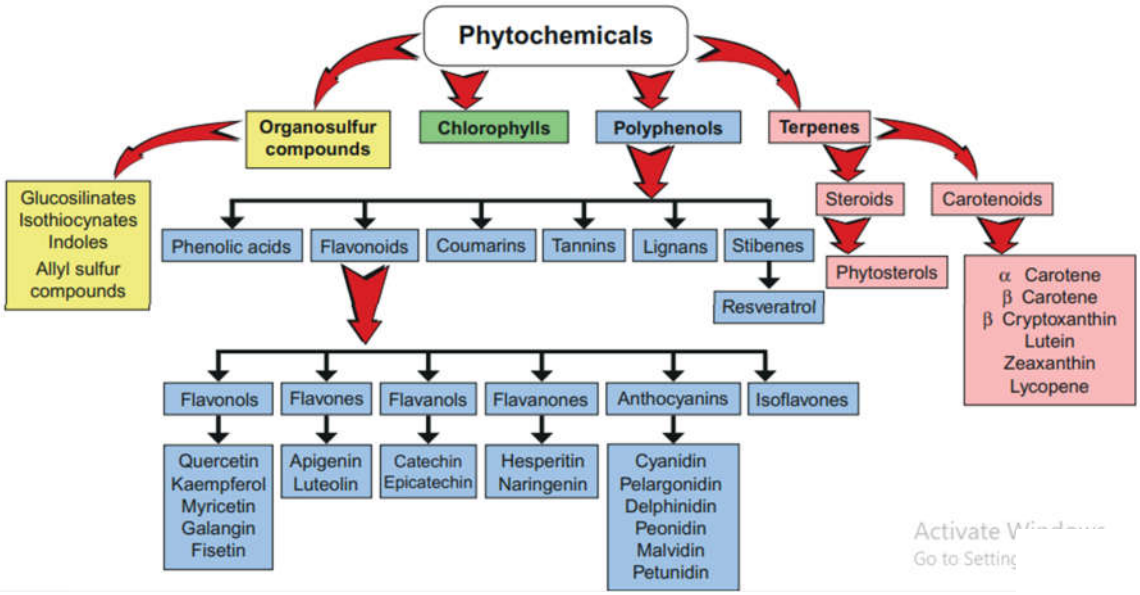


Figure 1.1.1. Classification of phytochemicals.

Source: (https://www.sciencedirect.com/science/article/pii/B9780128118160000142)

Secondary metabolites are further categorized into several types based on their chemical structure and biological functions (Figure 1.1.1):

1.1.6.1.2.1. Flavonoids

Flavonoids are a class of polyphenolic compounds produced by plants as bioactive secondary metabolites, playing key roles in imparting colour, flavour, and medicinal properties. These compounds are classified into six main types: isoflavanones, flavanols, flavones, and anthocyanidins, and they occur in various plant species. The primary sources of flavonoids in the human diet include fruits, vegetables, and beverages derived from plants, such as green tea, wine, and cocoa products. Studies

conducted by Korkina *et al.*, Rodriguez-Garcia *et al.*, Yahfoufi *et al.*, Abotaleb *et al.*, and Chirumbolo *et al.* have shown that flavonoids can neutralize free radicals, modulate cellular metabolism, and protect against diseases associated with oxidative stress (Korkina *et al.,*1997; Rodriguez-Garcia *et al.,* 2019; Yahfoufi *et al.,* 2018; Abotaleb *et al.,* 2018; Chirumbolo *et al.,* 2018) . Although substantial evidence indicates that many flavonoids have anticancer properties, the exact molecular pathways through which they exert these effects are not yet fully understood.

1.1.6.1.2.2. Terpenoids

Terpenoids, a vast group of secondary metabolites originating from plants, are constructed from multiple isoprene units. Their significant diversity has highlighted their potential for anticancer and pharmacological activities. Research has demonstrated that numerous terpenoids, such as menthol (Lin *et al.,* 2005) and limonene (Guo *et al.,* 2006), exhibit anticancer properties through various mechanisms of action.

1.1.6.1.2.3. Alkaloids

Alkaloids are organic compounds with naturally occurring nitrogen atoms. Typically derived from plants, these bases are characterized by their basic nature, inclusion of one or more nitrogen atoms (often within a heterocyclic ring), and significant physiological effects on humans and other animals (Verpoorte, 2005). Alkaloids constitute roughly 20% of the known secondary metabolites in plants (Kaur and Arora, 2015). Within plants, alkaloids serve protective functions against predators and play roles in growth regulation (Chik *et al.,* 2013). Therapeutically, alkaloids are well known for their use as cardioprotective agents, anesthetics, and anti-inflammatory agents. According to Kurek, prominent alkaloids utilized in clinical practice include morphine, strychnine, quinine, ephedrine, and nicotine (Kurek, 2019). Notably, the discovery of vinblastine and vincristine from Vinca rosea (*Catharanthus roseus*, Apocynaceae) marked the advent of significant anticancer alkaloids, ushering in a new era of anticancer drug development.

1.1.6.1.2.4. Polyphenols

Natural polyphenols, which are organic compounds derived from plants and characterized by the presence of multiple phenol units (Asensi, 2011), have been extensively studied for their potential health benefits. Research indicates that polyphenols offer protection against oxidative stress, diabetes, cardiovascular diseases, neurodegenerative disorders, and the effects of aging. These compounds exhibit anticancer properties through various mechanisms, including the modulation of signalling pathways, inhibition of cell cycle progression, induction of apoptosis, and regulation of enzymes involved in tumour cell proliferation (Abbas, 2017; Spatafora and Tringali, 2012).

1.1.7. Plants with anticancer molecules

To date, extensive research has explored the anticancer potential of numerous plants and plant-derived compounds. Certain plants and their constituents have demonstrated significant efficacy against various types of cancers. A comprehensive summary of key plants exhibiting anticancer properties, along with their respective compounds, is provided in the table 1.1.1 based on available data. Furthermore, these studies have elucidated the mechanisms underlying the anticancer activity of these plants and compounds, providing valuable insights into potential therapeutic targets. The identification of natural agents with anticancer properties holds promise for the development of novel treatments and preventive strategies against cancer. Additionally, ongoing research continues to explore the synergistic effects of combining plant-based compounds with conventional cancer therapies, aiming to enhance treatment efficacy and reduce adverse effects.

Table 1.1.1. Major plants exhibiting anticancer properties, along with their respective compounds.

Sl. No.	Plant Name	Parts Used	Extact Used	Cancer Cell Line Applied To
1	*Allium sativum*	Leaves	Aqueous extracts	Wehi-164 tumor cells
2	*Alpinia galangal*	Rhizomes	Ethyl acetate extract	Murine daltons lymphoma ascite (dla) and human lung cancer (a549) cells
3	*Alstonia scholaris*	Stem and bark	Ethanol extract	Hela cells lines
4	*Andrographis paniculata*	Aerial parts	Methanol extract	Cancer cell lines sw620 and a498
5	*Angelica archangelica*	Root and rhizome	Ethanol extract	Mcf7 and 4t1 cell lines
6	*Aralia elata*	Leaves	Ethanol extract	Mcf-7 cells
7	*Artemisia annua*	Stem and leaves	Ethanol extract	leukemia, melanoma, lung cance,colon cancer, renal cancer, ovarian cancer, and tumors of the CNS
8	*Asclepia scurassavica*	Leaves	Ethyl acetate and methanol extract	Human colo 320 dm
9	*Astragalus membranaceus*	--	--	Liver cancer
10	*Copaifera multijuga*	Trunk of the tree	Oil resin	B16f10 melanoma cells
11	*Coptidis rhizoma*	--	--	Human hepatic carcinoma cell lines HepG2 and mhcc97-l.
12	*Coptis chinensis*	--	--	Lung and blood cancer
13	*Curcuma longa*	--	--	Ht-29 colon cancer cells of human
14	*Elephantopus scaber*	--	Dimethyl sulfoxide extract	Murine ehrlich ascites carcinoma (eac)
15	*Fagonia indica*	--	Aqueous extract	MCF7, MDA - MB - 468,Caco - 2
16	*Fagonia schweinfurthii*	Whole plant	Ethanolic extract	HepG2 cell line
17	*Garcinia indica*	Fruits	Ethanol extract	HT29, HCT116, Hman leukemia cell line HL - 60
18	*Garcinia oblongifolia*	Branch	Methanol extract	HeLa - C3,MCF7
19	*Garcinia preussii*	Fruits and leaves	Meohextract	Du145, hela, ht-29, and a431 cell lines
20	*Hedyotis diffusa*	--	ethanol	MCF7, Hela, Ct - 26
21	*Hedyotis spp.*	Aerial parts, stem and leaves	Methanol extract	Cem-ss cell line
22	*Kaempferia parviflora*	Rhizomes	Ethanolic extract	Ovarian cancer cell line, skov3
23	*Litchi chinensis*	Fruit	Ethanolic extract	Human smmc-7721 hepatocellular carcinoma cell line
24	*Loranthus parasiticus*	--	Aqueous extract	ovary SKOV3, CAOV3, OVCAR - 3

25	*Menyanthes trifoliata*	Aerial part and root	Aqueous methanol extract	Grade iv glioma cells
26	*Morus alba*	leaves, root	N-hexane and methanolextracts.	HL-60 (human leukemia) andCrl1579 (human melanoma) cell lines,Pulmonary carcinoma, Calu - 6, Colon carcinoma HCT 116 , MCF7, gastric carcinoma SNU - 601, HL-60, HepG2, HCT-15
27	*Morus nigra*	Aerial parts	dimethyl sulfoxide extract	human prostate adenocarcinoma (PC-3)
28	*Nitraria retusa*	Leaves	Chloroform extract	B16-f10 cells lines
29	*Paeonia lactiflora*	Root	Aqueous extract	Human hepatoma cell lines (HepG2 and hep3b)
30	*Paris polyphylla*	Rhizomes	Methanol Aqueous and etanol	A549 cell lineHepG2 and SMMC-7721, LoVo, SW-116, CaES-17, ECA109
31	*Perilla frutescens*	Leaves	Meoh extractEthanolic extract	Huh-7 hepatoma cell carcinomaHepG2, DLD1
32	*Platycodon grandiflorus*	Root	Platycodin d was dissolved in	Human breast cancer cell line, mcf-7,HT80MCF7, SKOV3
33	*Pleurotus pulmonarius*	Edible part	Aqueous extract	Huh7 liver cancer cells
34	*Prunus armeniaca*	Fruit	Methanolic extract	HCT116, MCF7, HepG2
35	*Rabdosiae rubescens*	--	--	MDA - MB-231, MCF7, HepG2
36	*Rhodamnia rubescens*	--	--	Human breast (mcf-7 and mda-mb-231) cancer cells
37	*Scurrulus parasitica*	--	Ethanol	HL-60
38	*Scutellaria baicalensis*	Root	Aqueous extract	Human oral squamous cell carcinoma (oscc) cell line, BGC-823, MGC-803 gastric cancer cells
39	*Scutellaria barbata*	--	--	95-d cell line, MDA - MB-231, MDA-MB-468, HepG2
40	*Tripterygium wilfordii*	--	--	Neuroblastoma cell lines (n2a and sknsh)
41	*Tussilago farfara*	Flower buds	Methanol extract	Ht-29 human colon cancer cells,HT29, HCT116, Hman leukemia cell line HL - 60
42	*Wedelia chinensis*	Leaves	Essential oils	B16f-10 melanoma metastatic cell line
43	*Zuojin wan*	--	Aqueous extract	S180 tumor cells

Source: Khan *et al.,* (2019) Anticancer Plants: A Review of the Active Phytochemicals, Applications in Animal Models, and Regulatory Aspects. Biomolecules.

1.1.8. Research gap

The research gap in screening plant extracts for their potential anticancer properties assumes paramount importance, particularly within the context of India's rich herbal diversity, which presents an expansive reservoir of untapped therapeutic potential in cancer treatment. India's biodiversity, synergized with modern genomic insights, stands as a cornerstone in shaping bioscience endeavours in the 21st century. However, the realization of this potential necessitates a profound comprehension of taxonomy, a skillset that demands substantial time and expertise to cultivate.

The pharmaceutical and biotechnology sectors in India confront an urgent imperative for novel therapeutic interventions to combat clinical conditions that defy conventional therapeutic modalities. Addressing this challenge necessitates a critical exploration of the structure-activity relationships inherent in the active constituents sourced from India's diverse flora.

India’s natural products, characterized by their diverse chemical structures, represent invaluable reservoirs of lead components for drug development endeavours. Moreover, Indian natural metabolites frequently exhibit superior biochemical and pharmacological activities compared to their secondary metabolite counterparts. Nevertheless, the current paradigm of drug discovery in India, predominantly centered on the screening, isolation, purification, and identification of novel drug candidates from natural resources, demands modernization to sustain competitiveness in the global pharmaceutical research landscape.

To capitalize on India's herbal diversity in the realm of cancer treatment, there exists a pressing need to augment the efficiency of screening methodologies, encompassing computational methodologies alongside experimental processes such as isolation, robotic separation, and purification. By addressing these efficiency hurdles, Indian natural product research can catalyse innovation within the pharmaceutical sector and assert leadership in pioneering advanced drug discovery techniques tailored for cancer therapeutics.

The significance of screening a broader spectrum of plants for their potential anticancer properties cannot be overstated, particularly within the framework of India's abundant herbal diversity. In light of cancer's formidable global and national health burden, diversifying the range of plants screened facilitates the discovery of novel compounds harbouring distinctive mechanisms of action against malignant cells.

Furthermore, India's varied flora harbours a wealth of bioactive compounds yet to be exhaustively probed for their anticancer efficacy. A systematic exploration of additional plant species holds the potential to unveil hidden therapeutic treasures, fostering the development of novel and efficacious anticancer agents. This strategic approach resonates with the principles of biodiversity conservation and the sustainable exploitation of natural resources.

Moreover, as the pharmaceutical industry grapples with the challenges of drug resistance and the limitations inherent in existing therapeutic modalities, delving into plant-based compounds unveils novel avenues for drug discovery. These natural products, characterized by intricate chemical structures and diverse biological activities, emerge as promising candidates for therapeutic development.

Embracing traditional knowledge systems and indigenous practices can further guide the selection of plants for screening, thereby ensuring a comprehensive approach to drug discovery endeavours. Overall, the methodical screening of a wide array of plants for their anticancer properties holds immense potential in unearthing novel therapeutic agents, thereby mitigating the escalating burden of cancer within India and globally.

MATERIALS AND METHODS

1.2.1. Chemicals

HPLC-grade solvents, including petroleum ether, chloroform, ethyl acetate, acetone, and methanol, were used for plant extract preparation. They were sourced from Merck, Germany. Cell lines were grown in different media like DMEM, MEM and MC Coy's 5A, according to the cell type. DMEM (Dulbecco's Modified Eagle Medium) and McCoy's 5A medium were obtained from Sigma-Aldrich (USA), while Trypsin, MEM medium, MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide) and Dimethyl Sulfoxide (DMSO) were supplied by HiMedia Laboratories Pvt. Ltd., Mumbai, India. Fetal bovine serum (FBS) and the antibiotic-antimycotic cocktail solution were procured from Gibco, USA. EDTA was also purchased from Sigma-Aldrich (USA). All chemicals used for maintaining and treating the cell lines were tested and verified for animal tissue culture applications, and other chemicals and reagents were of analytical grade.

1.2.2. Collection of samples

Different plant parts for the study were collected from various parts of Wayanad, Malappuram and Palakkad districts in Kerala, India during the monsoon season (May- August, 2018-2022) for the study and were identified by Prof.(Dr.) Pradeep, Department of Botany, University of Calicut, Kerala, India. The details of the plants are listed in the table 1.2.1.

1.2.2.1. Plant extracts preparation

The collected plant materials were washed under flowing water, dried in the shade, and then ground into a coarse powder using a mixer grinder. The powdered samples of each plant were then stored in airtight containers for later use. Crude extracts were obtained using the sequential maceration technique using different organic solvents of different polarity.

Table 1.2.1. List of plants collected from various parts of Kerala, for the anti-cancer potential analysis.

Sl. No.	Plant	Family	Plant parts used	Collection Area
1	*Anamirta cocculus* (L.) Wight and Arn.	Menispermaceae	Leaves	Koottanad, Palakkad
2	*Acalypha fruiticosa* Forssk.	Euphorbiaceae	Leaves	Thenhippalam, Malappuram
3	*Alangium salviifolium* (L.f.) Wangerin	Cornaceae	Leaves	Koottanad, Palakkad
4	*Antigonon leptopus* Hook. and Arn.	Polygonaceae	Leaves	Malappuram
5	*Argyreia pomacea* Wall. Ex Choisy	Convolvulaceae	Leaves	Attappadi, Palakkad
6	*Bauhinia phoenicea* Wight and Arn.	Leguminosae	Leaves	Attappadi, Palakkad
7	*Bridelia retusa* (L.) A. Juss.	Euphorbiaceae	Leaves	Koottanad, Palakkad
8	*Calopogonium mucunoides* Desv.	Fabaceae	Seeds	Thenhippalam, Malappuram
9	*Cassia javanica* L.	Fabaceae	Leaves	Thenhippalam, Malappuram
10	*Chassalia curviflora* (Wall.) Thwaites	Rubiaceae	Leaves	Koottanad, Palakkad
11	*Cheilocostus speciosus* (J. Koenig) C. Specht	Costaceae	Leaves	Koottanad, Palakkad
12	*Chrysophyllum cainito* L.	Sapotaceae	Leaves	Mannarkkad, Palakkad
13	*Cipadessa baccifera* (Roth) Miq.	Meliaceae	Leaves, seeds	Attappadi, Palakkad
14	*Cissus discolour* Bl.	Vitaceae	Whole plant	Attappadi, Palakkad
15	*Cochlospermum religiosum* (L.) Alston	Bixaceae	Leaves	Thenhippalam, Malappuram
16	*Combretum indicum* (L.)	Combretaceae	Leaves, flower	Thenhippalam, Malappuram
17	*Conyza canadensis* (L.) Cronquist	Asteraceae	Leaves	Attappadi, Palakkad
18	*Cyclea peltata* Hook. F. and Thoms.	Menispermaceae	Leaves	Thenhippalam, Malappuram
19	*Drypetes venusta* (Wight) Pax and K.Hoffm.	Putranjivaceae	Leaves	Attappadi, Palakkad
20	*Entada rheedii* Spreng.	Fabaceae	Leaves	Thenhippalam, Malappuram
21	*Euphorbia heterophylla* L.	Euphorbiaceae	Leaves	Thenhippalam, Malappuram
22	*Euphorbia tithymaloides* L.	Euphorbiaceae	Leaves	Koottanad, Palakkad
23	*Gardenia jasminoides* J. Ellis	Rubiaceae	Leaves	Thenhippalam, Malappuram
24	*Getonia floribunda* Roxb.	Combretaceae	Leaves	Thenhippalam, Malappuram

25	*Hamelia patens* Jacq.	Rubiaceae	Leaves	Koottanad, Palakkad
26	*Hewittia malabarica* (L.) Suresh	Convolvulaceae	Aerial parts	Thenhippalam, Malappuram
27	*Impatiens minor* (DC.) Bennet	Balsaminaceae	Whole plant	Thenhippalam, Malappuram
28	*Jasminum grandiflorum* L.	Oleaceae	Leaves	Koottanad, Palakkad
29	*Justicia nagpurensis* V.A.W. Grah.	Acanthaceae	Whole plant	Thenhippalam, Malappuram
30	*Justicia wynaadensis* (Nees)	Acanthaceae	Aerial parts	Thenhippalam, Malappuram
31	*Laggera alata* Nanth.	Asteraceae	Aerial parts	Attappadi, Palakkad
32	*Lannea coromandelica* (Houtt.) Merr.	Anacardiaceae	Leaves	Thenhippalam, Malappuram
33	*Leea indica* (Burm.f.) Merr.	Vitaceae	Leaves	Koottanad, Palakkad
34	*Lecanthus peduncularis* (Royle) Wedd.	Urticaceae	Whole plant	Attappadi, Palakkad
35	*Leucas aspera* (Willd.)	Lamiaceae	Whole plant	Thenhippalam, Malappuram
36	*Lobelia alsinoides* Lam.	Campanulaceae	Aerial part	Koottanad, Palakkad
37	*Lophopetalum wightianum* Arn.	Celastraceae	Leaves	Attappadi, Palakkad
38	*Melia dubia* Cav.	Meliaceae	Leaves	Meenangadi, Wayanad
39	*Micrococca mercurialis* (L.) Benth.	Euphorbiaceae	Whole plant	Thenhippalam, Malappuram
40	*Murraya paniculata* (L). Jack	Rutaceae	Leaves	Thenhippalam, Malappuram
41	*Otacanthus caeruleus* Lindl.	Plantaginaceae	Leaves, flowers	Thenhippalam, Malappuram
42	*Pancratium triflorum* Roxb.	Amaryllidaceae	Leaves, bulb	Thenhippalam, Malappuram
43	*Pereskia aculeate* Mill.	Cactaceae	Leaves	Attappadi, Palakkad
44	*Phyllanthus simplex* Retz.	Phyllanthaceae	Whole plant	Thenhippalam, Malappuram
45	*Pithecellobium dulce* (Roxb.) Benth.	Fabaceae	Leaves	Attappadi, Palakkad
46	*Pothos scandens* L.	Araceae	Leaves	Koottanad, Palakkad
47	*Rhaphidophora pertusa* (Roxb.) Schott	Araceae	Leaves	Thenhippalam, Malappuram
48	*Ricinus communis* L.	Euphorbiaceae	Leaves	Thenhippalam, Malappuram
49	*Rauvolfia tetraphylla* L.	Apocynaceae	Leaves, seeds	Thenhippalam, Malappuram
50	*Schleichera oleosa* (Lour.) Oken	Sapindaceae	Leaves	Attappadi, Palakkad
51	*Senna alata* (L.) Roxb.	Fabaceae	Leaves	Koottanad, Palakkad

52	*Sauropus androgynus* L. Merr	Phyllanthaceae	Leaves	Koottanad, Palakkad
53	*Stachytarpheta indica* (L.) Vahl	Verbenaceae	Leaves	Koottanad, Palakkad
54	*Strobilanthes heyneanus* Nees	Acanthaceae	Aerial part	Attappadi, Palakkad
55	*Strychnos nux-vomica*L.	Loganiaceae	Leaves	Koottanad, Palakkad
56	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	Aerial part	Thenhippalam, Malappuram
57	*Talinum portulacifolium* (Forssk.) Asch. Ex Scweinf	Talinaceae	Leaves	Koottanad, Palakkad
58	*Terminalia arjuna* (Roxb. Ex DC) Wight and Arn.	Combretaceae	Leaves	Thenhippalam, Malappuram
59	*Thevetia peruviana* (Pers.) K. Schum.	Apocynaceae	Leaves	Attappadi, Palakkad
60	*Thunbergia erecta* (Benth)	Acanthaceae	Leaves	Koottanad, Palakkad
61	*Thunbergia grandiflora* (Roxb. ex Rottl.)	Acanthaceae	Leaves	Thenhippalam, Malappuram
62	*Torenia bicolor* Dalz.	Scrophulariaceae	Aerial part	Koottanad, Palakkad
63	*Trema orientale* (L.) Bl.	Ulmaceae	Leaves	Thenhippalam, Malappuram
64	*Vitex altissima* L.f.	Lamiaceae	Leaves	Thenhippalam, Malappuram
65	*Wattakaka volubilis* (L. fil) Stapf.	Apocynaceae	Leaves	Thenhippalam, Malappuram

1.2.2.1.1. Sequential maceration method

Each plant powder was precisely weighed and extracted sequentially with six organic solvents in order of increasing polarity: petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water. The powder, soaked in a solvent, was placed in a glass bottle and continuously stirred on a magnetic stirrer for 48 hours at room temperature. After this period, the mixture was filtered using Whatman filter paper No. 1, and the filtrate was dried using a rotary evaporator to obtain the crude extract, which was stored in glass bottles. The residue was then used for further extraction with the next solvent in sequence. Each extraction was repeated twice with the same solvent (Figure 1.2.1).

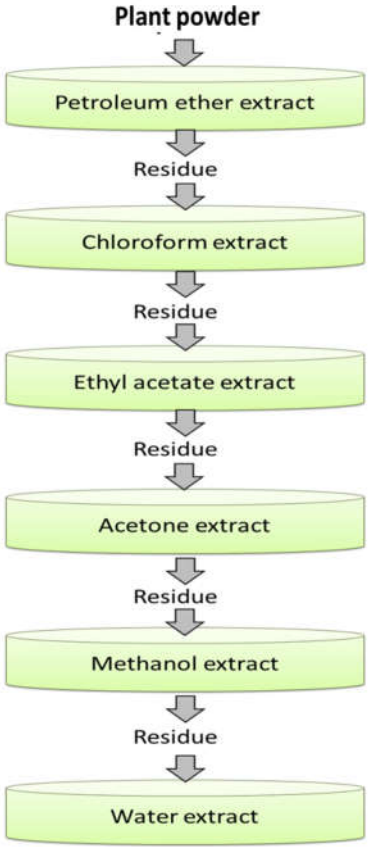


Figure 1.2.1. Schematic representation of plant extracts preparation by sequencial extraction using solvents in the increasing order of polarity. The residue of each step is used for extraction using the next solvent.

1.2.2.1.2. Plant extracts preparation for treatment

Each solvent extract was dried, accurately weighed and then dissolved in DMSO for use in cytotoxicity assays at a final concentration of 100µg/mL, with the final DMSO concentration being 0.1%.

1.2.3. Cell lines

The cell lines used in the present study were human colorectal adenocarcinoma cell lines (HT29, HCT116), human epidermoid carcinoma cell line (A431), human renal carcinoma cell line (A498), human cervical cancer cell line (HeLa), human breast adenocarcinoma cell line (MCF7), human osteosarcoma cell line (MG63), human ovarian teratocarcinoma cell line (PA1), human pancreatic epithelioid carcinoma cell line (PANC1), human squamous cell carcinoma cell line (UM-SCC-83B) and

human keratinocyte cell line (HaCaT) (Figure 1.2.2) and were purchased either from National Centre for Cell Science (Pune), India or other sources. HT29 was maintained and propagated in McCoy's 5A medium, while HCT116, HeLa, MCF7, PANC1 and UM-SCC-83B were cultured in DMEM and A431, A498, MG63, PA1 were cultured in MEM medium. All media were supplemented with 10% (v/v) foetal bovine serum, streptomycin (100µg/mL) and penicillin (100U/mL) (Table 1.2.2). All the cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. All assays were done after treating with the extracts for 48 hours except in experiments where it is mentioned otherwise.

Table 1.2.2. List of the cancer cell lines and the normal cell line used in the screening of plant extracts for cytotoxicity, their origin, morphology and growth medium used.

Sl. No.	Cell Line	Tissue	Disease	Morphology	Growth Medium
1	HT29	Colon	colorectal adenocarcinoma	Epithelial	McCoy's 5A +FBS
2	HCT116	Colon	colorectal adenocarcinoma	Epithelial	DMEM + FBS
3	A431	Skin; Epidermis	Epidermoid carcinoma	Epithelial	MEM + FBS
4	A498	Kidney	Renal carcinoma	Epithelial	MEM + FBS
5	HeLa	Uterus; Cervix	Adenocarcinoma	Epithelial	DMEM + FBS
6	MCF7	Breast; Mammary gland	Adenocarcinoma	Epithelial	DMEM + FBS
7	MG63	Bone	Osteosarcoma	Fibroblast	MEM + FBS
8	PA1	Ovary	Teratocarcinoma	Epithelial	MEM + FBS
9	PANC1	Pancreas; Duct	Epithelioid carcinoma	Epithelial	DMEM + FBS
10	UM-SCC-83B	Metastatic oral	Squamous cell carcinoma	Epithelial	DMEM + FBS
11	HaCaT	Skin; Epidermis	---	Keratinocyte	DMEM + FBS

1.2.4. Cell culture and maintenance

The cell lines were received in a moderately confluent condition. The medium was removed, later washed with 1XPBS and added 3-5 mL fresh medium along with the earlier medium. The cultures were kept inside a CO_2 incubator under 5% $_{CO2}$ at 37°C for further expansion of the culture.

1.2.4.1. Sub-culture

Adherent cells are sub-cultured/passaged when they are in log phase, before they reach confluence to keep the cell at an optimal density for continued growth which

would stimulate further proliferation. The following steps were followed for sub-culture:

1. Discarded the spent medium and
2. Washed the cells with 1XPBS.
3. Added 1-2 mL trypsin solution and kept inside the incubator for 2-5 minutes.
4. Then diluted the trypsin three times with medium as FBS inactivates trypsin.
5. Transferred the content into a 15 mL falcon tube.
6. It was centrifuged at 2000 rpm for 10 minutes.
7. Later the supernatant was discarded and the cell pellet was re-suspended in 1 mL fresh medium.
8. It was added into new tissue culture flasks containing 5 mL fresh medium in order to get a desirable population in it.

1.2.4.2. Storage

The excess cells were cryopreserved for future use according to the following procedure:

1. The spent medium was discarded.
2. Washed the cells with 1XPBS.
3. Added 1-2 mL trypsin solution and kept inside the incubator for 2-5 minutes.
4. Then diluted the trypsin three times with medium so that FBS in the medium inactivates trypsin.
5. Transferred the content into a 15 mL falcon tube.
6. It was centrifuged at 2000 xg for 10 minutes.
7. Later the supernatant was discarded and the cell pellet was re-suspended in 1 mL fresh medium.
8. The cells were counted on a hemocytometer, and the cell suspension was diluted in medium to achieve the desired cell count (5000-10000/mL).
9. Pipetted corresponding volume into a storage vial and centrifuged at 2000 rpm.

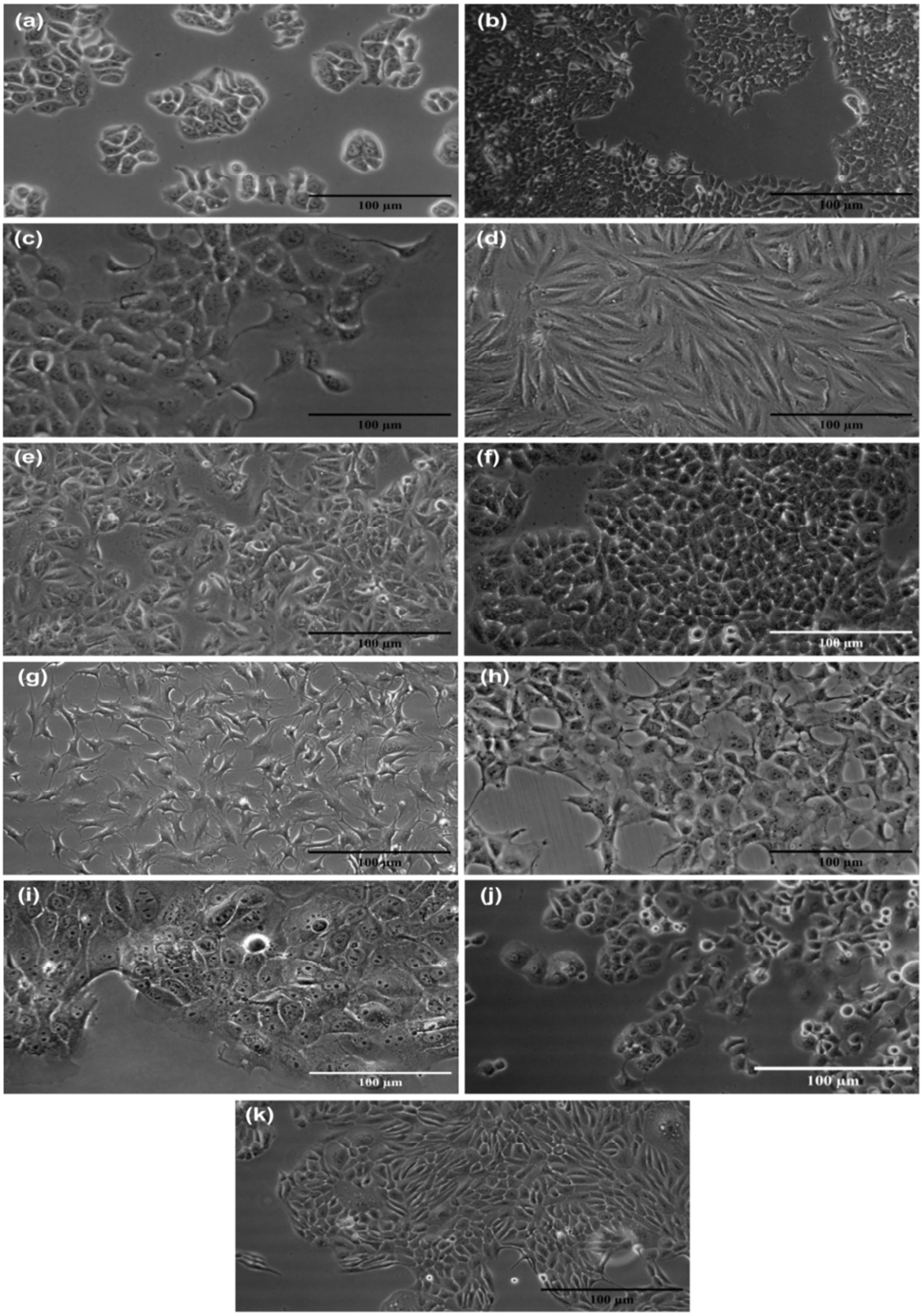


Figure 1.2.2. The cell lines used in the screening of plant extracts for anticancer activity. (a) Human colorectal adenocarcinoma cell line HT29, (b) Human colorectal adenocarcinoma cell line HCT116), (c) human epidermoid carcinoma cell line (A431), (d) human renal carcinoma cell line (A498), (e) human cervical cancer cell line (HeLa), (f) breast adenocarcinoma cell line (MCF7), (g) human osteosarcoma cell line (MG63), (h) human ovarian teratocarcinoma cell line (PA1), (i) human pancreatic epithelioid carcinoma cell line (PANC1), (j) human squamous cell carcinoma cell line (UM-SCC-83B) and (k) human keratinocyte cell line (HaCaT)

10. Discarded the supernatant and re-suspended the cell pellet in 900 µL medium. 100 µL DMSO was added and mixed.

11. Store at -80°C until further use.

1.2.4.3. Cytotoxicity assay/ MTT assay

The determination of cytotoxicity or anti-proliferative activity was based on the reduction of a water-soluble, yellow dye, MTT. In this assay (Mosmann, 1983) MTT was enzymatically reduced by mitochondrial dehydrogenases, leading to the formation of a purple-coloured formazan crystals. These crystals were subsequently solubilized in DMSO and subjected to spectrophotometric analysis.

Cancer cells and normal cells were each seeded at a density of $5X10^3$ cells/well in the 96-well tissue culture plates, followed by an overnight incubation. Subsequently, the cancer cells and normal cells were exposed to various concentrations of plant extracts, 100µg/mL at final, prepared from DMSO stock solution for 48 hours inside the incubator. Control cell populations received appropriate volumes of the vehicle control, DMSO.

Following the treatment and incubation phases, MTT was introduced into each well of the 96-well plate, achieving a final concentration of 500µg/mL. This was followed by incubation at 37°C in a dark environment for duration of 3-4 hours. Later, the formazan crystals generated in response to MTT were dissolved using the lysis buffer, and the absorbance was quantified at 570nm using micro plate reader. For calculation of percentage mortality, the viability of cells grown in medium containing vehicle alone was considered as 100%. The disparity in absorbance between the treated and untreated control samples was employed to calculate cell mortality rate using the formula:

$$\text{Cell mortality rate (in \%)} = 100 - \left\{ \frac{(\text{Absorbance}_{\text{Sample}} - \text{Absorbance}_{\text{Blank}})}{(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Blank}})} \right\}$$

1.2.4.3 Statistical analysis: The results were analysed using Microsoft excel and were expressed as mean±SEM (n=4).

RESULTS

Plant derived compounds have emerged as a promising avenue in the quest for innovative cancer treatments. These compounds, found abundantly in nature, offer potential solutions to the limitations of conventional therapies. Their diverse range of bioactive molecules, such as polyphenols, alkaloids, terpenoids, and flavonoids, have demonstrated the ability to selectively target cancer cells, induce cell death, and inhibit tumour growth. This intersection of traditional herbal wisdom and modern science holds the promise of more effective and less toxic cancer treatments, making it a focal point of on-going research in the field of oncology.

1.3.1. Screening of plant extracts for cytotoxicity against cell lines

In this research study, a total of 65 plant species were subjected to screening to assess their anticancer properties against a range of cancer cell lines. Additionally, the study meticulously examined the differential mortality rates of these plant derived compounds on normal cell lines. This comprehensive investigation serves as a critical step in the pursuit of identifying potential novel cancer therapeutics from natural sources, while also ensuring their selectivity and safety profile for healthy cells.

Plant parts, primarily leaves, were gathered from Palakkad, Malappuram and Wayanad districts of Kerala. Different solvent extracts derived from these plants displayed varying mortality rates when tested against multiple cancer cell lines and normal cell line.

The various cell lines demonstrated differing levels of susceptibility to the plant extracts, which may be attributed to the specific characteristics of each cell line. Notably, among the extracts tested, chloroform extracts exhibited higher toxicity towards many cell lines compared to other types of extracts. This suggests that the cytotoxic effects of the plant extracts may vary depending on both the chemical properties of the extracts and the inherent vulnerabilities of the specific cell lines.

1.3.1.1. Screening of plant extracts for cytotoxicity against HT29

When analysing the cytotoxicity of plant extracts against the colorectal carcinoma cell line (HT29), a total of 426 different extracts were examined. Of these, 59 extracts exhibited more than 50.00% mortality, indicating a high potential for anticancer activity. Notably, 18 extracts showed exceptionally high percentage mortality, exceeding 90.00%, after 48 hours of treatment at a concentration of 100 µg/mL. Among the most effective extracts, the methanol and acetone extracts of *Alangium salviifolium* demonstrated the highest percentage mortality of 99.70±0.11% and 99.70±0.98%, respectively. Following closely were the chloroform extracts of *Getonia floribunda* (99.12±1.62%) and *Strobilanthes heyneanus* (99.02±0.45%). These findings underscore the significant cytotoxic potential of certain plant extracts, particularly those of *Alangium salviifolium*, *Getonia floribunda*, and *Strobilanthes heyneanus*, as promising sources of anticancer compounds (Table 1.3.1).The other extracts with above 90.00% mortality were Leucas aspera chloroform extract (99.54±1.05%), *Chassalia curviflora methanol extract (*98.09±1.56%)*, Phyllanthus simplex* acetone extract *(*95.95±0.48%), *Stachytarpheta indica* acetne extract (95.23±1.02%), *Melia dubia* chloroform extract (94.23±0.35%), *Strobilanthes heyneanus* petroleum ether extract (92.95±0.75%), and *Thevetia peruviana* methanol extract (92.46±1.85%). *Alangium salviifolium* water extract, *Chassalia curviflora* acetone extract, chloroform and ethyl acetate extracts of *Cipadessa baccifera* leaf, *Combretum indicum* chloroform extract, *Conyza canadensis* chloroform extract, methanol and water extracts of *Euphorbia tithymaloides, Getonia floribunda* acetone extract, petroleum ether and chloroform extracts of *Hewittia malabarica,* ethyl acetate extract of *Melia dubia, Micrococca mercurialis* petroleum ether extract, *Otacanthus caeruleus* leaf chloroform extract, *Pereskia aculeate* ethyl acetate extract, *Schleichera oleosa* chloroform extract, petroleum ether and water extracts of *Thevetia peruviana* and chloroform extract of *Cissus discolor* also exhibited a mortality above 50.00%. At the same time, these plants were showing differential cytotoxicity with less than 50.00% mortality on HaCaT cell line, indicating their anticancer potential.

Table 1.3.1. Percentage mortality of the plant extracts at 100 µg/mL on the colorectal cancer cell line, HT29 after 48 hours.

Sl. No	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
1	*Acalypha fruiticosa*	0.99±1.14	29.88±1.93	15.81±1.50	19.73±0.87	7.69±1.31	6.36±0.86
2	*Alangium salviifolium*	9.42±1.06	**98.58±0.67**	56.25±1.35	**99.70±0.98**	**99.70±0.11**	55.33±1.58
3	*Anamirta cocculus*	0.63±1.03	16.75±1.34	**91.16±0.24**	25.01±1.83	15.33±2.37	12.29±1.78
4	*Antigonon leptopus*	3.95±1.26	14.81±2.14	1.14±1.08	15.97±1.10	5.49±0.83	20.38±2.44
5	*Argyreia pomacea*	4.01±1.58	31.78±2.66	20.63±2.35	25.24±5.15	1.64±0.67	3.26±1.51
6	*Bauhinia phoenicea*	6.86±1.62	20.05±1.53	10.22±0.52	2.18±0.76	2.43±0.80	1.93±0.55
7	*Bridelia retusa*	15.03±1.51	10.92±1.54	11.84±1.86	10.10±1.37	9.22±1.35	10.99±1.10
8	*Calopogonium mucunoides*	2.95±0.83	9.56±2.15	4.13±1.11	11.53±0.13	1.59±0.62	3.79±1.43
9	*Carl rheedii*	31.34±0.67	32.51±2.37	38.37±4.82	23.51±2.57	30.87±0.92	35.93±1.21
10	*Cassia javanica*	3.91±1.29	6.80±3.10	1.70±2.47	13.63±1.70	24.99±3.65	8.67±6.15
11	*Chassalia curviflora*	21.90±2.65	36.46±1.34	23.49±3.96	75.65±2.78	**98.09±1.56**	37.20±3.78
12	*Cheilocostus speciosus*	15.47±1.60	18.66±3.84	33.72±2.65	12.75±1.63	27.71±3.16	23.42±4.11
13	*Chrysophyllum cainito*	4.19±0.34	9.00±0.96	10.87±1.73	14.83±1.95	7.48±0.64	10.08±0.92
14	*Cipadessa baccifera* leaf	19.60±2.84	51.06±2.18	81.20±1.66	53.86±0.98	28.21±2.63	5.10±1.15
15	*Cipadessa baccifera* seed	**97.42±0.13**	34.16±1.76	0.85±1.18	16.44±2.41	15.50±4.41	30.69±2.42
16	*Cissus discolor*	26.55±2.03	53.18±1.70	3.81±2.19	9.44±4.01	8.10±4.31	20.37±3.16
17	*Cochlospermum religiosum*	5.34±1.09	6.02±2.18	7.54±0.55	1.90±0.64	5.05±0.67	2.95±0.72
18	*Combretum indicum*	3.29±2.33	65.35±1.47	15.54±5.99	31.72±7.33	16.57±4.44	41.39±3.84
19	*Conyza canadensis*	**95.04±0.58**	78.74±1.50	0.72±1.75	36.11±4.24	21.49±2.93	25.41±3.21
20	*Cyclea peltata*	4.55±1.07	2.83±1.62	4.14±0.85	2.15±2.65	30.45±3.61	10.29±3.12
21	*Drypetes venusta*	32.82±1.86	35.24±3.53	35.79±1.75	35.96±2.72	37.41±3.82	44.03±5.27
22	*Entada rheedii*	10.06±1.74	5.32±1.43	12.75±1.55	18.00±1.77	15.66±1.93	13.19±1.38
23	*Euphorbia heterophylla*	10.49±2.65	10.96±2.80	3.72±1.23	2.77±1.15	15.25±2.35	29.15±3.95

24	*Euphorbia tithymaloides*	34.63±1.54	28.01±4.84	9.19±2.03	46.15±7.01	65.06±6.23	84.33±4.09
25	*Gardenia jasminoides*	8.28±2.16	34.25±3.89	8.80±2.62	9.95±0.75	6.72±0.51	14.60±1.45
26	*Getonia floribunda*	71.54±2.36	**99.12±1.62**	32.50±1.05	71.70±1.58	15.56±1.48	14.54±1.90
27	*Globba schomburgkii*	15.69±1.58	3.03±1.05	17.01±2.67	55.46±1.50	36.98±1.61	29.49±2.66
28	*Hamelia patens*	24.94±2.39	38.64±2.40	18.96±0.72	24.62±1.75	31.10±2.26	11.83±0.56
29	*Hewittia malabarica*	65.38±0.94	81.59±4.90	21.21±1.30	12.62±2.54	19.91±3.69	22.47±0.77
30	*Impatiens minor*	30.01±2.04	28.67±1.84	26.05±1.35	49.41±0.58	37.57±0.27	19.18±4.04
31	*Jasminum grandiflorum*	13.23±0.66	4.44±1.49	1.10±1.58	33.83±3.93	46.96±4.10	21.18±2.21
32	*Justicia nagpurensis*	19.26±4.91	17.94±2.84	22.99±2.26	33.73±4.41	39.98±1.42	6.62±1.10
33	*Justicia wynaadensis*	12.80±3.93	22.46±4.93	17.18±3.02	25.38±4.35	18.44±3.41	10.45±4.99
34	*Laggera alata*	49.37±1.21	14.08±1.54	13.44±1.23	35.11±2.48	16.55±1.58	13.03±1.33
35	*Lannea coromandelica*	18.68±2.99	22.64±3.36	13.82±3.41	14.07±4.09	12.01±2.74	3.29±2.08
36	*Leea indica*	25.95±2.49	30.48±2.08	31.27±2.67	18.55±4.83	28.61±3.27	21.64±3.05
37	*Lecanthus peduncularis*	12.64±2.26	5.57±2.24	8.44±2.14	20.90±4.62	28.62±2.27	22.26±3.86
38	*Leucas aspera*	3.91±1.86	**99.54±1.05**	12.48±1.14	27.87±2.45	47.54±5.02	13.90±3.92
39	*Lobelia alsinoides*	10.86±3.79	13.22±3.82	32.59±2.91	38.04±5.94	44.79±2.72	20.81±1.55
40	*Lophopetalum wightianum*	18.62±1.46	6.48±0.73	1.22±0.84	23.10±3.25	6.51±2.85	6.61±2.10
41	*Melia dubia*	47.06±1.80	**94.23±0.35**	50.25±5.46	4.84±1.53	4.53±1.09	5.91±3.07
42	*Micrococca mercurialis*	86.82±1.50	39.08±2.68	21.58±1.69	39.97±2.48	38.01±1.93	23.86±1.85
43	*Murraya paniculata*	11.69±1.71	11.40±1.42	12.51±1.52	28.39±1.98	46.37±3.89	42.84±1.16
44	*Otacanthus caeruleus* flower	33.25±1.56	85.79±1.67	14.41±1.52	13.59±1.64	19.52±1.64	18.73±1.73
45	*Otacanthus caeruleus* leaf	16.21±0.53	68.16±3.74	26.65±0.50	7.47±0.95	7.00±0.81	7.45±1.12
46	*Pancratium triflorum* bulb	57.97±0.59	82.50±0.65	79.56±1.74	63.13±1.21	68.04±1.51	49.72±2.34
47	*Pancratium triflorum* leaf	51.21±1.99	58.08±1.96	51.00±2.24	58.65±0.78	55.17±1.18	61.59±0.51
48	*Pereskia aculeate*	26.82±1.88	15.10±6.65	51.58±1.69	20.84±8.65	18.01±3.66	28.86±1.85
49	*Phyllanthus simplex*	8.66±2.44	7.81±2.44	44.50±2.27	**95.95±0.48**	40.73±1.98	3.70±1.96
50	*Pithecellobium dulce*	11.63±3.79	2.20±1.94	5.71±3.01	2.50±0.59	7.27±1.57	8.66±4.07
51	*Pothos scandens*	19.37±2.66	8.08±1.46	13.00±1.04	16.60±1.63	6.84±1.73	22.98±2.63
52	*Ricinus communis*	8.59±2.78	9.62±3.38	28.51±3.36	35.68±4.40	20.78±1.51	23.44±1.64
53	*Rauvolfia tetraphylla* leaf	31.67±1.11	40.48±0.77	20.78±4.48	26.95±3.08	28.51±2.81	27.12±1.82

54	*Rauvolfia tetraphylla* seed	1.66±0.99	10.32±1.21	2.78±2.38	8.64±2.05	29.33±2.35	18.60±2.15
55	*Rhaphidophora pertusa*	19.61±2.52	20.57±7.83	18.97±6.50	12.27±3.64	8.09±1.51	2.03±0.98
56	*Schleichera oleosa*	59.38±2.20	57.90±4.22	15.53±1.31	19.84±2.99	3.03±3.26	10.06±3.08
57	*Senna alata*	24.83±2.89	19.17±4.02	14.08±3.88	22.35±1.83	36.62±3.37	28.43±1.37
58	*Sauropus androgynus*	11.63±3.79	2.20±1.94	5.71±3.01	25.53±2.05	4.62±0.90	6.35±1.18
59	*Stachytarpheta indica*	41.68±3.07	50.37±3.42	31.52±5.5	85.67±4.55	**95.23±1.02**	74.81±6.07
60	*Strobilanthes heyneanus*	**92.95±0.75**	**99.02±0.45**	22.73±2.83	4.13±1.49	3.22±2.99	4.70±2.66
61	*Strychnos nux-vomica*	37.38±0.83	7.70±1.33	10.17±1.27	20.38±2.25	14.40±1.59	50.57±5.25
62	*Synedrella nodiflora*	25.67±4.37	26.31±2.92	18.58±2.79	7.92±2.40	6.74±0.78	21.62±1.78
63	*Talinum portulacifolium*	10.01±1.28	14.78±1.53	29.14±1.40	15.01±0.73	11.09±2.34	15.00±2.12
64	*Terminalia arjuna*	3.32±1.15	7.78±2.60	4.12±0.54	25.53±2.05	4.62±0.90	6.35±1.18
65	*Thevetia peruviana*	56.91±2.69	**97.67±0.50**	**92.62±0.34**	**95.59±0.38**	**92.46±1.85**	53.49±2.56
66	*Thunbergia erecta*	36.84±1.88	31.10±1.39	15.69±3.08	34.09±1.68	30.26±2.92	37.11±1.55
67	*Thunbergia grandiflora*	13.25±2.67	19.95±4.87	6.75±1.16	8.98±0.36	2.41±0.87	34.74±1.12
68	*Torenia bicolor*	0.79±0.91	74.54±1.35	3.49±1.70	12.47±1.75	7.16±5.97	16.47±3.65
69	*Trema orientale*	5.39±1.18	6.71±1.74	10.59±2.87	17.00±1.21	6.85±0.81	6.02±1.55
70	*Vitex altissima*	5.53±3.93	6.90±1.77	3.43±1.36	11.60±3.16	10.46±3.96	8.26±2.37
71	*Wattakaka volubilis*	8.37±1.09	21.09±1.11	5.26±1.10	2.58±1.45	5.64±0.92	18.76±0.99

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.2. Screening of plant extracts for cytotoxicity against HCT116

The colorectal carcinoma cell line HCT116 was treated with 426 different plant extracts at a concentration of 100 µg/mL for 48 hours, resulting in varying levels of cytotoxicity across the extracts. Of these, 34 extracts demonstrated a mortality percentage above 50.00%. The methanol extract of *Chassalia curviflora* exhibited the highest cytotoxicity with a mortality percentage of 99.48±0.53%, followed closely by the methanol extract of *Alangium salviifolium* at 99.04±0.13%. Notably, 7 extracts displayed exceptionally high mortality percentage, exceeding 90.00%, highlighting their strong potential as anticancer agents (Table 1.3.2).

Table 1.3.2. Percentage mortality of the plant extracts at 100 µg/mL on the colorectal cancer cell line, HCT116 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
1	*Acalypha fruiticosa*	14.23±3.72	21.20±1.98	9.39±1.35	39.47±2.20	16.37±3.47	28.41±5.12
2	*Alangium salviifolium*	59.13±0.65	**98.29±0.28**	79.27±1.26	**90.76±0.38**	**99.04±0.13**	66.98±1.50
3	*Anamirta cocculus*	10.78±1.82	9.47±2.66	69.18±2.26	31.52±1.39	9.68±2.39	11.53±2.25
4	*Antigonon leptopus*	29.80±1.99	45.51±3.11	31.95±2.28	61.11±1.71	6.56±2.03	5.06±1.35
5	*Argyreia pomacea*	8.10±0.77	16.25±1.15	2.40±1.21	35.77±2.23	30.58±0.89	15.07±2.25
6	*Bauhinia phoenicea*	37.31±1.56	48.32±2.33	34.33±3.01	29.37±3.20	30.29±5.12	7.67±3.47
7	*Bridelia retusa*	12.56±1.62	27.36±2.03	42.85±3.78	39.53±1.45	12.08±2.05	22.78±3.11
8	*Calopogonium mucunoides*	15.43±1.06	15.85±1.06	17.12±1.27	27.13±3.17	23.42±0.82	13.06±1.21
9	*Carl rheedii*	23.56±2.16	39.47±4.12	21.61±5.06	44.14±0.34	17.94±3.38	8.60±3.11
10	*Cassia javanica*	2.92±0.93	12.06±0.89	8.41±0.79	5.86±1.21	1.51±1.00	10.56±1.34
11	*Chassalia curviflora*	13.22±6.89	22.35±4.43	51.72±3.90	66.07±2.59	**99.48±0.53**	28.31±7.83
12	*Cheilocostus speciosus*	13.45±5.20	38.13±0.61	51.42±2.79	42.23±4.06	35.51±5.79	30.87±1.63
13	*Chrysophyllum cainito*	39.30±3.00	13.54±4.12	17.46±5.05	20.24±7.13	9.57±2.06	22.77±6.72
14	*Cipadessa baccifera* leaf	52.04±2.18	64.00±1.47	38.39±1.37	21.07±1.65	37.21±4.70	36.01±1.77
15	*Cipadessa baccifera seed*	49.10±1.69	23.29±0.96	7.88±3.42	22.48±1.17	10.35±0.72	13.78±5.26
16	*Cissus discolor*	29.05±0.98	32.10±3.48	25.10±2.59	17.75±2.48	29.03±2.83	19.45±5.06
17	*Cochlospermum religiosum*	10.47±3.29	11.05±1.24	14.62±1.63	30.08±3.09	11.57±3.87	24.13±0.88
18	*Combretum indicum*	16.56±3.01	13.10±2.88	4.09±3.60	2.12±0.93	10.19±2.06	3.29±1.02
19	*Conyza canadensis*	7.85±1.51	34.62±2.38	21.13±2.62	39.51±4.61	40.59±4.12	23.67±4.35
20	*Cyclea peltata*	26.62±1.29	34.88±1.96	31.95±5.37	34.41±4.60	29.63±1.06	16.87±2.88
21	*Drypetes venusta*	23.48±1.03	27.37±1.63	27.59±4.78	36.77±0.96	33.25±1.50	14.94±1.30
22	*Entada rheedii*	4.12±2.09	10.47±1.81	10.45±1.78	7.17±2.07	1.05±2.06	3.17±0.97
23	*Euphorbia heterophylla*	6.56±1.27	24.23±2.45	20.23±2.19	13.84±1.03	2.17±2.01	4.91±1.76

24	*Euphorbia tithymaloides*	11.25±2.66	6.77±3.13	18.52±5.52	25.36±1.53	46.73±4.05	17.21±2.01
25	*Gardenia jasminoides*	10.93±1.44	5.23±3.03	15.55±1.73	14.28±3.35	4.46±1.04	9.02±3.49
26	*Getonia floribunda*	4.90±4.02	68.56±1.01	6.76±2.30	63.36±1.16	4.84±1.54	7.70±2.29
27	*Globba schomburgkii*	15.63±1.24	4.40±3.39	8.56±4.19	11.36±2.18	26.13±1.17	5.87±2.94
28	*Hamelia patens*	34.12±5.71	33.24±1.78	39.55±2.22	23.88±3.13	35.65±4.51	17.72±2.69
29	*Hewittia malabarica*	11.99±5.36	1.48±2.26	4.48±2.09	38.23±5.11	41.17±5.31	18.34±2.06
30	*Impatiens minor*	26.81±2.90	27.53±4.27	9.14±2.58	8.28±2.02	26.70±4.12	38.97±3.77
31	*Jasminum grandiflorum*	9.06±0.38	38.52±0.92	5.75±1.54	31.38±4.01	29.28±3.37	30.08±1.76
32	*Justicia nagpurensis*	35.61±2.19	31.39±2.02	30.38±3.45	31.63±2.25	23.09±1.15	45.73±3.23
33	*Justicia wynaadensis*	6.95±3.52	10.55±1.25	11.11±3.42	11.43±1.31	9.01±1.10	4.70±1.40
34	*Laggera alata*	45.23±2.36	**96.06±0.25**	64.36±8.29	64.39±5.61	45.73±3.38	19.45±2.04
35	*Lannea coromandelica*	15.68±2.64	24.38±1.52	21.42±1.78	22.03±2.31	19.90±1.72	21.46±2.16
36	*Leea indica*	2.59±2.29	10.42±2.92	4.47±1.98	3.38±1.79	7.01±1.24	4.34±3.51
37	*Lecanthus peduncularis*	4.98±2.47	10.29±2.21	3.08±1.38	8.15±2.36	14.01±3.07	24.26±2.06
38	*Leucas aspera*	1.50±1.77	**98.16±0.23**	25.15±0.94	16.91±2.90	20.02±3.39	14.62±3.80
39	*Lobelia alsinoides*	10.11±2.45	12.09±1.76	17.86±2.35	1.99±5.36	1.48±2.26	4.48±2.09
40	*Lophopetalum wightianum*	5.09±0.93	2.02±1.10	1.00±1.23	13.62±1.16	5.34±0.85	0.12±0.45
41	*Melia dubia*	17.16±0.31	29.63±0.99	13.94±1.59	19.09±1.35	13.08±1.00	8.97±2.00
42	*Micrococca mercurialis*	1.63±8.47	15.66±4.85	4.91±5.69	1.75±3.05	2.09±0.33	1.74±7.33
43	*Murraya paniculata*	5.33±0.91	14.87±0.71	13.21±1.21	68.25±2.85	6.99±1.20	11.31±1.62
44	*Otacanthus caeruleus* flower	1.44±1.65	2.52±1.28	1.36±1.06	0.20±1.64	89.76±1.21	26.59±1.56
45	*Otacanthus caeruleus* leaf	36.17±1.22	**97.94±0.20**	20.33±1.45	11.22±1.41	2.88±2.34	2.96±2.15
46	*Pancratium triflorum* bulb	46.82±0.80	47.27±1.40	49.12±2.39	48.57±2.24	44.44±2.06	47.08±2.03
47	*Pancratium triflorum leaf*	42.49±1.23	55.02±1.55	45.51±2.11	33.81±1.78	44.91±1.49	50.36±2.13
48	*Pereskia aculeate*	11.45±1.53	11.53±0.90	13.4±1.05	12.45±2.20	11.37±1.40	24.67±0.62
49	*Phyllanthus simplex*	34.87±2.03	15.61±2.82	28.33±1.83	32.71±2.50	37.29±5.77	36.15±2.35
50	*Pithecellobium dulce*	4.56±1.03	0.18±2.89	2.10±0.78	10.36±1.75	2.88±2.77	1.44±0.70

51	*Pothos scandens*	5.86±1.00	6.76±1.28	10.46±2.82	9.27±1.39	10.75±2.19	20.76±1.60
52	*Ricinus communis*	8.41±2.21	1.92±2.22	1.64±2.73	0.69±1.45	12.79±0.70	13.76±1.78
53	*Rauvolfia tetraphylla* leaf	7.15±1.27	5.31±0.99	3.22±0.70	12.36±5.26	38.40±4.01	14.93±2.99
54	*Rauvolfia tetraphylla* seed	8.01±0.54	4.75±1.31	8.16±1.02	15.23±3.37	41.60±4.94	35.88±2.34
55	*Rhaphidophora pertusa*	11.25±4.01	15.00±3.14	27.28±3.66	44.35±6.11	32.26±5.91	13.12±3.61
56	*Schleichera oleosa*	4.01±1.95	1.72±1.42	16.63±1.15	19.26±9.19	37.20±3.28	15.34±1.65
57	*Senna alata*	9.52±1.60	27.84±1.37	24.86±1.28	8.79±1.39	11.48±1.21	10.94±0.67
58	*Sauropus androgynus*	6.16±1.84	18.91±1.59	12.63±1.00	12.34±1.15	6.85±1.30	1.19±1.38
59	*Stachytarpheta indica*	12.87±2.95	32.85±3.07	46.38±2.00	68.66±3.21	60.33±1.67	51.12±3.19
60	*Strobilanthes heyneanus*	13.29±1.99	14.44±1.29	19.38±3.87	7.03±1.36	2.85±2.20	1.77±2.95
61	*Strychnos nux-vomica*	7.18±1.61	13.29±1.09	5.49±0.53	41.44±0.85	5.62±0.92	2.94±0.73
62	*Synedrella nodiflora*	11.98±2.99	1.45±3.87	0.69±1.78	1.32±2.35	49.67±1.61	45.34±4.09
63	*Talinum portulacifolium*	7.36±2.99	22.98±1.06	16.21±0.88	35.05±4.22	48.20±0.82	44.12±3.02
64	*Terminalia arjuna*	6.68±2.05	7.51±1.38	22.81±1.14	44.40±1.62	58.66±0.93	4.23±1.69
65	*Thevetia peruviana*	51.10±0.94	69.86±0.88	62.27±0.95	65.09±0.38	30.12±3.15	25.12±3.12
66	*Thunbergia erecta*	6.21±2.03	10.86±2.05	38.23±5.20	43.79±4.92	51.38±8.62	47.45±5.48
67	*Thunbergia grandiflora*	6.90±1.82	5.51±1.35	0.19±1.71	34.27±3.33	25.30±4.56	7.98±6.31
68	*Torenia bicolor*	20.77±3.31	66.83±2.30	22.56±3.41	12.56±5.06	19.86±7.83	23.91±1.65
69	*Trema orientale*	16.67±3.49	5.04±1.42	5.78±1.94	5.21±1.20	4.69±2.07	16.95±2.39
70	*Vitex altissima*	6.33±2.24	10.21±1.04	24.84±3.20	3.96±2.42	2.39±3.17	3.77±2.23
71	*Wattakaka volubilis*	5.32±1.52	2.87±1.45	19.79±2.43	0.92±2.27	3.67±2.78	6.28±2.80

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.3. Screening of plant extracts for cytotoxicity against A431

Out of the plant extracts tested, 48 extracts showed mortality above 50.00% in skin cancer cell line A431. Among these, 9 extracts exhibited high toxicity with percentage mortality exceeding 90.00%. The chloroform extract of *Otacanthus caeruleus* flower caused the

highest percentage mortality (99.69±0.63%), followed by the chloroform extract (99.37±0.24%) and methanol extract (99.24±0.09%) of *Alangium salviifolium* (Table 1.3.3).

Table 1.3.3. Percentage mortality of the plant extracts at 100 µg/mL on the skin cancer cell line, A431 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
1	*Acalypha fruiticosa*	17.18±1.27	7.03±1.15	19.05±1.33	12.66±1.29	10.77±1.53	16.84±2.24
2	*Alangium salviifolium*	20.43±1.64	**99.37±0.24**	79.56±1.53	**95.73±0.92**	**99.24±0.09**	53.08±1.56
3	*Anamirta cocculus*	5.74±0.87	4.17±1.04	57.54±2.93	51.35±3.18	0.75±2.92	6.58±0.98
4	*Antigonon leptopus*	24.20±3.94	67.84±1.96	18.22±4.26	73.80±1.83	11.14±4.79	9.49±3.10
5	*Argyreia pomacea*	1.80±1.55	29.23±1.60	10.30±2.53	16.99±3.59	24.54±1.72	12.37±2.37
6	*Bauhinia phoenicea*	4.65±1.46	2.14±0.54	1.78±1.01	13.15±3.92	26.65±2.24	9.65±1.53
7	*Bridelia retusa*	1.83±2.80	12.36±2.33	2.82±1.58	7.33±1.75	13.58±1.29	1.31±1.69
8	*Calopogonium mucunoides*	27.50±2.49	25.71±2.39	47.63±0.86	15.12±2.20	8.25±1.92	21.45±0.65
9	*Carl rheedii*	19.08±2.36	89.42±0.80	36.00±2.28	21.69±2.72	21.52±2.71	16.92±2.23
10	*Cassia javanica*	22.63±4.94	9.64±0.96	9.75±2.48	11.04±0.58	88.32±1.62	4.05±2.03
11	*Chassalia curviflora*	6.47±0.91	17.31±5.59	39.00±3.15	43.70±1.04	78.65±1.70	20.83±3.91
12	*Cheilocostus speciosus*	12.96±1.93	12.72±1.83	16.81±1.43	12.21±3.00	46.22±2.53	17.64±1.57
13	*Chrysophyllum cainito*	18.33±1.98	22.45±6.39	9.82±2.12	20.52±2.51	22.33±1.23	38.10±0.91
14	*Cipadessa baccifera* leaf	12.73±1.53	54.68±2.32	19.81±1.42	26.35±2.14	14.26±2.78	17.00±1.83
15	*Cipadessa baccifera* seed	13.34±2.37	12.44±2.10	3.29±1.92	6.31±0.62	12.11±1.41	18.38±3.90
16	*Cissus discolor*	21.19±8.58	6.79±2.94	31.32±1.74	49.23±1.07	26.03±2.07	9.61±1.34
17	*Cochlospermum religiosum*	4.22±1.52	88.09±0.67	47.51±2.46	8.44±1.30	5.23±1.07	5.51±1.32
18	*Combretum indicum*	27.50±2.49	62.28±2.54	13.77±1.67	7.02±1.18	3.87±0.84	12.35±0.86
19	*Conyza canadensis*	59.24±1.84	36.55±3.56	15.50±3.90	38.69±3.43	46.64±1.82	23.36±1.89

20	*Cyclea peltata*	0.30±0.42	0.34±0.34	63.93±0.72	44.83±1.34	0.49±0.40	0.26±0.44
21	*Drypetes venusta*	29.63±1.71	1.50±2.31	51.40±1.89	54.37±1.34	14.99±1.47	11.01±1.73
22	*Entada rheedii*	21.26±1.79	19.03±2.39	21.18±1.45	40.17±2.17	20.73±1.61	14.67±1.39
23	*Euphorbia heterophylla*	16.35±2.33	26.67±2.15	31.54±1.97	20.33±2.78	11.31±1.80	23.26±1.89
24	*Euphorbia tithymaloides*	18.86±3.52	2.21±1.69	12.00±0.90	1.91±5.86	2.08±3.04	12.38±0.83
25	*Gardenia jasminoides*	15.82±5.01	17.29±2.00	14.43±2.11	16.67±2.09	8.62±2.39	5.05±2.21
26	*Getonia floribunda*	88.95±1.38	**97.40±0.19**	53.76±0.75	90.7±0.89	24.76±2.66	18.49±3.20
27	*Globba schomburgkii*	25.77±5.09	33.95±0.94	40.31±4.66	49.85±3.64	12.38±0.83	31.08±0.54
28	*Hamelia patens*	3.15±2.18	22.56±2.54	17.61±3.98	29.92±2.53	31.70±2.82	8.29±1.65
29	*Hewittia malabarica*	16.34±2.54	8.68±2.20	1.45±2.02	38.99±2.38	31.90±2.53	21.95±1.21
30	*Impatiens minor*	3.01±3.17	0.13±1.87	9.78±1.92	3.64±0.83	5.62±0.75	18.43±0.92
31	*Jasminum grandiflorum*	2.65±1.76	8.77±1.27	19.21±1.34	28.84±2.61	29.71±8.29	13.23±1.85
32	*Justicia nagpurensis*	22.68±1.23	27.89±2.30	11.17±2.22	3.48±1.62	8.81±2.69	7.01±0.65
33	*Justicia wynaadensis*	12.96±2.48	6.07±4.22	2.35±3.21	2.26±1.43	15.57±2.70	19.11±2.93
34	*Laggera alata*	**97.11±0.36**	84.19±0.60	67.46±1.13	69.56±1.66	45.98±2.89	10.08±3.29
35	*Lannea coromandelica*	37.36±1.65	32.85±3.56	25.23±2.96	9.65±2.96	18.16±1.70	26.95±1.96
36	*Leea indica*	1.63±1.82	1.65±1.45	4.20±3.74	1.35±2.02	0.57±1.74	1.46±2.24
37	*Lecanthus peduncularis*	41.79±0.87	35.45±2.36	27.84±1.78	13.47±1.58	16.18±2.57	18.19±1.02
38	*Leucas aspera*	9.10±1.79	**96.55±0.30**	6.56±0.95	23.83±1.19	9.80±1.82	2.26±3.20
39	*Lobelia alsinoides*	3.78±1.26	3.72±0.90	24.09±3.05	7.86±2.41	13.10±2.70	11.57±1.47
40	*Lophopetalum wightianum*	8.08±1.70	12.35±1.70	8.03±1.90	19.33±1.82	5.73±0.90	3.69±2.58
41	*Melia dubia*	39.80±2.74	58.93±2.10	35.08±2.61	5.84±1.57	5.24±1.58	1.46±0.49
42	*Micrococca mercurialis*	4.54±2.21	14.28±2.13	27.34±2.47	1.11±1.77	3.92±1.95	36.47±1.07
43	*Murraya paniculata*	6.46±1.94	12.27±1.36	6.85±1.06	19.95±1.82	3.30±0.80	12.63±1.03
44	*Otacanthus caeruleus* flower	67.48±2.48	**99.69±0.63**	38.91±3.13	27.14±3.76	13.91±2.64	7.86±2.40
45	*Otacanthus caeruleus* leaf	5.22±1.46	97.65±0.15	3.35±0.96	6.20±0.74	3.64±0.88	5.39±1.28
46	*Pancratium triflorum* bulb	46.41±2.66	71.21±1.05	78.01±1.58	70.38±1.04	58.67±0.71	38.50±1.50

47	*Pancratium triflorum leaf*	27.51±2.75	66.94±1.31	76.87±1.36	58.82±2.09	39.39±1.23	57.53±1.62
48	*Pereskia aculeate*	17.95±3.05	22.65±4.80	13.35±4.09	2.01±2.18	5.38±3.98	35.10±2.35
49	*Phyllanthus simplex*	20.70±3.91	26.55±1.69	33.65±3.44	75.80±0.67	18.23±1.71	16.12±1.67
50	*Pithecellobium dulce*	9.37±1.37	23.65±3.59	35.15±1.44	29.49±4.13	13.34±3.62	39.18±7.01
51	*Pothos scandens*	10.40±2.36	6.13±2.53	1.16±1.13	1.71±0.49	3.07±1.58	5.06±1.24
52	*Ricinus communis*	21.62±3.38	24.08±5.13	28.22±2.47	40.06±1.49	18.84±1.01	17.07±3.40
53	*Rauvolfia tetraphylla* leaf	27.86±1.47	38.62±0.94	16.29±2.94	12.00±2.66	27.42±3.93	12.69±1.72
54	*Rauvolfia tetraphylla* seed	30.03±4.11	48.39±2.50	20.34±1.98	10.67±1.30	10.04±1.72	20.36±1.85
55	*Rhaphidophora pertusa*	31.06±2.05	22.34±1.85	16.50±3.82	24.03±1.05	31.59±1.70	39.59±4.00
56	*Schleichera oleosa*	1.77±1.66	2.52±1.33	11.49±1.57	28.05±3.79	51.85±2.45	77.93±1.31
57	*Senna alata*	22.53±2.13	45.05±1.02	29.19±1.72	35.14±2.69	29.06±1.64	6.49±1.17
58	*Sauropus androgynus*	18.48±1.72	26.62±1.20	26.47±2.35	27.74±2.89	19.48±4.12	18.32±5.93
59	*Stachytarpheta indica*	2.51±3.21	27.30±2.52	48.13±6.88	64.67±2.08	31.08±0.54	11.70±2.52
60	*Strobilanthes heyneanus*	29.98±1.59	19.55±1.70	31.74±2.10	26.47±2.50	27.29±2.95	25.72±4.77
61	*Strychnos nux-vomica*	4.67±1.09	2.04±0.94	3.85±1.02	36.26±1.13	4.18±0.57	0.68±0.93
62	*Synedrella nodiflora*	0.54±0.55	4.11±1.33	15.46±2.06	14.57±1.56	4.08±1.86	9.49±1.09
63	*Talinum portulacifolium*	24.79±2.27	1.17±2.73	16.11±2.34	14.20±2.30	11.09±2.23	13.45±3.11
64	*Terminalia arjuna*	38.88±1.24	31.61±1.56	40.31±3.60	57.13±2.90	10.71±3.65	8.02±4.81
65	*Thevetia peruviana*	4.29±1.38	74.16±1.40	62.91±1.67	71.56±1.33	2.08±0.83	2.94±1.32
66	*Thunbergia erecta*	25.53±1.20	20.98±0.72	14.02±1.22	4.14±1.26	19.29±2.46	15.97±2.20
67	*Thunbergia grandiflora*	0.34±1.86	0.81±1.09	1.29±2.22	19.71±1.56	13.49±1.95	12.69±1.72
68	*Torenia bicolor*	4.76±2.66	17.68±2.04	4.57±1.91	26.35±1.91	33.29±2.64	29.08±2.51
69	*Trema orientale*	0.72±0.23	4.25±0.76	1.15±0.56	13.08±1.40	11.04±1.70	6.44±0.82
70	*Vitex altissima*	5.21±2.98	25.49±2.79	30.82±3.53	22.45±3.81	14.19±4.54	5.40±2.73
71	*Wattakaka volubilis*	9.14±1.01	32.01±1.66	26.83±0.69	2.46±0.18	20.12±1.25	16.48±0.53

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.4. Screening of plant extracts for cytotoxicity against A498

Out of the 426 plant extracts screened, 35 exhibited a percentage mortality above 50.00% against the human kidney cancer cell line, A498. The highest percentage mortality was observed with the chloroform extract of *Otacanthus caeruleus* leaf (99.97±0.25%), followed by the methanol extract of *Alangium salviifolium* (99.64±0.37%) and the chloroform extract of *Alangium salviifolium* (96.57±0.91%). Notably, only four extracts demonstrated toxicity with a percentage mortality exceeding 90.00% (Table 1.3.4).

Table 1.3.4. Percentage mortality of the plant extracts at 100 µg/mL on the renal cancer cell line, A498 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	4.11±1.60	11.09±1.41	22.19±1.34	17.03±0.78	22.63±2.89	16.38±0.80
2	*Alangium salviifolium*	24.11±3.97	**96.57±0.91**	88.22±1.46	86.00±1.56	**99.64±0.37**	59.87±1.05
3	*Anamirta cocculus*	15.06±2.55	1.09±1.72	68.54±0.69	66.54±1.70	13.00±0.92	12.71±1.58
4	*Antigonon leptopus*	36.37±2.68	23.25±4.23	29.66±1.98	58.32±2.48	19.26±2.74	8.65±2.06
5	*Argyreia pomacea*	12.36±1.48	35.89±2.61	23.23±1.70	13.54±1.67	11.36±1.83	5.44±2.45
6	*Bauhinia phoenicea*	6.35±4.23	16.67±2.85	21.54±1.97	20.33±1.78	21.31±0.80	13.26±2.89
7	*Bridelia retusa*	31.97±2.23	26.05±1.44	16.73±2.65	38.46±3.71	27.68±3.63	17.69±1.24
8	*Calopogonium mucunoides*	6.50±1.75	12.45±1.74	21.04±3.34	14.28±3.15	15.28±3.18	46.57±3.88
9	*Carl rheedii*	23.59±2.68	28.64±2.98	11.45±1.65	28.85±1.24	21.89±2.34	35.13±2.65
10	*Cassia javanica*	38.33±1.86	27.53±1.56	25.92±1.85	45.88±7.65	14.55±1.68	10.25±1.69
11	*Chassalia curviflora*	20.70±6.29	15.56±3.71	17.53±3.23	16.89±4.20	66.47±3.74	22.63±2.98
12	*Cheilocostus speciosus*	6.76±3.85	2.27±0.72	1.96±1.15	2.76±1.24	7.20±1.82	11.68±2.71
13	*Chrysophyllum cainito*	19.68±3.90	35.42±1.04	20.96±6.42	23.86±2.17	34.54±3.76	38.77±1.23
14	*Cipadessa baccifera* leaf	40.53±6.40	62.61±4.37	55.56±2.85	39.36±3.81	28.48±6.08	19.10±4.12
15	*Cipadessa baccifera* seed	11.84±2.35	8.07±4.25	28.97±4.50	9.05±2.63	8.90±1.37	12.81±3.95

16	*Cissus discolor*	8.33±3.70	31.05±2.58	35.92±1.37	25.45±4.90	23.63±5.21	6.82±0.49
17	*Cochlospermum religiosum*	9.48±5.62	13.76±1.36	19.44±7.23	20.89±3.56	15.65±3.99	7.69±4.63
18	*Combretum indicum*	0.97±1.48	19.09±2.29	25.29±1.79	28.69±2.37	24.50±1.29	24.05±2.86
19	*Conyza canadensis*	27.17±7.99	87.23±1.74	41.84±6.91	14.75±2.62	43.43±3.37	22.30±1.94
20	*Cyclea peltata*	1.04±1.94	2.82±1.90	49.80±0.88	26.09±1.78	1.92±1.75	0.64±2.10
21	*Drypetes venusta*	16.48±3.57	33.33±4.09	21.35±2.99	47.13±2.33	35.17±6.21	10.49±0.70
22	*Entada rheedii*	22.37±1.46	37.26±2.56	33.67±1.67	30.84±0.57	26.45±1.75	12.48±1.79
23	*Euphorbia heterophylla*	23.16±2.68	24.65±1.01	28.86±0.67	9.11±1.64	15.33±0.36	23.15±5.54
24	*Euphorbia tithymaloides*	12.75±1.53	25.86±5.13	37.55±2.98	26.55±1.46	19.56±1.96	26.26±1.63
25	*Gardenia jasminoides*	15.98±1.65	41.63±3.00	39.13±3.62	28.45±2.76	9.63±1.96	49.15±2.36
26	*Getonia floribunda*	22.98±3.27	6.14±2.22	6.12±4.32	27.19±4.99	18.75±4.80	12.21±4.09
27	*Globba schomburgkii*	78.51±2.25	37.03±3.58	37.91±5.24	74.21±1.80	36.74±2.53	46.25±3.54
28	*Hamelia patens*	24.04±3.08	8.07±2.74	16.22±3.88	6.54±1.21	18.60±1.65	21.19±1.23
29	*Hewittia malabarica*	15.72±2.72	40.98±1.41	16.15±3.53	39.37±2.81	18.39±2.83	23.16±6.71
30	*Impatiens minor*	2.81±0.96	1.29±0.86	7.51±0.57	0.68±3.01	5.15±1.31	1.52±3.68
31	*Jasminum grandiflorum*	22.70±2.08	21.66±4.55	26.43±3.22	34.70±1.96	38.70±1.87	7.69±0.66
32	*Justicia nagpurensis*	30.12±1.55	31.68±1.38	14.46±1.08	14.47±4.83	12.61±6.40	9.57±3.75
33	*Justicia wynaadensis*	35.93±3.80	49.82±3.19	9.72±2.13	22.00±2.82	14.21±1.66	18.77±8.85
34	*Laggera alata*	81.71±1.36	80.87±1.62	48.68±2.46	63.36±3.37	37.19±1.42	18.96±2.86
35	*Lannea coromandelica*	4.21±0.73	2.80±0.37	7.35±2.66	12.98±3.60	17.12±2.98	61.14±1.85
36	*Leea indica*	36.75±6.07	22.26±4.69	18.10±2.89	26.36±1.83	32.67±1.77	23.46±8.32
37	*Lecanthus peduncularis*	8.08±2.85	21.62±3.79	22.29±2.08	21.73±4.22	26.91±3.25	6.26±4.28
38	*Leucas aspera*	5.15±1.39	51.55±4.27	5.01±1.53	0.95±3.46	1.46±1.30	6.57±1.87
39	*Lobelia alsinoides*	20.48±1.95	16.20±1.55	18.99±7.74	13.81±3.44	19.73±4.06	22.17±2.14
40	*Lophopetalum wightianum*	20.11±0.89	19.31±2.00	7.69±0.56	42.68±1.44	20.63±0.83	1.68±0.49
41	*Melia dubia*	7.68±1.25	12.06±1.43	3.55±0.86	9.14±1.53	10.12±1.35	9.25±1.50
42	*Micrococca mercurialis*	28.37±3.99	38.13±3.95	31.57±1.65	37.56±1.83	24.90±2.86	12.98±6.12

43	*Murraya paniculata*	14.13±1.65	25.55±0.65	19.15±1.85	19.62±0.85	28.46±2.78	23.84±1.55
44	*Otacanthus caeruleus* flower	49.18±4.33	**90.52±0.59**	33.48±3.78	20.12±2.69	27.67±3.63	7.58±2.45
45	*Otacanthus caeruleus* leaf	40.10±1.75	**99.97±0.25**	34.03±1.01	17.92±1.42	20.23±0.82	16.63±0.76
46	*Pancratium triflorum* bulb	33.17±2.22	75.73±0.99	57.29±1.24	56.70±1.98	43.25±1.41	33.79±2.27
47	*Pancratium triflorum* leaf	34.61±3.08	44.71±0.95	64.21±1.68	54.51±1.15	33.98±1.59	57.61±1.14
48	*Pereskia aculeate*	20.79±3.30	14.64±2.92	20.93±2.59	14.46±3.48	2.22±1.28	47.99±1.54
49	*Phyllanthus simplex*	2.86±1.06	22.56±5.58	34.02±4.51	20.31±1.98	21.26±2.09	22.18±1.91
50	*Pithecellobium dulce*	21.96±2.27	45.79±1.47	49.89±1.73	55.16±3.60	51.56±1.21	69.85±0.75
51	*Pothos scandens*	78.52±2.26	42.03±3.03	10.96±3.09	17.69±1.18	13.98±2.69	1.85±2.08
52	*Ricinus communis*	7.16±2.70	9.15±1.26	11.59±2.57	10.15±2.36	15.13±0.87	19.86±1.08
53	*Rauvolfia tetraphylla* leaf	19.93±2.52	36.74±2.65	22.50±3.44	26.04±2.83	30.25±2.99	7.98±3.87
54	*Rauvolfia tetraphylla* seed	8.41±4.27	14.60±4.75	4.62±4.03	24.92±4.46	23.17±3.59	28.94±4.44
55	*Rhaphidophora pertusa*	9.80±3.13	12.36±2.82	41.97±5.27	1.07±0.36	15.89±1.08	2.62±0.66
56	*Schleichera oleosa*	28.01±1.82	16.56±1.01	1.53±0.13	32.50±3.25	9.21±3.99	3.02±1.25
57	*Senna alata*	10.54±2.23	28.55±0.88	32.65±1.67	8.93±1.06	13.15±2.05	16.16±1.39
58	*Sauropus androgynus*	21.93±2.34	16.03±4.74	32.00±5.30	24.14±5.46	8.23±2.37	8.54±4.50
59	*Stachytarpheta indica*	13.85±3.98	22.66±1.99	36.85±1.76	42.23±2.34	40.70±2.99	37.98±1.63
60	*Strobilanthes heyneanus*	78.74±1.22	73.47±3.57	78.74±2.72	5.78±1.62	4.36±1.43	37.38±4.18
61	*Strychnos nux-vomica*	30.60±4.05	2.82±1.18	6.91±4.42	33.90±7.42	4.23±1.11	6.38±4.37
62	*Synedrella nodiflora*	3.81±1.13	4.75±2.41	6.94±1.12	3.53±1.39	2.99±1.58	6.10±1.18
63	*Talinum portulacifolium*	0.93±2.78	9.12±2.43	11.70±0.78	17.43±2.68	7.77±0.93	12.96±1.42
64	*Terminalia arjuna*	7.96±1.74	27.44±1.96	31.36±3.55	18.70±2.25	15.65±1.21	19.56±3.99
65	*Thevetia peruviana*	0.64±0.16	79.20±0.47	77.63±1.05	80.33±0.44	1.54±0.86	3.84±0.87
66	*Thunbergia erecta*	14.16±2.23	22.35±3.56	29.59±4.23	19.53±5.11	19.85±6.39	11.89±1.08
67	*Thunbergia grandiflora*	9.70±1.46	21.39±0.49	6.49±1.18	0.35±2.82	5.99±3.02	4.33±2.63
68	*Torenia bicolor*	16.89±0.25	23.11±3.56	25.63±4.24	9.37±1.82	13.05±2.90	17.57±5.63
69	*Trema orientale*	0.72±0.23	4.25±0.76	1.15±0.56	13.08±1.40	11.04±1.70	6.44±0.82

70	*Vitex altissima*	2.32±3.82	22.01±1.58	22.49±3.21	17.99±2.73	31.47±2.08	10.32±1.33
71	*Wattakaka volubilis*	4.86±1.76	21.27±2.41	29.62±4.99	31.89±1.13	24.95±0.64	16.96±3.03

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.5. Screening of plant extracts for cytotoxicity against HeLa

In a study evaluating the cytotoxic effects of various plant extracts on the HeLa cell line, 38 extracts demonstrated over 50% cell death, with 10 extracts showing over 90% mortality. The most potent extract was the ethyl acetate extract of *Pancratium triflorum* bulb, which induced 99.34±0.28% cell death. This was closely followed by the ethyl acetate extract of *Pancratium triflorum* leaf, which exhibited 99.26±0.50% mortality, and the methanol extract of *Alangium salviifolium*, with a mortality percentage of 99.21±0.39%. These findings highlight the potential of these extracts as strong anticancer agents (Table 1.3.5).

Table 1.3.5. Percentage mortality of the plant extracts at 100 µg/mL on the cervical cancer cell line, HeLa after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	13.74±0.70	9.03±0.96	2.51±0.82	7.51±1.24	8.77±1.06	12.39±1.03
2	*Alangium salviifolium*	51.63±2.32	**99.10±0.35**	75.36±1.36	**98.91±0.48**	**99.21±0.39**	68.96±0.88
3	*Anamirta cocculus*	18.21±3.47	9.18±1.71	60.11±1.24	37.85±2.31	15.91±3.40	6.78±3.07
4	*Antigonon leptopus*	13.07±0.90	26.26±3.47	14.50±2.15	46.92±0.37	9.71±3.03	4.59±1.98
5	*Argyreia pomacea*	33.66±1.33	25.66±6.82	31.39±3.86	36.84±3.31	12.24±2.05	8.50±0.23
6	*Bauhinia phoenicea*	12.83±1.84	22.34±1.32	16.62±3.55	14.96±2.42	19.45±1.03	17.13±1.06
7	*Bridelia retusa*	29.89±3.68	26.58±2.12	48.06±1.83	27.93±2.41	40.44±2.63	31.15±2.65
8	*Calopogonium mucunoides*	10.69±3.25	36.88±0.85	21.46±3.98	25.79±3.06	15.32±4.13	38.79±2.59
9	*Carl rheedii*	29.38±2.72	6.59±2.52	20.62±2.66	5.40±1.32	2.73±1.30	2.08±0.72

10	*Cassia javanica*	16.97±1.70	2.56±1.05	5.30±1.03	4.04±1.29	1.42±0.61	0.54±0.61
11	*Chassalia curviflora*	29.85±3.27	33.47±3.21	48.67±2.62	48.41±3.77	**98.98±0.59**	32.44±1.19
12	*Cheilocostus speciosus*	14.81±1.01	17.22±0.64	23.63±1.60	16.06±1.95	36.10±0.84	52.17±1.33
13	*Chrysophyllum cainito*	26.86±1.41	49.86±3.36	37.19±3.02	48.32±2.10	11.93±1.51	30.99±2.91
14	*Cipadessa baccifera* leaf	42.17±2.38	33.08±2.81	19.31±2.49	17.31±2.41	29.10±2.03	20.11±3.12
15	*Cipadessa baccifera* seed	93.66±1.04	36.85±3.39	11.24±2.91	39.96±1.78	28.19±1.82	19.77±1.36
16	*Cissus discolor*	30.57±4.86	17.44±0.57	34.01±1.39	40.94±1.74	36.86±0.06	25.05±4.86
17	*Cochlospermum religiosum*	18.85±1.70	28.05±2.22	17.36±1.85	26.16±1.12	20.19±7.46	33.96±1.75
18	*Combretum indicum*	8.73±0.96	14.45±1.72	8.42±0.74	44.03±0.84	39.56±1.70	19.34±0.62
19	*Conyza canadensis*	22.82±2.07	79.61±2.37	17.73±1.73	35.61±3.55	45.84±2.23	17.21±1.45
20	*Cyclea peltata*	33.36±1.88	30.56±1.66	29.75±1.99	17.54±5.99	32.64±1.33	33.49±1.99
21	*Drypetes venusta*	46.87±2.40	16.55±2.00	16.69±0.66	14.29±1.19	4.75±2.19	11.82±2.11
22	*Entada rheedii*	30.17±2.76	31.64±3.82	23.13±3.08	16.37±1.56	15.14±1.40	29.28±2.33
23	*Euphorbia heterophylla*	13.27±0.97	20.27±2.79	21.88±3.05	17.25±0.92	13.15±1.21	12.01±2.33
24	*Euphorbia tithymaloides*	23.42±1.70	18.96±1.99	9.85±1.03	24.63±2.21	10.71±0.56	8.15±1.91
25	*Gardenia jasminoides*	20.43±3.27	46.38±2.03	38.14±3.95	21.34±2.81	28.94±2.35	3.05±2.15
26	*Getonia floribunda*	37.38±0.60	**91.69±1.82**	47.20±1.93	68.57±1.91	27.36±2.16	19.83±2.63
27	*Globba schomburgkii*	16.71±2.23	7.84±1.40	13.65±0.88	9.24±1.25	12.00±1.30	13.96±2.02
28	*Hamelia patens*	22.70±1.88	35.24±1.87	33.55±0.96	21.56±2.35	14.32±3.42	21.63±1.24
29	*Hewittia malabarica*	31.70±4.24	63.29±2.46	29.76±1.76	23.15±1.87	18.54±1.33	20.87±1.40
30	*Impatiens minor*	19.65±2.56	44.15±1.25	31.54±2.02	18.06±1.74	27.37±2.92	30.08±1.56
31	*Jasminum grandiflorum*	36.36±0.78	27.16±3.92	34.15±1.87	20.05±3.35	45.64±2.24	11.18±1.43
32	*Justicia nagpurensis*	22.56±1.63	3.37±0.37	12.33±1.75	8.12±2.97	33.67±1.19	6.96±2.21
33	*Justicia wynaadensis*	12.57±2.91	5.79±1.18	6.43±3.96	6.59±1.75	3.01±5.05	8.11±5.05
34	*Laggera alata*	31.97±0.79	11.86±1.33	19.27±3.94	23.38±3.77	10.49±1.31	14.06±0.36
35	*Lannea coromandelica*	6.86±1.22	18.90±1.84	21.43±1.70	33.39±2.77	18.62±1.22	24.10±1.29
36	*Leea indica*	13.65±3.15	13.19±3.18	13.25±2.10	23.76±2.43	10.92±2.88	9.88±2.00

37	*Lecanthus peduncularis*	38.16±1.56	14.37±1.72	28.08±1.95	41.46±1.29	5.82±2.04	23.67±2.46
38	*Leucas aspera*	15.47±2.58	77.47±1.08	37.91±1.18	40.77±1.13	50.31±0.51	4.75±1.58
39	*Lobelia alsinoides*	9.65±1.54	16.56±1.35	8.44±0.67	27.18±3.41	36.60±1.24	44.56±3.99
40	*Lophopetalum wightianum*	38.82±2.48	44.30±2.93	30.97±1.65	14.11±2.62	24.05±2.45	13.17±3.57
41	*Melia dubia*	28.16±2.38	73.55±1.56	29.73±4.78	19.13±0.95	14.21±2.12	9.36±1.88
42	*Micrococca mercurialis*	8.17±2.53	14.84±1.16	25.41±1.94	19.35±0.81	12.55±0.97	27.31±1.40
43	*Murraya paniculata*	7.91±0.91	2.51±1.97	12.82±1.94	33.00±1.08	30.36±1.56	7.24±0.27
44	*Otacanthus caeruleus* flower	38.82±2.48	44.30±2.93	30.97±1.65	14.11±2.62	24.05±2.45	13.17±3.57
45	*Otacanthus caeruleus* leaf	0.80±2.56	2.84±1.12	0.99±3.69	0.35±2.27	15.90±3.22	1.65±1.38
46	*Pancratium triflorum* bulb	76.32±1.30	**92.36±0.81**	**99.34±0.28**	**97.59±0.52**	81.22±1.07	70.27±1.08
47	*Pancratium triflorum* leaf	55.30±3.68	81.93±0.96	**99.26±0.50**	89.89±0.57	60.13036	86.53±0.71
48	*Pereskia aculeate*	18.88±7.70	23.24±7.18	35.07±3.85	5.91±6.34	0.31±6.46	0.47±5.80
49	*Phyllanthus simplex*	15.52±1.49	57.03±1.29	55.89±0.47	19.25±1.56	10.96±1.64	9.21±2.17
50	*Pithecellobium dulce*	5.23±1.05	26.72±2.93	27.97±1.23	13.24±1.51	11.19±1.40	18.16±2.76
51	*Pothos scandens*	25.41±2.44	38.05±2.46	23.22±2.79	8.52±1.31	1.50±3.51	1.16±2.88
52	*Ricinus communis*	7.28±2.19	2.63±1.69	2.33±2.97	15.02±5.84	20.94±5.27	1.87±1.96
53	*Rauvolfia tetraphylla* leaf	34.65±1.83	39.76±2.61	42.97±1.32	8.26±2.11	19.08±2.38	33.46±0.93
54	*Rauvolfia tetraphylla* seed	40.48±0.28	32.37±1.74	51.66±2.10	19.73±1.65	2.98±2.06	35.90±2.17
55	*Rhaphidophora pertusa*	27.76±1.54	15.47±2.89	33.15±1.58	29.00±0.56	11.25±1.34	27.40±1.42
56	*Schleichera oleosa*	1.91±2.80	0.84±1.47	1.59±2.06	0.59±2.12	0.34±3.68	0.99±2.08
57	*Senna alata*	5.66±0.83	30.18±1.34	23.32±2.02	31.88±1.31	10.18±0.79	2.77±1.04
58	*Sauropus androgynus*	28.36±1.79	57.04±3.54	49.79±2.28	41.56±0.80	59.71±3.15	42.39±2.87
59	*Stachytarpheta indica*	31.63±2.30	21.04±1.08	16.98±1.87	27.22±2.55	48.15±1.67	17.48±4.23
60	*Strobilanthes heyneanus*	18.93±2.47	30.15±2.03	12.72±1.54	37.82±1.58	35.20±1.03	31.28±1.75
61	*Strychnos nux-vomica*	21.40±1.98	14.95±1.34	6.81±2.22	3.16±1.63	1.64±1.86	1.42±1.64
62	*Synedrella nodiflora*	6.37±1.68	13.12±2.37	36.47±0.99	32.66±2.78	13.68±0.75	28.15±4.90
63	*Talinum portulacifolium*	27.42±3.05	23.60±3.86	5.87±2.39	26.03±3.18	19.25±1.47	14.85±2.24

64	*Terminalia arjuna*	31.86±2.89	26.61±1.89	40.39±2.32	5.57±1.89	87.23±2.33	7.76±1.76
65	*Thevetia peruviana*	3.25±1.71	81.86±0.55	64.46±1.50	75.66±0.83	19.00±1.92	30.14±1.73
66	*Thunbergia erecta*	35.46±1.36	36.83±2.77	25.95±1.06	25.37±2.23	9.38±2.91	8.00±2.19
67	*Thunbergia grandiflora*	22.37±1.77	21.33±1.30	42.31±3.01	33.40±0.45	8.83±0.33	32.68±0.27
68	*Torenia bicolor*	33.01±0.59	6.47±1.52	39.89±1.70	5.66±1.55	7.70±1.67	14.12±1.79
69	*Trema orientale*	2.05±0.68	6.63±3.88	33.27±3.16	30.19±2.75	35.25±2.00	3.92±1.45
70	*Vitex altissima*	25.90±2.71	18.46±6.15	14.33±1.99	35.56±0.77	21.51±1.63	32.99±3.32
71	*Wattakaka volubilis*	46.24±3.57	65.42±2.46	32.243±2.51	26.91±3.11	26.70±2.60	50.99±1.37

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.6. Screening of plant extracts for cytotoxicity against MCF7

A total of 48 plant extracts demonstrated more than 50% cell mortality in the human breast cancer cell line MCF7, with four extracts inducing over 90% cell death. The methanol extract of *Alangium salviifolium* exhibited the highest mortality percentage at 99.47±0.23%. The chloroform extract of *Alangium salviifolium* also showed significant cytotoxicity, with 97.69±0.22% cell death, while the methanol extract of *Chassalia curviflora* caused 96.52±3.97% cell death in MCF7 cells. These extracts show strong potential as anticancer agents against breast cancer (Table 1.3.6).

Table 1.3.6. Percentage mortality of the plant extracts at 100 µg/mL on the breast cancer cell line, MCF7 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
1	*Acalypha fruiticosa*	12.05±1.50	8.81±1.84	1.20±1.34	14.58±1.64	11.34±0.30	12.95±0.91
2	*Alangium salviifolium*	65.60±1.69	**97.69±0.22**	78.24±1.10	85.21±0.76	**99.47±0.23**	72.03±0.48
3	*Anamirta cocculus*	5.42±2.69	1.17±1.57	67.23±1.47	20.84±2.55	12.61±2.58	6.18±6.67

4	*Antigonon leptopus*	15.45±2.99	45.59±3.12	33.67±2.18	24.33±2.63	15.65±0.87	31.65±5.32
5	*Argyreia pomacea*	5.69±0.87	12.34±1.41	15.88±1.68	10.55±2.21	5.34±8.00	6.37±1.16
6	*Bauhinia phoenicea*	5.13±1.85	11.26±3.15	20.66±2.25	23.66±4.30	10.02±0.91	21.17±0.30
7	*Bridelia retusa*	5.92±1.73	5.65±1.39	4.63±0.70	6.06±0.67	6.13±1.28	4.35±1.59
8	*Calopogonium mucunoides*	20.18±1.19	27.43±1.14	19.37±0.42	21.07±1.31	53.13±1.08	29.53±1.05
9	*Carl rheedii*	2.19±0.56	17.87±3.06	30.42±1.54	25.86±5.09	38.28±3.67	7.70±2.18
10	*Cassia javanica*	22.18±1.65	16.09±1.46	33.36±0.39	28.58±1.63	34.22±1.85	5.39±1.16
11	*Chassalia curviflora*	9.93±3.34	18.60±5.18	2.99±1.40	58.12±5.46	**96.52±3.97**	65.63±3.81
12	*Cheilocostus speciosus*	9.79±1.19	29.37±6.91	20.92±5.48	33.79±1.84	12.82±1.74	21.58±4.31
13	*Chrysophyllum cainito*	6.18±1.82	30.79±1.78	14.38±0.38	32.26±2.22	1.59±1.84	12.44±1.23
14	*Cipadessa baccifera* leaf	18.18±1.64	19.06±1.90	41.50±0.43	42.54±1.49	22.89±2.04	18.71±1.22
15	*Cipadessa baccifera* seed	79.98±4.81	**93.72±3.02**	72.09±3.63	43.38±3.56	28.16±2.56	15.81±4.31
16	*Cissus discolor*	3.91±2.48	13.63±2.67	20.83±1.56	18.52±1.31	30.41±2.76	14.77±1.49
17	*Cochlospermum religiosum*	5.77±1.16	5.70±1.27	5.66±1.22	6.09±0.98	21.44±1.27	5.68±1.10
18	*Combretum indicum*	81.34±4.56	73.97±2.97	66.66±1.55	6.68±2.96	35.15±1.95	8.87±0.62
19	*Conyza canadensis*	32.12±5.26	73.53±2.35	46.46±3.37	39.93±5.92	3.97±2.97	29.91±3.81
20	*Cyclea peltata*	0.53±2.33	19.37±2.63	8.64±1.93	1.67±0.83	4.35±0.90	13.09±0.60
21	*Drypetes venusta*	3.96±1.19	6.46±0.96	46.59±2.33	4.30±0.94	5.93±1.06	4.83±0.71
22	*Entada rheedii*	39.63±1.72	37.17±1.69	29.50±1.36	34.12±1.45	6.23±1.32	14.07±0.16
23	*Euphorbia heterophylla*	0.97±1.57	21.71±1.73	2.46±1.39	13.17±1.46	1.28±1.24	10.21±0.17
24	*Euphorbia tithymaloides*	13.63±1.66	22.67±1.68	33.50±1.64	29.49±1.51	22.68±1.42	0.96±0.17
25	*Gardenia jasminoides*	2.65±3.88	0.81±1.34	0.95±1.87	3.53±2.49	1.89±1.70	5.93±2.42
26	*Getonia floribunda*	27.83±2.34	68.35±4.44	30.16±2.02	67.11±2.14	24.41±2.62	9.73±3.98
27	*Globba schomburgkii*	10.11±5.23	34.97±2.26	48.07±1.33	49.30±4.49	33.95±2.31	30.60±4.84

28	*Hamelia patens*	13.51±1.95	26.12±1.06	19.05±1.72	37.37±0.99	29.65±1.66	38.76±1.26
29	*Hewittia malabarica*	35.56±2.59	15.69±1.14	33.08±1.80	28.63±1.77	41.70±0.74	30.38±1.17
30	*Impatiens minor*	2.48±1.59	16.34±1.01	40.36±2.64	15.56±1.12	27.79±1.77	22.78±1.07
31	*Jasminum grandiflorum*	21.55±6.73	25.01±0.94	29.04±1.00	41.53±0.96	29.11±4.76	49.38±0.99
32	*Justicia nagpurensis*	9.63±1.17	13.61±1.49	28.85±1.22	33.10±1.30	20.81±0.90	3.31±0.17
33	*Justicia wynaadensis*	8.10±2.70	8.81±2.69	2.69±1.23	7.27±1.42	4.64±1.35	24.09±2.35
34	*Laggera alata*	11.63±3.96	28.84±1.18	5.40±1.12	48.71±0.70	44.10±0.85	2.87±0.79
35	*Lannea coromandelica*	25.63±1.56	24.03±1.08	32.68±1.04	29.47±1.01	35.30±1.16	9.22±0.17
36	*Leea indica*	13.59±1.48	10.76±1.59	25.34±1.25	14.72±1.31	24.57±1.23	24.39±0.17
37	*Lecanthus peduncularis*	28.88±2.47	26.46±1.68	7.23±1.15	37.55±0.68	10.86±2.96	26.86±1.65
38	*Leucas aspera*	3.51±0.67	37.12±1.10	3.98±1.27	16.52±1.88	24.67±1.76	11.69±1.48
39	*Lobelia alsinoides*	24.68±1.58	25.65±1.12	30.84±1.79	26.22±1.78	31.68±1.72	34.11±1.17
40	*Lophopetalum wightianum*	36.76±1.62	25.95±1.03	34.16±1.45	35.07±1.75	8.50±1.62	2.25±1.20
41	*Melia dubia*	64.08±1.84	88.92±1.25	81.44±1.10	84.01±2.14	71.50±3.26	62.55±2.57
42	*Micrococca mercurialis*	35.51±1.57	14.07±1.14	66.11±1.52	25.14±0.91	13.36±1.67	27.91±1.16
43	*Murraya paniculata*	39.47±1.67	47.05±1.11	32.15±1.20	58.08±1.23	46.21±1.72	47.67±1.22
44	*Otacanthus caeruleus* flower	37.21±1.34	57.24±1.99	10.19±1.99	16.84±1.70	5.53±1.45	15.31±2.98
45	*Otacanthus caeruleus* leaf	8.85±2.39	11.76±1.85	14.10±2.50	17.60±1.47	9.01±2.54	18.02±1.87
46	*Pancratium triflorum* bulb	45.65±2.99	67.93±1.64	76.98±0.77	73.80±0.84	60.21±1.11	44.42±0.82
47	*Pancratium triflorum leaf*	81.75±2.52	48.78±4.83	81.44±3.23	69.58±0.50	40.80±1.16	65.23±0.66
48	*Pereskia aculeate*	22.98±1.68	23.80±1.25	3.77±1.70	9.98±2.93	2.99±2.38	21.59±2.36
49	*Phyllanthus simplex*	20.13±1.80	2.38±1.53	21.96±1.71	54.16±0.41	30.42±0.85	20.60±1.35
50	*Pithecellobium dulce*	23.45±3.02	61.36±2.26	56.59±1.12	23.46±2.59	10.75±3.37	30.26±2.62
51	*Pothos scandens*	13.69±2.83	9.46±1.77	10.30±2.14	7.18±1.15	23.33±1.98	2.18±1.94

52	*Ricinus communis*	0.88±1.57	2.44±1.36	12.67±2.97	8.72±2.17	5.18±1.19	0.28±1.81
53	*Rauvolfia tetraphylla* leaf	5.43±1.79	22.74±1.28	34.21±1.79	19.67±1.64	23.712±1.77	34.12±1.05
54	*Rauvolfia tetraphylla* seed	45.35±1.60	61.61±1.06	55.11±1.72	35.37±0.99	32.65±1.66	22.18±1.26
55	*Rhaphidophora pertusa*	7.20±1.73	14.80±3.99	37.39±7.81	56.10±2.54	9.85±5.29	41.99±4.26
56	*Schleichera oleosa*	9.21±1.86	13.18±2.04	7.16±1.60	6.21±1.09	3.70±1.80	1.77±2.19
57	*Senna alata*	6.35±1.79	32.74±1.28	40.21±1.79	26.74±1.64	17.17±1.77	31.18±1.05
58	*Sauropus androgynus*	12.35±1.59	26.63±1.01	31.04±1.64	25.56±1.12	29.79±1.77	1.08±1.07
59	*Stachytarpheta indica*	12.87±2.79	33.94±1.58	27.66±3.34	89.23±3.48	43.17±5.14	19.02±3.85
60	*Strobilanthes heyneanus*	13.65±2.28	10.64±3.39	13.04±3.45	15.04±3.23	20.63±3.05	10.13±1.37
61	*Strychnos nux-vomica*	27.90±1.90	31.23±2.85	34.53±2.31	36.13±1.49	25.06±1.19	18.54±2.36
62	*Synedrella nodiflora*	7.35±1.57	14.07±1.14	17.17±1.52	25.11±0.91	33.61±1.167	9.27±1.16
63	*Talinum portulacifolium*	10.87±2.15	5.35±1.38	8.00±1.92	5.25±0.73	2.51±0.94	0.99±0.81
64	*Terminalia arjuna*	3.08±1.15	15.33±0.80	38.12±2.31	30.71±1.12	46.97±2.48	32.13±3.22
65	*Thevetia peruviana*	2.45±1.83	81.96±1.15	77.70±0.57	78.45±0.62	39.10±2.35	11.66±1.67
66	*Thunbergia erecta*	20.52±1.26	33.60±1.25	12.11±1.32	11.68±1.18	30.93±1.07	41.01±0.68
67	*Thunbergia grandiflora*	25.33±0.73	21.28±1.25	32.37±1.27	23.83±1.23	31.11±1.21	28.22±0.69
68	*Torenia bicolor*	6.01±1.58	34.95±2.82	8.58±1.03	28.92±2.72	13.17±3.97	9.02±1.44
69	*Trema orientale*	10.53±1.23	31.12±1.16	33.33±1.21	20.99±1.01	21.03±1.02	7.67±0.68
70	*Vitex altissima*	11.27±1.25	23.56±1.18	32.13±1.17	7.36±0.93	6.51±1.30	2.13±1.44
71	*Wattakaka volubilis*	0.52±1.17	1.38±1.31	1.28±1.31	1.28±1.16	1.43±1.21	1.31±0.75

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.7. Screening of plant extracts for cytotoxicity against MG63

Of the plant extracts tested, several plant extracts demonstrated anticancer properties against the osteoblast cell line, MG63, by inducing significant cytotoxicity and cell death. Out of 426 plant extracts screened, 51 exhibited anticancer potential with more than 50.00% mortality. Of these, 17 extracts caused exceptionally high mortality, exceeding 90.00%. The petroleum ether extract of *Conyza canadensis* resulted in the highest mortality at 99.59±0.50%, followed closely by the ethyl acetate extract of *Hewittia malabarica* with a mortality percentage of 99.58±1.08% (Table 1.3.7).

Table 1.3.7. Percentage mortality of the plant extracts at 100 µg/mL on the osteoblast cancer cell line, MG63 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	11.68±4.21	2.87±2.09	15.06±1.36	11.86±2.65	20.59±2.26	6.22±1.86
2	*Alangium salviifolium*	22.44±1.37	76.25±0.84	69.45±0.90	75.79±2.47	**98.27±0.16**	67.17±1.97
3	*Anamirta cocculus*	11.56±2.45	5.48±2.20	86.76±1.04	81.33±1.33	8.10±2.69	17.33±2.22
4	*Antigonon leptopus*	59.37±1.59	**96.43±0.41**	43.28±1.80	65.62±1.34	42.43±1.90	44.29±1.21
5	*Argyreia pomacea*	7.90±1.63	45.83±0.84	5.17±1.53	10.87±1.32	7.90±4.02	11.59±3.48
6	*Bauhinia phoenicea*	7.45±2.34	19.35±1.89	13.65±4.61	32.66±2.07	13.64±1.86	5.25±2.26
7	*Bridelia retusa*	29.85±1.99	44.67±2.08	12.70±2.44	18.11±4.45	36.01±4.46	9.41±2.48
8	*Calopogonium mucunoides*	6.50±1.83	9.34±2.00	50.53±0.73	42.99±3.67	39.16±5.65	28.08±1.21
9	*Carl rheedii*	23.76±4.72	18.63±2.51	36.56±1.50	22.19±3.00	16.12±2.35	7.55±5.33
10	*Cassia javanica*	39.65±1.21	39.02±4.16	35.70±5.19	29.87±6.17	16.16±4.95	36.29±2.25
11	*Chassalia curviflora*	29.36±1.50	15.57±1.34	9.32±1.37	4.12±2.86	**99.02±2.33**	16.43±1.07
12	*Cheilocostus speciosus*	35.64±2.78	27.23±2.05	43.88±0.89	11.22±3.79	16.84±1.63	28.17±1.20
13	*Chrysophyllum cainito*	35.98±2.47	17.60±1.01	32.99±3.41	16.51±1.30	20.31±2.66	28.65±3.95

14	*Cipadessa baccifera* leaf	22.42±1.90	30.51±1.28	31.58±1.51	28.99±2.38	34.90±2.53	21.80±1.21
15	*Cipadessa baccifera* seed	95.18±0.69	21.80±1.21	0.30±2.03	12.18±1.79	23.60±1.80	5.22±2.37
16	*Cissus discolor*	15.95±0.95	19.49±2.76	44.81±3.85	12.91±5.41	25.67±0.94	41.05±3.71
17	*Cochlospermum religiosum*	34.12±4.95	41.01±6.33	12.70±3.04	11.29±1.44	20.57±1.09	27.94±1.25
18	*Combretum indicum*	22.27±7.44	49.41±3.26	27.04±1.38	18.11±7.94	5.67±1.45	22.09±2.48
19	*Conyza canadensis*	**99.59±0.50**	**98.57±0.40**	24.16±1.24	37.29±1.75	33.60±3.21	40.48±0.95
20	*Cyclea peltata*	0.72±2.47	17.86±1.65	62.46±2.12	52.22±1.09	13.61±1.55	6.75±2.95
21	*Drypetes venusta*	22.38±2.49	20.31±5.92	46.14±4.50	21.87±3.30	12.46±6.00	46.97±3.31
22	*Entada rheedii*	32.76±3.48	47.45±1.00	13.66±5.20	18.75±3.12	24.63±2.16	14.70±3.06
23	*Euphorbia heterophylla*	35.59±6.33	31.47±2.31	11.93±6.86	17.48±2.63	14.38±6.22	17.19±0.61
24	*Euphorbia tithymaloides*	37.55±3.56	45.25±2.00	16.56±1.97	47.66±1.19	31.44±5.99	34.80±6.07
25	*Gardenia jasminoides*	25.50±5.61	20.31±3.10	41.37±4.69	24.77±2.19	38.01±3.62	18.73±6.85
26	*Getonia floribunda*	13.34±2.63	34.75±1.71	26.36±2.53	26.63±1.30	31.44±5.04	44.47±4.89
27	*Globba schomburgkii*	7.29±1.07	**99.24±1.08**	38.77±3.98	39.11±1.99	44.65±1.87	33.14±1.60
28	*Hamelia patens*	5.69±0.89	8.05±2.23	1.98±1.13	3.94±0.98	6.92±0.82	3.98±1.38
29	*Hewittia malabarica*	6.58±2.17	45.94±2.71	**99.58±1.08**	13.75±2.70	52.56±1.96	43.85±1.47
30	*Impatiens minor*	3.05±1.30	6.05±1.35	11.33±1.60	9.15±3.10	3.49±1.80	2.47±3.05
31	*Jasminum grandiflorum*	13.38±2.22	34.53±1.62	10.28±2.76	21.66±1.63	14.78±1.45	22.86±1.25
32	*Justicia nagpurensis*	23.60±2.31	29.79±2.09	28.23±2.65	34.88±3.68	36.14±1.09	44.55±3.85
33	*Justicia wynaadensis*	5.54±1.05	0.39±1.47	1.65±1.96	0.94±1.00	0.30±1.67	0.58±2.96
34	*Laggera alata*	**90.94±0.57**	89.29±0.96	84.14±1.44	**93.74±0.56**	46.84±2.17	0.42±1.21
35	*Lannea coromandelica*	19.19±3.47	16.94±2.23	9.57±1.44	39.40±4.57	30.40±4.74	19.76±2.39
36	*Leea indica*	5.45±1.16	5.36±1.92	8.54±1.33	3.65±1.79	2.85±1.60	2.61±1.91
37	*Lecanthus peduncularis*	5.64±1.38	38.15±1.71	11.61±1.35	9.50±1.68	19.80±1.13	10.19±1.82
38	*Leucas aspera*	7.19±1.83	**98.48±0.23**	2.70±2.14	8.39±2.80	4.69±1.46	4.00±2.64
39	*Lobelia alsinoides*	19.19±3.47	8.17±2.26	15.71±1.44	39.40±1.46	1.30±2.66	9.76±3.82
40	*Lophopetalum wightianum*	5.27±1.13	8.05±1.65	11.55±2.28	20.09±2.07	4.65±1.11	11.03±2.43

41	*Melia dubia*	0.22±1.39	36.23±5.32	1.83±2.77	9.50±2.63	0.75±2.16	1.79±1.35
42	*Micrococca mercurialis*	21.69±2.92	41.53±3.19	27.86±0.99	13.16±1.36	35.76±1.16	36.92±2.96
43	*Murraya paniculata*	2.24±1.17	20.39±4.93	3.55±2.95	9.35±0.78	14.68±3.23	22.96±3.75
44	*Otacanthus caeruleus* flower	**96.73±0.81**	**99.24±0.08**	20.39±2.44	23.91±1.60	13.45±2.98	17.58±1.72
45	*Otacanthus caeruleus* leaf	68.84±1.42	**99.03±0.33**	15.49±1.31	12.06±1.20	6.88±1.90	6.80±0.98
46	*Pancratium triflorum* bulb	52.14±1.22	65.69±1.92	58.95±1.96	58.94±0.93	57.47±0.87	49.23±1.38
47	*Pancratium triflorum* leaf	39.05±2.43	55.38±1.42	62.79±1.22	59.31±0.85	43.56±1.01	58.48±0.95
48	*Pereskia aculeate*	3.30±1.49	1.15±1.72	1.12±1.36	7.80±3.68	3.77±1.13	16.47±2.29
49	*Phyllanthus simplex*	39.47±1.96	26.90±3.01	48.15±2.16	37.65±3.22	21.50±3.10	39.20±1.08
50	*Pithecellobium dulce*	29.36±1.15	33.54±1.89	31.60±2.90	58.56±5.55	35.99±3.23	19.56±0.99
51	*Pothos scandens*	13.23±1.97	7.61±2.04	3.66±1.22	4.34±2.27	9.53±0.98	1.28±1.48
52	*Ricinus communis*	19.07±3.22	22.43±2.89	11.35±2.27	9.85±1.98	13.58±1.63	23.07±2.05
53	*Rauvolfia tetraphylla* leaf	0.80±0.83	3.95±2.63	0.75±2.70	0.30±1.92	19.14±1.05	3.86±2.87
54	*Rauvolfia tetraphylla* seed	18.97±1.76	70.33±1.83	62.65±1.90	0.25±5.02	5.55±2.76	3.47±2.30
55	*Rhaphidophora pertusa*	14.62±4.97	48.01±2.00	27.59±3.51	33.21±3.45	8.31±3.74	21.30±2.11
56	*Schleichera oleosa*	28.05±1.34	21.29±1.61	35.20±1.30	48.09±1.43	38.35±1.73	31.27±1.62
57	*Senna alata*	33.47±1.14	28.63±2.34	39.77±1.33	35.49±2.75	24.32±1.41	20.48±4.95
58	*Sauropus androgynus*	28.64±3.68	81.36±0.65	68.56±2.52	46.32±1.19	34.26±1.62	27.60±2.23
59	*Stachytarpheta indica*	26.14±1.13	32.93±3.81	69.88±2.65	88.53±2.36	77.44±1.85	26.03±3.39
60	*Strobilanthes heyneanus*	15.24±2.31	84.97±1.90	51.13±0.64	40.59±3.25	22.44±3.39	14.62±1.67
61	*Strychnos nux-vomica*	38.16±1.09	45.89±3.74	36.46±1.06	24.76±2.40	14.89±2.34	8.56±1.23
62	*Synedrella nodiflora*	1.44±2.09	3.92±2.97	34.13±2.99	39.22±1.46	0.83±0.21	9.16±2.29
63	*Talinum portulacifolium*	22.81±1.87	9.42±0.99	8.23±1.19	7.90±0.94	17.05±0.98	16.98±1.82
64	*Terminalia arjuna*	33.73±2.53	49.86±2.90	36.27±4.00	37.51±1.19	0.86±1.28	39.61±1.23
65	*Thevetia peruviana*	59.39±1.65	**90.09±0.78**	**98.17±0.62**	**91.52±0.74**	14.57±0.78	35.61±4.16
66	*Thunbergia erecta*	15.75±2.48	2.39±1.98	1.63±2.36	6.22±2.55	1.68±1.51	0.33±1.64
67	*Thunbergia grandiflora*	20.14±1.56	15.45±1.40	21.37±1.70	8.53±3.24	27.44±1.80	7.26±1.34

68	*Torenia bicolor*	2.85±2.33	46.27±0.82	9.95±1.50	31.55±5.32	36.16±2.18	29.34±4.06
69	*Trema orientale*	1.06±1.18	17.91±0.84	3.05±2.58	20.77±3.11	33.14±6.03	35.61±1.87
70	*Vitex altissima*	36.88±2.58	14.42±3.25	30.48±3.99	4.92±1.76	5.67±0.84	10.44±2.41
71	*Wattakaka volubilis*	15.88±3.38	28.94±0.94	17.50±1.09	45.90±2.96	17.46±1.43	5.54±2.82

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.8. Screening of plant extracts for cytotoxicity against PA1

Table 1.3.8 presents the screening results of 426 extracts against the human ovarian cancer cell line, PA1. The study revealed that 86 extracts caused mortality exceeding 50.00% on PA1 cell line, with 35 extracts resulting in mortality above 90.00%. The acetone extract of *Alangium salviifolium* exhibited the highest toxicity with a percentage mortality of 99.91±0.18%. This was followed by the ethyl acetate extracts of *Pancratium triflorum* leaf (99.81±0.18%) and bulb (99.74±0.14%), as well as the chloroform extracts of *Otacanthus caeruleus* leaf (99.67±0.60%) *Leucas aspera* (99.52±0.31%) and *Alangium salviifolium* (99.42±0.38%).

Table 1.3.8. Percentage mortality of the plant extracts at 100 µg/mL on the ovarian cancer cell line, PA1 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	17.66±2.73	23.56±3.55	40.66±1.41	38.42±3.54	36.51±2.48	25.85±3.20
2	*Alangium salviifolium*	30.76±1.11	**99.42±0.38**	82.91±1.23	**99.91±0.18**	**99.13±0.38**	75.65±2.15
3	*Anamirta cocculus*	4.77±2.81	7.19±0.84	**98.64±0.40**	**92.71±0.58**	4.06±1.43	28.69±2.21
4	*Antigonon leptopus*	10.16±2.30	53.75±2.57	1.71±2.22	67.19±2.16	15.73±1.63	27.60±1.22
5	*Argyreia pomacea*	24.36±2.98	22.07±3.20	45.79±1.49	43.56±2.63	38.41±3.66	25.99±2.35

6	*Bauhinia phoenicea*	**96.74±0.54**	**99.28±0.16**	10.35±2.72	37.64±3.15	14.36±3.20	10.80±2.48
7	*Bridelia retusa*	33.39±4.86	55.91±0.55	43.23±4.00	41.91±3.66	32.73±4.70	18.48±1.57
8	*Calopogonium mucunoides*	2.29±1.96	8.11±1.46	57.76±0.97	43.72±3.25	42.07±1.81	7.61±2.98
9	*Carl rheedii*	7.33±4.86	35.59±0.98	24.32±1.64	34.19±1.57	27.39±5.26	18.48±3.16
10	*Cassia javanica*	8.19±2.36	34.37±2.00	33.40±5.82	10.94±1.36	15.96±2.93	31.55±1.67
11	*Chassalia curviflora*	6.23±2.23	2.94±1.00	15.65±0.52	23.40±1.51	**98.92±0.35**	24.56±1.36
12	*Cheilocostus speciosus*	26.28±3.96	12.27±1.71	28.13±4.578	21.94±2.26	22.92±2.59	17.50±5.45
13	*Chrysophyllum cainito*	28.35±3.08	22.74±1.71	26.56±2.11	46.00±5.16	22.92±2.59	17.50±4.47
14	*Cipadessa baccifera* leaf	47.55±0.79	77.89±1.21	49.25±1.25	54.11±3.24	58.06±2.25	39.71±2.04
15	*Cipadessa baccifera* seed	39.85±0.79	25.45±1.08	8.65±1.21	51.72±2.51	56.36±0.79	32.65±3.57
16	*Cissus discolor*	19.40±3.98	6.34±3.51	47.69±3.14	4.38±2.14	7.51±3.23	16.92±1.96
17	*Cochlospermum religiosum*	22.74±2.65	38.21±2.37	14.04±3.13	12.10±2.29	41.56±4.21	33.88±0.98
18	*Combretum indicum*	15.72±1.67	6.39±1.38	3.37±1.10	0.90±0.53	64.56±2.28	**95.71±1.25**
19	*Conyza canadensis*	41.09±3.24	98.06±2.53	70.99±6.78	49.26±4.48	51.59±3.26	56.75±5.99
20	*Cyclea peltata*	4.73±1.31	3.89±1.00	90.73±0.22	68.18±0.50	11.23±1.28	5.01±1.37
21	*Drypetes venusta*	15.84±1.01	42.32±2.85	32.89±2.29	16.04±0.65	35.19±4.21	26.50±0.54
22	*Entada rheedii*	**94.46±1.02**	**99.36±0.17**	**92.50±0.75**	**93.10±0.55**	13.10±1.78	31.19±0.75
23	*Euphorbia heterophylla*	6.42±2.03	27.89±2.01	0.83±1.23	4.91±1.91	1.89±1.05	50.99±2.96
24	*Euphorbia tithymaloides*	45.87±3.87	36.03±1.52	33.96±1.84	19.78±2.64	34.78±2.05	28.61±8.52
25	*Gardenia jasminoides*	3.42±1.16	4.74±1.08	3.41±0.67	27.16±1.69	6.75±1.82	6.92±0.99
26	*Getonia floribunda*	**94.46±1.02**	**99.36±0.17**	**92.50±0.75**	**93.10±0.55**	13.10±1.78	31.19±0.75
27	*Globba schomburgkii*	25.52±2.29	12.70±3.10	29.18±3.92	17.67±4.26	29.30±2.90	28.80±1.29
28	*Hamelia patens*	6.12±3.71	21.76±3.29	25.92±2.67	73.05±1.58	35.42±2.13	4.61±1.13
29	*Hewittia malabarica*	26.60±3.04	62.42±3.01	**97.23±0.82**	28.98±3.57	73.21±4.67	5.01±2.92

30	*Impatiens minor*	5.69±3.18	1.11±4.30	2.18±3.74	16.05±2.23	33.17±4.25	25.25±2.81
31	*Jasminum grandiflorum*	25.91±6.10	19.42±2.43	22.31±3.88	31.62±4.56	21.46±1.23	28.78±5.95
32	*Justicia nagpurensis*	23.56±6.10	50.58±1.02	22.40±1.68	34.09±1.01	35.77±2.82	25.06±1.57
33	*Justicia wynaadensis*	19.10±1.78	37.21±0.94	4.01±3.30	1.71±3.00	1.75±3.64	3.03±4.32
34	*Laggera alata*	**90.93±0.42**	**97.98±0.25**	**97.74±0.17**	**93.31±0.40**	**92.13±0.91**	14.03±2.78
35	*Lannea coromandelica*	34.49±7.42	25.97±2.71	50.81±3.47	40.27±1.44	35.01±3.85	20.99±1.42
36	*Leea indica*	10.32±1.56	18.61±3.00	3.59±1.71	13.79±0.85	2.56±0.45	1.35±0.92
37	*Lecanthus peduncularis*	18.51±2.11	24.40±1.31	5.90±2.35	23.89±1.12	27.01±1.09	32.15±1.90
38	*Leucas aspera*	24.26±1.70	**99.52±0.31**	38.73±1.54	52.09±2.68	45.66±3.06	17.29±2.60
39	*Lobelia alsinoides*	20.30±1.96	0.45±0.41	27.48±2.73	14.34±2.84	14.56±1.59	22.36±1.70
40	*Lophopetalum wightianum*	26.46±3.46	32.67±2.93	1.63±3.66	45.71±0.86	4.88±3.63	2.93±2.23
41	*Melia dubia*	17.70±2.19	**92.51±0.88**	7.33±1.76	39.93±1.84	14.26±1.17	3.48±1.71
42	*Micrococca mercurialis*	10.96±2.93	8.69±3.07	7.69±3.73	3.59±2.82	7.39±2.89	52.57089
43	*Murraya paniculata*	26.58±5.84	32.44±3.38	49.02±4.10	2.15±0.94	6.55±1.34	5.03±1.48
44	*Otacanthus caeruleus* flower	75.83±0.70	**97.53±0.58**	33.83±0.90	33.59±1.39	25.37±4.17	20.77±1.02
45	*Otacanthus caeruleus* leaf	**90.09±1.03**	**99.67±0.60**	68.48±2.81	52.54±3.05	1.87±2.02	15.30±2.06
46	*Pancratium triflorum* bulb	48.25±2.19	82.56±2.69	**99.74±0.14**	**99.41±0.52**	54.31±3.44	50.41±4.24
47	*Pancratium triflorum leaf*	57.09±3.16	55.94±2.79	**99.81±0.18**	50.02±4.53	53.47±3.90	60.10±2.49
48	*Pereskia aculeate*	37.34±1.28	5.67±3.68	8.77±1.03	1.69±1.66	11.35±2.61	39.47±4.90
49	*Phyllanthus simplex*	35.55±2.39	44.69±3.47	32.75±2.01	28.59±1.82	31.16±2.23	17.98±2.54
50	*Pithecellobium dulce*	9.22±3.50	42.72±0.92	63.59±2.26	51.33±2.93	46.92±1.29	25.37±3.96
51	*Pothos scandens*	72.36±1.52	43.26±3.56	16.69±2.17	14.50±3.33	18.98±4.64	11.38±2.99
52	*Ricinus communis*	39.43±2.96	21.25±6.23	19.33±3.69	28.12±1.81	45.60±5.38	16.63±3.24
53	*Rauvolfia tetraphylla* leaf	36.01±1.11	34.94±1.18	44.19±2.94	39.06±2.19	37.17±1.81	5.96±0.83

54	*Rauvolfia tetraphylla* seed	46.85±1.09	37.56±1.99	28.17±1.55	35.91±1.90	23.41±1.45	39.46±1.66
55	*Rhaphidophora pertusa*	30.00±3.89	46.92±2.60	33.88±4.15	43.47±4.42	37.57±4.50	24.62±3.79
56	*Schleichera oleosa*	9.56±2.94	17.93±2.29	14.23±1.78	88.78±0.79	4.67±2.14	21.68±2.28
57	*Senna alata*	24.76±3.25	64.51±2.88	65.12±4.57	40.52±5.20	31.33±3.69	42.40±4.05
58	*Sauropus androgynus*	21.54±3.16	42.72±1.91	63.59±2.02	51.33±2.93	45.12±1.29	25.37±2.40
59	*Stachytarpheta indica*	29.07±2.81	74.18±1.11	70.70±4.97	61.39±6.95	44.99±2.50	55.65±3.56
60	*Strobilanthes heyneanus*	22.89±1.28	56.67±2.71	50.81±2.54	40.27±3.59	35.01±6.39	20.99±1.42
61	*Strychnos nux-vomica*	46.53±6.92	21.11±1.78	23.88±1.55	45.84±1.86	37.57±1.45	46.16±3.79
62	*Synedrella nodiflora*	6.05±0.44	4.28±1.06	23.40±1.49	7.66±0.70	34.08±0.55	39.98±5.14
63	*Talinum portulacifolium*	27.93±3.58	22.44±2.82	29.16±4.22	39.70±6.42	40.28±4.22	33.11±1.86
64	*Terminalia arjuna*	1.20±0.35	23.51±0.86	44.32±2.56	53.65±1.80	5.88±1.85	5.09±2.14
65	*Thevetia peruviana*	51.06±0.86	**97.48±0.29**	**94.46±0.52**	86.67±0.78	57.60±0.35	29.12±1.90
66	*Thunbergia erecta*	23.51±5.36	40.64±1.28	35.97±1.98	40.05±2.04	37.40±1.74	11.56±1.39
67	*Thunbergia grandiflora*	21.51±2.66	17.43±3.54	13.53±1.75	39.07±0.83	14.44±2.16	38.43±3.39
68	*Torenia bicolor*	9.29±1.20	29.17±3.62	7.74±1.66	31.17±4.52	28.44±1.22	26.87±1.84
69	*Trema orientale*	25.52±2.92	12.70±1.01	29.18±2.00	20.18±2.62	29.29±2.13	28.80±2.91
70	*Vitex altissima*	5.49±1.73	0.72±2.49	19.97±2.57	49.84±1.98	16.74±2.10	5.16±1.07
71	*Wattakaka volubilis*	15.22±2.64	24.16±3.18	10.34±4.01	19.15±3.60	34.89±1.07	16.20±1.39

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

Screening of plant extracts for cytotoxicity against PANC1

Of the plant extracts tested, thirty-six plant extracts exhibited percentage mortality above 50.00% on the pancreatic cancer cell line, PANC1. Of these, seven extracts caused more than 90.00% mortality. The highest percentage mortality was observed with the *Otacanthus caeruleus* flower extract (99.71±2.65%), followed by the methanol extract of *Alangium salviifolium* (98.06±0.29%) (Table 1.3.9).

Table 1.3.9. Percentage mortality of the plant extracts at 100 µg/mL on the pancreatic cancer cell line, PANC1 after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	5.34±2.67	27.88±6.18	11.36±4.36	16.15±1.49	7.14±5.44	10.46±2.09
2	*Alangium salviifolium*	50.07±1.91	84.26±0.73	69.32±2.46	79.82±1.66	**98.06±0.29**	73.23±2.42
3	*Anamirta cocculus*	24.73±2.31	24.35±1.08	79.16±1.32	33.46±1.75	28.13±3.91	26.79±1.65
4	*Antigonon leptopus*	3.15±0.79	2.05±1.57	9.46±4.10	12.72±4.81	6.76±1.74	2.61±3.52
5	*Argyreia pomacea*	26.59±3.67	29.33±4.66	36.16±3.87	9.65±0.77	13.77±1.33	13.70±1.38
6	*Bauhinia phoenicea*	28.46±1.08	21.56±2.67	33.63±6.17	25.35±3.49	15.88±2.09	8.17±5.44
7	*Bridelia retusa*	4.93±2.25	7.18±3.34	12.11±2.85	18.59±1.72	18.94±3.76	9.77±1.64
8	*Calopogonium mucunoides*	9.36±1.60	12.49±2.69	23.85±2.48	13.37±1.86	22.65±3.96	26.49±3.10
9	*Carl rheedii*	12.36±1.46	25.75±1.98	21.36±2.65	18.74±2.33	19.36±0.99	13.49±1.86
10	*Cassia javanica*	13.99±3.08	24.56±2.46	30.16±1.00	24.50±4.02	26.11±1.99	34.62±2.30
11	*Chassalia curviflora*	34.85±1.23	29.09±1.83	28.45±0.49	37.46±1.89	69.85±2.39	50.96±0.58
12	*Cheilocostus speciosus*	26.98±1.85	40.68±0.76	37.95±1.69	31.57±0.67	26.67±5.58	21.58±1.86
13	*Chrysophyllum cainito*	8.85±0.46	9.47±1.06	18.54±7.49	30.94±0.90	11.49±6.03	30.34±3.79
14	*Cipadessa baccifera* leaf	4.27±1.01	6.24±2.76	14.45±2.00	19.28±2.96	5.41±0.69	13.32±1.69

15	*Cipadessa baccifera* seed	34.37±2.68	24.62±3.12	7.47±2.58	18.74±1.44	13.74±2.30	21.04±3.84
16	*Cissus discolor*	4.88±1.85	9.47±3.11	30.34±4.89	3.09±1.11	11.49±2.84	18.03±2.06
17	*Cochlospermum religiosum*	7.02±4.79	9.34±3.25	29.04±3.36	34.31±2.99	13.92±3.65	19.92±2.93
18	*Combretum indicum*	1.67±1.84	8.74±3.20	7.69±2.09	3.65±1.40	21.17±2.96	39.40±2.26
19	*Conyza canadensis*	25.57±3.21	38.67±2.04	21.67±3.08	21.50±3.36	32.11±1.42	8.71±3.05
20	*Cyclea peltata*	20.32±1.48	2.32±1.21	25.44±1.46	32.14±1.66	28.23±1.00	13.85±3.05
21	*Drypetes venusta*	31.57±2.09	5.06±2.34	31.06±2.67	44.14±2.96	32.57±2.03	21.56±2.48
22	*Entada rheedii*	23.79±1.67	46.13±5.61	48.53±1.22	30.95±1.76	24.87±1.23	25.65±3.69
23	*Euphorbia heterophylla*	9.08±1.20	0.97±0.95	1.85±2.17	30.94±1.11	3.88±0.86	8.19±0.77
24	*Euphorbia tithymaloides*	12.19±0.61	26.78±1.33	18.27±3.96	26.99±0.96	13.14±0.54	11.45±0.50
25	*Gardenia jasminoides*	4.27±1.01	6.24±2.76	14.45±2.00	19.28±2.96	27.32±0.69	13.32±1.69
26	*Getonia floribunda*	88.63±0.79	**93.10±0.59**	31.23±1.97	71.34±1.30	10.18±3.01	4.01±1.80
27	*Globba schomburgkii*	25.80±3.53	20.13±1.35	37.51±2.85	33.11±1.11	14.24±0.99	32.09±2.00
28	*Hamelia patens*	18.89±0.86	23.15±2.69	33.46±1.32	22.85±2.65	9.15±2.34	22.80±2.36
29	*Hewittia malabarica*	6.32±1.92	6.77±1.48	11.72±1.46	16.23±1.23	10.51±1.76	5.07±0.90
30	*Impatiens minor*	15.26±6.32	16.88±4.14	8.32±2.35	21.26±4.21	24.64±1.10	20.47±1.12
31	*Jasminum grandiflorum*	25.07±3.56	31.69±3.61	34.14±2.86	6.30±0.71	3.91±1.13	13.97±1.91
32	*Justicia nagpurensis*	16.56±1.97	20.34±3.77	18.79±1.53	32.87±2.46	8.32±2.14	15.85±2.09
33	*Justicia wynaadensis*	9.46±2.22	1.45±2.37	0.56±1.85	16.94±3.84	19.47±2.36	15.51±3.40
34	*Laggera alata*	64.81±1.24	56.86±2.57	35.81±3.03	56.52±1.40	28.44±2.13	12.17±3.07
35	*Lannea coromandelica*	6.86±2.22	5.58±2.63	20.79±2.61	23.13±2.15	10.16±2.80	10.75±4.19
36	*Leea indica*	14.78±1.90	15.99±0.82	20.16±1.59	42.57±2.84	38.90±1.33	33.45±1.86
37	*Lecanthus peduncularis*	2.49±2.27	29.55±0.77	4.05±1.16	19.05±3.59	8.74±2.02	28.04±3.06
38	*Leucas aspera*	28.88±1.56	86.27±0.46	58.17±2.10	37.15±1.30	25.49±1.96	31.26±1.86
39	*Lobelia alsinoides*	6.69±1.78	16.45±2.71	30.65±2.16	21.50±2.34	15.53±1.42	9.87±3.05
40	*Lophopetalum wightianum*	5.75±2.03	4.15±2.56	6.35±1.70	25.84±1.80	4.01±1.38	2.63±1.62
41	*Melia dubia*	**97.48±1.77**	48.85±3.02	24.17±3.48	26.87±3.69	42.59±3.54	22.51±2.25

42	*Micrococca mercurialis*	1.83±2.58	5.10±1.22	1.03±2.60	2.96±2.57	3.29±1.09	3.58±0.59
43	*Murraya paniculata*	19.65±1.89	12.37±2.79	34.63±0.98	1.20±2.55	4.15±3.00	1.69±2.84
44	*Otacanthus caeruleus* flower	57.35±2.90	**99.71±2.65**	5.20±1.20	3.91±1.23	4.98±1.05	6.07±0.73
45	*Otacanthus caeruleus* leaf	61.62±2.20	86.77±1.86	12.56±2.34	11.26±1.42	4.40±1.82	7.33±2.89
46	*Pancratium triflorum* bulb	63.33±0.95	82.96±1.10	89.27±1.59	82.55±2.16	68.57±2.68	61.05±2.27
47	*Pancratium triflorum* leaf	43.02±2.56	60.66±3.09	88.13±1.27	81.32±2.63	42.92±2.05	81.41±2.78
48	*Pereskia aculeate*	21.39±3.42	9.92±2.16	43.20±3.24	8.30±4.36	3.50±2.38	6.43±1.86
49	*Phyllanthus simplex*	19.05±1.34	4.27±1.98	28.04±3.06	5.20±2.19	31.82±1.56	26.41±2.94
50	*Pithecellobium dulce*	16.51±1.33	2.26±2.11	18.57±2.13	5.90±1.10	20.06±1.22	20.76±1.95
51	*Pothos scandens*	1.92±2.92	46.14±3.48	46.47±2.57	0.97±3.00	3.59±3.07	3.57±3.40
52	*Ricinus communis*	26.12±1.40	16.56±3.11	27.93±1.88	12.39±3.58	22.89±2.15	29.28±3.98
53	*Rauvolfia tetraphylla* leaf	20.72±1.41	17.97±3.39	30.18±3.51	21.93±1.33	35.34±5.60	27.73±3.27
54	*Rauvolfia tetraphylla* seed	77.35±1.79	35.80±3.67	20.20±4.36	23.90±6.74	17.23±4.88	1.90±1.86
55	*Rhaphidophora pertusa*	17.56±0.54	22.15±2.65	13.77±3.16	20.56±5.60	23.99±4.99	24.99±0.51
56	*Schleichera oleosa*	4.03±1.19	5.45±1.08	3.41±0.78	9.81±3.57	14.82±5.15	8.24±3.07
57	*Senna alata*	8.75±2.76	18.83±1.55	30.28±4.48	28.52±5.73	35.45±1.02	28.04±3.48
58	*Sauropus androgynus*	18.81±3.56	16.95±2.05	46.79±2.75	37.47±3.33	35.28±2.61	30.83±2.13
59	*Stachytarpheta indica*	29.35±1.07	20.37±2.33	39.42±1.99	48.87±1.00	24.36±1.86	18.88±1.31
60	*Strobilanthes heyneanus*	12.69±2.24	16.56±3.12	18.07±2.73	10.55±3.46	1.57±0.74	28.84±3.00
61	*Strychnos nux-vomica*	36.39±3.50	18.65±4.09	35.54±2.03	14.43±0.81	13.69±1.84	21.36±2.33
62	*Synedrella nodiflora*	17.79±6.36	40.04±2.17	20.20±3.21	5.90±2.96	14.45±3.55	40.95±1.19
63	*Talinum portulacifolium*	15.92±1.21	13.15±1.91	38.92±1.23	17.97±2.70	17.98±1.44	12.02±1.52
64	*Terminalia arjuna*	17.47±3.25	20.48±2.89	21.64±3.97	19.85±3.65	3.14±2.22	0.31±2.61
65	*Thevetia peruviana*	0.74±1.52	**95.39±0.41**	**95.37±0.26**	**92.81±0.36**	72.05±2.11	23.40±0.84
66	*Thunbergia erecta*	7.19±2.16	27.81±1.31	18.53±2.10	12.88±5.51	28.69±1.60	22.11±1.26
67	*Thunbergia grandiflora*	3.25±2.49	14.58±1.77	10.90±1.45	22.06±1.17	5.48±1.27	8.07±1.04
68	*Torenia bicolor*	12.82±3.98	46.17±3.37	6.11±2.20	5.80±1.03	5.66±1.02	8.18±2.89

69	*Trema orientale*	0.14±2.02	0.55±1.82	17.04±2.80	1.35±1.42	55.49±1.62	1.81±1.88
70	*Vitex altissima*	39.61±3.13	33.78±3.89	29.66±3.28	15.15±4.73	10.71±2.89	1.93±2.28
71	*Wattakaka volubilis*	16.66±2.66	9.35±1.60	22.83±2.80	13.91±1.01	17.09±1.40	9.37±1.22

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.10. Screening of plant extracts for cytotoxicity against UM-SCC-83B

More than 50.00% mortality was shown by 48 extracts, when tested on UM-SCC-83B. Among these, six extracts caused a percentage mortality exceeding 90.00%. The chloroform extracts of *Alangium salviifolium* and *Leucas aspera* showed the highest percentage mortality, both at 99.37±0.86% and 99.37±1.13%, respectively. Additionally, the ethyl acetate extract of *Pancratium triflorum* bulb exhibited a percentage mortality of 92.07±0.44% (Table 1.3.10).

Table 1.3.10. Percentage mortality of the plant extracts at 100 µg/mL on the oral cancer cell line, UM-SCC-83B after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	*Acalypha fruiticosa*	0.48±2.09	5.75±1.53	0.41±2.02	6.37±3.59	0.54±1.94	13.05±1.00
2	*Alangium salviifolium*	12.00±2.75	**99.37±0.86**	65.63±1.90	**91.26±0.51**	**91.23±0.62**	62.93±1.75
3	*Anamirta cocculus*	7.51±2.61	1.61±1.60	62.44±1.07	61.59±0.77	43.93±0.10	24.42±3.29
4	*Antigonon leptopus*	3.55±1.75	13.17±4.49	11.56±1.42	48.15±1.29	16.02±2.78	19.01±2.81
5	*Argyreia pomacea*	6.57±2.44	6.25±2.22	5.14±3.17	16.54±2.23	21.34±1.94	9.64±1.45
6	*Bauhinia phoenicea*	3.50±3.72	4.65±4.48	20.50±2.49	11.57±0.50	7.08±3.11	3.52±3.24
7	*Bridelia retusa*	9.91±2.44	3.80±1.92	6.85±1.34	32.84±1.66	21.67±1.31	1.93±0.44
8	*Calopogonium mucunoides*	21.95±3.30	17.85±2.74	21.69±2.84	35.85±2.65	26.35±2.53	14.64±1.89
9	*Carl rheedii*	7.97±0.93	16.29±0.79	12.69±0.85	18.55±1.47	19.42±1.63	5.07±0.37

10	*Cassia javanica*	17.26±1.56	25.51±1.13	38.51±3.12	41.83±1.95	30.18±2.37	22.00±1.26
11	*Chassalia curviflora*	17.51±1.94	12.77±2.05	40.68±2.75	69.47±3.08	85.54±3.26	30.83±2.13
12	*Cheilocostus speciosus*	25.40±1.98	18.43±2.04	17.56±3.65	23.75±1.88	52.50±3.34	55.47±3.36
13	*Chrysophyllum cainito*	13.27±2.60	14.66±2.59	13.05±3.54	35.41±2.14	11.78±0.46	27.94±1.73
14	*Cipadessa baccifera* leaf	33.65±1.99	38.26±1.91	25.92±0.76	28.82±1.17	20.28±1.84	29.49±2.96
15	*Cipadessa baccifera seed*	79.23±3.37	81.31±4.54	12.55±1.33	25.56±2.68	22.05±2.68	20.04±2.61
16	*Cissus discolor*	18.81±2.75	59.94±2.47	9.77±2.21	9.62±3.41	37.01±4.58	37.06±2.64
17	*Cochlospermum religiosum*	0.95±0.19	14.01±1.05	9.70±0.67	1.62±0.36	20.28±2.20	3.14±0.69
18	*Combretum indicum*	74.78±3.66	61.50±6.00	61.08±3.06	25.90±2.15	62.63±0.86	74.25±1.71
19	*Conyza canadensis*	54.87±2.64	14.77±2.68	19.29±2.41	21.59±3.76	31.26±3.15	38.75±1.52
20	*Cyclea peltata*	11.61±2.31	28.41±2.89	39.82±3.47	18.46±2.02	22.41±1.67	14.92±2.26
21	*Drypetes venusta*	9.43±1.30	6.92±1.59	43.58±1.49	14.03±2.93	7.72±1.34	13.50±2.32
22	*Entada rheedii*	10.25±2.85	2.17±1.55	9.59±3.11	4.98±0.97	3.27±1.86	5.25±1.97
23	*Euphorbia heterophylla*	32.50±1.59	33.90±3.16	41.63±1.25	21.64±2.56	16.06±4.42	13.60±3.68
24	*Euphorbia tithymaloides*	23.56±3.70	30.81±0.57	28.64±2.39	36.44±1.34	35.31±2.50	29.99±1.42
25	*Gardenia jasminoides*	15.95±2.04	1.97±1.11	4.36±1.63	6.72±4.37	1.39±0.62	40.82±2.93
26	*Getonia floribunda*	35.06±1.29	70.69±2.53	52.52±4.61	68.86±3.35	29.39±1.05	25.89±2.29
27	*Globba schomburgkii*	13.46±6.16	40.66±3.91	30.89±1.35	33.28±1.70	30.32±3.96	8.91±1.21
28	*Hamelia patens*	30.83±3.88	31.84±1.80	47.85±3.24	14.85±3.84	41.93±3.87	42.39±2.62
29	*Hewittia malabarica*	26.58±2.59	32.54±2.53	34.05±2.94	39.93±3.46	39.42±2.32	21.16±2.75
30	*Impatiens minor*	19.90±3.09	9.16±5.49	20.64±2.33	44.51±4.06	34.09±3.07	20.79±1.85
31	*Jasminum grandiflorum*	13.55±1.87	4.49±2.07	0.56±1.85	25.36±1.58	38.31±2.58	14.57±3.21
32	*Justicia nagpurensis*	7.92±2.21	12.20±1.85	11.72±0.61	8.83±1.87	7.60±2.34	11.19±2.05
33	*Justicia wynaadensis*	4.32±1.10	1.81±1.32	0.92±1.84	4.93±2.96	2.60±2.06	7.52±2.50
34	*Laggera alata*	30.01±2.24	42.67±1.69	3.78±3.26	11.82±2.99	6.40±3.86	6.34±2.17
35	*Lannea coromandelica*	6.45±1.78	3.49±1.04	4.55±1.05	22.01±1.96	22.64±1.59	22.14±3.22
36	*Leea indica*	32.46±1.80	20.20±3.36	33.79±2.88	20.42±2.23	21.07±3.86	38.00±4.09

37	*Lecanthus peduncularis*	14.80±±1.11	36.04±3.56	21.47±2.77	21.45±2.32	39.86±3.43	22.11±2.48
38	*Leucas aspera*	3.94±1.76	**99.37±1.13**	4.66±3.79	31.73±2.38	37.84±2.48	20.78±1.86
39	*Lobelia alsinoides*	36.04±2.61	13.73±2.45	41.88±1.76	23.20±2.19	16.88±1.55	36.89±1.65
40	*Lophopetalum wightianum*	20.05±3.40	26.54±3.09	27.63±1.30	57.76±0.78	35.51±3.91	32.14±1.73
41	*Melia dubia*	63.57±2.72	89.22±0.69	56.39±3.57	8.58±4.63	32.98±2.16	13.71±2.33
42	*Micrococca mercurialis*	20.23±4.27	2.95±2.45	20.30±1.23	25.29±3.41	14.52±3.86	40.49±3.13
43	*Murraya paniculata*	8.03±3.71	29.22±1.63	33.47±2.13	31.09±5.92	36.49±1.70	28.69±1.59
44	*Otacanthus caeruleus* flower	12.67±0.78	58.60±1.47	5.35±0.64	11.87±1.59	9.42±0.78	10.86±1.84
45	*Otacanthus caeruleus* leaf	0.85±3.09	0.40±2.44	2.61±1.76	3.27±6.94	2.58±4.30	6.89±4.62
46	*Pancratium triflorum* bulb	66.32±2.02	89.15±1.09	**92.07±0.44**	88.32±1.67	76.91±1.51	58.38±1.80
47	*Pancratium triflorum leaf*	46.34±1.59	78.52±1.46	**91.36±0.83**	79.45±0.48	55.58±1.48	81.50±0.96
48	*Pereskia aculeate*	7.08±3.11	3.52±3.24	12.27±2.91	5.57±5.18	7.59±2.97	28.14±1.80
49	*Phyllanthus simplex*	6.57±1.28	28.25±2.57	58.23±1.75	8.45±2.24	5.19±1.91	5.74±1.39
50	*Pithecellobium dulce*	14.66±0.87	9.27±0.80	14.09±0.64	3.42±0.61	12.01±3.37	23.25±3.55
51	*Pothos scandens*	6.63±1.88	14.63±2.84	21.62±1.59	1.71±1.75	1.33±1.82	0.94±2.13
52	*Ricinus communis*	7.48±4.21	3.66±2.02	0.93±3.00	1.87±2.45	5.63±2.46	1.25±3.55
53	*Rauvolfia tetraphylla* leaf	15.02±3.99	49.44±5.01	6.52±1.57	19.54±2.15	25.76±0.16	15.86±1.27
54	*Rauvolfia tetraphylla* seed	9.15±5.01	54.08±2.30	16.37±3.29	8.84±0.96	23.81±1.97	22.06±4.96
55	*Rhaphidophora pertusa*	11.68±2.73	33.03±3.00	35.09±3.64	28.11±1.61	18.81±4.53	5.24±2.08
56	*Schleichera oleosa*	16.01±1.32	5.41±0.96	4.85±1.18	1.69±1.80	23.13±2.21	7.10±1.57
57	*Senna alata*	2.08±3.37	1.94±5.34	3.18±0.85	0.09±3.74	3.28±3.63	0.75±4.16
58	*Sauropus androgynus*	28.00±5.01	28.88±1.47	16.46±2.16	52.15±1.45	19.66±3.17	36.00±2.94
59	*Stachytarpheta indica*	33.30±1.06	36.29±2.31	70.80±1.57	86.45±1.32	56.15±1.91	27.65±1.67
60	*Strobilanthes heyneanus*	1.42±2.61	2.87±1.55	4.45±1.76	0.04±1.63	4.58±1.00	1.32±2.61
61	*Strychnos nux-vomica*	19.68±2.75	17.13±1.95	24.00±3.12	7.01±1.78	5.47±1.49	3.17±0.72
62	*Synedrella nodiflora*	14.79±2.81	16.59±3.84	32.40±2.50	40.57±2.65	23.39±6.51	12.87±2.63
63	*Talinum portulacifolium*	23.09±2.76	40.88±1.55	30.28±2.60	28.52±1.68	35.45±1.02	48.04±3.48

64	*Terminalia arjuna*	10.65±2.85	18.03±4.28	39.75±2.51	27.64±1.36	5.84±0.86	1.13±1.43
65	*Thevetia peruviana*	15.40±2.50	89.58±0.78	82.95±1.15	75.32±1.17	37.13±2.27	31.32±3.24
66	*Thunbergia erecta*	12.44±3.36	46.58±3.05	22.09±3.57	35.37±4.88	45.33±1.27	29.82±3.46
67	*Thunbergia grandiflora*	11.19±0.94	12.77±3.46	9.09±0.75	46.95±3.31	35.28±2.39	30.83±4.21
68	*Torenia bicolor*	15.15±3.52	55.01±2.94	14.51±2.71	22.54±3.10	26.29±1.42	24.73±2.60
69	*Trema orientale*	15.63±3.10	13.39±3.92	10.46±2.57	19.66±2.16	33.74±3.73	23.13±3.86
70	*Vitex altissima*	31.57±2.13	36.91±2.08	24.93±2.11	6.36±1.62	5.21±0.98	8.60±2.47
71	*Wattakaka volubilis*	8.41±0.44	9.91±1.06	12.56±0.90	5.48±1.27	26.35±2.87	18.90±2.04

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

1.3.1.11. Screening of plant extracts for cytotoxicity against HaCaT

To assess the differential cytotoxicity of the plant extracts, their effects were also evaluated on the non-cancerous human keratinocyte cell line, HaCaT. Among the screened extracts, 50 extracts were found to be less toxic to the HaCaT cell line, with percentage mortality less than 20.00%. Additionally, 295 extracts exhibited a mortality rate below 50.00% (Table 1.3.11). Some of the plant extracts with high cytotoxicity on cancer cell lines displayed less toxicity to HaCaT revealing their specific toxicity to cancer cell lines. Such extracts are promising candidates for isolation of anticancer molecules as they have selective toxicity towards cancer cell lines.

Table 1.3.11. Percentage mortality of the plant extracts at 100 µg/mL on the normal keratinocyte cell line, HaCaT after 48 hours.

Sl. No.	Plant Sample	Percentage Mortality (48 hours)					
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
1	*Acalypha fruiticosa*	0.12±1.96	17.33±1.59	0.14±1.49	9.09±1.61	5.22±2.78	13.17±2.22
2	*Alangium salviifolium*	54.19±0.99	77.74±0.32	52.30±0.93	71.86±1.11	65.15±1.01	47.65±1.03
3	*Anamirta cocculus*	5.43±1.90	17.55±2.64	1.72±0.73	30.23±1.00	10.57±1.88	29.54±3.29

4	*Antigonon leptopus*	2.37±1.49	2.75±0.74	8.01±2.38	64.85±1.83	3.11±1.42	5.45±1.04
5	*Argyreia pomacea*	26.37±5.09	12.87±1.32	3.77±2.65	18.65±3.87	15.35±1.29	3.88±2.46
6	*Bauhinia phoenicea*	2.55±0.99	4.69±2.00	2.92±2.16	18.36±6.05	30.86±2.22	19.37±2.78
7	*Bridelia retusa*	7.20±9.35	11.55±9.86	56.66±9.01	41.35±9.95	52.55±9.34	45.57±3.65
8	*Calopogonium mucunoides*	33.47±4.13	33.11±2.52	42.36±1.39	36.35±4.74	61.46±6.46	12.32±5.32
9	*Carl rheedii*	6.51±1.58	4.10±1.36	3.19±2.53	2.25±1.70	4.84±2.26	7.53±2.42
10	*Cassia javanica*	37.74±5.49	5.69±7.34	16.64±6.34	8.54±8.65	11.36±1.55	45.35±9.46
11	*Chassalia curviflora*	33.47±6.55	52.43±4.24	10.12±1.32	9.35±8.55	42.66±1.36	11.27±9.16
12	*Cheilocostus speciosus*	22.64±4.55	66.65±8.52	65.35±6.63	46.65±1.55	63.55±4.66	44.47±6.13
13	*Chrysophyllum cainito*	5.89±8.24	55.58±5.46	8.16±4.65	62.35±1.34	8.54±9.12	13.23±4.33
14	*Cipadessa baccifera* leaf	23.84±5.44	8.22±1.37	21.31±9.64	55.64±6.48	9.52±6.53	48.53±1.56
15	*Cipadessa baccifera* seed	65.56±3.34	16.22±2.02	12.65±1.19	22.45±9.55	15.65±7.35	17.36±9.25
16	*Cissus discolor*	2.07±1.37	46.80±3.61	27.19±5.41	18.64±8.64	19.55±2.12	16.36±7.16
17	*Cochlospermum religiosum*	4.73±6.42	9.46±8.36	9.46±6.55	24.64±4.67	6.55±1.26	9.54±6.57
18	*Combretum indicum*	5.77±0.76	6.84±0.59	3.85±0.65	3.72±1.07	4.49±0.66	5.27±1.31
19	*Conyza canadensis*	55.70±3.97	25.07±2.95	4.91±1.34	66.44±9.45	62.64±6.16	7.66±7.76
20	*Cyclea peltata*	50.38±9.25	19.65±9.47	65.66±9.55	53.65±7.35	42.55±8.12	54.36±3.17
21	*Drypetes venusta*	1.74±5.45	12.55±1.36	6.65±6.55	44.63±1.45	61.65±9.12	62.54±8.22
22	*Entada rheedii*	44.84±7.55	1.65±9.67	9.45±2.64	15.64±5.55	18.61±6.14	28.62±9.46
23	*Euphorbia heterophylla*	6.90±0.69	4.96±0.75	5.59±1.12	6.12±1.08	4.38±1.04	6.82±0.74
24	*Euphorbia tithymaloides*	69.29±1.34	16.55±5.68	64.36±4.35	41.63±6.45	8.12±7.13	38.23±2.42
25	*Gardenia jasminoides*	8.53±2.44	38.64±3.05	5.37±1.92	0.91±1.55	0.69±1.02	17.16±1.52
26	*Getonia floribunda*	98.69±0.14	99.09±0.21	71.76±1.04	27.75±3.06	17.77±2.59	15.22±1.63
27	*Globba schomburgkii*	49.24±1.54	1.35±1.25	16.65±7.23	69.65±6.55	5.56±2.45	51.65±4.67
28	*Hamelia patens*	3.90±6.55	9.44±9.64	70.64±3.51	11.63±9.45	21.36±6.55	24.62±7.66
29	*Hewittia malabarica*	5.38±1.16	5.44±1.07	44.91±1.32	55.47±6.25	45.25±4.33	51.25±3.57
30	*Impatiens minor*	16.89±9.45	6.67±3.46	6.65±6.16	13.34±8.55	65.45±1.32	35.52±8.54

31	*Jasminum grandiflorum*	3.17±2.39	41.91±2.42	9.92±1.72	1.36±6.35	16.35±7.32	62.14±1.35
32	*Justicia nagpurensis*	11.29±5.45	69.56±6.34	11.64±8.64	56.35±4.67	14.35±6.13	41.12±9.47
33	*Justicia wynaadensis*	6.48±2.86	1.51±0.22	10.06±1.94	2.39±1.84	6.42±2.45	2.98±2.00
34	*Laggera alata*	9.84±9.45	25.26±7.33	3.13±7.64	67.65±9.65	6.55±9.11	11.13±4.36
35	*Lannea coromandelica*	15.40±2.79	17.34±2.90	8.79±1.25	6.23±1.18	11.12±2.49	22.98±3.53
36	*Leea indica*	8.91±4.59	15.39±4.38	25.13±3.15	3.11±4.07	1.38±1.00	14.49±3.10
37	*Lecanthus peduncularis*	5.28±0.90	35.48±2.32	0.60±0.20	11.35±9.35	65.45±7.62	22.16±6.45
38	*Leucas aspera*	65.74±4.22	3.15±9.64	56.65±9.31	61.66±5.45	55.44±6.16	34.51±1.57
39	*Lobelia alsinoides*	19.75±2.47	28.18±6.65	45.10±1.68	7.66±9.65	6.54±4.35	1.17±6.46
40	*Lophopetalum wightianum*	3.90±3.26	1.28±3.96	1.42±1.63	25.00±2.06	8.99±3.39	6.64±2.65
41	*Melia dubia*	48.81±2.04	28.70±1.21	22.38±1.98	17.14±2.78	20.40±2.37	2.63±2.07
42	*Micrococca mercurialis*	3.36±2.20	4.86±1.43	1.88±1.64	7.01±0.65	5.48±0.92	19.52±0.94
43	*Murraya paniculata*	45.38±6.15	65.64±9.62	19.32±4.12	0.50±2.15	11.08±1.48	13.53±1.50
44	*Otacanthus caeruleus* flower	54.39±9.15	6.54±5.36	6.17±5.53	9.66±8.55	55.64±7.34	9.47±9.43
45	*Otacanthus caeruleus* leaf	6.25±4.34	18.64±7.33	44.34±9.52	12.34±1.66	9.65±1.27	51.36±6.46
46	*Pancratium triflorum* bulb	81.05±0.71	43.47±0.42	68.49±0.33	94.18±0.54	85.06±0.90	76.95±1.01
47	*Pancratium triflorum* leaf	48.63±2.10	83.33±1.12	97.82±0.19	90.61±0.57	97.62±0.70	90.15±0.73
48	*Pereskia aculeate*	3.36±2.16	14.03±2.66	25.40±2.00	17.87±4.33	12.86±3.00	40.19±0.84
49	*Phyllanthus simplex*	55.64±3.45	36.45±9.35	9.35±3.15	41.31±2.76	50.94±2.18	32.10±1.84
50	*Pithecellobium dulce*	5.38±0.87	4.43±0.78	5.11±1.73	11.31±1.52	5.64±1.18	4.83±0.88
51	*Pothos scandens*	0.32±1.56	7.02±2.09	17.28±1.85	1.42±0.19	3.48±1.62	2.26±1.86
52	*Ricinus communis*	33.46±8.25	4.24±5.42	17.33±7.26	9.31±9.65	9.66±5.14	45.15±7.47
53	*Rauvolfia tetraphylla* leaf	1.00±9.46	9.64±2.72	52.34±6.17	17.31±5.65	16.34±4.33	41.17±6.47
54	*Rauvolfia tetraphylla* seed	9.05±0.68	9.49±1.83	7.50±2.73	52.33±6.24	55.35±1.12	6.12±1.36
55	*Rhaphidophora pertusa*	9.55±6.45	9.57±9.43	1.44±9.56	1.67±7.54	49.66±9.45	65.46±4.33
56	*Schleichera oleosa*	51.38±3.46	45.25±6.44	26.32±6.12	5.27±3.69	76.55±4.25	3.17±0.60
57	*Senna alata*	9.00±2.63	34.57±3.73	36.08±4.08	12.28±2.08	36.08±3.02	16.39±2.89

58	*Sauropus androgynus*	64.45±1.66	6.21±7.34	4.34±9.82	16.34±8.35	9.34±9.46	64.57±6.45
59	*Stachytarpheta indica*	53.28±3.45	62.46±1.63	12.47±3.35	19.35±7.45	1.25±4.33	61.62±4.36
60	*Strobilanthes heyneanus*	5.56±0.98	7.53±0.73	7.98±0.94	4.71±0.90	5.35±0.94	6.98±0.56
61	*Strychnos nux-vomica*	64.54±8.55	13.25±6.47	1.36±7.23	3.64±1.54	16.35±7.63	66.62±1.66
62	*Synedrella nodiflora*	59.25±3.46	48.24±5.65	36.31±1.62	45.35±6.35	58.35±1.36	31.47±4.33
63	*Talinum portulacifolium*	13.74±0.70	9.03±0.96	2.51±0.82	7.51±1.24	8.77±1.06	12.39±1.3
64	*Terminalia arjuna*	4.01±1.23	4.62±1.00	5.35±1.27	46.72±2.10	28.37±2.15	35.13±2.34
65	*Thevetia peruviana*	20.62±6.58	95.65±0.63	75.22±0.41	79.94±0.44	23.32±2.95	8.86±2.42
66	*Thunbergia erecta*	25.23±5.64	17.14±2.70	0.57±1.26	5.43±0.99	2.89±0.67	1.48±2.62
67	*Thunbergia grandiflora*	11.19±0.94	11.67±1.23	20.96±0.75	31.66±5.17	45.51±2.39	40.50±2.83
68	*Torenia bicolor*	10.71±2.43	70.39±2.80	6.88±2.37	1.66±9.55	64.35±5.12	1.57±6.57
69	*Trema orientale*	17.41±2.23	20.03±1.04	20.96±2.64	10.69±2.12	14.55±1.68	7.69±1.69
70	*Vitex altissima*	18.34±9.44	33.56±4.63	11.36±6.17	19.34±9.55	45.35±7.00	10.57±5.62
71	*Wattakaka volubilis*	6.51±1.59	4.10±1.36	3.19±2.53	2.25±1.61	4.84±2.26	7.53±2.42

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant ($p>0.05$). Percentage mortalities above 90.00% are shown in bold.

DISCUSSION

1.4.1. Differential cytotoxicity of plant- extracts against various cancer cell lines

This study involved the screening of 67 plants for their anticancer potential, using a total of 426 different extracts tested across various cancer cell lines. To ensure the safety of these extracts, their cytotoxicity was also evaluated on the normal human cell line HaCaT. This step is critical in understanding the effects of the extracts on non-cancerous cells, particularly in the context of drug development, where selective toxicity is highly desirable. Extracts that demonstrated high toxicity towards cancer cell lines but had minimal or no harmful effects on the normal HaCaT cells were considered potential candidates for anticancer drug development. This approach is crucial for identifying compounds that maximize efficacy while minimizing harm to normal tissues.

Among the cancer cell lines tested, the human colorectal carcinoma cell line HT29 showed sensitivity to 59 plant extracts, each of which caused more than 50% cell mortality. Another colorectal carcinoma cell line, HCT116, displayed vulnerability to 34 extracts, with more than 50% mortality. Additionally, 48 extracts caused over 50% cell death in the human skin cancer cell line A431, and 35 extracts had similar effects on the human renal carcinoma cell line A498. On human cervical cancer cell line HeLa was susceptible to 38 extracts, and on human breast cancer cell line MCF7, 48 extracts showed more than 50.00% mortality. On osteoblast cell line, MG63, 51 extracts caused above 50.00% cell death, and on ovarian teratocarcinoma cell line, PA1, 86 extracts showing over 50% cell mortality. On pancreatic carcinoma cell line, PANC1, 36 extracts and on metastatic oral cancer cell line, UM-SCC-83B, 47 extracts demonstrated cytotoxicity exceeding 50%. These results suggest the presence of potent anticancer compounds in these plant extracts.

In addition to their efficacy on cancer cells, the cytotoxicity of these extracts was tested on the normal human cell line HaCaT. Notably, 294 plant extracts exhibited less than 50.00% cell mortality, indicating their low toxicity towards non-cancerous cells. Fifty extracts were causing less than 20.00% cell death on this cell line. However, several plant extracts, such as the chloroform extract of *Melia dubia*, the

methanol extract of *Alangium salviifolium*, and the methanol extract of *Chassalia curviflora*, displayed selective cytotoxicity; these extracts caused significant mortality in various cancer cell lines while exhibiting reduced toxicity towards the normal HaCaT cells. This selective action suggests their potential for further research, particularly in the purification of anticancer compounds and their development as cancer therapies. The observed efficacy, coupled with their reduced toxicity to normal cells, underscores the value of these plant extracts in the ongoing search for new anticancer agents.

In this study, 28 extracts demonstrated over 50.00% mortality on HT29 cancer cells, while showing less than 50.00% mortality on normal HaCaT cells. Similarly, 18 extracts showed differential cytotoxicity on HCT116, 24 on A431, 21 on A498, 18 on HeLa, 28 on MCF7, 29 on MG63, 54 on PA1, 15 on PANC1, and 25 on UM-SCC-83B, with high mortality (above 50.00%) in cancer cells but low toxicity (below 50.00%) in HaCaT cells. These extracts exhibit potential as anticancer agents due to their selective cytotoxicity, suggesting the presence of bioactive molecules that target cancer cells while sparing normal ones.

Out of the 64 plants screened, the first reports of anticancer activity from plants with high toxicity are discussed below.

1.4.1.1. *Pancratium triflorum*

In the present study, *Pancratium triflorum* exhibited a high level of cytotoxicity against several cancer cell lines. The ethyl acetate extract of *P.triflorum* exerted the highest cytotoxicity on MG63 (99.74±0.28%) among the cell lines used in the present study. The acetone extract and the chloroform extracts also caused higher level of cell death in many cell lines, and the highest cell death were on MG63 (99.41±0.52%) and HeLa (92.36±0.81%) respectively. When considering the three extracts, their cytotoxicity on the normal cell line, HaCaT were varying and were 68.49±0.33%, 94.18±0.54% and 43.47±0.42% respectively for ethyl acetate, acetone and chloroform extracts. Both bulb and leaf extracts were investigated, with the bulb extracts causing over 50.00% cell death in all cancer cell lines tested except HCT116, and leaf extracts demonstrating over 50.00% cell death across all tested

cell lines. This comprehensive cytotoxicity suggests a broad-spectrum anticancer potential.

Interestingly, no previous data exist regarding the anticancer activity of *P. triflorum*. Even studies on its antioxidant properties are scarce, with only Johnson *et al.* reporting such effects. However, studies on a related species, *Pancratium maritimum*, provide a foundation for investigating the anticancer potential of *P. triflorum*. *P. maritimum* has been studied for its antiproliferative and antioxidant properties across various cancer cell lines, including C32, HeLa, MDA-MB-231, PC3, A549, MCF-7, and LNCaP, using the SRB assay. Notably, the ethanol extract from the stems of *P. maritimum* (IC_{50} of 27.1 µg/mL against C32 cells) and the aqueous extract from the fruits (IC_{50} of 36.5 µg/mL against MCF-7) showed considerable anticancer activity (Johnson *et al.,* 2018).

Given the close taxonomic relationship between *P. triflorum* and *P. maritimum*, it is reasonable to assume that *P. triflorum* may also possess similar bioactive compounds contributing to its cytotoxicity against cancer cells. The results of the current study align with this hypothesis, suggesting that *P. triflorum* holds potential as a source of novel anticancer agents. Further research should focus on identifying and isolating the active compounds responsible for the observed anticancer effects.

1.4.1.2. *Anamirta cocculus*

Anamirta cocculus demonstrated a broad range of cytotoxicity across various cancer cell lines, with notably lower toxicity on the human keratinocyte cell line, HaCaT. The ethyl acetate extract, in particular, exhibited significant cytotoxicity against cancer cells while maintaining a relatively low toxicity towards HaCaT cells (18.72±0.73%). The highest mortality rate was observed with the ethyl acetate extract on PA1 ovarian cancer cells (98.64±0.40%), followed by the acetone extract on PA1 (92.72±0.58%), the ethyl acetate extract on HT29 colorectal cancer cells (91.16±0.24%), and on MG63 osteosarcoma cells (86.76±1.04%) (The effect of the extracts from *A. cocculus* is discussed in detail in chapter 4).

Interestingly, no prior studies have reported the anticancer properties of *A. cocculus*, making the findings of this research particularly valuable. The significant cytotoxicity observed in this study suggests that *A. cocculus* extracts, especially the ethyl acetate extract, hold promise as source of potential anticancer agents. Further exploration of its bioactive compounds and mechanisms of action is warranted for developing therapeutic applications.

1.4.1.3. *Chassalia curviflora*

Chassalia curviflora, a medicinal plant traditionally utilized in folklore for treating various ailments and infections due to its anti-inflammatory properties, demonstrated substantial cytotoxicity across all cancer cell lines evaluated in this study. Notably, the methanol extract of *C. curviflora* exhibited the highest potency, inducing 99.48±0.53% cell death in the HCT116 colorectal cancer cell line and 99.02±2.33% mortality in the MG63 osteosarcoma cell line. The mortality observed on HaCaT was considerably lower (42.66±1.36%), indicating the cell line specificity of the extract.

Despite its recognized anti-inflammatory, antihepatotoxic, and analgesic activities, the anticancer potential of *Chassalia curviflora* has not been extensively explored until recent studies. Gopa *et al.* investigated the anticancer effects of *C. curviflora* by isolating total alkaloids from its roots. Utilizing the MTT assay, they assessed the antiproliferative activity of these alkaloids in three cancer cell lines: liver cancer (A549) with an IC_{50} value of 3.59±0.14 µg/mL, breast cancer (MCF-7), and ovarian cancer (HeLa). Their findings revealed that the total alkaloid extract significantly inhibited cell proliferation and induced apoptosis in these cancer cell lines, suggesting a promising anticancer potential of *C. curviflora* (Gopa *et al.,* 2020).

Further supporting these results, additional studies have demonstrated the antioxidant and anticancer activities of *C. curviflora* extracts. These studies have highlighted the plant's ability to inhibit cancer cell proliferation, motility, and adhesion, thereby reinforcing its potential as a therapeutic agent against cancer (Farabe *et al.*, 2024). The observed antiproliferative and apoptotic effects align with

our findings, underscoring the significant cytotoxicity of *C. curviflora* extracts on multiple cancer cell lines.

With its long-standing use in traditional medicine and growing scientific support, *Chassalia curviflora* emerges as a promising subject for continued research. Islam *et al.* demonstrated that ethyl acetate, acetone, and ethanol extracts of *C. curviflora* exhibit neuropharmacological activity in mouse models (Islam *et al.,* 2022). Future studies should aim to isolate and identify the bioactive compounds responsible for its anticancer potential, while also clarifying the mechanisms involved. This research could pave the way for the development of new anticancer therapies based on *C. curviflora*, contributing to more precise and effective cancer treatments.

1.4.1.4. *Antigonon leptopus*

Antigonon leptopus has demonstrated promising differential cytotoxicity, indicating its potential for cancer treatment. The chloroform extract of *A. leptopus* showed the highest cell mortality in the MG63 cell line (96.43±0.41%), followed by A431 (67.84±1.96%) and PA1 (53.75±2.57%). The acetone extract displayed cytotoxicity with 73.80±1.83% mortality in the A431 cell line and over 50% mortality in the PA1, MG63, HCT116, and A498 cancer cell lines. The petroleum ether extract caused 59.37±1.59% mortality in the MG63 cancer cell line. All extracts, except for the acetone extract (64.85±1.83%), did not cause considerable cell mortality in the HaCaT cell line. The percentage mortality of HaCaT caused by the chloroform extract and petroleum ether extract were 2.75±0.74% and 2.37±1.49% respectively. However, the acetone extract caused 64.85±1.83% mortality in HaCaT cells, indicating potential toxicity to non-cancerous cells.

In the Philippines, *A. leptopus* is traditionally used as an herbal remedy for pain and gout-like symptoms. The methanol extract of this plant has shown a potent inhibitory effect on xanthine oxidase, with the isolated compound acting as a noncompetitive inhibitor of xanthine with an IC_{50} of 1.79 μg/mL. Furthermore, a study identified the IC_{50} of the ethanolic extract of *A. leptopus* on normal cell lines to be 289.60 μg/mL, indicating its non-toxic nature to healthy cells (Elhaj *et al.,* 2015).

Despite these promising findings, there is a lack of extensive studies supporting the anticancer properties of *A. leptopus*. Therefore, this study emphasizes the need for deeper investigations into the specific components of *A. leptopus* extracts that contribute to their cytotoxic effects. Understanding these mechanisms could lead to the development of new anticancer therapies, leveraging the rich ethnobotanical background and emerging scientific evidence surrounding *A. leptopus*. The plant's demonstrated efficacy against cancer cells, coupled with its significant antioxidant properties, makes it a compelling candidate for further research in cancer treatment.

1.4.1.5. *Otacanthus caeruleus*

Otacanthus caeruleus, a known ornamental plant, demonstrated significant cytotoxic potential across various cancer cell lines. The chloroform extract of *O. caeruleus* flowers exhibited high levels of cell death in several cell lines, including PANC1 (99.71±2.65%), A431 (99.69±0.63%), MG63 (99.24±0.08%), PA1 (97.53±0.58%), A498 (90.52±0.59%), HT29 (85.79±1.67%), UM-SCC-83B (58.60±1.47%), and MCF7 (57.24±1.99%). Similarly, the petroleum ether extract also induced cytotoxicity, especially on A431 (67.48±2.48%), MG63 (96.73±0.81%), PA1 (75.83±0.70%), and PANC1 (57.35±2.90%). The cell death on HaCaT by the chloroform extract and petroleum ether extract were 6.54±5.36% and 54.39±9.15% respectively. Interestingly, leaf extracts exhibited broader cytotoxic effects compared to flower extracts. The chloroform leaf extract caused cell death in a variety of cell lines, with high cytotoxicity in HCT116 (97.94±0.20%), A431 (97.65±0.15%), A498 (99.97±0.25%), MG63 (99.03±0.33%), PA1 (99.67±0.60%), and PANC1 (86.77±1.86%). At the same time, a lesser percentage of death was observed on HaCaT (18.64±7.33%).

The pronounced cytotoxicity of both flower and leaf extracts of *Otacanthus caeruleus* suggests the presence of potent bioactive compounds. Given that there are no previous studies investigating the anticancer properties of this plant, these findings are of considerable importance. They not only provide a foundation for future research but also highlight the potential of *O. caeruleus* as a promising candidate for anticancer drug development. Further exploration of its bioactive

constituents and their mechanisms of action could pave the way for new therapeutic applications, especially in targeting cancers such as osteosarcoma (MG63), ovarian teratocarcinoma (PA1), and skin cancer (A431).

In this study, *Melia dubia*, *Strobilanthes heyneanus*, *Stachytarpheta indica*, and *Globba schomburgkii* demonstrated significant cytotoxicity across various cancer cell lines, showing high mortality percentages. Notably, none of these plants had prior reports of anticancer potential in the literature, making these findings particularly relevant.

While the anticancer activity of these plants remains unexplored until now, other biological activities have been studied. For instance, *Melia dubia* is known for its antioxidant and antibacterial properties. Similarly, *Strobilanthes heyneanus*, *Stachytarpheta indica*, and *Globba schomburgkii* have been studied for their potential in treating infections, as well as their anti-inflammatory, antioxidant, and antibacterial activities. The findings from this study suggest that these plants not only possess other therapeutic properties but may also have promising anticancer potential. Future research should focus on isolating and characterizing the bioactive compounds in these plants to better understand their mechanisms of action and anticancer drug development.

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Following are some of the plants with reported anticancer activity from other parts of the plant or studies have been done on cell lines other than the cell lines we used.

1.4.1.6. *Alangium salviifolium*

Alangium salviifolium leaf extracts have shown notable anticancer properties across various extracts and cancer cell lines. Among the six extracts tested, the methanol extract demonstrated the most significant potential, achieving over 99.00% mortality in all cancer cell lines studied. Notably, this extract exhibited lower toxicity to the normal cell line HaCaT, with a mortality rate of 65.15±1.01%. The chloroform extract was the second most potent, showing the highest mortality in the PA1 cell line (99.42±0.38%), followed by significant effects on the UM –SCC-83B and A431 cell lines. The acetone extract also showed substantial activity, with high mortality rates in several cell lines, including PA1 (99.91±0.18%), HT29 (99.70±0.09%), and HeLa (98.91±0.48%). The ethyl acetate extract was effective, particularly against A498 (88.22±1.46%), PA1 (82.91±1.26%), and A431 (79.56±1.53%) cell lines. Both the aqueous and petroleum ether extracts demonstrated cytotoxicity to certain cell lines, with the aqueous extract exhibiting more than 50% mortality across all tested cell lines and the highest toxicity on PA1 (75.65±2.15%), while the petroleum ether extract showed the highest toxicity on the MCF7 cell line (65.57±1.69%).

Phytochemical investigations have identified two octahydro–protoberberine alkaloids, alangiifoliumines A and B, and two protoemetine derivatives, alangiifoliumines C and D, along with 11 known compounds isolated from the stems of *A. salviifolium*. These compounds exhibited inhibitory effects against the A-549, HeLa, and SKOV-3 human cancer cell lines, with IC_{50} values ranging from 3 nM to 9.4 μM (Cai *et al.,* 2019). Further studies have shown that the methanolic extract of *A. salviifolium* possesses anticancer activity, with an IC_{50} of 42.20 μg/mL on Dalton's Ascitic Lymphoma (DAL) cells. This activity is attributed to the presence

of flavonoids, alkaloids, and tannins, with deoxytubulosine identified as a significant anticancer alkaloid (Venkateshwarlu *et al.,* 2012).

Additionally, eleven undescribed isoquinoline alkaloids and their analogues were isolated from the bark of *A. salviifolium*. These alkaloids demonstrated cytotoxicity against various cancer cell lines, including SGC-7901, HeLa, K562, A549, BEL-7402, HepG2, and B16, with IC_{50} values ranging from 0.01 to 48.12 μM. β-Carboline-benzoquinolizidine and cepheline-type alkaloids exhibited remarkable cytotoxicity (Wei *et al.,* 2024).

Bama *et al.* reported the anticancer activity of *A. salviifolium* against the lung cancer cell line A549 and the breast cancer cell lines MCF7 and MDA-MB-468, with IC_{50} values of 3.7±5.7, 41±1.2, and 36±20 μg/mL, respectively (Bama *et al.,* 2012). Additionally, the antioxidant activities of *A. salviifolium* have been documented, with flavonoids and tannins acting as primary antioxidants and free radical scavengers (Tanwer and Vijayvergia, 2014; Ayoola *et al.,* 2008). These antioxidant properties may contribute to the higher anticancer potentials of *A. salviifolium*.

1.4.1.7. *Thevetia peruviana*

In the present study, ***Thevetia peruviana*** leaf extracts exhibited significant cytotoxicity across multiple cancer cell lines. The chloroform, ethyl acetate, and acetone extracts demonstrated pronounced cytotoxic effects, with the ethyl acetate extract showing 98.17±0.62% cell death in the PANC1 pancreatic cancer cell line. Similarly, the methanol extract resulted in 92.46±1.85% cell death in the HT29 colorectal cancer cell line. However, while the chloroform, ethyl acetate, and acetone extracts showed substantial toxicity towards cancer cells, they also displayed notable toxicity towards the normal human HaCaT cell line. The methanol extract, in contrast, exhibited reduced toxicity against HaCaT cells (23.32±2.95%), indicating its potential for selective cytotoxicity. This selective effect suggests the methanol extract could serve as a promising anticancer agent, warranting further investigation into its bioactive components and mechanisms of action.

Previous studies align with our findings, reinforcing the anticancer potential of *T. peruviana*. For instance, Cheng *et al.,* identified cardiac glycosides from *T. peruviana* seeds that triggered intrinsic apoptosis and cell cycle arrest in cancer cells, notably demonstrating selective cytotoxicity with an IC_{50} ranging from 0.05 to 0.15 μM. Compound 1, isolated from *T. peruviana* seeds, induced apoptosis in gastric cancer cells in a dose-dependent manner (Cheng *et al.,* 2016). Similarly, Ramos-Silva *et al.* reported the methanol extract of *T. peruviana* fruits exhibited antiproliferative and pro-apoptotic activity, significantly affecting cell proliferation, motility, and adhesion across various cancer cell lines (Ramos-Silva *et al.*, 2017). The methanolic extract of aerial parts and roots displayed IC_{50} values of 1.91 μg/mL for prostate cancer, 5.78 μg/mL for breast cancer, and 6.30 μg/mL for colorectal cancer (Ramos-Silva *et al.,* 2017). Similarly, Rehman Khan *et al.* emphasized the anticancer activity of *T. peruviana* bark *in silico* and in vitro studies (Rehman Khan *et al.,* 2024). El Gizawy *et al.,* further supported these findings, reporting an IC_{50} of 39.3 μg/mL for colon cancer cells (El Gizawy *et al.,* 2023).

The cytotoxic, pro-apoptotic, and antiproliferative effects of *T. peruviana* demonstrate its promising potential in cancer treatment. Future research should focus on isolating specific bioactive compounds for therapeutic application and elucidating the underlying mechanisms of action. These findings support the potential of *T. peruviana* as a source for developing novel anticancer agents.

1.4.1.8. *Cipadessa baccifera*

Cipadessa baccifera, a plant traditionally used in folk medicine, has demonstrated significant anticancer potential in this study, especially through its extracts derived from seeds and leaves. Various extracts, including petroleum ether, chloroform, ethyl acetate, and acetone, exhibited cytotoxic activity across a range of cancer cell lines. For instance, the petroleum ether extract of the seeds induced 97.42±0.13% mortality in the HT29 colorectal cancer cell line, while it also caused 95.18±0.69% and 93.66±1.04% cell death in MG63 and HeLa cell lines, respectively. Furthermore, the petroleum ether extract was effective against other cancer cell lines such as UM-SCC-83Ba and MCF7, achieving over 70% mortality. These extracts

showed lower cytotoxicity in the normal HaCaT cell line, suggesting that they possess selective anticancer properties.

This selective cytotoxicity is a key observation as it indicates the ability of *C. baccifera* extracts to distinguish between cancerous and non-cancerous cells. A high degree of specificity toward cancer cells with minimal impact on normal cells is a fundamental requirement for potential chemotherapeutic agents. Many existing cancer treatments, such as chemotherapeutic drugs, often suffer from the drawback of harming normal, healthy tissues, which leads to severe side effects. Therefore, the reduced toxicity of *C. baccifera* extracts towards HaCaT cells is particularly encouraging and positions this plant as a promising candidate for further exploration in cancer therapy.

In the broader context of research on *Cipadessa baccifera*, various phytochemicals have been isolated that contribute to its observed bioactivity. The most prominent of these are limonoids, a class of tetranortriterpenoids known for their biological activities. Previous studies identified several limonoid compounds, such as cipatrijugin E, which has demonstrated significant cytotoxic effects on various cancer cell lines, including MCF-7, SW480, and HL-60, with IC_{50} values ranging from 4.5 to 21.6 μM (Ning *et al.,* 2010). This aligns with our findings, suggesting that the bioactive compounds in *C. baccifera* may be responsible for the selective cytotoxicity observed in the study.

Moreover, the chloroform extract of the leaves showed notable cytotoxicity in the A431, HCT116, and A498 cell lines, while the chloroform, acetone, and methanol extracts were effective against the PA1 ovarian teratocarcinoma cell line. These results provide a broad spectrum of cytotoxic activity across different types of cancers, indicating that the active compounds in *C. baccifera* have the potential to target various cancer pathways.

Research by Siva *et al.* further supports the cytotoxic potential of limonoids from *C. baccifera*. In their study, two newly identified limonoids exhibited potent anti-proliferative activity against cancer cell lines such as HepG2 and IMR32, with GI_{50} (growth inhibition) values as low as 0.013 and 0.01 μM, respectively (Siva *et al.,*

2017). These findings underscore the importance of limonoids in *C. baccifera* and their potential as powerful cytotoxic agents that could be further developed into cancer therapeutics.

Additionally, another investigation revealed the moderate cytotoxicity of a newly identified limonoid (compound 1) against glioma cell lines U251 and BT-325, with IC_{50} values of 7.32±0.21 and 13.25±0.35 μM (Kun *et al.,* 2024). While the glioma cells showed a moderate response, this study provides a foundation for further research into the structural optimization of these limonoid compounds to enhance their potency and therapeutic potential. This is especially relevant considering gliomas' high malignancy and resistance to treatment, highlighting the need for novel therapeutic agents.

The cytotoxic effects of *C. baccifera* extracts, especially on the HT29, HeLa, and MG63 cell lines, highlight its role as a promising source of anticancer agents. The selective cytotoxicity of these extracts—demonstrating high mortality rates in cancer cells and reduced toxicity in normal cells—is an essential criterion for the further exploration of this plant in anticancer drug development. The results suggest that the bioactive compounds, particularly limonoids, are likely responsible for inducing cell death through mechanisms that warrant further investigation, such as apoptosis or cell cycle arrest.

1.4.1.9. *Conyza canadensis*

The chloroform extract of *Conyza canadensis* demonstrated significant cytotoxicity across various cancer cell lines in this study. Notably, it caused 98.06±2.53% cell death in PA1, 87.23±1.74% in A498, 79.61±2.37% in HeLa, 78.74±1.50% in HT29, and 73.53±2.35% in MCF7. These results suggest that the chloroform extract of *C. canadensis* is highly effective against a broad range of cancer cell types, particularly ovarian teratocarcinoma (PA1), renal cancer (A498), and cervical cancer (HeLa).

This study's findings align with previous research on *C. canadensis*, which has been investigated for its potential anticancer properties. For example, Ayaz *et al.* explored the cytotoxic effects of *C. canadensis* extracts on human lung adenocarcinoma cell

lines. The study assessed the toxicity of various extracts, including n-hexane, chloroform, and n-butanol. Among these, the n-hexane extract from the roots was found to be the most cytotoxic, with IC_{50} values of 94.73 and 84.85 μg/mL (Ayaz *et al.,* 2016). This highlights the plant's moderate cytotoxicity against lung cancer cells, pointing toward its potential therapeutic applications.

In another study, isolated compounds from *C. canadensis* were evaluated for their antiproliferative activity against human cancer cell lines, including HeLa (cervix adenocarcinoma), A431 (skin carcinoma), and MCF-7 (breast adenocarcinoma). The results indicated that these compounds exhibited significant growth inhibition in cancer cells while being less toxic to non-cancerous human fetal fibroblasts (MRC-5), indicating their potential as selective antiproliferative agents (Csupor-Löffler *et al.,* 2011). This selectivity is critical for the development of anticancer drugs, as it reduces the risk of harming healthy cells.

Additionally, the antitumour activity of *C. canadensis* essential oils has been explored. A study by Aragão *et al.* revealed that leaf oil from *C. canadensis* exhibited potent inhibitory effects on neoplastic cell lines, including K562 (leukemia) and NCI-ADR/RES (ovarian cancer with multidrug resistance). The study reported TGI (total growth inhibition) values of 16.8 and 19.0 mg/mL, respectively, suggesting that essential oils from *C. canadensis* leaves have strong potential as natural anticancer agents (Aragão *et al.,* 2019).

Furthermore, a study on the phytochemical profiles of traditional medicinal plants, including *C. canadensis*, evaluated their antioxidant, cytotoxic, and anti-inflammatory properties. The results demonstrated that *C. canadensis* exhibited notable antioxidant activity, which could contribute to its anticancer effects by mitigating oxidative stress, a known factor in cancer development (Polat *et al.,* 2022).

The chloroform extract of *Conyza canadensis* showed high cytotoxicity in several cancer cell lines, complementing previous studies that have demonstrated the plant's antiproliferative, antioxidant, and selective cytotoxic activities. These findings highlight the potential of *C. canadensis* as a source of bioactive compounds with

promising anticancer properties, warranting further investigation into its mechanisms of action and possible therapeutic applications in cancer treatment.

1.4.1.10. *Getonia floribunda*

In the current study, *Getonia floribunda* chloroform extract demonstrated significant cytotoxicity across several cancer cell lines. The extract exhibited potent effects on PA1 (99.36±0.17%), HT29 (99.12±1.62%), A431 (97.40±0.19%), PANC1 (93.10±0.59%), HeLa (91.69±1.82%), UM-SCC-83B (70.69±2.53%), HCT116 (68.56±1.01%), and MCF7 (68.35±4.44%). Both ethyl acetate and acetone extracts also showed cytotoxic effects, although their activity was limited to certain cell lines.

The potential anticancer properties of *Getonia floribunda* have been further supported by previous studies. In research conducted by Ren *et al.*, amentoflavone, a compound with known anticancer properties, was identified from this plant, reinforcing its potential in cancer therapy (Ren *et al.,* 2019). Additionally, phytochemical investigations of the ethyl acetate and methanol extracts from the flowers of *Getonia floribunda*, commonly used in Thai herbal medicine, resulted in the isolation of two new flavonols: 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol and 4′-hydroxy-6,7,8-trimethoxyflavonol, alongside the known compound pachypodol. Of these, 4′-hydroxy-6,7,8,3′-tetramethoxyflavonol exhibited notable cytotoxicity against the oral cavity cancer (KB) cell line, with an IC_{50} value of 8.99±2.00 µg/ml (Suttaratrungse, 2015).

These findings highlight the significant potential of *Getonia floribunda* as a source of bioactive compounds with anticancer properties. The chloroform extract's broad cytotoxic effects, combined with the previously identified flavonols and amentoflavone, suggest that further research is needed to explore the plant’s bioactive constituents and their mechanisms of action. This could lead to the development of novel cancer therapies based on *G. floribunda*.

1.4.1.11. *Laggera alata*

The chloroform extract from *Laggera alata* leaves demonstrated significant cytotoxicity in various cancer cell lines, causing mortality in PA1 (97.98±0.25%),

HCT116 (96.06±0.25%), MG63 (89.29±0.96%), A431 (84.19±0.60%), A498 (80.87±1.62%), and PANC1 (56.86±2.57%). Additionally, the petroleum ether, ethyl acetate, acetone, and methanol extracts also exhibited notable cell death in selected cell lines.

Laggera alata is recognized for its medicinal properties, particularly its potential in cancer treatment. The plant contains bioactive compounds such as eudesmane-type sesquiterpenes (ETSs) and flavonoids, which have been reported to possess anticancer activity. Two key ETSs, 5α-hydroxycostic acid and hydroxyisocostic acid, isolated from *Laggera alata*, have demonstrated anti-angiogenic effects, inhibiting the formation of new blood vessels that fuel tumour growth (Liang *et al.,* 2017). Despite these promising findings, the chemical composition and specific bioactive constituents of *Laggera alata* have not been thoroughly investigated.

Given the significant cytotoxicity observed in this study, it would be valuable to explore each extract of *Laggera alata* separately to identify the specific compounds responsible for its anticancer activity. Further phytochemical analysis and mechanistic studies are necessary to fully understand its potential as a source of anticancer agents.

1.4.1.12. *Bauhinia phoenicea*

In contrast, ***Bauhinia phoenicea*** extracts demonstrated significant anticancer potential. The chloroform extract showed the highest mortality rate on the PA1 cell line (99.28±0.16%), followed by the petroleum ether extract on the same cell line (96.74±0.54%). Other cell lines, including a normal cell line, exhibited high tolerance to *B. phoenicea* extracts, indicating the selective cytotoxic potential of these extracts against cancer cells.

Previous research has further highlighted the efficacy of *B. phoenicea* extracts. Among the six various extracts tested, the water extract exhibited the lowest IC_{50} value (34 µg/mL) on Dalton's lymphoma ascites (DLA) cell lines. Maximum cytotoxicity for the chloroform extract was achieved at a concentration of 200

µg/mL, while the petroleum ether and acetone extracts also showed high cytotoxicity at 100 µg/mL (Baby and Raphael, 2018).

Given these findings, further exploration of the anticancer potential of *B. phoenicea*, particularly on the PA1 cell line, is highly warranted. The current study's results underscore the promise of *B. phoenicea* extracts as selective anticancer agents, meriting additional research to fully understand their mechanisms and efficacy across different cancer cell lines.

1.4.1.13. *Leucas aspera*

The chloroform extract of *Leucas aspera* was found to exhibit significant anticancer activity in the current study, causing cell mortality in all cancer cell lines tested, with the exception of MCF7. These findings align with several existing studies supporting the anticancer potential of *Leucas aspera*.

Research on *Leucas aspera* has highlighted its potential in various cancer models. For instance, Augustine *et al.,* (2014) delved into the antitumour activity of *Leucas aspera* by studying the ethyl acetate fraction in Dalton's lymphoma (DAL) bearing mice. The study revealed promising results in tumour growth inhibition, suggesting that *L. aspera* may be effective in treating certain cancers (Augustine *et al.,* 2014). Augustine and colleagues further investigated its immunomodulatory activity, demonstrating that the plant could stimulate macrophage cells. This immunomodulation could enhance the body's defense against cancer cells, potentially explaining part of the plant's anticancer efficacy (Augustine *et al.,* 2014).

In more recent studies, Jayanthi examined the ethanol extract of *Leucas aspera* flowers in *in vitro* and *in vivo* models against Ehrlich ascites tumours. The study showed significant anticancer potential, supporting the plant's broader therapeutic applications (Jayanthi, 2020). Additionally, Mohan *et al.,* investigated the use of *Leucas aspera* nanoparticles in prostate cancer cells, highlighting that the nanoparticle formulations exhibited superior toxicity and cell migration inhibition compared to crude extracts (Mohan *et al.,* 2017). This research underscores the

potential benefits of nanoformulations in enhancing the anticancer efficacy of herbal medicines like *Leucas aspera*.

The cumulative evidence suggests that *Leucas aspera* possesses a wide array of anticancer properties, making it a promising natural candidate for further development in cancer therapy. These studies emphasize not only its direct tumour-suppressing effects but also its ability to modulate the immune system and inhibit cell migration, both of which are key factors in cancer progression.

1.4.1.14. *Cassia javanica* and *Combretum indicum*

In the present study, both *Cassia javanica* and *Combretum indicum* exhibited significant cytotoxic effects on various cancer cell lines. The methanol extract of *Cassia javanica* resulted in a mortality percentage of 88.32±1.62% on the A431 cell line, indicating strong cytotoxic potential. Similarly, *Combretum indicum* extracts displayed mortality percentage above 50% on several cell lines. For instance, the chloroform extract showed cytotoxic effects on MCF7 (73.97±2.97%), HT29 (65.35±1.47%), A431 (62.28±2.54%), and UM-SCC-83B (61.50±6.00%). The petroleum ether extract was also effective against MCF7 (81.34±4.56%) and UM-SCC-83B (74.78±3.66%), while the ethyl acetate extract impacted MCF7 (66.66±1.55%). Notably, the water extract demonstrated a high level of cytotoxicity on PA1 (95.71±1.25%).

Previous research, such as the study by Abd Elkarim and Taie (2023), further supports these findings. They investigated the antioxidant and antitumour activities of *C. indicum* using aqueous methanol extracts (AME) from the aerial parts. The extract was tested on Ehrlich ascites carcinoma cells, showing a dose-dependent inhibition of cell viability (Abd Elkarim and Taie 2023). This study concluded that *C. indicum* could serve as a potential antioxidant and antitumour agent, which aligns with the results of the current research.

Out of the 426 plant extracts screened for anticancer properties, many exhibited cell line-specific anticancer effects, with several showing reduced cytotoxicity on HaCaT, a non-cancerous human keratinocyte cell line. This differential cytotoxicity

is a critical feature in drug development, as an ideal anticancer drug should selectively target cancer cells without harming normal, healthy cells. The ability of certain plant extracts to exhibit such selectivity highlights their potential as promising sources of anticancer molecules.

Given the increasing need for more effective and selective cancer treatments, identifying plant extracts with these properties is vital. While traditional medicinal plants have been studied for their therapeutic potential, many still remain unexplored. As a result, the discovery and investigation of these plant-derived compounds hold significant promise for the development of novel anticancer drug candidates, contributing to the ongoing search for more targeted and less toxic cancer drug development.

CONCLUSIONS

In the current study, several plant extracts demonstrated potent cytotoxic effects across various cancer cell lines after 48 hours of exposure:

- **Human colorectal carcinoma cell line (HT29)**: Twenty-six extracts showed heightened cytotoxicity. The most active were the acetone and methanol extracts of *Alangium salviifolium* (99.70±0.10%, 99.70±0.11%), and chloroform extracts of *Getonia floribunda* (99.12±0.16%), *Leucas aspera* (99.54±0.05%), and *Strobilanthes heyneana* (99.02±0.45%).

- **Human colon cancer cell line (HCT116)**: Only eight extracts showed significant cytotoxicity, with the methanol extract of *A. salviifolium* leaf (99.04±0.13%) being the most effective, followed by chloroform extract of *A. salviifolium* leaf (98.29±0.28%) and chloroform extract of *Leucas aspera* (98.16±0.23%).

- **Human epidermoid carcinoma cell line (A431)**: The chloroform extract of *Alangium salviifolium* leaf (99.37±0.24%), methanol extract of *A. salviifolium* leaf (99.24±0.09%), and chloroform extract of *Otacanthus caeruleus* flower (99.69±0.63%) exhibited the most significant cytotoxic effects. Additionally, 22 other extracts showed over 70% cytotoxicity against this cell line.

- **Human renal carcinoma cell line (A498)**: Eighteen plant extracts showed cytotoxicity exceeding 70%. The methanol extract of *A. salviifolium* leaf (99.64±0.37%) and the chloroform extract of *Otacanthus caeruleus* flower (99.97±0.25%) had the highest activity.

- **Human cervical cancer cell line (HeLa)**: Twenty-three extracts showed anticancer potential. The chloroform and methanol extracts of *A. salviifolium* leaf (99.10±0.35%) were highly effective, as were the ethyl acetate extracts of *Pancratium triflorum* bulb (99.34±0.28%) and leaf (99.26±0.50%).

- **Human breast cancer cell line (MCF7)**: Sixteen extracts demonstrated significant anticancer potential, with the methanol extract of *Alangium*

salviifolium leaf (99.47±0.23%) being the most effective, followed by the chloroform extract of *Alangium salviifolium* leaf (97.69±0.22%) and the chloroform extract of *Melia dubia* leaf (88.92±1.25%).

- **Human osteosarcoma cell line (MG63)**: Twenty-four extracts showed promising cytotoxicity, including the petroleum ether extract of *Conyza canadensis* (99.59±0.50%), acetone extract of *Hewettia malabarica* (99.58±1.08%), and the chloroform extracts of *Otacanthus caeruleus* flower (99.24±0.08%) and leaf (99.03±0.33%).

- **Human ovarian cancer cell line (PA1)**: Forty extracts showed more than 70% cytotoxicity. The chloroform and methanol extracts of *Alangium salviifolium* leaf (99.42±0.38%, 99.13±0.38%), chloroform extracts of *Bauhinia pheonacia* (99.28±0.16%), *Getonia floribunda* (99.36±0.17%), *Leucas aspera* (99.52±0.31%), *Otacanthus caeruleus* leaf (99.67±0.60%), ethyl acetate extracts of *Pancratium triflorum* bulb (99.74±0.14%) and leaf (99.81±0.18%), and the acetone extract of *Pancratium triflorum* bulb (99.40±0.52%) were particularly effective.

- **Human pancreatic cancer cell line (PANC1)**: Twenty-one extracts showed anticancer potential with more than 70% mortality. The chloroform extract of *Otacanthus caeruleus* flower was the most active (99.71±2.65%), followed by the methanol extract of *Alangium salviifolium* leaf (98.06±0.29%) and the petroleum ether extract of *Melia dubia* leaf (97.48±1.77%).

- **Human oral cancer cell line (UM-SCC-83B)**: Seventeen plant extracts displayed significant cytotoxicity, with the chloroform extracts of *Alangium salviifolium* (99.37±0.68%) and *Leucas aspera* (99.37±1.13%), and methanol extract of *Alangium salviifolium* (99.23±0.62%) being the most active.

Many of the above extracts showed minimum cytotoxicity to HaCaT cell line indicating their potential for isolating anticancer molecules with selective toxicity to cancer cells.

Chapter 2

Study of differential cytotoxicity of selected plant extracts and analysis of its composition by GC-MS

- *To explore the differential cytotoxicities of Hewittia malabarica, Pancratium triflorum, Conyza canadensis, Anamirta cocculus, and Melia dubia against various cancer cell lines.*
- *Characterization of the plant extract with anticancer properties via GC-MS analysis.*

REVIEW OF LITERATURE

2.1.1. *Hewittia malabarica*

Hewittia malabarica L. Suresh, a species within the Convolvulaceae family, has been identified in regions such as Uttar Pradesh and Kerala in India (Chaurasia *et al.,* 2021). Despite its presence, this plant remains largely unexplored, particularly concerning its potential as a source of anticancer compounds. It is among the least studied plants regarding its bioactivities, making it a promising candidate for future research.

The Convolvulaceae family**,** commonly referred to as the morning glory family, includes a wide variety of plants with significant potential in anticancer research. Recent studies have shed light on the anticancer properties of numerous species within this family, underscoring their potential as natural remedies for cancer prevention and treatment.

One notable study by Budiman *et al*. focused on the anticancer properties of Purple Sweet Potato (PSP) phytochemicals. This research evaluated the effects of PSP across multiple cancer types, including breast, bladder, colorectal, liver, gastric, and cervical cancers **(**Budiman *et al*., 2021**).** The findings highlighted the significance of PSP as a natural cancer-preventive and therapeutic agent, suggesting that its phytochemical constituents could play a crucial role in cancer management.

Further research explored the anticancer potential of merromoside from *Ipomoea aquatica***,** which demonstrated promising activity against breast cancer by downregulating reactive oxygen species (ROS) (M S *et al.,* 2022). This study emphasized the value of secondary metabolites from Convolvulaceae plants in targeted cancer therapies, particularly for breast cancer.

Another significant contribution came from a study investigating the synergistic anticancer activity of *Ipomoea batatas* (sweet potato) combined with caffeic acid (Johnson and Pace, 2010) The combination was found to enhance apoptosis in non-

small-cell lung cancer cells, highlighting the potential of Convolvulaceae plants when used in conjunction with other agents for cancer treatment.

Salehi *et al.* highlighted the importance of exploring plants with the anticancer potential within the Convolvulaceae family. It highlighted compounds such as capsaicin**,** found in some Convolvulaceae roots, and trypsin inhibitor proteins, which possess notable anticancer properties (Salehi *et al.*, 2020).

2.1.2. *Pancratium triflorum*

Pancratium triflorum, a species from the Amaryllidaceae family, is renowned for its extensive use in traditional medicine. Historically, it has been employed by various ethnic communities to manage ailments like abdominal pain, impotence, and tumours. This plant's medicinal value is well-documented in folk medicine, where it is recognized for its therapeutic potential. Over the past few years, *Pancratium triflorum* has attracted increasing scientific attention due to its pharmacological properties, particularly its anti-inflammatory effects (Elgorashi, 2019).

Modern research has begun to validate the medicinal values, with numerous studies exploring its pharmacological effects. Toxicological assessments have also been conducted, and findings suggest that the plant has a favorable safety profile, further bolstering its potential for therapeutic applications. Comparative studies examining ethnomedicinal plants used by different communities highlight *Pancratium triflorum* as a significant source of bioactive compounds with therapeutic potential. Its inclusion in these studies emphasizes its role as a potent medicinal plant. Beyond its historical significance, its phytochemical and pharmacological properties have been well-researched, revealing several key bioactivities. Specifically, studies have shown that compounds isolated from *Pancratium triflorum* possess anti-inflammatory, antioxidant, and anticholinesterase properties, making it an attractive candidate for drug development (Elgorashi, 2019).

Additionally, *Pancratium triflorum* has been studied for its ability to treat common colds and other respiratory conditions. Herbal remedies like this plant have shown

promise in relieving symptoms of these illnesses, further demonstrating its versatility in treating both acute and chronic conditions (Mammari *et al.,* 2023). Given its wide range of bioactive properties, this plant offers significant potential for future research aimed at developing novel treatments for a variety of diseases, including cancer and inflammatory disorders.

Several alkaloids within the Amaryllidaceae family are recognized for their cytotoxic effects, with members of the lycorine group standing out due to their potent and selective antiproliferative activity on various cancer cell lines. Among these, phenanthridone alkaloids like narciclasine and pancratistatin have garnered significant attention for their highly selective cytotoxicity, and are currently progressing through different stages of development. It is anticipated that one of these compounds may reach the clinical market in the next decade (Elgorashi, 2019). In addition to the lycorine group, the crinane subgroup has produced several bioactive molecules, including crinamine and haemanthamine, which also exhibit promising cytotoxic properties. Nair *et al.,* identified two rare alkaloids from the Amaryllidaceae family, β-crinane distichamine and phenanthridone narciprimine, as novel antiproliferative agents. Distichamine, in particular, has demonstrated apoptosis-inducing effects in human acute lymphoblastic leukemia (CEM) cells (Nair *et al.,* 2012).

Pancratium triflorum is a valuable medicinal plant with diverse therapeutic properties. Its traditional use is supported by modern research, which highlights its pharmacological potential. Continued studies are needed to fully elucidate the mechanisms of its medicinal effects and expand its use in modern medicine.

2.1.3. *Conyza canadensis*

Conyza canadensis, commonly known as horseweed or Canada fleabane, is a plant species from the Asteraceae family, native to North America. The plant is characterized by an upright, hairy stem, reaching heights of up to 1.5 meters. Its branches form terminal clusters of small flower heads, which feature white or pale purple ray florets and yellow disc florets. The plant flowers from spring to autumn.

Traditionally, the aerial parts of *C. canadensis* have been used in folk medicine for treating dental pain, headaches, kidney issues, gastrointestinal problems, rheumatism, respiratory tract infections, wounds, and burnscal Composition Various studies have revealed the phytochemical profile of *C. canadensis*, highlighting the presence of secondary metabolites, including polyacetylene derivatives , triterpenes ,and phenolic search has investigated the volatiral *Conyza* species, there has been a notable lack of research into the chemical and bioactivity profiles of *C. canadensis* (Hayet *et al.,*2009; Polat *et al.,*2022; Barhoumi *et al.,* 2024).

Various studies have revealed the phytochemical profile of *C. canadensis*, highlighting the presence of secondary metabolites, including polyacetylene derivatives, triterpenes, flavones, and phenolic compounds (Barhoumi *et al.,* 2024). Although previous research has investigated the volatile composition of several *Conyza* species, there has been a notable lack of research into the chemical and bioactivity profiles of *C. canadensis* growing in Jordan. This prompted a recent investigation aimed at characterizing the chemical composition and bioactivity of the plant's aerial parts, including inflorescence heads, leaves, and stems.

A study by Barhoumi *et al.,* (2024) analyzed the hydro-distilled essential oil (HD-EO) and methanolic extracts from the aerial parts of *C. canadensis* collected in Jordan. The chemical analysis using GC-MS identified (2E, 8Z)-matricaria ester as the predominant compound in the essential oil. The methanolic extract, referred to as CCM, demonstrated a high content of phenolic compounds (95.59±0.40 mg Gallic acid equivalents/g extract) and flavonoids (467.0±10.5 mg Quercetin equivalents /g extract). Additionally, the extract exhibited moderate DPPH radical scavenging activity (IC_{50} of 23.75±0.86 µg/mL) and low iron chelating activity (IC_{50} = 5396.07±15.05 µg/mL).

CCM extract upon LC-MS/MS profiling revealed the presence of twenty-five phenolic compounds and flavonoids, including significant concentrations of rosmarinic acid (1441.1 mg/kg plant) and caffeic acid phenethyl ester (231.8 mg/kg

plant) (Barhoumi *et al.,* 2024). These findings suggest that *C. canadensis* contains a rich variety of bioactive compounds with potential antioxidant properties.

Barhoumi *et al.* also assessed the antimicrobial potential of the methanolic extract of *C. canadensis* against several Gram-positive and Gram-negative bacteria, as well as two fungal species, *Candida* and *Cryptococcus*. The results indicated significant antibacterial activity, particularly against *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) value of 3.125 µg/mL. This demonstrates the potential of *C. canadensis* extracts for use as natural antimicrobial agents. The high phenolic and flavonoid content, combined with the moderate DPPH radical scavenging activity, suggests that *C. canadensis* possesses antioxidant properties, which may be beneficial in preventing oxidative stress-related diseases. Moreover, the extract's antibacterial efficacy against *S. aureus* reinforces its potential as an antimicrobial agent (Barhoumi *et al.,* 2024). The results lay the groundwork for future studies on the medicinal uses of *C. canadensis*, with a focus on its potential to combat microbial infections and alleviate oxidative stress.

The Asteraceae family, to which *C. canadensis* belongs, is renowned for its bioactive compounds, especially sesquiterpene lactones. Numerous studies have demonstrated the cytotoxic and pro-apoptotic effects of Asteraceae species against cancer cells. For instance, Gach *et al.,* highlighted the anticancer properties of sesquiterpene lactones, which act by influencing intracellular redox signaling pathways (Gach *et al.,* 2015). Moreover, traditional knowledge and ethnobotanical insights have guided the identification of medicinal plants from this family with therapeutic potential. Bouyahya *et al.* reviewed Moroccan Asteraceae species, emphasizing their cellular and molecular anticancer efficacy (Bouyahya *et al.,* 2020).

The recent studies on *Conyza canadensis* from Jordan has provided the first detailed phytochemical and bioactivity assessment of this plant, revealing its significant antioxidant and antimicrobial potential. The presence of phenolic compounds and flavonoids, particularly rosmarinic acid and caffeic acid phenethyl ester, suggests

that *C. canadensis* holds promise for future research aimed at exploring its therapeutic applications. Additionally, the antimicrobial efficacy of the plant against *S. aureus* highlights its potential as a natural antimicrobial agent. This study contributes to the growing body of evidence supporting the medicinal potential of Asteraceae plants, with implications for the development of novel antioxidant and antimicrobial therapies. Further studies are needed to fully understand the mechanisms underlying these bioactivities and to explore the clinical relevance of *C. canadensis* extracts.

2.1.4. *Anamirta cocculus*

Anamirta cocculus (L.) Wight and Arn., a member of the Menispermaceae family, has long been used in traditional medicine throughout Asia. Its stems and roots are rich in alkaloids with antibacterial, antimicrobial, and sympatholytic properties (Jijith *et al.,* 2016). The seeds contain picrotoxin, a known compound with a history of use as an antidote for barbiturate and morphine poisoning, as well as a nerve tonic for treating neurological disorders, including schizophrenia and epilepsy. Picrotoxin functions as a universal chloride (Cl^-) channel blocker, producing effects similar to bicuculline, with convulsions and seizures at higher doses (Pluskal and Weng, 2018). While picrotoxin has limited clinical use due to its toxicity, it is still employed in research as a tool for studying GABAergic mechanisms.

The Menispermaceae family has attracted interest for its bioactive compounds with potential in cancer research. Alkaloids derived from Menispermaceae plants, such as bisbenzylisoquinoline (BBIQ), have shown promising anticancer properties. Chan *et al.* explored the effects of two BBIQ alkaloids, tetrandrine (TET) and fangchinoline (FAN), extracted from *Stephania tetrandra* roots. These compounds demonstrated the ability to reverse multi-drug resistance (MDR) in cancer cells, a major challenge in chemotherapy. The study indicated that TET and FAN enhance drug sensitivity in resistant cancer cells, making them viable candidates for further development in cancer treatment (Chan *et al.,* 2021).

Magnoflorine, another alkaloid from the Menispermaceae family, has garnered attention for its antitumour activity. A review by Okon *et al.* emphasized magnoflorine's ability to induce apoptosis, inhibit cancer cell proliferation, and disrupt cell cycle progression. These anticancer properties make magnoflorine a promising lead compound for drug development (Okon *et al.,* 2020).

Research on other Menispermaceae species continues to uncover additional bioactive compounds with potential medicinal applications. Baharuddin *et al.* conducted a comprehensive review of *Dicranopteris linearis*, research on its medicinal properties and anticancer potential. This highlighted the need for more exploration of the plant's bioactive compounds and their roles in oncology (Baharuddin *et al.,* 2021).

Pérez *et al.* investigated the anticancer properties of Thiadiazinthione, a compound isolated from Menispermaceae species. The study showed that the decomposition products of Thiadiazinthione had cytotoxic effects on cancer cells, suggesting a new mechanism for exploiting this compound in cancer therapy (Pérez *et al.,* 2000).

Luteolin, a flavonoid found in several Menispermaceae plants, was reviewed by Lin *et al.* for its anticancer properties. Luteolin was found to promote apoptosis and inhibit proliferation, metastasis, and angiogenesis in cancer cells. These multi-targeted mechanisms highlight luteolin's potential as part of cancer prevention and therapy strategies, reinforcing its value in oncology (Lin *et al.,* 2008).

The growing body of research into *Anamirta cocculus* and the broader Menispermaceae family underscores their potential as sources of bioactive compounds with significant anticancer properties. Alkaloids such as tetrandrine, fangchinoline, and magnoflorine, along with flavonoids like luteolin, offer promise in reversing drug resistance, inducing cancer cell death, and inhibiting tumour progression. While preclinical studies have demonstrated the pharmacological potential of these compounds, further research is necessary to clarify their mechanisms of action and evaluate their clinical applicability. The diverse bioactive compounds found in Menispermaceae plants represent an exciting frontier for

developing novel cancer therapies, and continued research will be essential in unlocking their full therapeutic potential.

2.1.5. *Melia dubia*

Melia dubia, commonly known as Malabar neem, has gained interest in cancer research as a potential source of novel anticancer compounds. In Indian ayurvedic medicine, the leaf extract of *M. dubia* is used to treat a variety of skin disorders and gastro-intestinal tract ailments (Kumar and Sami, 2022).The ethanolic extract of seeds contains alkaloids, and the seeds exhibit both antimicrobial and antioxidant properties (Prasad, 2024). Wang *et al.* demonstrated the inhibitory effects of *Melia toosendan* fruit extract on colon cancer in both *in vitro* and *in vivo* studies, showing promising therapeutic potential (Wang *et al.,* 2015). In another study, Trung *et al.* successfully isolated new triterpenoids from *M. dubia* bark, which exhibited significant anti-inflammatory and antifungal activities against phytopathogenic fungi (Trung *et al.,* 2023). Despite these advances, the exploration and isolation of anticancer compounds from *M. dubia* leaves remain an important area of research, highlighting the need to examine all plant parts for therapeutic agents.

The Meliaceae family has garnered significant attention in anticancer research due to the identification of numerous bioactive compounds with therapeutic potential. In a study by Sirisoma *et al.* a structure-activity relationship (SAR) analysis led to the discovery of N-(4-methoxyphenyl)-N,2-dimethylquinazolin-4-amine, a potent inducer of apoptosis and a promising anticancer agent (Sirisoma *et al.,* 2009). This compound demonstrated substantial blood-brain barrier permeability, marking it as a candidate for further oncological research. Similarly, Belluti *et al.* focused on the design, synthesis, and biological evaluation of stilbene-coumarin hybrid compounds, identifying novel pro-apoptotic agents capable of triggering apoptosis in cancer cells (Belluti *et al.,* 2010). This work underscores the significance of discovering novel molecules for cancer drug development.

Ren *et al.* identified niclosamide as a small-molecule inhibitor of the STAT3 signaling pathway, providing a key insight into the development of molecularly

targeted therapies by inhibiting crucial cancer-related pathways (Ren *et al.,* 2010). Çiftçi *et al.* and Chung *et al.* discovered a piperazine derivative that induces caspase-dependent apoptosis while also inhibiting several cancer-related signaling pathways, reinforcing the importance of targeting multiple pathways for effective treatment outcomes (Çiftçi *et al.,* 2022; Chung *et al.,* 2013).

Tyagi *et al.* isolated β-sesquiphellandrene from turmeric, demonstrating its anticancer activity comparable to curcumin, thus illustrating the potential of natural products in oncology (Tyagi *et al.,* 2015). Moreover, Shoaib *et al.* studied sesquiterpene lactones, highlighting their role as potent antioxidants and anticancer agents, reinforcing the importance of exploring natural products for novel anticancer therapies (Shoaib *et al.,* 2017).

The Meliaceae family and the compounds present in Meliaceae family have played a significant role in advancing anticancer research. From small-molecule inhibitors to natural product derivatives, various compounds are being explored, offering promising potential for the development of more effective cancer therapies.

2.1.6. Research gap

Despite the established medicinal value and various biological activities such as antioxidant, anti-inflammatory, and antibacterial effects of *Hewittia malabarica*, *Pancratium triflorum*, *Conyza canadensis*, *Anamirta cocculus*, and *Melia dubia*, there remains a significant research gap regarding their anticancer potential. While these plants have been traditionally used in medicine, their specific anticancer properties have not been thoroughly investigated. There is a critical need for comprehensive studies on phytochemical composition of these plants to identify and characterise their anticancer agents. Addressing this gap could contribute to the development of safer and more effective anticancer drugs, emphasizing the necessity of further exploration in this area.

MATERIALS AND METHODS

2.2.1. Chemicals

Animal tissue culture tested DMEM, MEM, Mc Coy's 5A, DMSO, FBS and antibiotic antimycotic solution (from Sigma-Aldrich, Himedia and Gibco) were used for cell line culture maintenance and plant extract dilution. Organic solvents including petroleum ether, chloroform, ethyl acetate, acetone, and methanol, used for preparation of sample for GC-MS and HPTLC were of HPLC grade.

2.2.2. Plant collection and sample preparation

Hewittia malabarica and *Pancratium triflorum* were collected from Thenhippalam, Malappuram district, Kerala. *Conyza canadensis* was collected from Attappadi hills, Palakkad district, Kerala. *Anamirta cocculus* was collected from Koottanad, Palakkad district. *Melia dubia* were collected from Meenangadi, Wayanad districts. The plant parts were identified by Dr A. K. Pradeep, Department of Botany, University of Calicut. The plant materials were processed and samples were prepared by sequential extraction as discussed in the chapter 1.

2.2.3. Cytotoxicity Assay

The plant extracts were analysed for their cytotoxicity on ten different cancer cell lines and a normal cell line using the MTT assay as per Mosmann, 1983 (Mosmann, 1983). Each extract was treated at 100 µg/mL concentration and mortality after 48 hours of treatment was recorded.

2.2.4. IC_{50} calculation

IC_{50} values for 48 hours of the extracts were determined for cell lines exhibiting more than 50% mortality by treating them at different concentrations on the corresponding cell lines. Probit analysis was carried out to calculate the IC_{50} value.

2.2.5. Identification of compounds in extracts by GC-MS analysis

GC-MS analysis was performed on the chloroform extracts of *Hewittia malabarica*, *Pancratium triflorum* bulb, *Conyza canadensis*, *Melia dubia*, as well as ethyl acetate

and acetone extracts of *Anamirta cocculus* using a Shimadzu gas chromatography system. The system was equipped with an ELITE-5MS column (30 m length, 0.25 mm ID, 0.25 µm film thickness). The oven temperature was initially set at 80°C for 1 minute and then gradually increased to 260°C at a rate of 5°C per minute. Helium was used as the carrier gas, with a flow rate of 1 mL/min. A sample volume of 1 µL was injected for analysis.

Identification of the components was achieved by comparing the mass spectral fragmentation patterns with the MS library databases, specifically NIST11 (National Institute of Standards and Technology, USA) and WILEY 8, which helped in recognizing and characterizing the chemical constituents present in the extracts.

2.2.6. Statistical analysis

IC_{50} was calculated using probit analysis in IBM SPSS statistics 24 software. Data were expressed as mean±SEM. Data visualization was conducted using Origin (Version 9) and GraphPad Prism 10.2.2 (397). Statistical analysis to determine the significance of differences between group means was performed using one-way ANOVA, followed by Dunnett's multiple comparison tests, to compare experimental groups with the control. All analyses were carried out in GraphPad Prism, with statistical significance set at $p < 0.05$.

RESULTS

2.3.1. *Hewittia malabarica*

2.3.1.1. *Hewittia malabarica* extracts exhibit differential cytotoxicity

The cytotoxic effects of *Hewittia malabarica* extracts (aerial parts) were evaluated using the MTT assay across various cell lines, including HT29, HCT116, A431, A498, PANC1, UM-SCC-83B, and HaCaT. Extracts prepared with different solvents—petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water—were tested. Among these, the chloroform extract exhibited the highest cytotoxicity across most cell lines. The ethyl acetate extract was found to cause the highest cytotoxicity among the six extracts tested on PANC1 pancreatic cancer cell line (99.58±1.08%) and it was followed by the same extract on MG63 (97.23±0.82%). On HaCaT, the extract caused 44.91±1.32% mortality. HT29 cell line showed the strongest response to the chloroform extract, with a mortality rate of 81.59±4.90%, followed by HeLa cell line, which had a mortality rate of 63.29±2.46%. Additionally, the petroleum ether extract displayed significant cytotoxicity against HT29 cells, with a mortality rate of 65.38±0.94% after 48 hours of exposure. Notably, the petroleum ether extract and chloroform extract didn't show substantial cytotoxicity, even at 100 µg/mL, towards the normal human keratinocyte cell line (HaCaT) with a mortality percentage of 5.38±1.16% and 5.44±1.07% respectively, indicating a selective cytotoxic effect, particularly against the human colorectal carcinoma cell line HT29 (Table 2.3.1).

2.3.1.2. Determination of IC_{50} of chloroform extract and petroleum ether extract on HT29 cell lines

Both the petroleum ether and chloroform extracts exhibited concentration-dependent cytotoxicity against the HT29 cell line. Notably, the chloroform extract had the lowest IC_{50} value of 31.97±3.07 µg/mL against HT29 cells (Table 2.3.2.), indicating it contains potentially potent anticancer agents. This result underscores the need for further research to isolate and characterize these anticancer compounds from the chloroform extract of *Hewittia malabarica*. Additionally, the petroleum ether extract

also showed considerable sensitivity to HT29 cells, with an IC_{50} value of 76.58±4.74 µg/mL (Table 2.3.2). Both extracts exhibited increased cytotoxicity with higher concentrations and extended incubation periods (Figure 2.3.1).

Table 2.3.1. Percentage mortality of *H. malabarica* extracts on various cell lines.

Cell Line	Percentage Mortality (48 hrs) Mean±SEM					
	Petroleum ether extract	**Chloroform extract**	**Ethyl acetate extract**	**Acetone extract**	**Methanol Extract**	**Water extract**
HT29	**65.38±0.94**	**81.59±4.90**	21.21±1.30	12.62±2.54	19.91±3.69	22.47±0.78
HCT116	11.99±5.36	1.48±2.26	4.48±2.09	38.23±5.11	41.17±5.31	18.34±2.06
A431	16.34±2.54	8.68±2.20	1.45±2.02	38.99±2.38	31.90±2.53	21.95±1.21
A498	15.72±2.72	40.98±1.41	16.15±3.53	39.37±2.81	18.39±2.83	23.16±6.71
HeLa	31.70±4.24	63.29±2.46	29.76±1.76	23.15±1.87	18.54±1.33	20.87±1.40
MCF7	35.56±2.59	15.69±1.14	33.08±1.80	28.63±1.77	41.70±0.74	30.38±1.17
MG63	26.60±3.04	62.42±3.01	**97.23±0.82**	28.98±3.57	73.21±4.67	5.01±2.92
PA1	6.32±1.92	6.77±1.48	11.72±1.46	16.23±1.23	10.51±1.76	5.07±0.90
PANC1	6.58±2.17	45.94±2.71	**99.58±1.08**	13.75±2.70	52.56±1.96	43.85±1.47
UM-SCC-83B	26.58±2.59	32.54±2.53	34.05±2.94	39.93±3.46	39.42±2.32	21.16±2.75
HaCaT	5.38±1.16	5.44±1.07	44.91±1.32	55.47±6.25	45.25±4.33	51.25±3.57

Cytotoxic activity of the extracts was assessed by considering cytotoxicity of DMSO control as 0.00%. Percentage mortality was calculated after 48 hours of treatment and the values are expressed as mean±SEM (n=6).

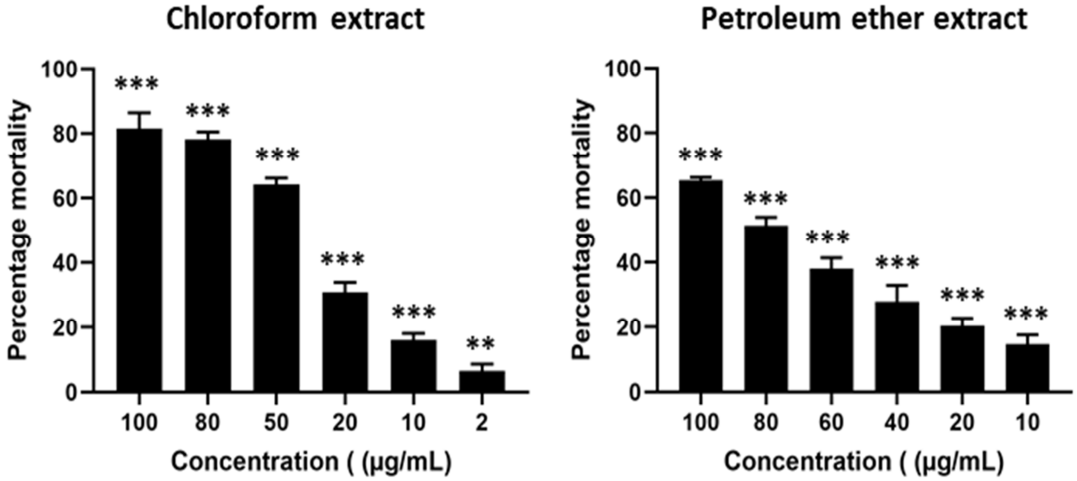


Figure 2.3.1. The percentage mortality of HT29 cell line exposed to different concentrations of chloroform and petroleum ether extracts from *H. malabarica* after 48 hours. Mortality of DMSO control treated was zero percentage. The values are plotted as mean±SEM (n=6). Level of significance: ***p<0.001, **p<0.01.

Table 2.3.2. IC_{50}Values of chloroform and petroleum ether extracts on HT29 and HaCaT cell lines.

Sl. No.	Plant Extract	IC_{50}Value (µg/ml)		
		HT29	HaCaT	p value
1	Chloroform extract	31.97±3.07	>100	>0.001
2	Petroleum ether extract	76.58±4.74	>100	>0.001

The values are expressed as mean±SEM (n=6). Level of significance: ***p<0.001, **p<0.01.

2.3.1.3. Identification of compounds in chloroform extract of *Hewittia malabarica* aerial parts by GC-MS analysis

The bioactive chloroform extract of *Hewittia malabarica* subjected to GC-MS analysis to identify its organic constituents. The analysis revealed the presence of several compounds, including Sulfurous acid, hexyl octyl ester, 2,4-Di-tert-butylphenol, Cetene, Palmityl acrylate, Neodene, Cyclododecanol, Neophytadiene, 9-eicosene, N-pentadecane, (E)-phytol, 1-Heneicosanol, O,o'-biphenol, 4,4',6,6'-tetra-t-butyl-, Tritetracontane, 1,2-benzenedicarboxylic acid, Oleyl alcohol, trifluoroacetate, Tetratetracontane, and Bis(2-ethylhexyl) terephthalate (Figure 2.3.2 and Table 2.3.3).

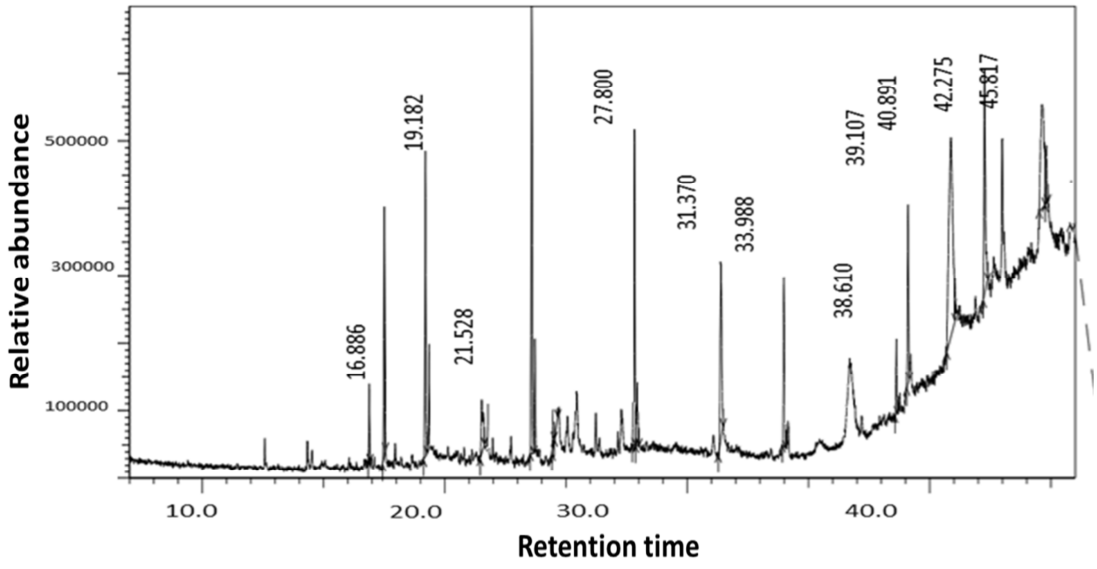


Figure 2.3.2. Chromatogram of the chloroform extract of *H. malabarica* obtained via GC-MS analysis.

Table 2.3.3. List of phytochemicals identified in the chloroform extract of *H. malabarica* via GC-MS analysis.

Peak	Area%	Height%	Name
1	1.28	2.55	Sulfurous acid, hexyl octyl ester
2	5.4	8.35	2,4-Ditert-Butylphenol
3	7.01	10.18	Cetene
4	2.39	1.78	Palmityl acrylate
5	9.78	14.87	Neodene
6	2.35	2.55	Cyclododecanol
7	5.56	5.91	Neophytadiene
8	8.59	10.49	9-eicosene
9	2.08	2.1	N-pentadecane
10	7.51	6.03	(E)-phytol
11	5.44	5.62	1-Heneicosanol
12	1.72	2.53	O o'-biphenol, 4,4',6,6'-tetra-t-butyl-
13	4.51	5.97	1-heneicosanol
14	17.37	6.57	Tritetracontane
15	6.95	7.48	1,2-benzenedicarboxylic acid
16	8.59	3.47	Oleyl alcohol, trifluoroacetate
17	1.77	1.5	Tetratetracontane
18	1.72	2.05	Bis(2-ethylhexyl) terephthalate

2.3.2. *Pancratium triflorum*

2.3.2.1. *Pancratium triflorum* bulb extracts exhibit differential cytotoxicity

The cytotoxic effects of *Pancratium triflorum* extracts were evaluated using the MTT assay across various cell lines, including HT29, HCT116, A431, A498, HeLa, PA1, MG63, MCF7, PANC1, UM-SCC-83B, and HaCaT. Extracts prepared with different solvents-petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water-were tested. The ethyl acetate extract of *P. triflorum* bulb produced the highest percentage mortality in the MG63 cell line, achieving 99.74±0.14%. It also resulted in a percentage mortality of 99.34±0.28% in the HeLa cell line, while the acetone extract induced 99.41±0.52% percentage mortality in the MG63 line. The chloroform extract demonstrated significant percentage mortality in various cell lines: 92.36±0.81% in HeLa, 89.15±1.09% in UM-SCC-83B, 82.96±1.10% in PA1,

82.56±2.69% in MG63, 82.50±0.65% in HT 29, 75.73±0.99% in A498, 71.21±1.05% in A431, 67.93±1.64% in MCF7, and 65.69±1.92% in PANC1. In contrast, the chloroform extract exhibited a percentage mortality of 43.47±0.42% in the normal cell line, while the ethyl acetate extract had a percentage mortality of 68.49±0.33%, and the acetone extract showed a percentage mortality of 94.18±0.54% (Table 2.3.4.).

Table 2.3.4. *In vitro* cytotoxicity of *Pancratium triflorum* bulb on cancer cell lines-MTT assay.

Cell Line	Percentage Mortality (48 hrs) Mean±SEM					
	Petroleum ether extract	**Chloroform extract**	**Ethyl acetate extract**	**Acetone extract**	**Methanol Extract**	**Water extract**
HT29	57.97±0.59	82.50±0.65	79.56±1.74	63.13±1.21	68.04±1.51	49.72±2.34
HCT116	46.82±0.80	47.27±1.40	49.12±2.39	48.57±2.24	44.44±2.06	47.08±2.03
A431	46.41±2.66	71.21±1.05	78.01±1.58	70.38±1.04	58.67±0.71	38.50±1.50
A498	33.17±2.22	75.73±0.99	57.29±1.24	56.70±1.98	43.25±1.41	33.79±2.27
HeLa	76.32±1.30	92.36±0.81	**99.34±0.28**	97.59±0.52	81.22±1.07	70.27±1.08
MCF7	45.65±2.99	67.93±1.64	76.98±0.77	73.80±0.84	60.21±1.11	44.42±0.82
MG63	48.25±2.19	82.56±2.69	**99.74±0.14**	**99.41±0.52**	54.31±3.44	50.41±4.24
PA1	63.33±0.95	82.96±1.10	89.27±1.59	82.55±2.16	68.57±2.68	61.05±2.27
PANC1	52.14±1.22	65.69±1.92	58.95±1.96	58.94±0.93	57.47±0.87	49.23±1.38
UM - SCC -83B	66.32±2.02	89.15±1.09	92.07±0.44	88.32±1.67	76.91±1.51	58.38±1.80
HaCaT	81.05±0.71	43.47±0.42	68.49±0.33	94.18±0.54	85.06±0.90	76.95±1.01

Cytotoxic activity of the extracts was assessed by considering cytotoxicity of DMSO control as 0.00%. Percentage mortality was calculated after 48 hours of treatment and the values are expressed as mean±SEM (n=6).

Among these, the ethyl acetate extract exhibited the highest cytotoxicity across most cell lines. On MG63, it caused the highest cell death of (99.74±0.14%), followed by acetone extract on MG63 and ethyl acetate extract on HeLa with percentage mortality of 99.41±0.52% and 99.34±0.28% respectively. Additionally, the chloroform extract and acetone extract produced higher mortality on cancer cell lines indicating its anticancer potential when treated at 100 µg/mL (Table 2.3.4.).

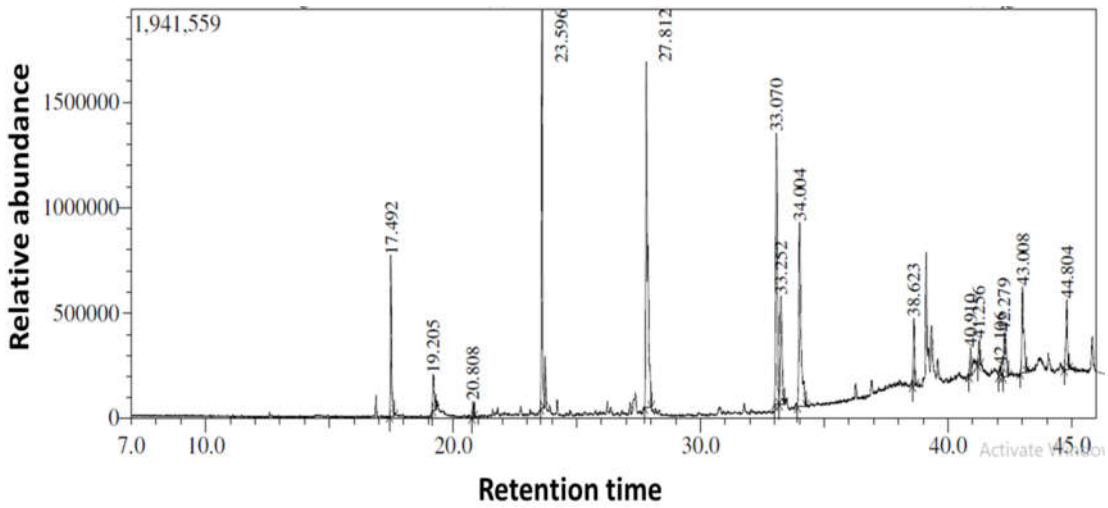


Figure 2.3.3. Chromatogram of the chloroform extract of *Pancratium triflorum* bulb obtained via GC-MS analysis.

Table 2.3.5. List of phytochemicals identified in the chloroform extract of *P. triflorum* bulb via GC-MS analysis.

Peak	Area%	Height%	Name
1	6.41	8.69	2,4-Ditert-butylphenol
2	1.98	1.91	1-Hexadecene
3	0.45	0.86	3,4-Dihydroxymandelic acid-tetratms
4	14.14	21.59	E-15-Heptadecenal
5	22.47	18.69	3-Eicosene, (E)-
6	15.39	14.68	Linoleic acid ethyl ester
7	6.77	5.84	(E)-9-Octadecenoic acid ethyl ester
8	13.78	9.97	1-Heneicosanol
9	2.79	3.65	O O'-Biphenol, 4,4',6,6'-tetra-T-butyl-
10	1.35	1.5	4-[(Trimethylsilyl)oxy]cyclohexanol, (Z)-
11	0.97	1.33	Tetratetracontane
12	0.43	0.54	3-Trifluoroacetoxypentadecane
13	3.55	2.36	Dioctyl phthalate
14	5.66	4.63	Octadecyl trifluoroacetate
15	3.85	3.74	Heptacosane

2.3.2.2. Identification of compounds in chloroform extract of *Pancratium triflorum* bulb by GC-MS analysis

The GC-MS analysis of the chloroform extract from *Pancratium triflorum* bulb revealed a total of 15 compounds, with 3-Eicosene, (E) being the most abundant based on peak area. The analysis identified several other notable compounds,

including 2,4-Ditert-butylphenol, 1-Hexadecene, 3,4-Dihydroxymandelic acid-tetratms, E-15-Heptadecenal, Linoleic acid ethyl ester, (E)-9-Octadecenoic acid ethyl ester, 1-Heneicosanol, O O'-Biphenol, 4,4',6,6'-tetra-T-butyl-, 4-[(Trimethylsilyl)oxy]cyclohexanol (Z), Tetratetracontane, 3-Trifluoroacetoxy pentadecane, Dioctyl phthalate, Octadecyl trifluoroacetate, and Heptacosane. This diverse range of compounds highlights the potential of *P. triflorum* for further exploration due to its complex chemical profile, which may contribute to its biological activities (Table 2.3.5, Figure 2.3.3).

2.3.3. *Conyza canadensis*

2.3.3.1. *Conyza canadensis* leaf extracts exhibit differential cytotoxicity

The study reveals that only a few extracts of *Conyza canadensis* demonstrated over 90% mortality in select cancer cell lines within 48 hours. The petroleum ether extract exhibited the highest cytotoxicity, achieving a 99.59±0.50% mortality rate on the PANC1 pancreatic cancer cell line and a 95.04±0.58% mortality rate against the HT29 colorectal cancer cell line. The chloroform extract showed strong cytotoxic effects as well, with a 98.57±0.40% mortality rate against PANC1 and a 98.06±2.53% mortality rate against the MG63 bone cancer cell line. On other cancer cell lines, like A498 (87.23±1.74%), HeLa (79.61±2.37%), HT29 (78.74±1.50%) and MCF7 (73.53±2.35%) the chloroform extract exhibited higher cytotoxicity. These results emphasize the potent anticancer potential of the petroleum ether extract on HT29 and PANC1, and the chloroform extract on MG63 and PANC1 cell lines. Both these extracts caused less cytotoxicity on HaCat cell line, with a mortality percentage of 55.70±3.97% and 25.07±2.95% (Table 2.3.6.).

2.3.3.2. Identification of compounds in chloroform extract of *Conyza canadensis* leaf by GC-MS analysis

The GC-MS analysis of the chloroform extract of *Conyza canadensis* revealed 11 prominent compounds, with 2,5-Etheno[4.2.2]propella-3,7,9-triene and 2,4-Hexadiyn-1-one, 1-phenyl- being the most abundant, showing the highest area percentages in the chromatogram. These two compounds dominated the extract's composition. Other key compounds identified in the extract, in decreasing order of

peak area, include (-)-Spathulenol, known for its anti-inflammatory and antimicrobial properties; Caryophyllene oxide, a compound with potential anticancer activity; (E)-β-Famesene, a natural hydrocarbon; Humulene epoxide 2; α-Curcumen; Andrographolide, recognized for its medicinal properties; Ledene oxide-(II); E-15-Heptadecenal; and Caryophyllene. The diverse chemical profile of these compounds suggests potential biological activities that warrant further exploration (Figure. 2.3.4, Table 2.3.7.).

Table 2.3.6. *In vitro* cytotoxicity of *Conyza canadensis* on various cell lines - MTT assay.

Cell Line	Percentage Mortality (48 hrs) Mean±SEM					
	Petroleum ether extract	**Chloroform extract**	**Ethyl acetate extract**	**Acetone extract**	**Methanol Extract**	**Water extract**
HT29	**95.04±0.58**	78.74±1.50	0.72±1.75	36.11±4.24	21.49±2.93	25.41±3.21
HCT116	7.85±1.51	34.62±2.38	21.13±2.62	39.51±4.61	40.59±4.12	23.67±4.35
A431	59.24±1.84	36.55±3.56	15.50±3.90	38.69±3.43	46.64±1.82	23.36±1.89
A498	27.17±7.99	87.23±1.74	41.84±6.91	14.75±2.62	43.43±3.37	22.30±1.94
HeLa	22.82±2.07	79.61±2.37	17.73±1.73	35.61±3.55	45.84±2.23	17.21±1.45
MCF7	32.12±5.26	73.53±2.35	46.46±3.37	39.93±5.92	3.97±2.97	29.91±3.81
MG63	41.09±3.24	**98.06±2.53**	70.99±6.78	49.26±4.48	51.59±3.26	56.75±5.99
PA1	25.57±3.21	38.67±2.04	21.67±3.08	21.50±3.36	32.11±1.42	8.71±3.05
PANC1	**99.59±0.50**	**98.57±0.40**	24.16±1.24	37.29±1.75	33.60±3.21	40.48±0.95
UM-SCC-83B	54.87±2.64	14.77±2.68	19.29±2.41	21.59±3.76	31.26±3.15	38.75±1.52
HaCaT	55.70±3.97	25.07±2.95	4.91±1.34	66.44±9.45	62.64±6.16	7.66±7.76

Cytotoxic activity of the extracts was assessed by considering cytotoxicity of DMSO control as 0.00%. Percentage mortality was calculated after 48 hours of treatment and the values are expressed as mean±SEM (n=6).

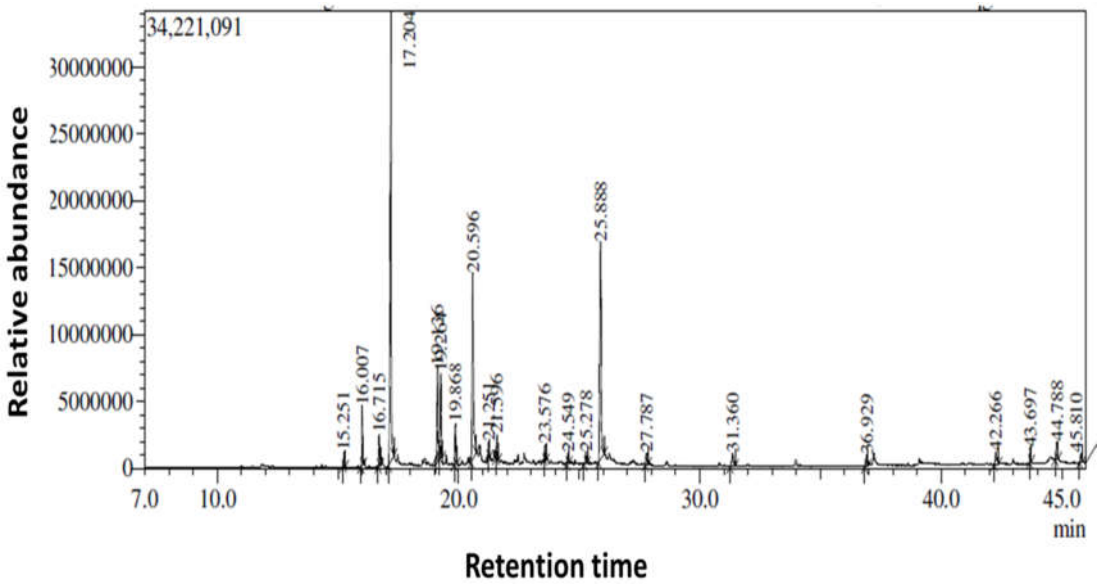


Figure 2.3.4. Chromatogram of the chloroform extract of *Conyza canadensis* leaf obtained via GC-MS analysis.

Table 2.3.7. List of phytochemicals identified in the chloroform extract of *Conyza canadensis* leaf by GC-MS analysis.

Peak	Area%	Height%	Name
1	0.71	1.03	Caryophyllene
2	3.17	4.46	(E)-.beta.-Famesene
3	1.77	2.4	Alpha.-curcumen
4	34.21	33.77	2,5-Etheno[4.2.2]propella-3,7,9-triene
5	6.00	6.74	(-)-Spathulenol
6	5.21	6.32	Caryophyllene oxide
7	2.79	3.01	Humulene epoxide 2
8	15.46	13.96	2,4-Hexadiyn-1-one, 1-phenyl-
9	0.78	1.01	Ledene oxide-(II)
10	1.43	1.75	Andrographolide
11	0.73	1.08	E-15-Heptadecenal

2.3.4. *Anamirta cocculus*

2.3.4.1. *Anamirta cocculus* leaf extracts exhibit differential cytotoxicity

The cytotoxic effects of various *Anamirta cocculus* leaf extracts were evaluated using the MTT assay on several cancer cell lines and a non-cancerous human keratinocyte cell line (HaCaT). Extracts were prepared with petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water. The highest mortality percentage was observed for the ethyl acetate extract of *Anamirta cocculus* on PA1 (98.64±0.40%) followed by the acetone extract on PA1 (92.72±0.58%) and the ethyl acetate extract on HT29 (91.16±0.24%). The ethyl acetate extract was also toxic to the other cancer cell lines like, MG63 with mortality of 86.76±1.04%, PANC1 with mortality of 79.16±1.32%, HCT116 with 69.18±2.26% mortality, A498 with 68.54±0.69% mortality, MCF7 with 67.23±1.47% mortality, and UM-SCC-83B with 62.44±1.07% mortality. Besides the PA1, the acetone extract also exhibited 81.33±1.33% mortality on MG63 cell line (Table 2.3.8.). At the same time, these extracts caused 18.72±0.73% and 30.23±0.10% mortality on HaCaT. The mortality rates after 48 hours of incubation are shown as mean±SEM.

Table 2.3.8. *In vitro* cytotoxicity of *Anamirta cocculus* on various cell lines - MTT assay.

Sl. No	Cell Line	Percentage Mortality (48 hrs) Mean±SEM					
		Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Acetone Extract	Methanol Extract	Water Extract
1	A431	5.74±0.87	4.17±1.04	57.54±2.93	51.35±3.18	0.75±2.92	6.58±0.98
2	A498	15.06±2.55	1.09±1.72	68.54±0.69	66.54±1.70	13.00±0.92	12.71±1.58
3	HCT116	10.78±1.82	9.47±2.66	69.18±2.26	31.52±1.39	9.68±2.39	11.53±2.25
4	HeLa	18.21±3.47	9.18±1.71	60.11±1.24	37.85±2.31	15.91±3.40	6.78±3.07
5	HT29	0.63±1.03	16.75±1.34	**91.16±0.24**	25.01±1.83	15.33±2.37	12.29±1.78
6	MCF7	5.42±2.69	1.17±1.57	67.23±1.47	20.84±2.55	12.61±2.58	6.19±2.57
7	MG63	11.56±2.45	5.48±2.20	86.76±1.04	81.33±1.33	8.10±2.69	17.33±2.22
8	PA1	4.77±2.81	7.19±0.84	**98.64±0.40**	**92.72±0.58**	4.06±1.43	2.87±2.21
9	PANC1	24.73±2.31	24.35±1.08	79.16±1.32	33.46±1.75	28.13±3.91	26.79±1.65
10	UM-SCC-83B	7.51±2.61	1.61±1.60	62.44±1.07	61.59±0.77	43.93±0.10	24.42±3.29
11	HaCaT	5.43±1.90	17.55±2.64	18.72±0.73	30.23±0.10	10.57±1.88	29.54±3.29

Cytotoxic activity of the extracts was assessed by considering cytotoxicity of DMSO control as 0.00%. Percentage mortality was calculated after 48 hours of treatment and the values are expressed as mean±SEM (n=6).

2.3.4.2. Identification of compounds in ethyl acetate extract of *Anamirta cocculus* leaf by GC-MS analysis

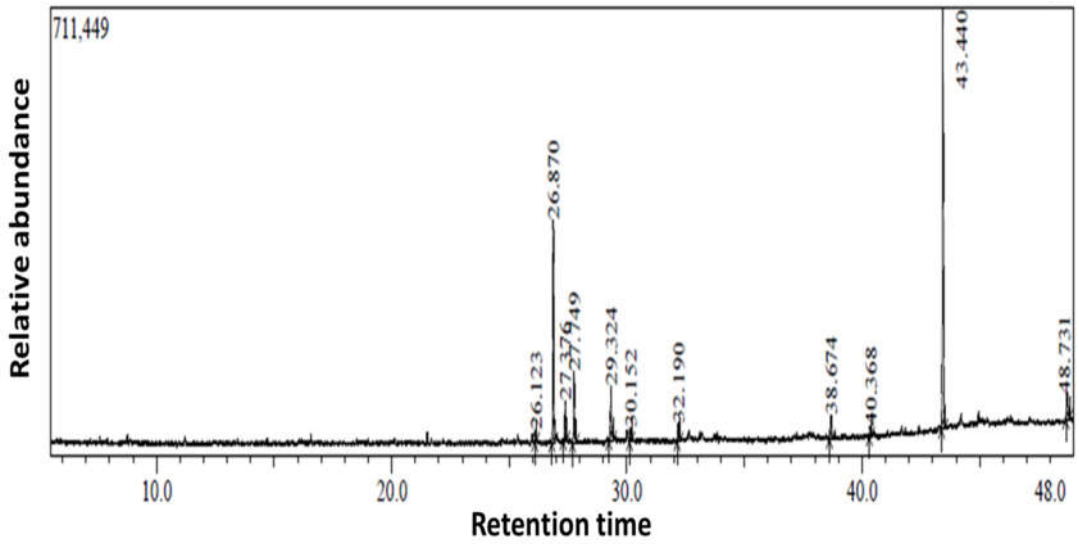


Figure 2.3.5. Chromatogram of the ethyl acetate extract of *Anamirta cocculus* leaf obtained via GC-MS analysis.

The bioactive ethyl acetate extract of *Anamirta cocculus* was analyzed using GC-MS to identify its organic constituents. The analysis revealed the presence of several

compounds, including octadecane, neophytadiene, n-hexadecanoic acid, heneicosane, phytol, epoxylathyrol, dotriacontane, squalene, and vitamin E (Figure 2.3.5 and Table 2.3.9). Notably, neophytadiene was identified as the most prominent compound, exhibiting the largest area under the peak in the chromatogram. The presence of these bioactive compounds suggests that the extract may possess a variety of biological activities that could be further investigated for therapeutic potential.

Table 2.3.9. List of phytochemicals identified in the ethyl acetate extract of *Anamirta cocculus* leaf by GC-MS analysis.

Peak	Area%	Height%	A/H	Name
1	1.11	1.33	2.34	Octadecane
2	22.73	24.4	2.61	Neophytadiene
3	3.7	4.28	2.42	Neophytadiene
4	7.73	7.75	2.8	Neophytadiene
5	6.23	5.97	2.92	n-Hexadecanoic acid
6	1.28	1.48	2.41	Heneicosane
7	2.86	2.02	3.96	Phytol
8	3.24	2.63	3.44	Epoxylathyrol
9	0.96	1.15	2.34	Dotriacontane
10	45.3	46.16	2.75	Squalene
11	4.87	2.81	4.85	Vitamin E

Potential of the ethyl acetate extract of *A. cocculus* leaf on cytomorphology and apoptosis were discussed in the fourth chapter in detail.

2.3.5. *Melia dubia*

2.3.5.1. *Melia dubia* leaf extracts exhibit differential cytotoxicity

Melia dubia leaf extracts demonstrated significant anticancer potential with varying levels of cytotoxicity across ten cancer cell lines, while showing reduced toxicity towards the normal HaCaT cell line (Table 2.3.10). The petroleum ether extract exhibited the highest mortality rate of 97.48±1.77% against the PANC1 pancreatic cancer cell line. Similarly, the chloroform extract achieved notable mortality rates of 94.36±0.93% on HT29 and 93.47±1.29% on PA1. Both extracts displayed comparatively lower mortality rates on HaCaT cells (48.81±2.04% and

28.70±1.21%), indicating a selective cytotoxic effect on cancer cells with minimal impact on normal, non-cancerous cells.

Table 2.3.10. *In vitro* cytotoxicity of *Melia dubia* on various cell lines - MTT assay.

Cell Line	Percentage Mortality (48 hrs) Mean±SEM					
	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
A431	39.80±2.74	58.93±2.10	35.08±2.61	5.84±1.57	5.24±1.58	1.46±0.49
A498	7.68±1.25	12.06±1.43	3.55±0.86	9.14±1.53	10.12±1.35	9.25±1.50
HCT116	17.16±0.31	29.63±0.99	13.94±1.59	19.09±1.35	13.08±1.00	8.97±2.00
HeLa	28.16±2.38	73.55±1.56	29.73±4.78	19.13±0.95	14.21±2.12	9.36±1.88
HT29	47.06±1.80	**94.36±0.93**	50.25±5.46	4.84±1.53	4.53±1.09	5.91±3.07
MCF7	64.08±1.84	88.92±0.87	81.44±1.10	84.01±2.14	71.50±3.26	62.55±2.57
MG63	0.22±1.39	36.23±5.32	1.83±2.77	9.50±2.63	0.75±2.16	1.79±1.35
PA1	17.70±2.19	**93.47±1.29**	7.33±1.76	39.93±1.84	14.26±1.17	3.48±1.71
PANC1	**97.48±1.77**	48.85±3.02	24.17±3.48	26.87±3.69	42.59±3.54	22.51±2.25
UM-SCC-83B	63.57±2.72	88.15±1.09	56.39±3.57	8.58±4.63	32.98±2.16	13.71±2.33
HaCaT	48.81±2.04	28.70±1.21	22.38±1.98	17.14±2.78	20.40±2.37	2.63±2.07

Cytotoxic activity of the extracts was assessed by considering cytotoxicity of DMSO control as 0.00%. Percentage mortality was calculated after 48 hours of treatment and the values are expressed as mean±SEM (n=6).

2.3.5.2. Identification of compounds in chloroform extract of *Melia dubia* leaf by GC-MS analysis

The gas chromatographic analysis of the chloroform extract from *Melia dubia* revealed ten prominent peaks on the chromatogram (Figure 2.3.6), each corresponding to different compounds in varying proportions. The most abundant compound identified was tetratetracontane, followed by (E)-Phytol. Other notable compounds included 3, 5-Di-Tert-Butylphenol, [E]-5-Eicosene, E-14-Hexadecenal, Ethyl palmitate, Linolenic acid, 1-Heneicosanol, and Heptadecyl trifluoroacetate (Table 2.3.11).

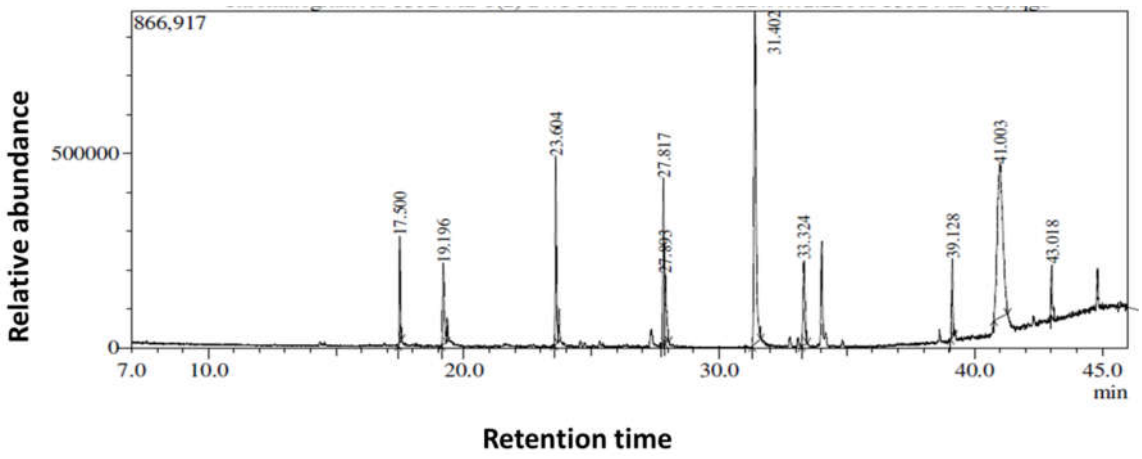


Figure 2.3.6. Chromatogram of the chloroform extract of *Melia dubia* leaf obtained via GC-MS analysis.

Table 2.3.11. List of phytochemicals identified in the Chloroform extract of *Melia dubia* leaf by GC-MS analysis.

Peak	Area%	Height%	Name
1	4.17	8.14	3, 5-Di-Tert-Butylphenol
2	4.54	6.2	[E]-5-Eicosene
3	8.18	14.35	E-14-Hexadecenal
4	7.82	12.61	E-15-Heptadecenal
5	4.41	5.2	Ethyl palmitate
6	25.73	25.4	(E)-Phytol
7	5.72	6.44	Linolenic acid
8	3.71	6	1-Heneicosanol
9	32.92	11.64	Tetratetracontane
10	2.8	4.01	Heptadecyl trifluoroacetate

Further fractionation, characterization and the effect of active fraction on cytomorphology, apoptosis, and EMT pathway were separately dealt within the chapters 3 and 4.

DISCUSSION

Cancer is a highly aggressive disease marked by high morbidity and mortality rates, a poor prognosis, and limited treatment options. Although chemotherapy is a cornerstone of cancer treatment, its effectiveness is often hindered by severe side effects and the development of drug resistance, which diminishes its overall efficacy. This situation highlights the urgent need for novel therapeutic agents that offer both improved efficacy and reduced side effects.

Natural products, especially those derived from plants, have emerged as promising candidates for new anticancer drugs. Medicinal and aromatic plants, known for their diverse bioactive compounds, not only provide potential therapeutic benefits but also tend to have lower toxicity to normal cells. Plants such as *Hewittia malabarica*, *Pancratium triflorum*, *Conyza canadensis*, *Anamirta cocculus*, and *Melia dubia* have traditionally been used to treat various ailments including arthritis, conjunctivitis, endocrine disorders, neurological disorders, and respiratory conditions. Recent studies suggest these plants hold significant promise in cancer therapy due to their anticancer properties. In vitro research has demonstrated the anticancer potential of extracts from these plants, revealing varying degrees of cell death across ten different cancer cell lines. The evaluation of these plant extracts highlights their considerable promise for cancer treatment, showcasing their unique bioactive compounds and potential therapeutic applications.

2.4.1. Differential cytotoxicity of *Hewittia malabarica* on cancer cell lines

The cytotoxic effects of *Hewittia malabarica* aerial parts extracts were evaluated on ten different cancer cell lines using the MTT assay. Among the six extracts tested, the ethyl acetate extract demonstrated the highest cytotoxicity, particularly against the PANC1 pancreatic cancer cell line, with a mortality percentage of 99.58±1.08%. This was followed closely by its effect on the MG63 osteosarcoma cell line, showing a mortality percentage of 97.23±0.82%. However, when tested on normal human keratinocytes (HaCaT), the ethyl acetate extract exhibited a lower cell mortality of 44.91±1.32%, indicating reduced toxicity toward normal cells.

The chloroform and petroleum ether extracts of *H. malabarica* aerial parts also showed concentration-dependent cytotoxicity, especially against the HT29 colorectal cancer cell line. After 48 hours of treatment at a concentration of 100 µg/mL, the chloroform extract induced 81.59±4.90% cell death, while the petroleum ether extract resulted in 65.38±0.94% cell mortality. The chloroform extract exhibited the most potent anticancer activity, with an IC_{50} value of 31.97±3.07 µg/mL against HT29 cells, indicating its strong efficacy. The petroleum ether extract also showed significant cytotoxicity, with an IC_{50} value of 76.58±4.74 µg/mL on the same cell line.

Interestingly, both the chloroform and petroleum ether extracts displayed minimal toxicity toward normal cells (HaCaT), suggesting selective action against cancer cells. At 100 µg/mL, the mortality rates for these extracts on HaCaT cells were 5.44±1.07% for the chloroform extract and 5.38±1.16% for the petroleum ether extract, highlighting their relative safety for normal cells. The lower toxicity of the chloroform extract on normal keratinocytes and its higher cytotoxicity on cancer cells suggest that the extract is more specific to cancer cells, with minimal adverse effects on normal cells. This selective cytotoxicity of the chloroform extract towards cancer cells, particularly HT29, with reduced toxicity toward normal cells, presents it as a promising candidate for further investigation in cancer treatment research.

There are no prior studies on the anticancer properties of *Hewittia malabarica*, making this research a pioneering effort. However, a related species, *Convolvulus arvensis L.*, has been shown to exhibit cytotoxic effects on the HepG2 liver cancer cell line when its extracts were incorporated into nanoparticles, as reported by Osman *et al.* (Osman *et al.,* 2024). The present study on *H. malabarica* could potentially lead to the identification of novel anticancer compounds within this genus.

In another study by Sadeghi-Aliabadi *et al.*, the cytotoxic activities of chloroform, ethyl acetate, and hydroalcoholic extracts from *Convolvulus arvensis* were tested on the HeLa human tumour cell line. Among the extracts, chloroform showed the

strongest cytotoxic effect, likely due to the presence of lipophilic glycosides, which are non-polar compounds efficiently isolated by chloroform (Sadeghi-Aliabadi *et al.,* 2009). This highlights the potential role of non-polar compounds in enhancing the cytotoxic properties of plant extracts, which could be relevant to the findings in *H. malabarica* as well.

The biological activities of *Hewittia malabarica* leaf extracts is further explored by analysing the chemical composition of each extract using GC-MS. In this study, GC-MS analysis of the bioactive chloroform extract revealed several notable compounds, including 2,4-Di-tert-Butylphenol, Sulfurous acid hexyl octyl ester, Cyclododecanol, Cetene, Palmityl acrylate, Neodene, Neophytadiene, 9-eicosene, N-pentadecane, (E)-phytol, 1-Heneicosanol, O,o'-biphenol, Tritetracontane, 1,2-benzenedicarboxylic acid, Oleyl alcohol, trifluoroacetate, Tetratetracontane, and Bis(2-ethylhexyl) terephthalate. This marks the first GC-MS analysis of *H. malabarica* extract.

Previous studies on the related species *Convolvulus arvensis* have analyzed its phytochemical composition. In the leaf extract of *C. arvensis*, seventeen compounds including Neophytadiene, hexadecanamide, 9-octadecanamide, and 1,2-benzenedicarboxylic acid were detected. The stem extract contained thirteen compounds, like, hexadecanamide, 9-octadecanamide, 1,2-benzenedicarboxylic acid, and stigmasterol. In the roots, twelve compounds were detected, with three major peaks: hexadecanamide, 9-octadecanamide, and 1,2-benzenedicarboxylic acid. Additionally, the dry extract of *C. arvensis* contained 25 compounds, with eight major compounds, including Neophytadiene, stearic acid, Vitamin E, stigmasterol, and 5-beta-pregn-7-en-3,20-dione (Abu-Shandi *et al.,* 2015). The presence of Neophytadiene, 1,2-benzenedicarboxylic acid, and phytol in *H. malabarica* parallels these findings.

Neophytadiene, a diterpene, has been reported as an effective analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound (Raman *et al.,* 2012). Phytol, a common terpenoid found esterified to chlorophyll in all plants, is used in

the production of synthetic vitamin E (Netscher, 2007) and vitamin K1 (Daines *et al.,* 2003). Its cytotoxic effects have been demonstrated against MCF7 breast cancer cells (IC_{50}= 8.79±0.41 μM) (Pejin *et al.,* 2014). Additionally, 1,2-benzenedicarboxylic acid, mono 2-ethylhexyl ester has shown potential cytotoxicity against HepG2 and MCF7 cancer cell lines (Krishnan *et al.,* 2014).

Recent research by Kaari *et al.* isolated and identified 2,4-Di-tert-Butylphenol (2,4-DTBP) as a potent anticancer agent. At a concentration of 1 mg/mL, 2,4-DTBP inhibited 88% of HT-29 colon cancer cells and 89% of HeLa cervical cancer cells, suggesting that this compound contributes to the anticancer properties of *H. malabarica* (Kaari *et al.,* 2023). Various physicochemical properties, such as lipophilicity, water solubility, pharmacokinetics, and drug-likeness, are important for evaluating compounds like 2,4-DTBP in the drug discovery process (Daina *et al.,* 2017). In this context, the detailed study of *H. malabarica* finds relevance in developing anticancer molecule based treatment strategies.

In this context, a detailed study of *Hewittia malabarica* becomes highly relevant for the development of anticancer molecule-based treatment strategies. By identifying bioactive compounds with cytotoxic effects, such as those found in its chloroform extract, this research may pave the way for novel therapies targeting specific cancer cells while minimizing harm to normal tissues. The findings from this study, particularly with compounds like 2,4-Di-tert-Butylphenol, phytol, Neophytadiene, or other compounds contribute valuable insights toward advancing plant-derived anticancer treatments.

2.4.2. Differential cytotoxicity of *Pancratium triflorum* on cancer cell lines

The study assessed the cytotoxicity of *Pancratium triflorum* bulb extracts against multiple cancer cell lines, utilizing the MTT assay. Extracts prepared using solvents such as petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water were tested on cell lines including HT29, HCT116, A431, A498, HeLa, MG63, MCF7, PA1, PANC1, and UM-SCC-83B. Among the extracts, the ethyl acetate extract demonstrated the most potent cytotoxic effects, achieving a percentage

mortality of 99.74±0.14% in the MG63 cell line and 99.34±0.28% in HeLa cells. Additionally, the acetone extract caused 99.41±0.52% mortality in the MG63 line.

The chloroform extract exhibited substantial cytotoxicity across a range of cell lines, with HeLa showing 92.36±0.81% mortality, UM-SCC-83B at 89.15±1.09%, PA1 at 82.96±1.10%, and HT29 at 82.50±0.65%. Lesser but still significant effects were seen in cell lines such as A498, A431, MCF7, and PANC1. These results indicate a broad-spectrum anticancer potential for the chloroform extract.

Notably, the selectivity of the chloroform extract is highlighted by its relatively lower toxicity towards normal cell lines. It caused 43.47±0.42% mortality in normal cells, compared to the higher toxicity of the ethyl acetate (68.49±0.33%) and acetone (94.18±0.54%) extracts. This suggests that the chloroform extract might have better specificity in targeting cancer cells with reduced harm to healthy cells, making it a promising candidate for further anticancer investigations.

Importantly, this study represents the first report of the anticancer properties of *P. triflorum*, filling a gap in the scientific literature. Previous studies, such as Johnson *et al.*, have focused on the antioxidant and cytotoxic activities on brine shrimp, but *P. triflorum* has been underexplored in terms of its anticancer potential (Johnson *et al.,* 2018).

GC-MS analysis of the chloroform extract revealed the presence of a variety of chemical constituents, such as 3-Eicosene (E), 2,4-Ditert-butylphenol, 1-Hexadecene, and Linoleic acid ethyl ester, among others. The intricate composition of these compounds suggests multiple biologically active molecules that may contribute to the extract's cytotoxic properties. Comparisons with related species, like *Pancratium maritimum*, which has demonstrated antiproliferative effects in cancer models, underscore the potential of *P. triflorum* for drug discovery.

The study by Tayoub *et al.* examined the antiproliferative effects of *Pancratium maritimum* extracts on MDA-MB-321 breast cancer cells. Extracts from the bulbs and leaves were tested, showing significant inhibition of cancer cell growth through

the induction of cell cycle arrest at the S and G2/M phases. This disruption in cell cycle progression effectively halted cell proliferation. Furthermore, the extracts modulated the expression of critical regulatory proteins, including Cyclin B1 (which governs the G2/M transition), Bcl-2 (an anti-apoptotic protein), and Ki67 (a proliferation marker). The root extract exhibited IC_{50} values of 0.051 mg/mL after 72 hours and 0.03 mg/mL after 96 hours, while the flower extract had an IC_{50} value of 0.048 mg/mL after 96 hours (Tayoub *et al.,* 2018). These findings emphasize the strong cytotoxic potential of *Pancratium maritimum*, particularly across different plant parts, highlighting its value in anticancer research.

In another related study, Adewusi *et al.* investigated the cytotoxic effects of a crinine-type alkaloid isolated from *Boophane disticha*. This research focused on the toxicological profile of *Boophane disticha*, a plant known for its traditional medicinal use, but also recognized for its potential toxicity if improperly administered. Methanolic extracts from the bulbs were tested for cytotoxicity in cancer cells using the MTT assay, a standard method for evaluating cell viability. The MTT assay relies on the conversion of MTT salt into formazan by mitochondrial enzymes in active cells (Adewusi *et al.,* 2012). The results clearly demonstrated the plant's cytotoxic properties, underscoring the need to balance its therapeutic benefits with safety concerns when considering its use in drug development.

Both studies—by Tayoub *et al.* on *Pancratium maritimum* and by Adewusi *et al.* on *Boophane disticha*—provide critical insights into the cytotoxic mechanisms of plant extracts against cancer cells. These investigations highlight the importance of identifying compounds that induce cell cycle arrest and modulate key regulatory proteins. Moreover, the attention to toxicity is crucial for minimizing the risks associated with therapeutic applications. Together, these findings contribute to the growing body of research into the anticancer potential of plants from the Amaryllidaceae family, bolstering the case for further exploration of *Pancratium triflorum* as a promising candidate in cancer therapy (Tayoub *et al.,* 2018 and Adewusi *et al.,* 2012).

This research adds to the previous findings regarding other *Pancratium* species, such as *P. maritimum*, which also demonstrated the ability to inhibit cancer cell proliferation by inducing cell cycle arrest and modulating key proteins. The current findings on *P. triflorum* offer a preliminary understanding of its anticancer potential, paving the way for deeper investigations into its bioactive constituents and therapeutic efficacy.

2.4.3. Differential cytotoxicity of *Conyza canadensis* on cancer cell lines

The cytotoxicity of *Conyza canadensis* leaf extracts was evaluated across various cancer cell lines and normal cell line using the MTT assay. Among the tested extracts—petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water—the petroleum ether extract exhibited the highest cytotoxicity. It achieved a 99.59±0.50% mortality rate against the PANC1 pancreatic cancer cell line and a 95.04±0.58% mortality rate against the HT29 colorectal cancer cell line. The chloroform extract also demonstrated potent cytotoxicity, with 98.57±0.40% mortality in PANC1 cells and 98.06±2.53% in the MG63 bone cancer cell line. These results highlight the strong anticancer potential of both the petroleum ether and chloroform extracts, with notable effectiveness in the HT29, PANC1, and MG63 cell lines. Both these extracts caused less cytotoxicity on HaCaT cell line, with a mortality percentage of 55.70±3.97% and 25.07±2.95%.

Ayaz *et al.* studied the cytotoxic effect of *Conyza canadensis* (*L.*) Cronquist (Asteraceae) on human lung adenocarcinoma cell lines (A549 and H1299). They tested methanol extracts from the aerial parts and roots of *C. canadensis*, showing cytotoxic effects in a dose-dependent manner. The n-hexane extract from the roots exhibited higher cytotoxicity than the aerial parts, with IC_{50} values of 94.73 µg/mL for the A549 cell line and 84.85 µg/mL for the H1299 cell line. Since these IC_{50} values are close to 100 µg/mL, the findings suggest significant anticancer potential for the chloroform extract of *C. canadensis* leaves, which could be considered for therapeutic use (Ayaz *et al.,* 2016).

The GC-MS analysis of the chloroform extract of *Conyza canadensis* revealed 11 prominent compounds, with 2,5-Etheno[4.2.2]propella-3,7,9-triene and 2,4-Hexadiyn-1-one, 1-phenyl- being the most abundant, showing the highest area percentages in the chromatogram. These two compounds dominated the extract's composition. Other key compounds identified in the extract, in decreasing order of peak area, include (-)-Spathulenol, known for its anti-inflammatory and antimicrobial properties; Caryophyllene oxide, a compound with potential anticancer activity; (E)-β-Famesene, a natural hydrocarbon; Humulene epoxide 2; α-Curcumen; Andrographolide, recognized for its medicinal properties; Ledene oxide-(II); E-15-Heptadecenal; and Caryophyllene. The diverse chemical profile of these compounds suggests potential biological activities that warrant further exploration.

Masuda *et al.* isolated a compound from the essential oil of *Artemisia capillaris* Thunb. flower, which inhibited the growth of human leukemia HL-60 cells. The active compound was identified as capillin (2,4-Hexadiyn-1-one, 1-phenyl-) (Masuda *et al.,* 2015). Research has attributed the antibacterial properties of essential oils to their enriched terpenoid content (Burt, 2004). Sesquiterpenes like spathulenol, α-curcumene, α-bisabolol, and nerolidol were the main components of the essential oil of *A. ordosica*. These terpenoids, with their high hydrophobicity, facilitate penetration across the plasma membrane and interaction with intracellular proteins and/or intraorganelle sites (Silva *et al.,* 2015).

Spathulenol has also been shown to induce apoptosis in melanoma cells, specifically in SKMEL-28 cells, according to research by Santos *et al.* (Santos *et al.,* 2020). Caryophyllene oxide has substantial inhibitory effects on the HCCLM3 and HUH7 liver cancer cell lines by modulating oxidative stress, autophagy, and iron metabolism, as reported by Xiu *et al.* (Xiu *et al.,* 2022). The natural bicyclic sesquiterpene β-caryophyllene oxide, found in numerous plants, possesses significant anticancer activities. It affects the growth and proliferation of several cancer cell types by influencing key signaling pathways involved in cancer development, including the mitogen-activated protein kinase (MAPK), PI3K/AKT/mTOR/S6K1, and STAT3 pathways. Moreover, treatment with this

compound reduces the expression of procancer genes while enhancing the levels of proapoptotic proteins (Fidyt *et al.,* 2016).

α-Curcumen, a bioactive bisabolane-type compound, has demonstrated potent anticancer effects against Sarcoma 180 ascites in mice (Itokawa *et al.,*2008). Additionally, the diterpene lactone andrographolide, isolated from *Andrographis paniculata*, exhibits a wide range of biological activities, including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, cytotoxic, and cardioprotective effects. Andrographolide has also shown therapeutic potential in treating major diseases like Parkinson's, rheumatoid arthritis, and colitis, as well as exerting anticancer effects through mechanisms involving oxidative stress, cell cycle arrest, apoptosis, and inhibition of cancer cell proliferation and migration (Islam *et al.,* 2018).

Furthermore, *Andrographis paniculata* plant extracts have been linked to various pharmacological activities, with andrographolide identified as the main active constituent. This compound has been studied for its effects on cancer cells and the immune system. It inhibits in vitro proliferation of several tumour cell lines, including those representing different cancer types, by causing cell cycle arrest in the G0/G1 phase through induction of cell-cycle inhibitory protein p27 and suppression of cyclin-dependent kinase 4 (CDK4). The compound's in vivo anticancer activity has also been confirmed in B16F0 melanoma syngenic and HT-29 xenograft models (Rajagopal *et al.,* 2003).

Finally, Csupor-Löffler *et al.* isolated various compounds, such as conyzapyranone A and B, 4Z,8Z-matricaria-γ-lactone, 4E,8Z-matricaria-γ-lactone, 9,12,13-trihydroxy-10(E)-octadecenoic acid, epifriedelanol, friedeline, taraxerol, simiarenol, spinasterol, stigmasterol, β-sitosterol, and apigenin from *Conyza canadensis*. These compounds were evaluated for their antiproliferative properties and demonstrated significant cell growth-inhibitory activity against human cervix adenocarcinoma (HeLa), skin carcinoma (A431), and breast adenocarcinoma (MCF-7) cell lines. Some components, such as conyzapyranone B, 4E,8Z-matricaria-γ-lactone, and

spinasterol, were notably more potent against these cancer cell lines than against non-cancerous human fetal fibroblasts (MRC-5), highlighting their potential as selective antiproliferative agents (Csupor-Löffler *et al.,* 2011).

The presence of well-established anticancer compounds within the extract strongly suggests its potential for anticancer activity. These bioactive molecules, which have been previously documented for their cytotoxic and antiproliferative effects, provide a foundation for the extract's ability to target cancer cells. Their known mechanisms of action, such as inducing apoptosis, regulating oxidative stress, and inhibiting critical cancer-related pathways, reinforce the extract's potential for therapeutic use in cancer treatment.

2.4.4. Differential cytotoxicity of *Anamirta cocculus* on cancer cell lines

The cytotoxic effects of *Anamirta cocculus* leaf extracts were assessed using the MTT assay on several cancer cell lines, including PA1 and HT29, as well as the non-cancerous human keratinocyte cell line (HaCaT). The extracts were prepared using different solvents, including petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water. Among these, the ethyl acetate extract demonstrated the highest cytotoxicity, showing a mortality rate of 98.64±0.40% on PA1 cells, followed by the acetone extract, which caused 92.72±0.58% mortality on PA1 cells, and the ethyl acetate extract, which also exhibited 91.16±0.24% mortality on HT29 cells. In contrast, these extracts showed considerably lower cytotoxicity on the non-cancerous HaCaT cell line, with mortality rates of 18.72±0.73% and 30.23±0.10%, respectively. Overall, the ethyl acetate extract was found to exhibit the strongest cytotoxic activity against the cancer cell lines tested.Notably, this is the first anticancer study conducted on *A. cocculus*, with no prior research available, making the current investigation highly valuable. The detailed mechanisms of the anticancer activity, including cytomorphological changes and apoptosis induction, are further discussed in the fourth chapter of the thesis.

GC-MS analysis of the bioactive ethyl acetate extract revealed several key compounds, including octadecane, neophytadiene, n-hexadecanoic acid,

heneicosane, phytol, epoxylathyrol, dotriacontane, squalene, and vitamin E. Neophytadiene was the most abundant compound based on peak area in the chromatogram. The presence of these bioactive compounds suggests potential biological activities, meriting further therapeutic exploration.

Among these, vitamin E is well known for its antioxidant properties, protecting cell membranes and exhibiting anticancer effects by delaying or inhibiting tumour development, reducing tumour size, and promoting apoptosis. Squalene has been shown to suppress abnormal hyperproliferation in human mammary epithelial cells (MCF10A) (Thozhukkad Moosaripparambil and Vadakkadath Meethal, 2024). Epoxy lathyrol, recognized for its anti-inflammatory and anticancer properties, has a derivative—lathyrol-3-phenyl-acetate-5,15-diacetate—that has demonstrated potent cytotoxicity against A549 lung cancer cells (Zhang *et al*., 2017). This derivative induces apoptosis by elevating reactive oxygen species (ROS), reducing mitochondrial membrane potential, releasing cytochrome C, and activating caspase-9 and caspase-3. Additionally, phytol, a constituent of chlorophyll, has demonstrated anticancer properties and immune-enhancing effects. The presence of these anticancer compounds suggests that the observed growth-inhibitory effects on cancer cell lines are likely due to an additive or synergistic action of these components.

2.4.5. Differential cytotoxicity of *Melia dubia* on cancer cell lines

The investigation into the cytotoxicity of *Melia dubia* leaf extracts underscores their significant potential as anticancer agents, with the petroleum ether and chloroform extracts showing particularly high levels of cytotoxicity across various cancer cell lines. The petroleum ether extract displayed the highest mortality rate of 97.48±1.77% against the PANC1 pancreatic cancer cell line, while the chloroform extract exhibited substantial cytotoxicity, with mortality rates of 94.36±0.93% on HT29 and 93.47±1.29% on PA1 cells. Importantly, both extracts demonstrated reduced toxicity on normal HaCaT cells (48.81±2.04% for the petroleum ether extract and 28.70±1.21% for the chloroform extract), highlighting their selective

action on cancer cells while minimizing harm to healthy tissues. Eventhough the related plants of this species were well studied for their anticancer potential and other biological activities, *M. dubia* is the least explored

The GC-MS analysis of the chloroform extract of *M.dubia* leaf indicated the presence of 10 compounds. Tetratetracontane was the most abundant compound with 32.92% peak area, and was followed by (E)-Phytol. The other compounds in the order of abundance were E-14-Hexadecenal, E-15-Heptadecenal, Linolenic acid, [E]-5-Eicosene, Ethyl palmitate, 3, 5-Di-Tert-Butylphenol, 1-Heneicosanol and Heptadecyl trifluoroacetate.

Phytol (Pyt), a diterpenoid, possesses a wide range of bioactivities, including anticancer effects. It has been shown to significantly reduce the viability and proliferation of sarcoma 180 (S-180) and human leukemia (HL-60) cell lines, with IC_{50} values of 18.98±3.79 μM and 1.17±0.34 μM, respectively. Phytol's anticancer effects are likely mediated through the induction of apoptosis, as demonstrated in various studies (de Alencar *et al.,* 2023; de Alencar *et al.,* 2019). Phytol has also demonstrated antitumour activity in a 7,12-dimethylbenzanthracene (DMBA)-induced cancer model (de Alencar *et al.,* 2019). Gamma-linolenic acid (GLA) and its derivatives have shown selective tumouricidal effects with little to no side effects. GLA induces apoptosis in tumour cells by promoting free radical generation in cancer cells, while sparing normal cells (Das, 2006). Conjugated linolenic acids (CLA) possess various biological activities, including antioxidant, anti-carcinogenic, and immunomodulatory effects. CLA has been studied extensively for its anticancer potential in vitro and in vivo, particularly in colon and breast cancer models. CLA isomers have been shown to inhibit proliferation and induce apoptosis in HT-29, MIP-101, and Caco-2 colon cancer cells, with studies demonstrating its effect on the ErbB3 signaling pathway (Cho *et al.,* 2003). Moreover, CLA has been shown to suppress tumour growth and induce apoptosis in breast cancer cells (MCF-7, MDA-MB-231) and prostate cancer cell lines (DU145 and PC-3) (Wang *et al.,* 2006; Kim *et al.,* 2006). It has also demonstrated antimutagenic and anticancer effects in rat hepatoma dRLh-84 cells (Yamasaki *et al.,* 2002).

The identification of these bioactive compounds, many of which have demonstrated anticancer properties, supports the potential of *Melia dubia* leaf extracts as effective anticancer agents. The detailed mechanism of action of the extract and the isolation of its active components are discussed further in Chapters 3 and 4.

Further supporting the anticancer potential of plant extracts, past studies have reported various extracts with selective activity against different cancer cell lines. For example, methanol extracts of *Chromolaena odorata* (Yusuf *et al.,* 2021) and *Stachys pilifera* and ethanol extracts of *Ficus hispida***,** *Cnidium officinale***,** and *Cynodon dactylon* have shown significant cytotoxicity. *Adhatoda vasica* and *Pergularia daemia* extracts also demonstrated notable cytotoxicity against specific cancer cell lines. Some plant extracts, such as those from *Eucalyptus sp.***,** *Clausen sp.***,** *Trichopus zeylanicus***,** and *Begonia floccifera***,** were cytotoxic against PA1 cells, with IC_{50} values ranging between 40 and 100 μg/mL. Extracts with even lower IC_{50} values, such as *Garcinia morella* (0.9 μg/mL), *Plectranthus urticoides* (1.0 μg/mL), *Aristalochia tagala* (13 μg/mL), and *Arlnychia pedunculata* (20 μg/mL), were identified as particularly promising candidates for further exploration.

The role of specific phytochemicals in anticancer activity was also examined. Cardiac glycosides, such as digitoxin, are known for their potent and selective cytotoxic effects against various cancer cells, including lung cancer. Isoquinoline alkaloids might contribute to the cytotoxicity and selectivity of *Pancratium maritimum* extracts. *Taxus baccata* extracts' anticancer activity is likely mediated by taxane-type diterpenes, including paclitaxel. Similarly, the cytotoxicity of *Tetraclinis articulata* extracts may involve diterpenes like ferruginol and sandaracopimaric acid, monoterpenes such as limonene and carveol, and sesquiterpenes like β-caryophyllene and humulene.

Our research aligns with these findings and further underscores the potential of the selected plant extracts (chloroform extracts of *H. malabarica aerial parts, P. triflorum bulb, C. canadensis leaf, M. dubia leaf and ethyl acetate extract of A. cocculus)* in anticancer drug development. The observed differences in IC_{50}

values between cancer and normal cell lines reinforce the selective anticancer activity of these extracts. For example, the higher IC_{50} values for normal cell lines like HaCaT (>100 µg/mL) and lower IC_{50} values for cancer cell lines highlight the differential anticancer potential of the extracts under study.

The findings of the current study position the studied plant extracts as promising candidates for the development of novel anticancer drugs. Their favorable molecular properties and selective cytotoxicity towards cancer cells, coupled with lower toxicity to normal cells, suggest potential for improved therapeutic profiles. These compounds offer a valuable avenue for developing targeted cancer therapies with enhanced efficacy and minimized side effects. Further research, including *in vivo* studies, is essential to fully validate their therapeutic potential and establish their role in cancer treatment.

CONCLUSIONS

Hewittia malabarica

- The chloroform extract of *Hewittia malabarica* aerial parts, at a concentration of 100 µg/mL, exhibited the highest differential cytotoxicity, causing a significant mortality rate of 81.59±4.90% in the human colorectal carcinoma cell line HT29, while showing minimal cytotoxicity (5.44±1.07%) in the normal HaCaT cell line.
- The IC_{50} value of the chloroform extract against HT29 was determined to be 31.97±3.07 µg/mL.
- GC-MS analysis of the extract revealed the presence of 17 compounds, with tritetracontane being the most abundant, followed by neodene.

Pancratium triflorum

- Among the six extracts of *Pancratium triflorum* bulb, the ethyl acetate extract showed the highest cytotoxicity against the human osteoblast cell line MG63, followed by its effect on the human cervical cancer cell line HeLa. The chloroform extract also demonstrated strong cytotoxicity, particularly on HeLa cells (92.36±0.81%), while showing lower cytotoxicity on the HaCaT cell line (43.74±0.42%), indicating its selective cytotoxic potential compared to the other extracts.
- GC-MS analysis of the chloroform extract identified 15 compounds, with 3-eicosene, (E)- being the most abundant.

Conyza canadensis

- Among the tested extracts—petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water—the petroleum ether extract displayed the highest cytotoxicity, achieving a mortality rate of 99.59±0.50% against the PANC1 pancreatic cancer cell line and 95.04±0.58% against the HT29 colorectal cancer

cell line. The chloroform extract also exhibited strong cytotoxic effects, with 98.57±0.40% mortality in PANC1 cells and 98.06±2.53% in the MG63 bone cancer cell line.

- GC-MS analysis of the chloroform extract identified 11 prominent compounds, with 2,5-Etheno[4.2.2]propella-3,7,9-triene and 2,4-Hexadiyn-1-one, 1-phenyl- being the most abundant.

Anamirta cocculus

- The *Anamirta cocculus* extract exhibited the highest cytotoxicity on the PA1 ovarian cancer cell line (98.64±0.40%), followed by the acetone extract on PA1 (92.72±0.58%) and the ethyl acetate extract on HT29 colorectal cancer cells (91.16±0.24%).

- GC-MS analysis confirmed the presence of 11 compounds in the extract.

Melia dubia

- The petroleum ether and chloroform extracts of *Melia dubia* leaves exhibited significant cytotoxicity across multiple cancer cell lines. The petroleum ether extract showed the highest mortality rate of 97.48±1.77% against the PANC1 pancreatic cancer cell line, while the chloroform extract demonstrated strong cytotoxic effects with 94.36±0.93% on HT29 and 93.47±1.29% on PA1. Importantly, these extracts displayed lower toxicity towards the normal HaCaT cell line (petroleum ether extract: 48.81±2.04%, chloroform extract: 28.70±1.21%), indicating their selective targeting of cancer cells over healthy cells.

- GC-MS analysis of the chloroform extract identified a diverse range of ten prominent compounds, including tetratetracontane and (E)-Phytol.

Chapter 3

Bioactivity guided enrichment of anticancer molecules from the leaf extract of *Melia dubia* Cav.; Characterisation and *in silico* studies

- *To enrich potent anticancer compound/s from Melia dubia; the most effective against human colon cancer cell line HT29.*
- *To compare the IC_{50} values of different extracts and purified fractions of M. dubia.*
- *To identify the phytochemicals in the enriched active fraction by GC-MS analysis.*
- *In silico docking studies to identify potential anticancer compounds and their targets from the enriched active fraction.*

REVIEW OF LITERATURE

Among the myriad of medicinal plants, those belonging to the Meliaceae family, commonly known as the Mahogany family, are particularly noteworthy. This family encompasses approximately 51 genera and about 575 species, predominantly found in tropical and subtropical regions (Mabberley, 2010). While members of the Meliaceae family are economically valuable for their timber, they are also recognized for their rich content of bioactive secondary metabolites, especially limonoids. Limonoids, which are highly oxygenated triterpenoids, are found primarily in the Meliaceae and Rutaceae (citrus) families (Fan *et al.,* 2022). These compounds exhibit a wide range of biological activities, including anti-cancer, anti-malarial, anti-inflammatory, and anti-feedant effects.

The genus *Melia* within the Meliaceae family has attracted considerable scientific attention due to its diverse pharmacological properties. Species such as *Melia azedarach* and *Melia dubia* stand out for their potential therapeutic applications. *Melia azedarach*, known as the Chinaberry tree, has been used in traditional Chinese medicine for its broad-spectrum pesticidal properties. Beyond its commercial value, *M. dubia* demonstrates promising medicinal properties, including antioxidant, anticancer, antimicrobial, and antidiabetic activities (Wajdy *et al.,* 2021).

The therapeutic potential of *Melia dubia* is largely attributed to its rich phytochemical profile, which includes a variety of bioactive compounds such as limonoids, flavonoids, tannins, and alkaloids. The antioxidant properties of *M. dubia* are associated with its phenolic content, which helps neutralize free radicals and mitigate oxidative stress-related diseases (Goswami *et al.,* 2020). Additionally, *M. dubia* has shown antimicrobial properties against a range of pathogenic microorganisms, further underscoring its potential in treating infectious diseases. The observed hypoglycemic activity in *M. dubia* extracts also points to its potential role in managing diabetes, a major global health concern.

Recent studies have further expanded the scope of research on *Melia dubia*. For instance, Tang *et al.* investigated the inhibitory effects of *Melia toosendan* fruit

extract against colon cancer, demonstrating promising results in both in vitro and in vivo models (Tang *et al.,* 2012). Trung *et al.* isolated new triterpenoids from *M. dubia* bark, revealing anti-inflammatory and antifungal properties against phytopathogenic fungi (Trung *et al.,* 2023). These studies highlight the importance of exploring various parts of the *Melia dubia* plant for therapeutic agents. Notably, the isolation of anticancer molecules from *M. dubia* leaves remains an area of significant interest and necessity, underscoring the need for comprehensive research into all parts of this plant.

Our research interest in *M. dubia* is driven by its traditional medicinal uses and its emerging potential in cancer treatment. Historically used for various ailments, *M. dubia* has shown promise in cancer research due to its ability to target critical pathways involved in cancer progression. Our research aims to purify extracts from *M. dubia* and explore their potential in controlling cancer, with a particular focus on targeting angiogenesis and the epithelial-mesenchymal transition (EMT) pathway and apoptosis. This approach underscores the importance of conducting a thorough investigation of *M. dubia* to fully harness its therapeutic potential against cancer.

3.1.1. Role of phytochemicals in plants

Phytochemicals are naturally occurring compounds in plants that contribute to their colour, flavour, and aroma, and play significant roles in both plant biology and human health. While not essential nutrients, they have been recognized for their health benefits and potential in disease prevention. In plants, phytochemicals function as phytoalexins, defending against pathogens like bacteria, viruses, and fungi, and as antifeedants, deterring herbivores by making plants less palatable or toxic (Gupta and Roy, 2021). Additionally, they possess allelopathic properties, reducing competition by inhibiting the growth of nearby plants. Phytochemicals also aid in plant reproduction by attracting pollinators with vibrant colours and fragrances, ensuring species survival.

Phytochemicals provide a wide range of health benefits, with antioxidant activity being one of their most well-known. Compounds like polyphenols, carotenoids, and

vitamins C and E neutralize free radicals, preventing oxidative stress linked to chronic diseases like cancer and cardiovascular conditions (Engwa, 2018). Flavonoids, a type of polyphenol, are particularly effective in protecting cells from oxidative damage. In addition to antioxidant effects, many phytochemicals possess anti-inflammatory properties, with curcumin from turmeric being a prime example (Menon and Sudheer, 2007). Curcumin reduces inflammation by modulating inflammatory enzymes and cytokines, potentially lowering the risk of inflammation-driven diseases such as diabetes and heart disease (Zia *et al.,* 2021). Phytochemicals are also valuable in cancer prevention and treatment; compounds like resveratrol and sulforaphane have been shown to inhibit cancer cell growth and induce apoptosis. These phytochemicals may also disrupt cancer-related signalling pathways, making them promising candidates for both cancer prevention and therapy (Jiang *et al.,* 2010). Cardiovascular health benefits are notable as well, with phytochemicals like flavonoids improving endothelial function by increasing nitric oxide availability, aiding in blood vessel relaxation and circulation. Polyphenols, found in foods like berries and wine, help reduce LDL oxidation and improve HDL levels, contributing to lower heart disease risk (Sánchez *et al.,* 2019). For metabolic health, phytochemicals such as anthocyanins can improve insulin sensitivity and regulate blood sugar, aiding in the prevention of type-2 diabetes (Zhao *et al.,* 2018). Neuroprotective effects of phytochemicals, especially polyphenols and flavonoids, are being explored for their ability to reduce oxidative stress and inflammation in the brain, potentially offering protection against neurodegenerative diseases like Alzheimer's and Parkinson's. Overall, phytochemicals are promising agents in promoting health and preventing a range of chronic diseases.

3.1.2. Clinical applications

3.1.2.1. Potential applications

Phytochemicals hold promise for various clinical applications, including use as dietary supplements and incorporation into functional foods and nutraceuticals. They are being investigated for their potential to treat and prevent a range of conditions,

including cancer, cardiovascular diseases, metabolic disorders, and neurodegenerative diseases. Despite their potential benefits, the safety and efficacy of phytochemicals require careful evaluation (Rudzińska *et al.,* 2023).

3.1.2.2. Medicinal value of phytochemicals

Phytochemicals, especially phenolic compounds, are crucial in combating oxidative stress – a condition linked to chronic diseases like cancer and diabetes (Rudzińska *et al.,* 2023; Nani *et al.,* 2021). These compounds neutralize free radicals, reducing cellular damage and lowering disease risk. For example, flavonoids and polyphenols are well-studied for their free radical scavenging abilities and protective effects against oxidative damage (Bell *et al.,* 2015; Rana *et al.,* 2022; Obrenovich *et al.,* 2010).

In addition, some plant extracts, including those from *Melia dubia*, possess antimicrobial properties that help fight infections from bacteria, fungi, and viruses (Paritala *et al.,* 2015; Ravichandiran *et al.,* 2013). They also have potential antidiabetic effects, aiding in blood glucose management. The antimicrobial and antidiabetic activities of these phytochemicals highlight their value in both prevention and therapy.

3.1.3. Phytochemicals in traditional and modern medicine

3.1.3.1. Traditional medicine

Phytochemicals have long been utilized in traditional medicine systems for their therapeutic properties. Traditional Persian medicine, along with other ancient practices, employs various plant-based compounds to treat a wide range of ailments. These include kidney diseases, digestive disorders, and respiratory issues. The historical use of phytochemicals underscores their importance in traditional healing practices and their continued relevance in contemporary herbal medicine.

3.1.3.2. Applications in modern medicine

In modern medicine, phytochemicals are increasingly recognized for their potential health benefits. They are incorporated into pharmaceuticals, cosmetics, and functional foods. The roles of phytochemicals in preventing chronic diseases and enhancing overall health are gaining attention, leading to their inclusion in dietary supplements and therapeutic products (Zhang *et al.,* 2015). Their application in these fields reflects the growing appreciation of their medicinal value and efficacy.

3.1.4. Cancer research and phytochemicals

As cancer incidence continues to rise, there is an increasing need for innovative approaches to cancer control, including chemoprevention. Chemoprevention involves using natural, synthetic, or biological agents to prevent, suppress, or reverse the development of cancer before it becomes invasive. Lifestyle modifications, including dietary changes, could prevent a significant portion of cancer cases (López-Plaza, *et al.,* 2022).

Phytochemicals have shown significant promise in cancer research. Studies on cell lines and animal models have demonstrated their anticarcinogenic and antimutagenic properties. Phytochemicals can reduce cell proliferation, induce apoptosis, inhibit metastasis, and prevent angiogenesis. For example, garlic has been found to inhibit the growth of the Hep-2 human larynx carcinoma cell line (Bagul *et al.,* 2015). Extracts from *Hibiscus sabdariffa* have shown potential in inducing autophagic cell death in melanoma cells, inhibiting tumor growth in xenograft studies (Laskar and Mazumder, 2020) and inducing apoptosis in leukemia and gastric carcinoma cells. In rat models, *Shemamruthaa* has demonstrated significant anti-cancer effects by reducing lipid peroxidation, preventing membrane damage, and restoring membrane integrity (Purushothaman *et al.,* 2016). Extracts from *Viola tricolor* have shown antitumor effects against neuroblastoma N2a cells, reducing tumor cell proliferation and inhibiting angiogenesis (Sadeghnia *et al.,* 2014). Rosemary species are recognized for their potential chemopreventive properties due

to their high antioxidant content, and *Vitex rotundifolia* has shown cytotoxic effects on human breast and colon cancer cell lines (Sitarek *et al.,* 2020).

Despite these promising laboratory findings, clinical trials and human studies are still limited. Phytochemicals are categorized into five major groups: phenolics, carotenoids, organosulfur compounds, nitrogen-containing compounds, and alkaloids. These compounds can influence carcinogenesis at various stages, and ongoing research continues to explore their potential in cancer prevention.

3.1.4.1. Alkaloids

Alkaloids are a diverse class of nitrogenous compounds renowned for their significant anticancer properties. These compounds are crucial in cancer therapy due to their ability to exert antiproliferative and antimetastatic effects. For instance, camptothecin inhibits topoisomerase I, an enzyme essential for DNA replication, and is used to treat cancers such as ovarian and lung cancer (Pommier, 2006; Dancey and Eisenhauer, 1996). Vincristine and vinblastine, derived from *Catharanthus roseus*, disrupt microtubule formation, thereby preventing cell division and treating conditions like leukemia, lymphoma, and other cancers (Dhyani *et al.,* 2022). Berberine, found in various plants, induces apoptosis and inhibits cell proliferation in several types of cancer (Chidambara Murthy and Jayaprakasha, 2012). Sanguinarine, extracted from *Sanguinaria canadensis*, shows promise by inducing cell death and inhibiting tumor growth (Achkar *et al.,* 2017). Evodiamine, derived from *Evodia rutaecarpa*, suppresses tumor growth and induces apoptosis (Jiang J and Hu C, 2009). Piperine, present in black pepper, enhances drug bioavailability and exhibits anticancer properties by inhibiting cancer cell growth (Stojanović-Radić *et al.,* 2019). Matrine and tetrandrine, sourced from *Sophora* and *Stephania* species, modulate cell cycle progression and induce apoptosis (Lu, *et al.,* 2012). Alkaloids represent a significant portion of low molecular weight organic compounds found in terrestrial and marine environments. Their structural diversity results from various amino acids and building blocks, leading to numerous functional groups that interact with biological targets. Approximately 17,000

alkaloids exhibit significant biological and pharmacological activities, including anti-inflammatory, antibacterial, and antitumor effects (Ballout *et al.,* 2019).

Anticancer alkaloids play crucial roles in targeting cellular processes such as proliferation, cell cycle progression, and metastasis. For instance, paclitaxel stabilizes microtubules to prevent cell division, and vincristine disrupts microtubule formation (Abal *et al.,* 2003). Despite their efficacy, alkaloids can induce DNA damage and apoptosis, which may result in toxic side effects, highlighting the need for novel compounds with selective cancer targeting and reduced toxicity.

Emerging cytotoxic alkaloids include those with apoptotic strategies, such as hirsutine from *Uncaria*, which induces apoptosis in breast cancer cells, and α-tomatine from *Lycopersicon esculentum*, which inhibits cancer signaling pathways. Alkaloids like cathachunine, subditine, and rohitukine generate reactive oxygen species, leading to DNA damage and apoptosis. Caspase activators such as subditine and scutebarbatine A also show promise by inducing apoptosis in cancer cells through various pathways (Habli *et al.,* 2017).

Anti-proliferative alkaloids target cell cycle regulation, which is crucial for maintaining cellular balance and preventing cancer. Alkaloids like noscapine, liriodenine, and isogravacridone chlorine induce cell cycle arrest at specific phases, leading to cell repair or apoptosis (Habli *et al.,* 2017). Ongoing research aims to further explore these compounds and identify their therapeutic potential, with some alkaloids requiring additional investigation to fully elucidate their anticancer mechanisms.

3.1.4.2. Flavonoids

Flavonoids are polyphenolic compounds derived from phenylalanine, tyrosine, and acetate units, and they play a critical role in photosynthesis by catalyzing electron transport. They are widely present in fruits, vegetables, nuts, and beverages like tea and red wine. Despite their common occurrence in the diet, only about 10% of flavonoids reach peak concentrations in the bloodstream due to low bioavailability.

Flavonoids are renowned for their pharmacological properties, particularly their significant anti-cancer activity. Their antitumor effects are largely attributed to their antioxidant and anti-inflammatory properties. Flavonoids scavenge free radicals and inhibit the formation of reactive oxygen species (ROS), which are associated with cancer development (Slika *et al.,* 2022). They also exhibit pro-oxidative effects, inhibit fatty acid synthase activity, and modulate several key cellular pathways such as NF-κB, MAPK, FAK, PI3K-Akt, inflammasome, and STAT3. Additionally, flavonoids can reduce angiogenesis by decreasing VEGF expression and suppress metastasis by downregulating MMP expression (Oršolić N and Jazvinšćak Jembrek, 2022).

Flavonoids demonstrate significant potential in cancer prevention and treatment across various types of cancer. Continued research is essential to further elucidate their mechanisms of action and enhance their therapeutic efficacy. The relationship between flavonoids and lung cancer presents a complex and evolving picture. While some studies suggest a beneficial effect of flavonoid intake on lung cancer risk, others have found no significant associations. Further research is needed to understand the mechanisms underlying flavonoids' potential anticancer properties and their specific effects on lung cancer. Future studies should focus on the molecular mechanisms of flavonoids in lung cancer cells and consider their interactions with other treatment modalities to fully explore their therapeutic potential in lung cancer treatment.

3.1.4.3. Phytosterols

Phytosterols, plant-derived compounds structurally similar to cholesterol, are found in a variety of foods including vegetable oils, cereal grains, nuts, legumes, vegetables, and fruits. They are well-known for their cholesterol-lowering effects, achieved by reducing cholesterol absorption in the intestine. Beyond their role in cholesterol management, phytosterols have been studied for their potential protective effects against various chronic diseases, particularly cancer. These compounds exhibit anticancer properties through several mechanisms, including

inhibition of cell cycle progression, induction of apoptosis, and modulation of cholesterol synthesis (Shahzad *et al.,* 2017).

Research has increasingly focused on the relationship between phytosterols and cancer risk. Epidemiological and clinical studies suggest that phytosterols may offer protective benefits against multiple types of cancer. For instance, high phytosterol intake has been associated with a reduced risk of breast cancer. Studies indicate that diets rich in plant-based foods, which are high in phytosterols, are linked to lower incidences of breast cancer. This is supported by findings from a hospital-based case-control study in Mexico City, which reported that dietary phytochemicals, including phytosterols, were associated with a decreased risk of breast cancer among postmenopausal women. Additionally, the Mediterranean diet, known for its high phytosterol content, has been associated with a lower risk of breast cancer, further suggesting the potential protective role of these compounds (Shahzad *et al.,* 2017).

The protective effects of phytosterols extend to other types of cancer as well. For example, research suggests that phytosterols may help reduce the risk of prostate cancer. A notable study involving vegetarians found a 54% lower risk of prostate cancer compared to non-vegetarians, with higher intakes of dried fruits, which are rich in phytosterols, correlating with reduced prostate cancer risk. Similarly, phytosterols have shown potential in reducing colorectal cancer risk. While some animal studies suggest a chemoprotective effect of dietary phytosterols against colorectal cancer, findings from the Netherlands Cohort Study on Diet and Cancer did not show a significant association. However, a 2017 study in China found an inverse relationship between phytosterol intake and colorectal cancer risk, indicating a potential protective effect (Shahzad *et al.,* 2017).

Phytosterols may also be beneficial in the context of stomach and pancreatic cancers. A comprehensive case-control study in Uruguay found a significant inverse relationship between phytosterol intake and stomach cancer risk, with higher phytosterol levels associated with reduced incidence of this malignancy. Additionally, studies indicate that dietary sources rich in phytosterols, such as

legumes and dried fruits, are linked to a decreased risk of pancreatic cancer. These effects are thought to stem from phytosterols' influence on cholesterol metabolism and inflammation, both of which are crucial factors in cancer development (Shahzad *et al.,* 2017).

Despite the promising evidence, the full extent of phytosterols' anticancer potential remains under investigation. The mechanisms through which phytosterols exert their protective effects are not yet fully understood. Research has highlighted the need to further elucidate these mechanisms and confirm the findings through more extensive and rigorous studies. Additionally, challenges related to the bioavailability and metabolism of phytosterols must be addressed to optimize their therapeutic potential. Enhancing the stability and absorption of these compounds through novel formulations may improve their effectiveness as anticancer agents (Shahzad *et al.,* 2017).

Phytosterols, with their cholesterol-lowering properties and potential anticancer benefits, represent a significant area of interest in cancer prevention and therapy. Current research supports their role in reducing the risk of several cancers, including breast, prostate, colorectal, stomach, and pancreatic cancers. However, further studies are needed to confirm these benefits, understand the underlying mechanisms, and explore ways to enhance their bioavailability and therapeutic efficacy. As research continues to advance, phytosterols may become integral components in strategies for cancer prevention and treatment, offering valuable insights into dietary and therapeutic approaches to managing cancer risk.

3.1.4.4. Phenolic acid

Phenolic acids, which include hydroxybenzoic and hydroxycinnamic acids, are a diverse group of polyphenolic compounds prevalent in many common foods such as coffee, berries, nuts, tea, wine, and olive oil. Key phenolic acids like gallic, ellagic, caffeic, and ferulic acids are known for their significant biologically active properties and have long been used in traditional medicine. These compounds are recognized for their potential anticancer effects, which are thought to arise from

various mechanisms such as detoxification, prevention of cellular damage, inhibition of cell proliferation, and induction of apoptosis. Notably, recent research suggests that the pro-oxidant actions of caffeic and ferulic acids may be crucial to their anticancer activities, beyond their well-known antioxidant properties (Kumar and Goel, 2019).

Studies indicate that high dietary intake of phenolic acids, particularly caffeic and ferulic acids, is associated with a reduced risk of several cancers. For example, increased consumption of these acids has been linked to a lower risk of prostate and breast cancers. The mechanisms by which these phenolic acids exert their effects include inhibiting cancer cell proliferation and promoting programmed cell death, which is often disrupted in cancer cells. In breast cancer, a diet rich in phenolic acids has been shown to decrease cancer risk, likely due to their antioxidant and anti-inflammatory properties (Kumar and Goel, 2019).

In the context of lung cancer, phenolic acids, particularly when included in traditional Chinese medicine formulations, have demonstrated benefits such as improved quality of life and symptom relief for patients with non-small cell lung cancer (NSCLC). These formulations help in managing symptoms and may potentially slow disease progression. Additionally, topical formulations containing ferulic acid have shown promise in reducing skin cancer-related mutations, specifically thymine dimers, which are markers of UV-induced skin damage (Kumar and Goel, 2019).

Despite these encouraging findings, the full therapeutic potential of phenolic acids requires further exploration. While preliminary results are promising, especially in prostate and breast cancers, ongoing research is needed to confirm these benefits and understand the underlying mechanisms more clearly. Clinical trials are also underway to assess the effectiveness of phenolic acids, such as caffeic acid, in preventing other cancers like oesophageal cancer, though results are still pending.

Phenolic acids are emerging as significant components in cancer prevention and management strategies. Their potential benefits, driven by both their antioxidant and

pro-oxidant actions, highlight the need for continued research to fully leverage their therapeutic potential and integrate them into effective cancer prevention and treatment protocols.

3.1.4.5. Stilbenes

Stilbenes, a class of polyphenolic compounds with a 1,2-diphenylethylene structure, are present in a variety of plant-based foods, including fruits, vegetables, whole grains, chocolate, wine, olive oil, and tea. Notable stilbenes, such as resveratrol, pterostilbene, piceatannol, and pinosylvin, are recognized for their potential anticancer properties through antioxidant, anti-inflammatory, and pro-apoptotic effects. Resveratrol, in particular, has shown promise in modulating cancer-related pathways: it influences the Wnt signaling pathway in colorectal cancer, enhances Notch-1 activation in neuroendocrine tumors, and affects IGF-II levels in breast cancer. Despite these advances, the effectiveness of stilbenes, especially resveratrol, can be limited by rapid metabolism and low bioavailability. Research has shown mixed results, with some studies indicating potential benefits in cancer prevention and treatment, while others, such as those involving prostate cancer and multiple myeloma, suggest that higher doses or alternative formulations may be necessary. Clinical trials are ongoing to better understand these compounds' mechanisms and to improve their therapeutic efficacy, highlighting stilbenes' ongoing importance in cancer research and treatment (Al-Khayri *et al.,* 2023).

3.1.4.6. Carotenoids

Carotenoids are a diverse group of pigments prevalent in colorful fruits and vegetables, renowned for their antioxidant properties. Prominent carotenoids, including lycopene, β-carotene, α-carotene, lutein, and zeaxanthin, have been extensively researched for their potential role in cancer prevention. In prostate cancer, carotenoids such as lycopene, β-carotene, and α-carotene have been scrutinized for their protective effects. Meta-analyses indicate that higher intakes of lycopene and α-carotene are associated with a reduced risk of prostate cancer. Specifically, increased dietary and blood levels of lycopene correlate with a lower

risk of developing this cancer. Conversely, β-carotene's impact on prostate cancer risk is mixed, with some studies showing no significant association (Wang *et al.,* 2015).

For breast cancer, carotenoids like β-carotene, α-carotene, and lutein are linked to a decreased risk. Increased intake of β-carotene and higher blood levels of α-carotene and lutein exhibit protective effects against breast cancer. However, the relationship between lycopene and breast cancer risk remains unclear, with some studies failing to find a significant link (Dehnavi *et al.,* 2024).

Carotenoids also contribute to lung cancer prevention, with higher blood concentrations and dietary intake of lycopene and β-carotene associated with a reduced risk. However, high-dose β-carotene supplementation has been linked to an increased risk of lung cancer, particularly in smokers and individuals exposed to asbestos, highlighting that excessive β-carotene can be harmful (Böhm *et al.,*2021).

In gastrointestinal cancers, carotenoids such as β-carotene and lycopene are associated with reduced risks of esophageal, pancreatic, and gastric cancers. For colorectal cancer, higher serum levels of α-carotene, β-cryptoxanthin, and lycopene are linked to lower risk, with β-cryptoxanthin showing a particularly strong inverse association (Institute of Medicine (US), 2000).

The phytochemicals including carotenoids, phenolic acids, phytosterols, and stilbenes exhibit diverse anticancer properties through mechanisms such as neutralizing free radicals, reducing inflammation, inducing apoptosis, and inhibiting cell proliferation and invasion. Despite these benefits, high doses or isolated supplements may pose risks or offer limited advantages, emphasizing the importance of a balanced, plant-based diet.

The evidence suggests that dietary intake of these phytochemicals offers more substantial cancer prevention benefits compared to isolated supplements. The synergistic effects of various phytochemicals within whole foods are likely more effective in reducing cancer risk than individual compounds consumed separately.

As research progresses, ongoing and future clinical trials will be essential to investigate the safety, efficacy, and optimal dosing of phytochemicals for cancer prevention and treatment. These trials will provide valuable insights into the potential risks and benefits of these compounds, enhancing our understanding of their role in cancer prevention strategies.

While phytochemicals show promising anticancer properties in various studies, the complexity of their interactions and the risk of adverse effects from high-dose supplements necessitate careful consideration. A diet rich in these compounds, obtained from whole foods, may offer the most beneficial approach to leveraging phytochemicals for cancer prevention.

3.1.5. *Melia dubia*: an overview of its medicinal significance

Systematic position of *Melia dubia*:

Order: Sapindales

Family: Meliaceae

Genus: Melia

Species: dubia

Melia dubia, commonly referred to as Malabar Neem (Figure 3.1.1), is a plant species of significant interest due to its multifaceted applications in both commercial and medicinal fields. This tree, native to India and parts of Southeast Asia, has gained prominence for its rapid growth, versatile utility, and promising health benefits. This overview highlights the commercial importance and medicinal value of *Melia dubia*, underscoring its potential contributions to various industries and therapeutic areas.

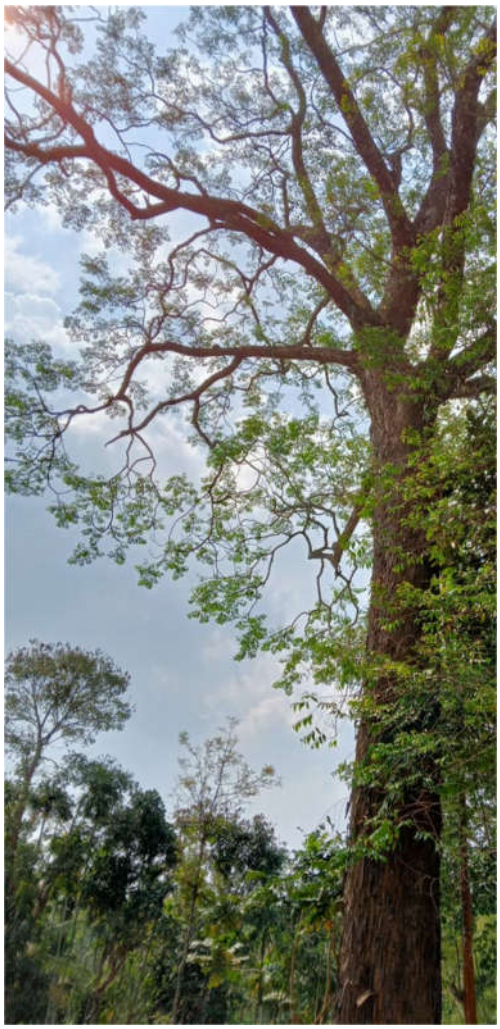


Figure 3.1.1. *Melia dubia* tree in its habitat

3.1.5.1. Antioxidant activity

Melia dubia is recognized for its potent antioxidant properties, which are crucial in addressing oxidative stress linked to various health conditions. Oxidative stress, caused by an imbalance between free radicals and antioxidants, is a contributing factor to diseases such as cancer, diabetes, and inflammation. Research has demonstrated that the ethanolic extract of *Melia dubia* exhibits strong antioxidant activity, with an IC_{50} value of 16.89 μg/ml in the nitric oxide radical scavenging assay. This activity is attributed to the plant's rich content of phenolic compounds and flavonoids, which play a key role in neutralizing harmful free radicals (Valentina *et al.,* 2013; Ahmad *et al.,* 2008; Charde *et al.,* 2010; Shah *et al.,* 2016).

The antioxidant properties of *Melia dubia* not only support its potential in preventing oxidative damage but also highlight its possible use in developing supplements and therapeutic agents aimed at reducing oxidative stress-related diseases.

3.1.5.2. Anticancer activity

The anticancer potential of *Melia dubia* is a significant area of interest in current research. No significant studies are to substantiate the notable anticancer properties of the plant. Further research supports the plant's anticancer effects, with various studies confirming its potential as a valuable component in cancer therapy (Anusuya *et al.,* 2011; Husseiny *et al.,* 2015; Saber *et al.,* 2018). The ongoing exploration of *Melia dubia*'s anticancer properties underscores its potential to contribute to the development of novel cancer treatments and preventive strategies.

3.1.5.3. Antimicrobial activity

Melia dubia also exhibits significant antimicrobial activity, making it a valuable resource for addressing infectious diseases. The essential oil extracted from the leaves of *Melia dubia* demonstrates both bacteriostatic and fungistatic properties against a range of pathogens, including *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Fusarium oxysporum*, and *Candida albicans* (Nagalakshmi

et al., 2003). This broad-spectrum antimicrobial activity highlights the plant's potential as a natural remedy for infections.

The leaf and root extracts have also demonstrated effectiveness against a variety of bacterial pathogens, including *Klebsiella pneumoniae*, *E. coli, Staphylococcus aureus, Salmonella typhi*, and *Salmonella paratyphi* (Chanthuru *et al.,* 2014; Malarvannan *et al.,* 2009). These findings emphasize *Melia dubia*'s potential in developing antimicrobial agents for treating bacterial and fungal infections.

3.1.5.4. Antidiabetic activity

The antidiabetic properties of *Melia dubia* are supported by several studies indicating its potential as a natural treatment for diabetes. The alcoholic extract of *Melia dubia* fruits has demonstrated significant hypoglycemic effects in animal models, with a reduction in blood glucose levels at a dose of 300 mg/kg body weight (Susheela *et al.,* 2008). This effect supports the plant's role as an effective hypoglycemic agent and highlights its potential for managing diabetes.

Additional research reinforces the antidiabetic properties of *Melia dubia*, suggesting that it could be integrated into diabetes management strategies as a natural therapeutic option (Mamun-or-Rashid *et al.,* 2014). The plant's ability to regulate blood glucose levels underscores its significance in addressing one of the most prevalent health challenges of our time.

In terms of medicinal value, *Melia dubia* exhibits impressive antioxidant, anticancer, antimicrobial, and antidiabetic properties. The ongoing research into these aspects highlights the plant's potential in developing therapeutic agents and natural remedies. As studies continue to explore its benefits, *Melia dubia*'s role in enhancing health and promoting sustainability is likely to grow, further establishing its multifaceted value in both commercial and medicinal contexts.

3.1.6. Research gap

Melia dubia, commonly known for its significant economic value and applications in traditional medicine, has been studied extensively for its anti-inflammatory,

antibacterial, and other therapeutic activities. However, there remains a notable research gap regarding its anticancer properties. Despite the plant's established pharmacological effects, there is a scarcity of studies investigating its potential as an anticancer agent.

Current research primarily focuses on *Melia dubia*'s utility in areas such as pest control, timber production, and traditional medicinal uses. Although some bioactive compounds from the plant have been isolated and studied, their anticancer properties have not been thoroughly explored. This lack of comprehensive research represents a missed opportunity to uncover and characterize novel anticancer compounds that could lead to the development of new cancer therapies.

Addressing this gap involves conducting systematic investigations to identify and isolate specific compounds from *Melia dubia* with potential anticancer activity. This includes screening extracts for cytotoxic effects against cancer cell lines, elucidating their mechanisms of action, and evaluating their efficacy in preclinical models. By bridging this research gap, it is possible to harness the full therapeutic potential of *Melia dubia* and contribute to the discovery of new and effective cancer treatments.

MATERIALS AND METHODS

3.2.1. Chemicals

Solvents of HPLC/NMR grade (n-Hexane, chloroform, petroleum ether, ethyl acetate, acetone, methanol, and toluene) were purchased from SRL or Merck. Precoated aluminium Silica gel plates were obtained from Merck. Silica gel for column chromatography with three mesh sizes was purchased from SRL. Animal tissue culture tested Mc Coy's 5A medium and trypsin were obtained from Himedia, FBS and antibiotic antimycotic solution was obtained from Gibco. All other chemicals used in tissue culture were of animal tissue culture tested grade and others are of analytical grade.

3.2.2. Sample collection

M. dubia leaves (Figure 3.2.1) were collected from Meenangadi, Wayanad (11.6603º N, 76.1629º E). The plant material was authenticated by Dr A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut. A voucher specimen (Voucher number: 7187) was deposited in the Herbarium of the Department of Botany, University of Calicut.

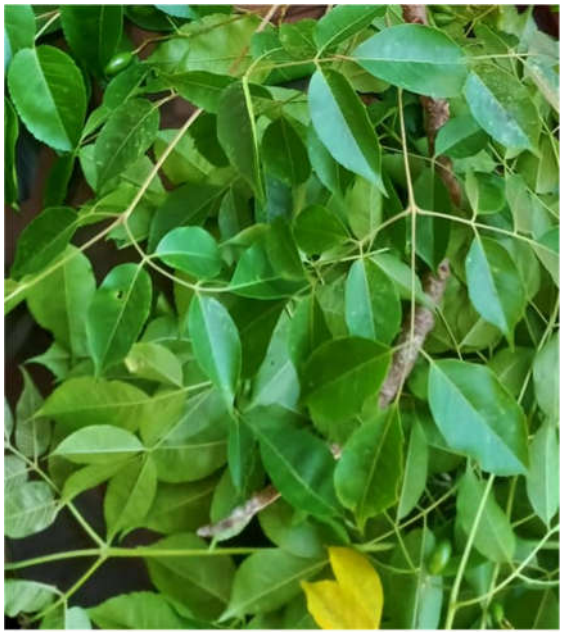


Figure 3.2.1. Twig of *M. dubia* with leaves collected for sample preparation.

3.2.3. Crude extract preparation

The collected leaves were thoroughly washed under running water and then dried in an oven at 40°C. Once dried, they were ground into a fine powder. Initially, 200 grams of the powdered leaf material were subjected to extraction using n-hexane, a non-polar solvent capable of dissolving a broad spectrum of organic compounds. Following the n-hexane extraction, the residue was further extracted with chloroform followed by ethyl acetate, acetone, methanol, and water as described in

the first chapter to obtain compounds with potentially anticancer properties. The chloroform extract, rich in various phytochemicals, was subsequently processed through a series of chromatographic techniques. These techniques aimed to purify and fractionate the extract, thereby isolating and enriching the fractions containing bioactive molecules with potential anticancer activity.

3.2.4. Enrichment of anticancer fractions from *M. dubia* leaf extract

The initial step involved silica gel column chromatography, a technique widely used for separating mixtures of compounds based on their different affinities to the stationary phase (silica gel) and the mobile phase (solvent system). The silica gel used here had a specific particle size range of mesh, which provided an optimal balance between separation efficiency and flow rate. The column was packed meticulously to avoid air bubbles, which could disrupt the flow and lead to uneven separation.

The elution process commenced with non-polar n-hexane and progressively transitioned through a gradient of increasingly polar solvents. This gradient included chloroform, ethyl acetate, acetone, and methanol, each mixed with water in varying ratios. The gradual increase in polarity of the solvents facilitated the elution of compounds from the column based on their polarity, allowing for the separation of the complex mixture into distinct fractions. Sub-fractionation was carried out multiple times to refine the separation further and ensure the removal of non-specific compounds, thus enhancing the purity and specificity of the fractions.

3.2.4.1. First column chromatography

The first stage of column chromatography involved a precise and methodical procedure. A total of 2.1 grams of the *M. dubia* leaf chloroform extract was carefully combined with silica gel (mesh size 60-120), ensuring that the sample was uniformly distributed and free of air bubbles before being applied to the pre-packed silica gel column (300 x 25 mm). The elution began with pure n-hexane (100%, 100 mL), a non-polar solvent ideal for eluting non-polar compounds. Following the n-

hexane, the column was eluted with a series of solvent mixtures with increasing polarity: n-hexane and chloroform (80:20, 60:40, 40:60, and 20:80). Each mixture was prepared to a specific ratio to fine-tune the separation process. This step was followed by pure chloroform (100%, 100 mL), a slightly more polar solvent that helped to elute more polar compounds that did not elute with n-hexane. The process continued with mixtures of chloroform ethyl acetate (80:20, 60:40, 40:60, 20:80), followed by pure ethyl acetate (100%, 100 mL). As the polarity increased, the ability of the solvents to elute more polar compounds also increased. The next step involved eluting the column with ethyl acetate and acetone mixtures (80:20, 60:40, 40:60, 20:80), followed by pure acetone (100%, 100 mL), and then acetone and methanol mixtures (80:20, 60:40, 40:60, 20:80). The final elution was done with pure methanol (100%, 100 mL), the most polar solvent used in this sequence, to elute the most polar compounds. The fractions collected were numbered as SC1-F1, SC1-F2, SC1-F3 etc.

3.2.4.2. Second column chromatography

The fractions collected from the first column chromatography were analysed, and the fractions numbered 8 and 9 were identified for further processing due to their promising bioactivity profiles. These fractions, weighing a combined total of 800 mg, were again mixed with silica gel to ensure proper application onto the next chromatography column. This column, measuring 300 mm in length and 25 mm in diameter and filled with 100-200 mesh silica gel, was similarly packed with care to prevent air bubble formation. Elution in the second column chromatography was again performed with an increasing polarity gradient. It started with pure n-hexane (100%, 100 mL), followed by a series of n-hexane and toluene mixtures (80:20, 60:40, 40:60, 20:80). The elution continued with pure toluene (100%, 100 mL), a solvent that, while non-polar, has a different solvation power compared to n-hexane, potentially eluting different compounds. Subsequent elutions involved toluene and ethyl acetate mixtures (90:10, 80:20, 60:40, 40:60, 20:80), followed by pure ethyl acetate (100%, 100 mL). The process further included ethyl acetate and acetone mixtures (80:20, 60:40, 40:60, 20:80), pure acetone (100%, 100 mL), acetone and

methanol mixtures (80:20, 60:40, 40:60, 20:80), and concluded with pure methanol (100%, 100 mL). This sequence allowed for the gradual and selective elution of compounds based on their affinity to the stationary phase versus the mobile phase. The fractions collected were numbered as SC2-F1, SC2-F2, SC2-F3 etc.

3.2.4.3. Third column chromatography

The third stage of purification was focused on fraction F8 from the second chromatography, which had shown potential bioactivity. This fraction, now weighing 250 mg, was dissolved in chloroform and prepared for further fractionation. The column used was smaller (200 mm x 15 mm) and packed with a finer silica gel (200-400 mesh) to achieve a higher resolution separation. The elution process followed a similar pattern to previous steps, beginning with pure n-hexane (100%, 100 mL) and moving through a gradient of increasing polarity. This included n-hexane and toluene mixtures (80:20, 60:40, 40:60, 20:80), pure toluene (100%, 100 mL), toluene and ethyl acetate mixtures (90:10, 80:20, 60:40, 40:60, 20:80), pure ethyl acetate (100%, 100 mL), ethyl acetate and acetone (50:50), pure acetone (100%, 100 mL), acetone and methanol (50:50), and pure methanol (100%, 100 mL).

This thorough and systematic approach ensured that the compounds within the fractions were increasingly purified and that compounds with similar polarities were grouped together, simplifying further analysis and characterization. The collected fractions were numbered as SC3-F1, SC3-F2, SC3-F3, etc.

3.2.4.4. Thin Layer Chromatography (TLC)

Following the third round of column chromatography, fraction F8 was subjected to thin layer chromatography (TLC) for additional separation. The TLC plate was developed using a mobile phase of toluene and ethyl acetate in an 80:20 v/v ratio. This solvent system was chosen based on preliminary trials to effectively separate the components of the fraction. After development, the TLC plate was visualized under ultraviolet (UV) light, which revealed distinct bands representing different

compounds. These bands were indicative of different components present in the fraction. The individual bands were carefully scraped off, and the compounds were eluted using the same solvent mixture used for the TLC. This step provided further purification and allowed for the isolation of individual compounds for subsequent analysis. The eluted bands were designated as TLC-B1, TLC-B2, TLC-B3, etc.

Yield of each bioactive fraction obtained at each step of purification was also calculated based on the initial weight used for the purification.

3.2.5. High-Performance Thin-Layer Chromatography (HPTLC)

High-performance thin-layer chromatography (HPTLC) was employed for a more refined analysis the isolated fractions with activity. HPTLC offers enhanced resolution and sensitivity compared to traditional TLC, making it ideal for both qualitative and quantitative analysis of complex mixtures. The HPTLC analysis was conducted using a CAMAG system equipped with pre-coated aluminium-backed silica gel plates (Merck 60 F254, 0.2 mm thickness). The application of samples onto the HPTLC plates was performed with high precision using the CAMAG ATS 4 system, which ensured uniform application of the samples as 10 mm bands positioned 10 mm from the bottom of the plate. This precise application is crucial for obtaining reproducible results. The development of the HPTLC plates was carried out in a Twin Trough Chamber pre-saturated with the mobile phase, a mixture of toluene and acetate in an 80:20 v/v ratio. The development process was performed in ascending mode, with the solvent front allowed to reach a height of 80 mm. The development was conducted at room temperature, a condition carefully maintained to ensure consistent solvent evaporation rates and, consequently, consistent separation results. Post-development, the plates were air-dried and subsequently scanned at two different wavelengths—254 nm and 366 nm—using a CAMAG TLC Scanner 3 equipped with a deuterium lamp. The use of these specific wavelengths is based on the absorption characteristics of the compounds present in the samples. The scanning process was controlled and analysed using winCATS software, which provided detailed densitometric data on the separated components.

For additional visualization, the plates were derivatized with anisaldehyde-sulfuric acid reagent, a common staining agent that reacts with a wide range of organic compounds, producing coloured products visible under white light (550 nm). This derivatization step allowed for the visual identification of compounds that might not have been detectable under UV light, thereby providing a comprehensive profile of the chemical constituents.

3.2.6. GC-MS analysis

To further characterize the chemical composition of the chloroform extract from M. dubia leaves, gas chromatography-mass spectrometry (GC-MS) was employed. The analysis was performed using a Shimadzu GC system equipped with an ELITE-5MS capillary column, which has a length of 30 meters, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm. The choice of this column provided a good balance between separation efficiency and analysis time. The temperature program was carefully optimized to ensure effective separation of the components. It started at a relatively low temperature of 80°C, held for 1 minute, then ramped up at a rate of 5°C per minute to a final temperature of 260°C. This temperature program was designed to gradually elute compounds based on their volatility and thermal stability. Helium was used as the carrier gas at a constant flow rate of 1 mL per minute, providing an inert and stable environment for the separation. A sample volume of 1 μL was injected into the system, a standard volume that ensures adequate detection sensitivity while preventing column overloading.

The mass spectrometric analysis was performed using an electron ionization (EI) source, a method that ionizes the compounds, allowing for their detection and identification based on their mass-to-charge ratios (m/z). The identification of the compounds was achieved by comparing the obtained mass spectra with reference spectra in the NIST11 and WILEY 8 libraries. These libraries contain extensive databases of known compounds, facilitating the accurate identification of the components present in the extract. The GC-MS analysis provided detailed information on the molecular structure and composition of the compounds in the

extract, contributing to the understanding of the bioactive constituents responsible for the observed anticancer activity.

3.2.7. Cytotoxicity analysis and IC_{50} calculation

The bioactivity-based purification of the compounds was conducted using the HT29 cancer cell line at a concentration of 100 µg/mL. Dimethyl sulfoxide (DMSO) served as the vehicle control and the cell death in the vehicle control was considered as 0.00%. Cytotoxicity analysis was performed using the MTT assay, following the protocol detailed in the first chapter (Mosmann, 1983). To determine the efficacy of the crude extract and its active fractions, the HT29 cells were treated with varying concentrations of these substances. The IC_{50} values, indicating the concentration at which 50% of cell viability is inhibited, were calculated for each treatment. All assays were conducted over a 48-hour period.

3.2.8. Molecular docking studies

In silico molecular docking studies were performed to investigate the binding interactions between ligands and target proteins using AutoDockVina software (Trott and Olson 2010). Native 3D structures of target proteins were downloaded from the RCSB PDB database, (https://www.rcsb.org/) (Berman *et al*., 2000) followed by preparation for docking through Discovery Studio 2021 client software. Ligand structures, identified from GC–MS analysis, were obtained from the PubChem database (Kim *et al*., 2016) and prepared using Open Babel GUI (O'Boyle *et al.*, 2011) for virtual molecular screening. Multiple ligand docking was conducted using PyRx software, and the resulting protein-ligand interaction models were visualized in 3D using Discovery Studio 2021. The compounds from the active fraction, TLC-B3 were converted into PDBQT format with MGLTools 1.5.7, while crucial apoptosis-related proteins, including caspase-3 (PDB ID-3DEI), caspase-9 (PDB ID-1JXQ), and TP53 (PDB ID-3DCY), were prepared by removing water molecules, adding Kollman charges and polar hydrogens, and converting them into pdbqt format. PyRx (Dallakyan and Olson, 2015) software was used to generate

receptor grids and execute molecular docking, with the best docking scores visualized using Discovery Studio Visualizer.

3.2.9. Statistical analysis

The data for continuous variables were presented as mean±SEM, obtained from multiple independent experiments. Statistical significance was assessed using one-way ANOVA with Tukey's post hoc test, utilizing IBM SPSS Statistics 24 software and Dunnett's method using Graphpad Prism. The IC_{50} values were determined through Probit regression analysis. A p-value of less than 0.05 was considered to indicate statistical significance, reflecting a significant difference between the treatment groups and the controls.

RESULTS

3.3.1. Purification using silica gel column chromatography

In this study, the anticancer potential of *M. dubia* was explored through serial exhaustive extraction of the oven-dried leaf powder using six different organic solvents, including water, arranged in increasing polarity. The resulting extracts were tested for their cytotoxic effects on the HT29 colorectal carcinoma cell line using the MTT assay. The chloroform extract demonstrated the highest cytotoxic activity among the tested extracts, prompting further investigation to isolate the active anticancer components.

3.3.1.1. First column chromatography

The chloroform extract subjected to silica gel column chromatography, and the resulting fractions were assessed for their cytotoxicity against the HT29 cell line. Among the fractions obtained from the first column chromatography, the fraction eluted with chloroform and ethyl acetate in a ratio of 80:20 showed the highestn mortality rate of 84.48±1.94% and the fraction is designated as **SC1-F8 (Silica gel Column fraction 8)**. This was followed by the fraction eluted with chloroform and ethyl acetate in a 60:40 ratio, which exhibited a mortality rate of 80.33±2.05%. Other fractions eluted showed significant but lower mortality rates (Table 3.3.1).

3.3.1.2. Second silica gel column chromatography

Further fractionation of the most active fractions (SC1-F8 and SC1-F9) from the first column chromatography revealed that the fraction eluted with toluene and ethyl acetate in a ratio of 80:20 exhibited the highest cytotoxicity, with a mortality rate of 99.46±1.33% and the fraction is designated as **SC2-F8 (Silica gel Column fraction 8)**. Other fractions showed much lower cytotoxic effects (Table 3.3.2).

Table 3.3.1. Percentage mortality of fractions obtained from silica column chromatography 1 against HT29 cell line.

Elute No.	Solvent Mixture used	Ratio in % (v/v)	Percentage Mortality (48 hours)
1	n-Hexane	100	21.35±3.53
2	n-Hexane: Chloroform	80::20	20.89±4.10
3	n-Hexane: Chloroform	60::40	26.66±1.08
4	n-Hexane: Chloroform	40::60	18.75±5.49
5	n-Hexane: Chloroform	20::80	15.52±2.41
6	Chloroform	100	30.41±3.33
7	Chloroform: Ethyl acetate	90::10	69.59±6.28
8	**Chloroform: Ethyl acetate**	**80::20**	**84.48±1.94**
9	**Chloroform: Ethyl acetate**	**60::40**	**80.33±2.05**
10	Chloroform: Ethyl acetate	40::60	74.93±2.13
11	Chloroform: Ethyl acetate	20::80	70.56±1.67
12	Ethyl acetate	100	64.12±2.58
13	Ethyl acetate:: Acetone	90::10	36.21±4.64
14	Ethyl acetate:: Acetone	80::20	23.64±5.25
15	Ethyl acetate:: Acetone	60::40	25.07±3.94
16	Ethyl acetate:: Acetone	40::60	21.55±7.12
17	Ethyl acetate:: Acetone	20::80	20.53±4.67
18	Acetone	100	22.83±3.33
19	Acetone:: Methanol	90::10	26.19±3.75
20	Acetone:: Methanol	80::20	23.76±4.55
21	Acetone:: Methanol	60::40	29.44±3.70
22	Acetone:: Methanol	40::60	19.96±3.67
23	Acetone:: Methanol	20::80	18.63±9.19
24	Methanol	100	10.63±2.75

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

3.3.1.1. Third silica gel column chromatography

A third round of column chromatography was conducted to further purify the active components from the bioactive fraction from second column chromatography (SC2-F8). The fraction eluted with toluene and ethyl acetate in an 80:20 ratio remained the most cytotoxic, with a mortality rate of 98.86±1.35% and the fraction is designated as **SC3-F8 (Silica gel Column fraction 8)**. This result was consistent with the

previous findings, indicating the presence of a highly potent anticancer compound in this fraction (Table 3.3.3).

Table 3.3.2. Percentage mortality of fractions obtained from column chromatography 2.

Elute No.	Solvent Mixture used	Ratio in % (v/v)	Percentage Mortality (48 hours)
1	n-Hexane	100	2.61±2.45
2	n-Hexane: Toluene	80::20	0.91±3.32
3	n-Hexane: Toluene	60::40	9.91±1.33
4	n-Hexane: Toluene	40::60	11.55±3.85
5	n-Hexane: Toluene	20::80	11.99±2.03
6	Toluene	100	23.81±4.41
7	Toluene: Ethyl acetate	90::10	20.61±4.61
8	**Toluene: Ethyl acetate**	**80::20**	**99.46±1.33**
9	Toluene: Ethyl acetate	60::40	62.97±2.45
10	Toluene: Ethyl acetate	40::60	21.48±1.03
11	Toluene: Ethyl acetate	20::80	11.72±5.13
12	Ethyl acetate	100	10.6±3.36
13	Ethyl acetate:: Acetone	90::10	21.69±0.67
14	Ethyl acetate:: Acetone	80::20	19.46±2.43
15	Ethyl acetate:: Acetone	60::40	37.06±4.42
16	Ethyl acetate:: Acetone	40::60	36.48±3.70
17	Ethyl acetate:: Acetone	20::80	13±8.18
18	Acetone	100	25.08±6.46
19	Acetone:: Methanol	90::10	37.63±6.82
20	Acetone:: Methanol	80::20	24.55±7.38
21	Acetone:: Methanol	60::40	23.79±2.55
22	Acetone:: Methanol	40::60	5.25±7.17
23	Acetone:: Methanol	20::80	8.78±1.78
24	Methanol	100	24.35±6.11

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

3.3.1.1. Thin layer chromatography

The fraction from the third column chromatography (SC3-F8) was further separated using thin layer chromatography (TLC). The bands obtained were tested for cytotoxicity against HT29 cells. Band 3 showed the highest cytotoxicity with a mortality rate of 94.70±1.09% and the fraction is designated as **TLC-B3 (Thin**

layer chromatography band 3), the band containing the most active component (Table 3.3.4).

Table 3.3.3. Percentage mortality of fractions obtained from column chromatography 3.

Elute No.	Solvent Mixture used	Ratio in % (v/v)	Percentage Mortality (48 hours)
1	n-Hexane	100	8.72±4.18
2	n-Hexane: Toluene	80::20	14.35±1.38
3	n-Hexane: Toluene	60::40	32.16±1.56
4	n-Hexane: Toluene	40::60	23.08±3.34
5	n-Hexane: Toluene	20::80	36.52±4.91
6	Toluene	100	35.11±3.69
7	Toluene: Ethyl acetate	90::10	31.89±4.38
8	**Toluene: Ethyl acetate**	**80::20**	**98.86±1.35**
9	Toluene: Ethyl acetate	60::40	9.17±4.70
10	Toluene: Ethyl acetate	40::60	12.98±4.44
11	Toluene: Ethyl acetate	20::80	14.34±1.32
12	Ethyl acetate	100	9.06±4.19
13	Ethyl acetate:: Acetone	50::50	0.85±1.78
14	Acetone	100	14.70±1.88
15	Acetone:: Methanol	50::50	32.57±3.41
16	Methanol	100	18.19±8.31

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

Table 3.3.4. Percentage mortality of fractions obtained from thin layer chromatography.

Sl. No.	Extracted bands	Percentage Mortality (48 hours)	p value
1	Band 1	19.16±4.27	<0.001
2	Band 2	28.25±1.88	<0.001
3	**Band 3**	**94.70±1.09**	**<0.001**
4	Band 4	32.57±3.46	<0.001
5	Band 5	19.18±6.53	<0.001

The percentage mortality above 3.50% compared to control are significant with a p value <0.05. Difference in percentage mortality of the DMSO control compared to cell alone (without DMSO) is not significant (p>0.05). Percentage mortalities above 90.00% are shown in bold.

3.3.2 Determination IC_{50} concentration

The IC_{50} values for the crude extract and the fractions were determined to quantify the potency of the active components. The crude extract had an IC_{50} value of 61.26±2.55 µg/mL. Among the fractions, TLC-B3 exhibited the lowest IC_{50} value of 7.87±1.32 µg/mL, indicating it as the most potent fraction. This was followed by SC3-F8, SC2-F8, and SC1-F8, with IC_{50} values of 10.27±0.79 µg/mL, 17.86±1.29 µg/mL, and 31.07±4.46 µg/mL, respectively (Table 3.3.5, Figure 3.3.1).

Table 3.3.5. Yield and fold changes in IC_{50} concentrations on HT29 cell line, at each step of purification

Sl. No.	Sample/Fraction	Yield (g%)	IC_{50} in µg/mL on HT29-48 hours	Fold decrease in IC_{50}
1	Leaf powder	-	-	-
2	Chloroform extract	26 .00	61.26±2.55	-
3	SC1-F8	12.25	31.07±4.46	1.97
4	SC2-F8	3.44	17.86±1.29	1.74
5	SC3-F8	0.94	10.27±0.79	1.74
6	TLC-B3	0.01	7.87±1.32	1.30

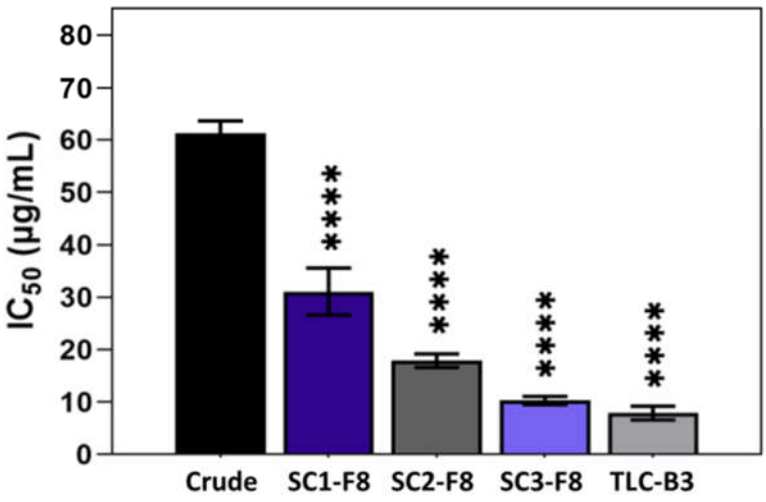


Figure 3.3.1. IC_{50} concentrations of the extract and fractions at each step of purification on HT29 cell line.

3.3.3. Characterization of the bioactive fractions by High-performance thin-layer chromatography (HPTLC)

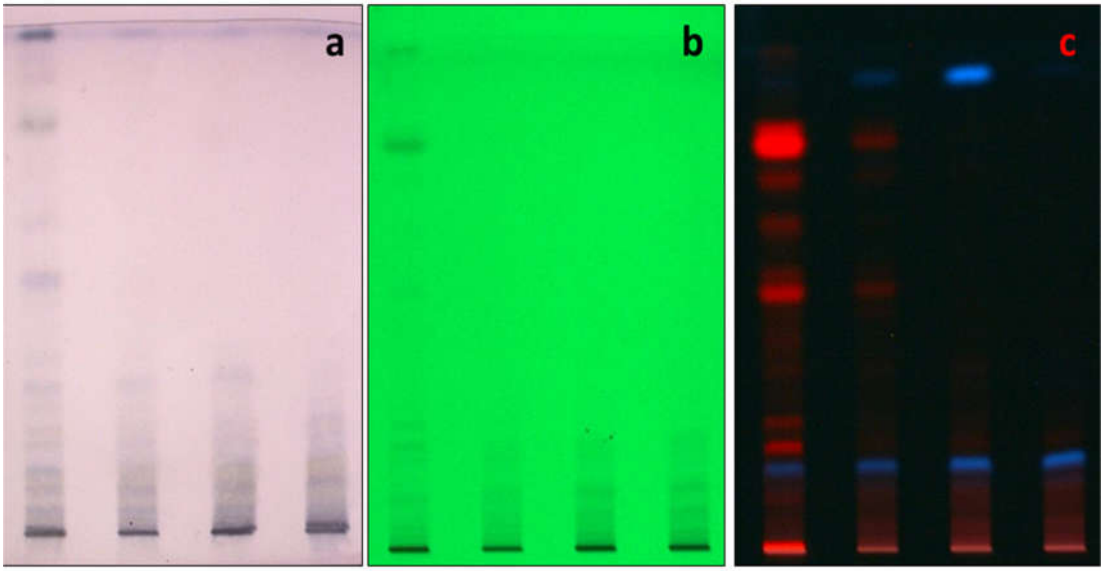


Figure. 3.3.2. HPTLC chromatogram of the crude and bioactive fractions at each stage of purification visualised (a) under white light after derivatization, (b) UV 254 nm prior to derivatization and (c) UV 366 nm prior to derivatization. Tracks: (1) *M. dubia* chloroform crude extract; (2): Active fraction obtained from first column chromatography- SC1-F8 (F1); (3): Active fraction obtained from second column chromatography- (SC2-F8); (4): Active fraction obtained from third column chromatography- SC3-F8).

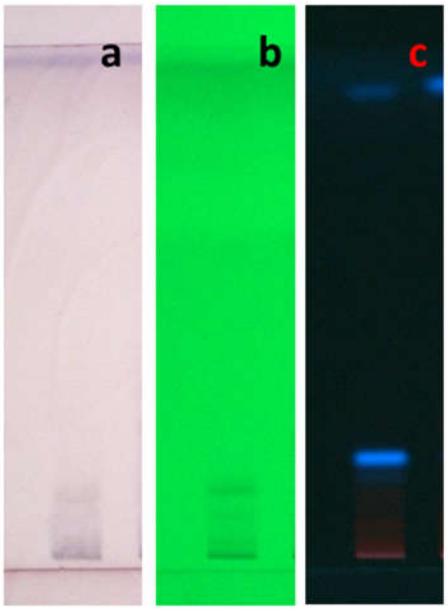


Figure. 3.3.3. HPTLC chromatogram of the bioactive fractions of TLC (TLC-B3) visualised (a) under white light after derivatization, (b) UV 254 nm prior to derivatization and (c) UV 366 nm prior to derivatization.

Crude extract, bioactive fractions from column chromatography1, 2 and 3 and the eluted band from TLC were loaded on to HPTC and separated This study focuses on optimizing HPTLC and separated using a mobile phase of toluene::ethyl acetate (80::20 v/v). The HPTLC chromatograms were examined under UV light (366 nm and 254 nm) and white light (following derivatisation with sulphuric acid/p-anisaldehyde). The bioactive fraction TLC-B3 from TLC when loaded on to HPTLC gave 6 prominent bands (Figures 3.3.2, 3.3.3).

3.3.4. Characterization of the bioactive fraction by GC-MS analysis

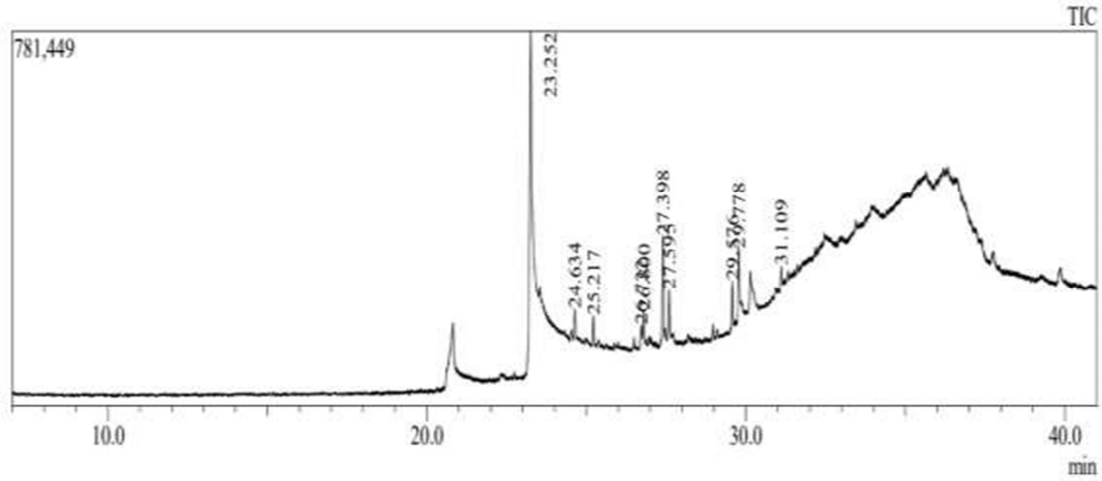


Figure 3.3.4. Chromatogram of the TLC-B3 obtained via GC-MS analysis.

Table 3.3.6. List of phytochemicals identified in the chloroform extract of TLC-B3 via GC-MS analysis.

Peak	Area%	Height%	Name
1	68.07	45.1	cis-13-Eicosenoic acid
2	2.13	3.76	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
3	2.17	3.95	Glycidyl palmitate
4	2.30	3.12	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester
5	1.99	4.31	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
6	7.32	13.99	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
7	3.82	6.36	Stearic acid, TMS derivative
8	3.45	6.50	3,11-Dioxa-2,12-disilatridecane,2,2,4,4,8,12,12-heptamethyl-
9	6.86	9.65	Petroselinic acid, TMS derivative
10	1.88	3.26	Oleanan-29-oic acid, 3-(acetyloxy)-12,13-epoxy-11-oxo-, methyl ester, (3.be

The GC-MS analysis of the bioactive fraction from TLC (TLC-B3) identified a diverse range of compounds with various retention times and concentrations. The most prominent compound was cis-13-Eicosenoic acid, making up 68.07% of the total area and 45.1% of the total height. Other significant compounds included 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-, Glycidyl palmitate, 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester, 3,11-Dioxa-2,12-disilatridecane-2,2,4,4,8,12,12-heptamethyl-, Petroselinic acid, Oleanan-29-oic acid, 3-(acetyloxy)-12,13-epoxy-11-oxo-, methyl ester.

3.3.5. Molecular docking studies

For molecular docking studies, the ligands identified from the TLC-B3 fraction in the GC–MS analysis were docked against key apoptotic proteins, including caspase-3 (PDB ID-3DEI), caspase-9 (PDB ID-1JXQ), and P53 (PDB ID-3DCY). The 3D structures of these proteins were obtained from the PDB database, and the docking results were visualized and analysed using Discovery Studio Client 2021. The in silico docking study revealed the interactions between the ligands and caspase-3, caspase-9, and P53, with the binding scores detailed in Table 6. Among the tested ligands, Petroselinic acid exhibited the highest binding energy towards caspase-3 (-6.1 kcal/mol), while 9-Octadecenoic acid displayed a binding energy of -6.0 kcal/mol towards caspase-3. Petroselinic acid also showed the strongest binding affinity for P53 (-5.7 kcal/mol) and caspase-9 (-5.7 kcal/mol). These findings suggest these compounds may be responsible for the observed cytotoxicity mediated through apoptosis.

Table 3.3.7. Binding energy of the ligand molecules docked on to the major apoptotic proteins.

Ligand	Binding Affinity (kcal/mol)		
	P53	Caspase 3	Caspase 9
Petroselinic acid	-5.7	-6.1	-5.7
9-Octadecenoic acid	-4.6	-6.0	-5.2
Eicosenoic acid	-3.9	-5.5	-5.2
Stearic acid	-5.6	-5.4	-4.0

DISCUSSION

To identify bioactive fraction from the crude chloroform extract of *M. dubia* leaves, sequential chromatography was conducted. This includes three column chromatography steps followed by a thin layer chromatography. During the purification step, the enrichment of bioactive fractions is reflected in the decrease in IC_{50} values in the bioactive fractions from different steps of purification.

In the present study, the IC_{50} values of both the crude extract and various fractions of *Melia dubia* were evaluated to determine the potency of their active compounds. The crude extract exhibited an IC_{50} value of 61.26±2.55 µg/mL. Among the fractions, TLC3-B3 had the lowest IC_{50} value of 7.87±1.32 µg/mL, indicating it as the most potent fraction. Following this, the fractions SC3-F8, SC2-F8, and SC1-F8 displayed IC_{50} values of 10.27±0.79 µg/mL, 17.86±1.29 µg/mL, and 31.07±4.46 µg/mL, respectively. These results underscore the significant cytotoxic potential of TLC-B3, suggesting it contains highly active compounds that promote cancer cell death. As purification progressed, the IC_{50} values continued to decrease, falling within the National Cancer Institute (NCI) criteria (IC_{50} < 30 µg/mL) for anticancer drugs.

The HT29 cell line was used to measure bioactivity and calculate the IC_{50} values. The crude chloroform extract showed an IC_{50} that was 1.97 times higher than the IC_{50} of the active fraction (SC1-F8) obtained through the first round of silica gel column chromatography. The IC_{50} of SC1-F8 was 1.74 times higher than that of the active fraction obtained from the second round of silica gel chromatography (SC2-F8), which, in turn, was 1.74 times greater than the active fraction from the third round of silica gel chromatography (SC3-F8). The IC_{50} of the final purified fraction TLC-B3 was 1.30 times lower than SC3-F8 and 7.78 times lower than the crude chloroform extract. This progressive reduction in IC_{50} values highlights the effectiveness of the purification process, resulting in a 7.78-fold purification of the active components.

The purification technique used by Umthong *et al.* to partially purify bioactive fractions with anticancer activity from the ethanol extract of *Trigona laeviceps* propolis, a stingless bee, is comparable to the method employed in this study. In their research, the extract's cytotoxicity was tested on five cancer cell lines (Chago, Kato-III, SW620, BT474, and Hep-G2) and two normal cell lines (CH-liver cells and HS27 fibroblast cells), with IC_{50} values of 19.9 µg/mL for SW620 and 29.1 µg/mL for CH-liver cells. Following solvent partitioning, the hexane fractions displayed the most potent antiproliferative activity against the cancer cell lines and the least toxicity toward normal cells (Umthong *et al.,* 2011). These findings demonstrate that increased purification resulted in greater antiproliferative effects, similar to the current study's outcomes.

Soumya *et al.* reported comparable findings, where a diterpenoid was isolated from the petroleum ether extract of *Curcuma mutabilis* rhizomes through bioactivity-guided fractionation using silica gel column chromatography. The compound was tested against nine human cancer cell lines, yielding IC_{50} values between 5.4-10.6 µg/mL (Soumya *et al.,* 2021). Similarly, Athikkavil *et al.* purified a bioactive fraction from the ethyl acetate extract of *Plectranthus vettiveroides*, which demonstrated an IC_{50} of around 25 µg/mL on the HCT116 colon cancer cell line and the HeLa cervical cancer cell line. Further silica gel fractionation produced a bioactive fraction with an IC_{50} of 5 µg/mL (Athikkavil *et al.,* 2023). Consistent with the current study, IC_{50} values decreased as purification progressed, highlighting the similarity in results.

TLC3-B3 showed a promising ability to induce cell death in cancer cells, comparable to the significant cytotoxic activity observed in triterpenoids isolated from *Melia dubia* bark, such as meliastatins 1-5, which exhibited significant inhibition of P388 lymphocytic leukemia cell growth (ED_{50} 1.7-5.6 µg/mL) (Pettit *et al.,*2002). This observation strengthens the case for exploring TLC-B3 as a source of anticancer agents, particularly in the search for new triterpenoid-based therapies. Moreover, this finding aligns with the cytotoxic triterpenoids identified in *Melia toosendan*, where compounds like meliasenins I-R demonstrated significant

cytotoxicity against U20S human osteosarcoma and MCF-7 human breast cancer cells, with some IC_{50} values below 10 μg/mL (Wu *et al.,* 2010).

GC-MS analysis of the bioactive fraction revealed a diverse range of compounds with varying retention times and concentrations. The most prominent compound was cis-13-Eicosenoic acid, comprising 68.07% of the total area and 45.1% of the total height. Other notable compounds included 9-Octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E)-, Glycidyl palmitate, 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester, 3,11-Dioxa-2,12-disilatridecane, 2,2,4,4,8,12,12-heptamethyl-, Petroselinic acid, Oleanan-29-oic acid, and 3-(acetyloxy)-12,13-epoxy-11-oxo-, methyl ester. The comprehensive profiling of these compounds underscores the complexity of *Melia dubia***'s** chemical composition and provides a valuable basis for further research into their potential anticancer applications. The HPTLC analysis of the TLC-B3 fraction revealed six prominent bands, which align with the GC-MS results, where several compounds identified have known anticancer or antitumor properties. However, the major component found in TLC-B3, cis-13-Eicosenoic acid, does not have any documented anticancer activity, limiting its potential role in contributing to the anticancer effects observed in this study. On the other hand, 9-Octadecenoic acid, which has been isolated from various sources, has been well-established to possess anticancer potential. Yu *et al.* investigated the cytotoxic and antitumor activities of an octadecenoic acid extract, mainly comprising oleic and linoleic acids, from *Euphorbia kansui* on human cancer cell lines, including gastric (SGC-7901), hepatocellular carcinoma (BEL-7402), and leukemia (HL-60) strains (Yu *et al.,* 2008). They found significant, dose-dependent antiproliferative effects on tumor cells starting at a dose of 3.2 μg/mL, which were either comparable to or exceeded those of the widely-used antitumor agent, 5-fluorouracil. Additionally, Hayshi *et al.* examined the anticancer potential of 9-Octadecenoic acid variants in P388 mouse leukemia cells, further supporting the role of this compound in cancer treatment (Hayshi *et al.,* 1998). Another compound, Petroselinic acid, identified in the fraction, was shown to act as a topoisomerase inhibitor of the cleavable complex-

nonforming type, interacting directly with the enzyme molecule in a noncompetitive manner without DNA intercalation, as noted by Suzuki *et al.,* (Suzuki *et al.,* 2000).

Thus, the presence of bioactive compounds like 9-Octadecenoic acid and its esters, as well as Petroselinic acid, is likely responsible for the cytotoxicity observed in this study, despite the absence of anticancer properties in cis-13-Eicosenoic acid. The combination of these active compounds, particularly the well-documented anticancer effects of 9-Octadecenoic acid and the unique enzyme-targeting mechanism of Petroselinic acid, underscores the therapeutic potential of the TLC-B3 fraction. The findings from this study align with previous research, which has shown that as purification of bioactive compounds progresses, their anticancer efficacy improves. Therefore, even though the major component lacks anticancer activity, the synergistic presence of other bioactive molecules likely enhances the overall cytotoxic effect, contributing to the anticancer potential of this fraction.

To explore the cytotoxic mechanisms mediated by apoptosis, in silico docking studies were conducted on key apoptosis-related proteins, including caspase-3, caspase-9, and P53. Compounds identified via GC–MS analysis exhibited an affinity for these apoptotic targets, with Petroselinic acid demonstrating the strongest binding affinity to caspase-3 (-6.1 kcal/mol), P53 (-5.7 kcal/mol), and caspase-9 (-5.7 kcal/mol). 9-Octadecenoic acid showed the second-highest affinity for caspase-3. These results suggest that these compounds may contribute to apoptosis induction. The consistently high binding scores for caspase-3 across all ligands indicate their potential individual or synergistic roles in promoting apoptosis.

Petroselinic acid, a rare fatty acid, has not yet been studied for its anticancer properties. The docking data suggest that it may be worth investigating for its anticancer potential. Research has highlighted the anticancer activity of 9-Octadecenoic acid. Two hydroxy acids, (10E,12Z)-9-hydroxy-10,12-octadecadienoic acid and (9Z,11E)-13-hydroxy-9,11-octadecadienoic acid, were identified as cytotoxic compounds from a water extract of rice bran against P388 mouse leukemia cells (Hayshi *et al.,*1998). Bioactive compounds identified in the

methanol and aqueous extracts of *Azanzagarckeana* seeds, including 9-Octadecenoic acid and its derivatives, such as methyl oleate and oleic acid, have also demonstrated anticancer potential (Momodu *et al.,* 2022). Additionally, these compounds possess antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties.

Studies have further shown that fatty acids like 9-Octadecenoic acid exhibit antioxidant properties and may function as anticancer agents (Youssef *et al.,* 2023). Other fatty acids, such as hexadecanoic acid and 9,12-Octadecadienoic acid, have also been identified as having anticancer properties (Mostofa *et al.,* 2023; Youssef *et al.,* 2023). These compounds have been shown to induce apoptosis and inhibit cancer cell proliferation, highlighting their potential as therapeutic agents (Mostofa *et al.,* 2023; Youssef *et al.,* 2023). The presence of 9-Octadecenoic acid and related compounds in various plant extracts underscores their potential as natural sources of anticancer agents.

Thus the observed high cytotoxicity of the purified fraction TLC-B3 towards HT29 cancer cell line may be contributed by Petroselinic acid individually or in combination with 9-Octadecenoic acid by additive or even synergistic mode. It is worth investigating the potential of these compounds as anticancer agents.

The potent cytotoxic activity observed in the *M. dubia* fractions mirrors the bioactivity of limonoids isolated from *Melia toosendan*, where compounds such as meliatoosenins E-S showed significant antiproliferative effects on A-549 and HL-60 cell lines (Zhang *et al.,* 2011). The consistency in bioactivity across different *Melia* species underscores the therapeutic potential of this plant genus, marking it as a valuable resource for developing novel anticancer agents.

Additionally, Kurniasih *et al.,* isolated the sesquiterpenoid compound senecrassidiol from the n-hexane extract of *Aglaia simplicifolia* stembark, which showed cytotoxic activity against HeLa cervical cancer cells with an IC_{50} value of 2.18 μM (Kurniasih *et al.,* 2019).

The Meliaceae family is renowned for its bioactive triterpenes, particularly limonoids, with promising anticancer properties. Gedunin, a limonoid isolated from *Trichilia pallida* Sw. (Carvalho *et al.,* 2011), crystallizes in the orthorhombic space group P212121, with two independent molecules in the asymmetric unit (α-Gedunin and β-Gedunin). Gedunin has been studied for its anticancer activities and its ability to modulate cancer hallmarks and oncogenic signaling. This limonoid is also found in various Meliaceae genera, including *Azadirachta*, *Cabralea*, *Carapa*, *Cedrela*, *Chukrasia*, *Entandrophragma*, *Guarea*, *Trichilia*, and *Xylocarpus*. Gedunin is abundant in the fruit epicarp of *Azadirachta indica* A. Juss., with higher concentrations in young green fruits (Braga *et al.,* 2020).

Kamath *et al.,* investigated gedunin's in vitro effects on SKOV3, OVCAR4, and OVCAR8 ovarian cancer cell line proliferation, both alone and in combination with cisplatin. The effects on other cancer cell lines, including breast, cervical, colon, lung, leukemia, and oral cancers, were also studied (Kamath *et al.,* 2009). The findings from this and other studies in the Meliaceae family emphasize the therapeutic versatility and significance of limonoids, reinforcing the importance of our study in advancing the discovery of potential anticance**r** compounds from *M. dubia***.**

CONCLUSIONS

- The chloroform extract of *Melia dubia* leaves was fractionated using silica gel column chromatography to enrich its anticancer compounds.
- Bioactivity-guided purification resulted in IC_{50} values of 61.26±2.55, 31.07±4.46, 17.86±1.29, 10.27±0.79, and 7.87±1.32 µg/mL for the crude extract, SC1-F8, SC2-F8, SC3-F8, and TLC-B3, respectively.
- GC-MS analysis of the most purified fraction, TLC-B3, identified ten compounds.
- *In silico* docking studies revealed that petroselinic acid individually or in combination with 9-Octadecenoic acid by additive or even synergistic mode

Chapter 4

Study of anticancer activity of leaf extracts from *Anamirta cocculus* and active fraction from *Melia dubia*

- *To study the effects of anticancer compounds present in the ethyl acetate extract of Anamirta cocculus.*
- *To study the effect of anticancer compounds present in the purified fractions of Melia bubia on apoptosis and cell migration.*
- *Study of cytomorphological changes in cancer cell lines on treatment with purified fractions of Melia bubia.*
- *Study of level of expression of mRNA of genes related to apoptosis, migration and angiogenesis by RT-qPCR.*
- *Study of level of expression of proteins involved in apoptosis by flow cytometry.*
- *Study of level of expression of proteins in EMT (epithelial – mesenchymal transition) pathway by western blotting.*

REVIEW OF LITERATURE

Cancer is a complex, multi-stage process in which cells undergo significant metabolic and behavioural transformations. These changes cause cells to grow uncontrollably and evade immune system detection, ultimately allowing them to spread to distant tissues and form metastases. These alterations occur due to the accumulation of mutations in genetic pathways that regulate cell growth, lifespan, interactions with neighbouring cells, and immune evasion. As a result, a mass of deregulated cells forms, behaving abnormally and bypassing the mechanisms that typically govern normal cell function. Cancer development involves various cellular factors, including gene mutations, altered cell communication, disrupted cell cycle regulation, angiogenesis, metastasis, and the epithelial-to-mesenchymal transition (Arley and Eker, 1962).

4.1.1. Genetic changes and cancer risk

Cancer develops from specific gene alterations or damage, primarily through mutations that disrupt normal gene functions, leading to uncontrolled cell growth. This understanding is crucial for grasping the disease's complexity and advancing treatments. Gene mutations, which can arise from various sources, play a key role in cancer risk (Bertram, 2000).

4.1.2. Sources of gene mutations

Mutations can originate from several sources. Inherited mutations are passed down from one or both parents and can increase cancer risk from birth or early development. Environmental factors, such as exposure to tobacco smoke, radiation, and chemicals, can also damage genes and contribute to cancer. Additionally, aging plays a role as cumulative environmental exposures and natural cellular processes can lead to gene mutations over time, further increasing cancer risk (Lewandowska *et al*., 2019).

Cancer often arises when multiple mutations accumulate in a cell, disrupting the normal regulation of cellular growth and leading to uncontrolled proliferation.

Although some cancers are directly linked to specific genetic mutations, the mechanisms behind many other cancers are less understood. Current research is focused on determining whether mutations associated with one type of cancer might also contribute to other cancers.

4.1.3. Cancer-related genes

Several types of genes are crucial in cancer development**.** Oncogenes and proto-oncogenes are normal genes that regulate cell growth. When mutated, proto-oncogenes can become oncogenes, leading to unregulated cell proliferation. Tumour suppressor genes normally control cell division, repair DNA, and initiate apoptosis. Mutations in these genes can deactivate their function, removing critical regulatory checks and allowing uncontrolled cell growth. DNA repair genes are responsible for correcting errors during DNA replication. Mutations in these genes can lead to the accumulation of genetic errors and increase the likelihood of cancer development (Brown, 2021).

4.1.4. Specific genetic mutations linked to cancer

Certain genetic mutations are well-established in cancer development. *BRCA1* and *BRCA2* mutations are linked to an increased risk of breast, ovarian, prostate, and pancreatic cancers (Greer and Whitcomb, 2007). The *TP53* gene, involved in DNA repair and apoptosis, is frequently mutated in various cancers, found in over 50% of cases (Giglia-Mari and Sarasin, 2003). DNA mismatch repair (MMR) genes**,** such as *MLH1* and *MSH2*, are associated with Lynch syndrome and increase the risk of colorectal and endometrial cancers. *APC* gene mutations are related to familial adenomatous polyposis (FAP) and colorectal cancer (Boland, *et al.,* 2018). The *HER2/ERBB2* gene is linked to aggressive forms of breast, esophageal, and stomach cancers, with targeted therapies available. The *BCR-ABL* fusion gene results from chromosomal translocation and is commonly associated with chronic myelogenous leukemia (CML) and some cases of acute lymphocytic leukemia (ALL) (Ye, *et al.,* 2014). *KRAS* mutations are found in some colorectal cancers and influence therapy effectiveness (Teo, *et al.,* 2022). *EGFR* mutations are linked to non-small cell lung

cancers and respond to tyrosine kinase inhibitors. *ALK* gene mutations are associated with a subset of non-small cell lung cancers and can be treated with ALK inhibitors (Gridelli *et al.,* 2014). *BRAF* mutations are common in melanoma and are targeted by therapies like vemurafenib (Kim *et al.,* 2016). Lastly, *NTRK* gene fusions are associated with various cancers, including thyroid and colon cancers, and are targeted by drugs such as larotrectinib (Ricciuti *et al.,* 2019).

4.1.5. The role of the mutator phenotype

Many cancer cells accumulate mutations at a much higher rate than normal cells, a property known as the "Mutator Phenotype." This characteristic is thought to be critical for cancer development and the emergence of resistance to cancer treatments. The Mutator Phenotype arises from mutations in genes that control DNA repair and genomic integrity, leading to an increased accumulation of mutations that drive cancer progression (Loeb, 2001).

4.1.5.1. *TP53* tumour suppressor: A key genetic change

Among all cancer-related genes, *TP53* is the most extensively studied. The *TP53* gene encodes the P53 protein, a tumour suppressor that is mutated in about half of all human cancer cases. The P53 protein plays a critical role in halting cell division and inducing apoptosis in abnormal cells, making it a key player in preventing cancer (Steele, 1998).

4.1.6.1.1. Tumour suppressor genes

Tumour-suppressor genes often referred to as "gatekeepers," encode proteins that inhibit cell proliferation. The loss of one or more of these "brakes" on cell division contributes to the development of many cancers. There are five broad classes of proteins typically recognized as being encoded by tumour-suppressor genes:

Unlike oncogenes, tumour-suppressor genes are targeted by genetic alterations in the opposite way, with mutations reducing the activity of the gene product (loss-of-function). Cells that are heterozygous for a tumour suppressor gene are usually

phenotypically normal due to the phenomenon of haploinsufficiency. Therefore, mutations in both the maternal and paternal alleles of a tumour-suppressor gene are generally required to confer a selective advantage to the cell.

The first tumour-suppressor gene was identified in patients with inherited retinoblastoma, caused by the loss of function of the *RB* gene, which regulates cell cycle progression. Other examples of tumour-suppressor genes include *BRCA1*, *APC*, and *TP53* (Cowell, 1992; Zhu, 2005).

4.1.5.1.2. Commonly inherited cancers and associated tumour suppressor genes

Certain cancers are linked to inherited mutations in tumour suppressor genes, which are crucial for regulating cell growth and maintaining genomic stability. Retinoblastoma is associated with mutations in the *RB1* gene, which is vital for controlling cell division, DNA replication, and cell death. Li-Fraumeni Syndrome is linked to mutations in the *TP53* gene, affecting cell division, DNA repair, and apoptosis. Melanoma often involves mutations in the *CDKN2A* (*p16*) gene, which regulates cell division and DNA repair. Wilms Tumour is associated with mutations in the *WT1* gene, responsible for regulating cell division. Neurofibromatosis Type 1 and 2 result from mutations in the *NF1* or *NF2* genes, which are involved in cell signalling pathways that control proliferation and differentiation. Familial Polyposis Coli is linked to mutations in the *APC* gene, which regulates cell division. Breast and ovarian cancers are associated with mutations in the *BRCA1* and *BRCA2* genes, crucial for DNA repair and maintaining genomic integrity. Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome) is linked to mutations in *MSH2*, *MLH1*, and *MSH6*, involved in DNA mismatch repair (Boland, *et al.,* 2018). Xeroderma Pigmentosum is associated with mutations in the *XPA* gene, which plays a role in DNA repair (Lehmann, 2003). Ataxia Telangiectasia results from mutations in the *ATM* gene, important for the DNA damage response. Bloom's Syndrome is linked to mutations in the *BLM* gene, also involved in DNA repair (Beamish *et al.,* 2002).

4.1.6. Cell growth signalling pathways and cancer

Genetic alterations in cancer cells often affect signalling pathways that regulate cell growth and tumour development. These changes lead to dysregulated signalling, bypassing the normal control mechanisms that keep cell proliferation in check. Oncogenic mutations can cause overexpression of certain genes (e.g., through gene amplification) or result in the production of mutated proteins with abnormal activity (e.g., through point mutations, truncations, or fusions). Key examples include proteins involved in signalling pathways like growth factor receptor tyrosine kinases (e.g., EGFR), small GTPases (e.g., Ras), serine/threonine kinases (e.g., Raf and Akt), and lipid kinases (e.g., PI3Ks). Additionally, developmental signalling pathways such as Wnt, Hedgehog, Hippo, and Notch, as well as nuclear targets like transcription factors (e.g., Myc and NF-κB) and cell cycle regulators (e.g., cyclins), can also be affected (Bianco et al., 2005; Glaviano et al., 2023; Wee and Wang, 2017).

Conversely, deletions or other mutations can inactivate tumour suppressors-proteins that normally act as negative regulators of cell growth. A prominent example is the tumour suppressor gene *p53*, known as the "guardian of the genome," which plays a crucial role in controlling cell proliferation and responding to stress signals like DNA damage and apoptosis. Other tumour suppressors include *pRB* and *CKI*s (e.g., *p16*), which regulate the cell cycle, and proteins like APC and PTEN, which inhibit the Wnt and PI3K-Akt pathways, respectively (Bianco et al., 2005; Glaviano et al., 2023; Wee and Wang, 2017).

Interestingly, hyperactivated oncogene pathways can trigger cellular senescence, a state of irreversible cell cycle arrest that serves as a fail-safe mechanism to prevent abnormal cell proliferation. However, for oncogenic transformation to occur, cells must acquire additional alterations that bypass senescence, such as the loss of *p53* or *PTEN*. This underscores the complex interplay between genetic mutations and signalling pathways in driving cancer progression.

4.1.6.1. The PI3K-Akt and Ras-ERK Pathways as examples of oncogenic signalling pathways

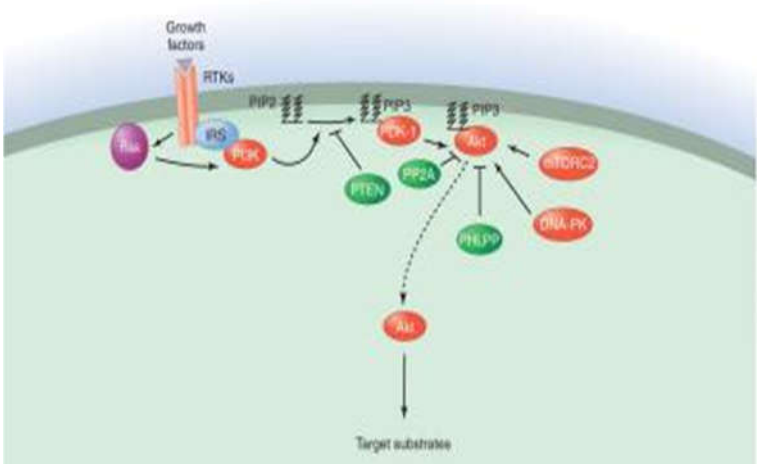


Figure 4.1.1. PKB/Akt activation downstream of RTKs via the P13K pathway (Hemmings *et al.,* 2012)**.**

Many genes commonly mutated in cancer are involved in the PI3K-Akt and Ras-ERK signalling pathways. Under normal conditions, these pathways are briefly activated in response to growth factors, cytokines, or integrin adhesion receptors. However, genetic alterations can cause these pathways to become continuously active, even without growth factor stimulation **(**Hemmings *et al.,* 2012**).**

In the PI3K-Akt pathway, amplification or activating mutations in components like *PIK3CA* (p110α), *Akt*, and *PIK3R1* can drive this abnormal signalling. Additionally, mutations in tumour suppressors like *PTEN* and *INPP4B*, which normally inhibit the pathway by dephosphorylating its products, can lead to uncontrolled activation. Downstream mutations in *TSC1* and *TSC2* further enhance signalling through *mTORC1*, a key target of PI3K-Akt signalling (Figure 4.1.1.) (Mendoza *et al.,* 2011).

Similarly, the Ras-ERK pathway can be constitutively activated by mutations in *Ras* or *Raf*, or by the inactivation of GTPase-activating proteins (GAPs) such as NF1, DAB2IP, and RASAL2, which normally deactivate Ras. The transcription factor Myc, a significant downstream target of Ras-ERK signalling, is often amplified or

overexpressed in cancer, amplifying the expression of a wide range of genes (Figure 4.1.2) **(**Hemmings *et al.,*2012)**.**

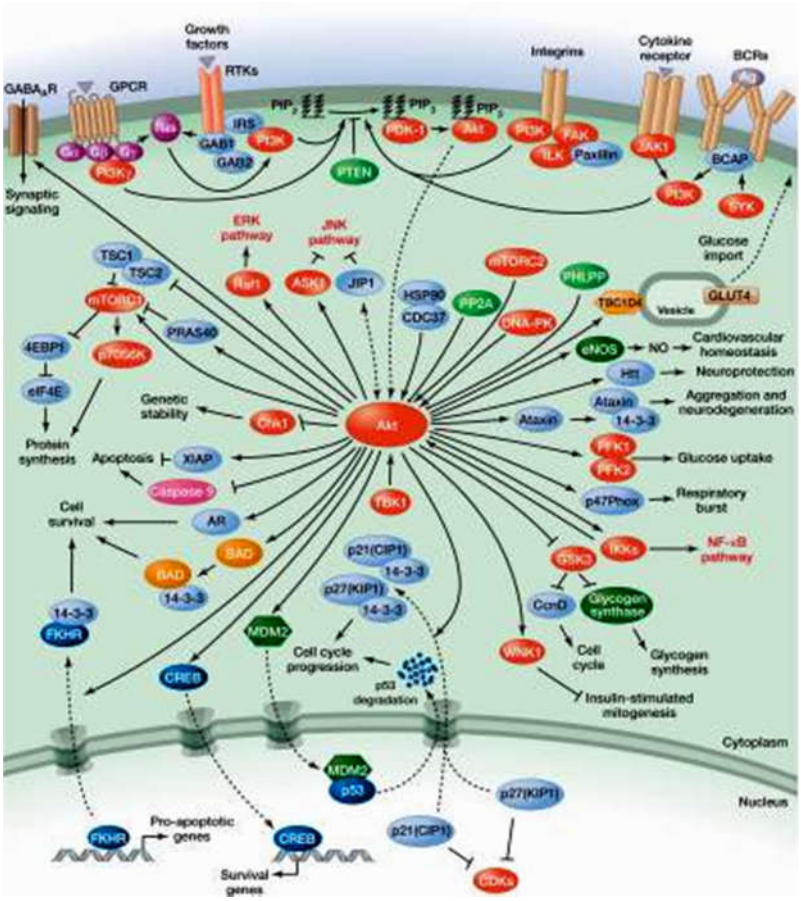


Figure. 4.1.2. Signalling events activating PKB/Akt and cellular functions regulated by PKB/Akt **(**Hemmings *et al.,* 2012)**.**

Oncogenic mutations, gene amplifications, or fusions in upstream tyrosine kinases, like *EGFR*, *ErbB2*, *FGFR*, and *PDGFR*, can lead to persistent activation of both the Ras-ERK and PI3K-Akt pathways. Similarly, mutations in G-protein-coupled receptors (GPCRs) can also activate these pathways **(**Hemmings *et al.,* 2012)**.**

Moreover, deregulated growth factor synthesis plays a crucial role in many cancers. Cells may produce growth factors that bind to their own receptors, creating an autocrine loop that drives signalling. This can also occur through the cleavage and release of anchored soluble growth factors by surface ADAM proteases, activated downstream of oncogenic pathways. Alternatively, growth factors may be produced

by neighbouring cells (paracrine stimulation), further enhancing signalling through the Ras-ERK and PI3K-Akt pathways.

4.1.6.2. Dysregulation of cellular processes by oncogenic signalling

Dysregulation of cellular signalling is a central factor in cancer progression, driving the distinct characteristics of tumour cells. Key signalling pathways, such as the Ras-ERK and PI3K-Akt pathways, become altered, playing a major role in controlling tumour behaviour. These pathways influence processes like cell proliferation, survival, metabolism, polarity, and migration, making them integral to tumour development (Wu *et al.,* 2013).

4.1.6.3. Cell proliferation

Excessive cell proliferation is a hallmark of most cancers and is normally regulated by growth factors, nutrient availability, contact inhibition, and feedback mechanisms. However, genetic mutations in proto-oncogenes, tumour suppressors, and ligand or receptor synthesis disrupt these regulatory pathways, leading to uncontrolled cell division. Mutations in key cell cycle regulators, including *cyclin D*, *cyclin E*, *CDK4*, and inhibitors like *pRB* and *p16*, are common across many cancers (MacLachlan *et al.,* 1995).

The Ras-ERK and PI3K-Akt pathways are essential in controlling cell proliferation. The Ras-ERK pathway stabilizes Myc, a transcription factor that promotes the cell cycle while inhibiting cycle inhibitors. PI3K-Akt signalling regulates proliferation through mTORC1, which drives protein synthesis and cell growth, while also stabilizing key proteins like cyclin D and Myc. These pathways also influence other regulators, such as cytokines, receptor tyrosine kinases (RTKs), Notch, and Wnt/β-catenin, all of which contribute to tumour cell proliferation when dysregulated (Figure 4.1.3) **(**Molina and Adjei, 2006).

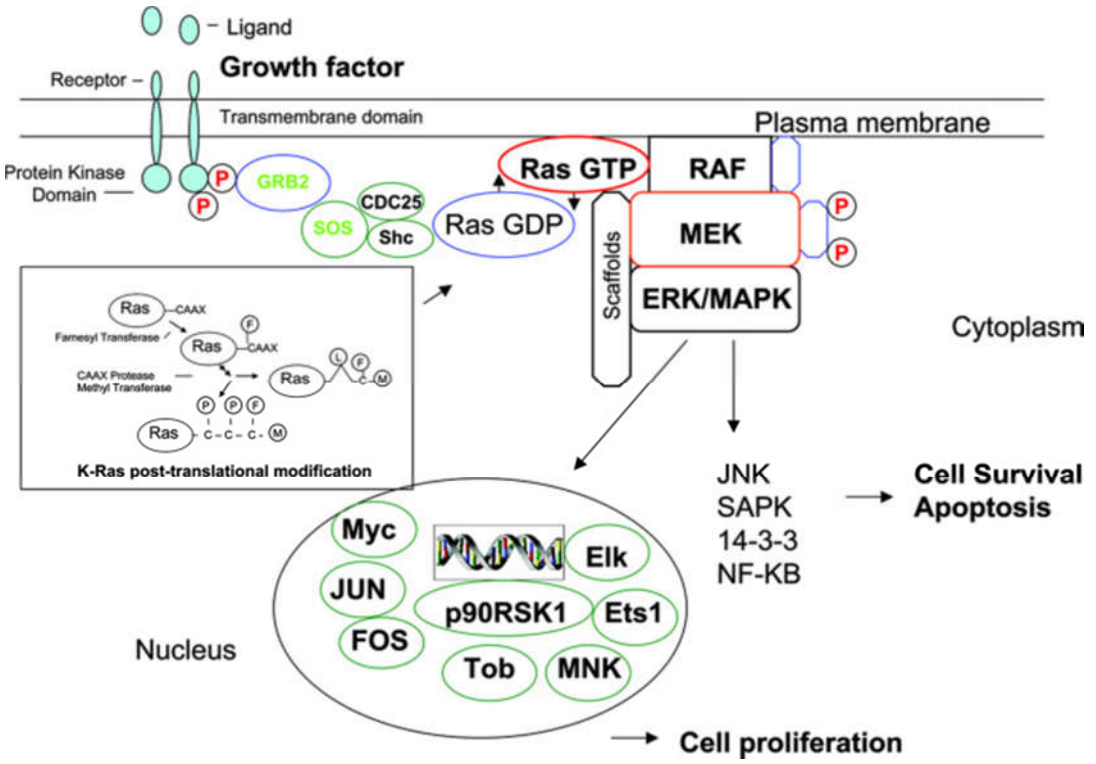


Figure 4.1.3. Ras/Raf/MAPK pathway (Molina and Adjei, 2006).

4.1.6.4. Cell survival

Cell death, particularly apoptosis, is a key mechanism for maintaining homeostasis and preventing tumourigenesis. Mutations that impair cell death signals, such as those involving *Bcl2* or *p53*, can lead to cancer by allowing abnormal cells to survive and proliferate. Oncogenic signalling in Ras-ERK and PI3K-Akt pathways disrupts apoptosis by tipping the balance toward anti-apoptotic signals.

Akt interferes with apoptotic signalling by phosphorylating FoxO3A, inhibiting pro-apoptotic proteins like Bim, and stabilizing anti-apoptotic proteins like XIAP and NF-κB. Similarly, ERK regulates apoptosis by targeting proteins like Bim for degradation, ultimately preventing apoptosis in tumour cells (Fulda, 2013).

4.1.6.5. Cell metabolism

Cancer cells show altered metabolism to meet the demands of rapid growth. These cells increase glucose uptake and glycolysis, supporting biosynthesis of

macromolecules essential for proliferation. The PI3K-Akt pathway promotes glycolysis and lipid synthesis, while Ras-ERK signalling enhances glutamine metabolism.

Akt drives metabolic reprogramming by regulating glucose transporters and enzymes involved in glycolysis. It also impacts lipid and amino acid synthesis through mTORC1 signalling. Loss of tumour suppressors like *p53* further disrupts normal metabolic regulation, pushing cancer cells toward oxidative phosphorylation, and altered metabolism (Chae and Hong, 2022).

4.1.6.6. Cell polarity and migration

As cancer progresses, cells often become migratory and invasive. This transition involves changes in cell adhesion, cytoskeletal dynamics, and polarity, regulated by growth factors, chemokines, and adhesion receptors. Both the Ras-ERK and PI3K-Akt pathways play central roles in controlling these processes (Cuesta *et al.,* 2021).

These pathways regulate Rho-family GTPases, integrins, and matrix-degrading enzymes, which enable cancer cells to invade surrounding tissues. Changes in cell polarity, controlled by proteins like Scribble and Par, further drive tumour invasion. Oncogenic signalling disrupts these polarity proteins, leading to enhanced migration and loss of contact inhibition.

Epithelial-mesenchymal transition (EMT), a process regulated by Ras-ERK and PI3K-Akt signalling, enables cancer cells to become more invasive. Transcription factors like Snail, Twist, and ZEB drive EMT, contributing to tumour spread. However, not all tumour cells undergo full EMT, as some migrate as epithelial sheets or push through tissue boundaries without losing their epithelial characteristics (Karimi *et al.,* 2019).

Advances in cancer treatment now focus on targeted therapies, using next-generation sequencing to map cancer pathways. This has led to treatments that specifically target oncogenic signalling, such as receptor tyrosine kinases (RTKs), with many therapies approved by regulatory agencies like the FDA and EMA.

4.1.6.10. Current therapies and challenges

KRAS inhibitors, such as AMG510 and MRTX849, selectively target KRAS G12C mutations by forming covalent bonds with the mutant protein, offering a new approach for previously untargetable cancers. BRAF inhibitors, including dabrafenib, encorafenib, and vemurafenib, are effective against BRAF V600E/K mutations but may activate wild-type Raf-1, potentially leading to resistance (Degirmenci *et al*., 2001). Newer "paradox breaker" inhibitors like PLX704 and PLX8394 target B-Raf dimers and block signalling in non-BRAF V600E tumours (Yip and Papa, 2021). MEK inhibitors, like binimetinib, trametinib, and cobimetinib, are approved for BRAF V600E/K tumours, while next-generation inhibitors, including RO5126766 and ERK inhibitors like ulixertinib, are under development to address resistance from ERK reactivation.

4.1.7. Cell cycle and its regulation

The cell cycle is a fundamental biological process that governs cellular growth, replication of genetic material, and division into two daughter cells. It ensures the precise transmission of genetic information from one generation to the next, maintaining cellular integrity and function. This process is tightly regulated to prevent errors that could lead to genetic instability and diseases such as cancer. The cell cycle comprises two main phases: interphase and mitosis, each with distinct stages and regulatory mechanisms. Understanding these phases and their regulation is crucial for elucidating how disruptions in cell cycle control contribute to cancer development and for developing targeted therapies.

4.1.7.1. Phases of the cell cycle

The phases of cell cycle include the stages as depicted in the figure (Alberts *et al.,* 2002)

4.1.7.2.1. Interphase

Interphase is the preparatory phase of the cell cycle where cells grow, duplicate their genetic material, and prepare for division. It is subdivided into three distinct stages (Figure 4.1.4):

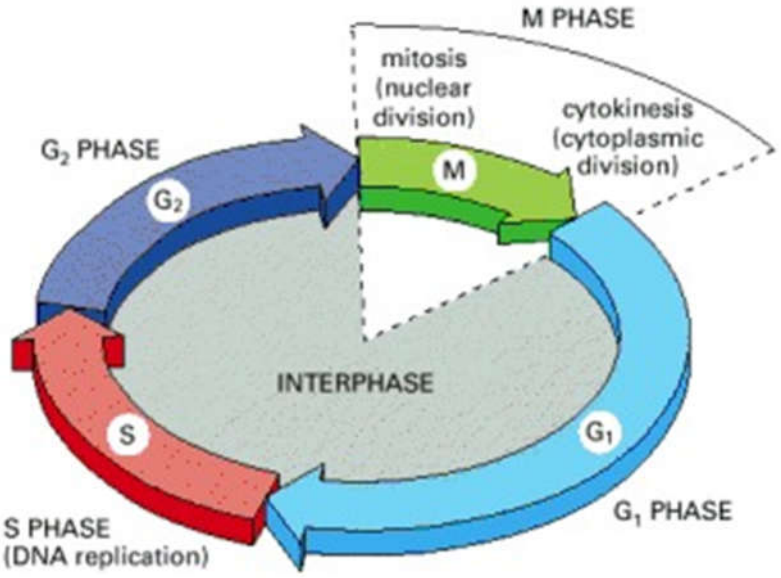


Figure 4.1.4. cell cycle phases. Source:
(https://www.ncbi.nlm.nih.gov/books/NBK26869/figure/A3171/?report=objectonly)

4.1.7.2.1.1. G1 Phase (Gap 1)

Growth and preparation: During the G1 phase, cells grow in size and synthesize proteins necessary for DNA replication. This phase is crucial for assessing the availability of resources and ensuring that the cell is prepared for DNA synthesis.

Quiescent state (G0): Cells that are not destined to divide may enter a quiescent state known as G0. In this state, cells remain metabolically active but do not proceed through the cell cycle. The G0 phase can be temporary or permanent, depending on the cell type and the needs of the organism.

4.1.7.2.1.2. S Phase (Synthesis)

DNA replication: The S phase is characterized by the duplication of the cell's entire genome. This process produces sister chromatids, which are essential for accurate chromosome separation during mitosis. DNA replication involves the unwinding of the double helix, synthesis of new DNA strands, and the reassembly of chromatin.

5. 4.1.7.2.1.3. G2 Phase (Gap 2)

Preparation for mitosis: After DNA replication, the cell continues to grow and prepares for mitosis. During G2, cells check for errors in DNA replication and repair any mistakes to ensure genome stability before entering mitosis. This phase involves additional growth and the synthesis of proteins required for chromosome segregation and cell division.

4.1.7.2.1.4. Mitosis

Mitosis is the phase where replicated chromosomes are divided into two identical nuclei, followed by the division of the cytoplasm. Mitosis is divided into several stages (Figure 4.1.5):

4.1.7.2.1.4.1. Prophase

Chromosome Condensation: Chromosomes condense and become visible under a microscope. The mitotic spindle, a structure made of microtubules, begins to form, and the nuclear envelope starts to break down.

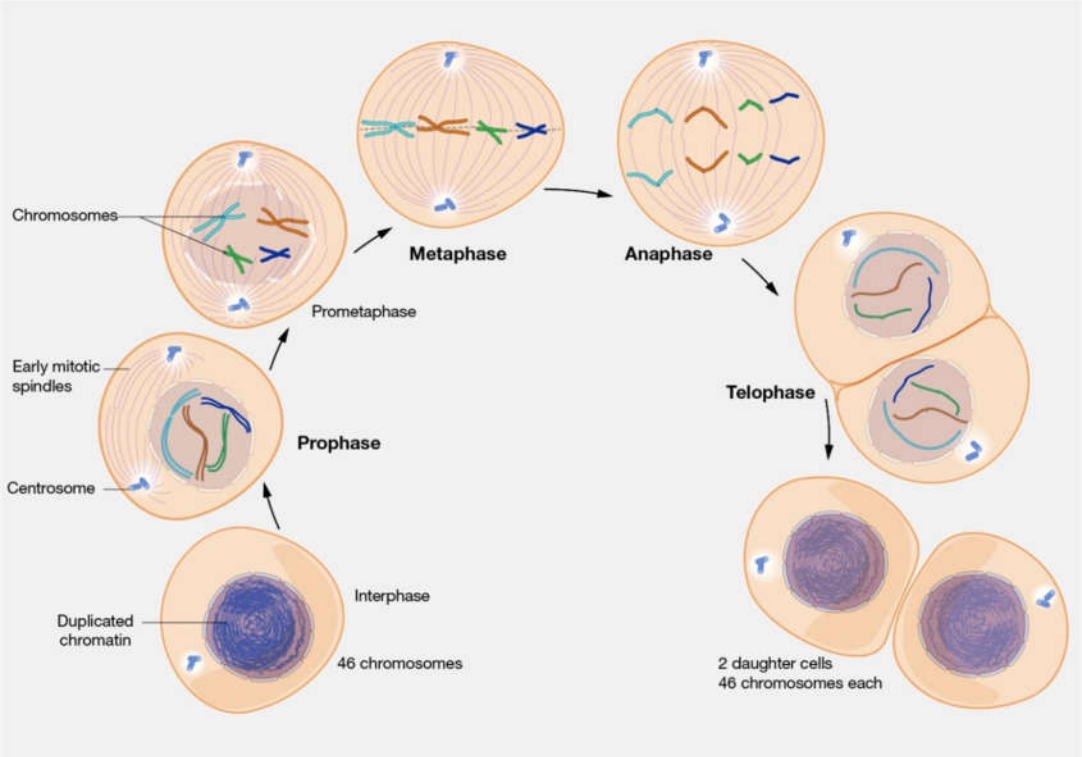


Figure 4.1.5. Mitosis – stages. Source: (https://www.genome.gov/genetics-glossary/Mitosis

4.1.7.2.1.4.2. Metaphase

Chromosome alignment: Chromosomes align along the cell's equatorial plane, known as the metaphase plate. This alignment ensures that each chromosome is properly attached to spindle fibres from opposite poles of the cell.

4.1.7.2.1.4.3. Anaphase

Separation of chromatids: Sister chromatids are pulled apart toward opposite poles of the cell by the spindle fibres. This separation ensures that each daughter cell will receive an identical set of chromosomes.

4.1.7.2.1.4.4. Telophase

Chromosome De-condensation: Chromosomes reach the poles and begin to de-condense. The nuclear envelope reforms around each set of chromosomes, resulting in the formation of two distinct nuclei within the cell.

Following mitosis, cytokinesis occurs, dividing the cytoplasm and resulting in two separate daughter cells. The cell cycle then restarts with interphase.

4.1.7.3. Regulation of the cell cycle

The regulation of the cell cycle is a complex process involving various proteins and signalling pathways that ensure cells progress through the cycle in an orderly and controlled manner. Key regulators include cyclins, cyclin-dependent kinases (CDKs), tumour suppressors, and checkpoint mechanisms (Łukasik *et al.,* 2021).

4.1.7.3.1. Cyclins and cyclin-dependent kinases (CDKs)

Cyclins and cyclin-dependent kinases (CDKs) are essential for regulating the cell cycle. CDKs, which are enzymes, become active when bound to cyclins and subsequently phosphorylate target proteins crucial for cell cycle progression. Cyclins are regulatory proteins whose levels change throughout the cell cycle, modulating CDK activity (Nigg, 1995).

4.1.7.3.1.1. Cyclin D–CDK4/6 Complex: This complex regulates the G1 phase of the cell cycle by driving the transition from G1 to S phase. Cyclin D binds to CDK4 or CDK6, leading to the phosphorylation of the retinoblastoma protein (pRb). Phosphorylated pRb releases E2F transcription factors, which then activate genes necessary for DNA synthesis and cell cycle progression (Gao *et al.,* 2020).

4.1.7.3.1.2. Cyclin E–CDK2 Complex: This complex facilitates the transition from G1 to S phase. Cyclin E binds to CDK2 and further phosphorylates pRb, enabling continued progression into the S phase (Payton and Coats, 2002).

4.1.7.3.1.3. Cyclin A–CDK2 Complex: During the S phase, Cyclin A binds to CDK2 to regulate DNA replication, ensuring that DNA replication is completed accurately (Coverley, *et al.,* 2022).

4.1.7.3.1.4. Cyclin B–CDK1 Complex: This complex is critical for the transition from G2 to M phase. Cyclin B binds to CDK1 to initiate mitosis and ensure proper chromosome segregation. The activation of CDK1 is regulated by the removal of inhibitory phosphorylations through the CDC25C phosphatase (Lindqvist, *et al.,* 2007).

4.1.7.3.2. Tumour suppressors and checkpoints

Tumour suppressors and checkpoint mechanisms are essential for maintaining genomic integrity by regulating the cell cycle and ensuring that cells only advance through the cell cycle when conditions are optimal.

4.1.7.3.2.1. P53 tumour suppressor

P53 is a key transcription factor that plays a pivotal role in cell cycle regulation. It induces growth arrest, facilitates DNA repair, and promotes apoptosis in response to cellular damage. By regulating proteins like P21 and GADD45, P53 inhibits CDKs and controls cell cycle progression. Mutations in *P53* can impair these functions, allowing damaged cells to bypass checkpoints and proliferate uncontrollably (Giono and Manfredi, 2006).

4.1.7.3.2.1.1. CDK Inhibitors (CDKIs): There are two main families of CDK inhibitors. The INK4 family, which includes p16INK4A, p15INK4B, p18INK4C, and p19INK4D, specifically inhibits CDK4 and CDK6. The Cip/Kip family, including p21Waf1/Cip1, p27Kip1, and p57Kip2, inhibits CDKs across all cell-cycle phases. P53 activates the transcription of p21 in response to DNA damage, which inhibits cyclin-E–CDK2 complexes and induces G1 arrest (Wang *et al.,* 2007).

4.1.7.3.2.1.2. DNA Repair Mechanisms: Base Excision Repair (BER) is one of the critical mechanisms for correcting mismatched bases and single-strand breaks in DNA. DNA polymerase plays a role in proofreading and correcting errors during replication. Effective DNA repair mechanisms are vital for preventing genetic instability, which can lead to cancer development.

4.1.7.3.2.2. Cell cycle checkpoints

Checkpoints are crucial surveillance mechanisms that monitor the integrity of the cell cycle, ensuring that cells only proceed to the next phase when conditions are favourable. These checkpoints are essential for maintaining genomic stability and preventing errors (Murray, 1994).

4.1.7.3.2.2.1. G1/S checkpoint: The G1/S checkpoint regulates the transition from the G1 phase to the S phase and is critical for maintaining genomic stability. Cyclin-CDK activity at this checkpoint is modulated by CDK inhibitors (CDKIs), which are categorized into two families: INK4 inhibitors and Cip/Kip inhibitors. INK4 inhibitors, such as p16INK4A, inhibit CDK4 and CDK6, while Cip/Kip inhibitors, such as p21Waf1/Cip1, inhibit CDKs across all cell-cycle phases. In response to genotoxic stress, the tumour suppressor P53 activates the transcription of *p21*, which binds to and inhibits cyclin-E–CDK2 complexes. This action leads to hypophosphorylation of retinoblastoma protein (pRb) and G1 arrest. Additionally, p16INK4A can mediate G1 arrest independently of P53 by inhibiting cyclin-D–CDK4 and cyclin-D–CDK6 (Murray, 1994).

4.1.7.3.2.2.2. G2 checkpoint: The G2 checkpoint monitors DNA integrity and ensures that cells do not enter mitosis with damaged DNA. Key regulators include ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) kinases. ATM activates checkpoint kinase 2 (Chk2) in response to ionizing radiation, while ATR activates Chk1 in response to ultraviolet radiation. Both Chk1 and Chk2 phosphorylate CDC25C, leading to its sequestration in the cytoplasm and inhibition of CDK1, thereby inducing G2 arrest. P53 supports G2 checkpoint function by upregulating the transcription of 14-3-3 proteins and p21, which inhibit CDK1 activity and disrupt G2 progression. Additionally, p21 can interfere with the interaction between proliferating cell nuclear antigen (PCNA) and CDC25C, further enhancing G2 arrest (Murray, 1994).

4.1.7.3.2.2.3. Mitotic spindle sheckpoint: The mitotic spindle checkpoint ensures accurate chromosome segregation by monitoring spindle assembly and chromosome attachment. Key proteins involved include MAD2, BUBR1, BUB1, and BUB3, which regulate the anaphase-promoting complex (APC) and delay anaphase if spindle defects are detected. MAD2 and BUBR1 inhibit APC activity, preventing anaphase onset in the presence of spindle dysfunction. BUB1 and BUB3 contribute to mitotic arrest by responding to microtubule disruptions, ensuring that chromosomes are properly aligned before anaphase begins (Murray, 1994).

4.1.7.4. Cell cycle dysregulation in cancer

Disruptions in cell cycle regulation are a hallmark of cancer. Genetic alterations in cell-cycle regulators often lead to uncontrolled cell proliferation and genomic instability, which contribute to tumour development and progression (Stewart *et al.,* 2003).

4.1.7.4.1. Alteration in cell-cycle machinery

4.1.7.4.1.1. Cyclins and CDKs

Overexpression and Amplification: Tumour cells frequently exhibit alterations in cyclins and CDKs. Increased levels of cyclin D and cyclin E, amplification of *CDK4*

and *CDK6* genes, and loss of *p16INK4A* are common in cancers such as breast, lung, and retinoblastoma. Elevated cyclin D expression is observed in approximately 50% of invasive breast cancers, and transgenic mice overexpressing cyclin D1 or cyclin E develop mammary tumours. Similarly, amplification of *CDK4* and *CDK6* is noted in various cancers, including sarcomas and gliomas (Amani *et al.,* 2021).

4.1.7.4.1.2. Tumour suppressor mutations

***P53* Mutations:** *P53*, a critical tumour suppressor, is frequently mutated in cancers, highlighting its importance in maintaining genomic integrity. Germline mutations in *P53* are associated with Li-Fraumeni syndrome, which predisposes individuals to multiple cancers, including brain tumours and breast cancers. In tumours with wild-type *P53*, disruption may occur through overexpression of *Mdm2*, which targets *P53* for degradation (Goi *et al.,* 1997).

CDKI Mutations: Mutations in *CDKI* genes are also common in tumours. Aberrant expression of *P27* is linked to aggressive breast cancers, while decreased expression of *P57* is associated with bladder cancers. Loss or inactivation of *p15INK4B* and *p16INK4A* is observed in melanomas and pancreatic cancers (Shin *et al.,* 2000).

4.1.8. Apoptosis and cancer

Apoptosis, or programmed cell death, is a crucial, evolutionarily conserved process essential for embryonic development and maintaining tissue homeostasis. This physiological response is triggered by various stimuli, including irreparable DNA damage. The key morphological features of apoptosis are nuclear shrinkage and chromatin condensation in which the nucleus of the cell becomes smaller, and chromatin condenses, cytoplasmic shrinkage, dilated endoplasmic reticulum, membrane blebbing and apoptotic bodies formation**.** The cell membrane forms bulges, or blebs, and eventually breaks into small, membrane-bound apoptotic bodies. These bodies are typically engulfed by phagocytic cells and digested in lysosomes (Brown and Attardi, 2005; Elmore, 2007).

4.1.8.1. Apoptosis: mechanisms, implications, and therapeutic strategies

Apoptosis, a form of programmed cell death, is integral to maintaining cellular homeostasis and orchestrating developmental processes. This highly regulated process ensures the removal of damaged or unnecessary cells while minimizing inflammation. Dysregulation of apoptosis, however, can contribute to pathological conditions such as cancer. This essay elucidates the mechanistic underpinnings of apoptosis, its implications in oncogenesis, and the potential for targeting apoptotic pathways in cancer therapy (Jan and Chaudhary, 2019).

4.1.8.1.1. Mechanisms of apoptosis

Apoptosis is characterized by a series of well-defined morphological and biochemical changes culminating in cell death. It is primarily mediated through two major pathways: the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway. These pathways converge on the activation of executioner caspases, which drive the cellular dismantling process (Elmore, 2007).

The intrinsic pathway is initiated by intracellular stressors such as DNA damage, oxidative stress, or growth factor deprivation. Central to this pathway is the Bcl-2 family of proteins, which includes both pro-apoptotic and anti-apoptotic members. Pro-apoptotic proteins, such as BAX and BAK, induce mitochondrial outer membrane permeabilization (MOMP), facilitating the release of cytochrome c and other pro-apoptotic factors from the mitochondria into the cytosol. Cytochrome c, in conjunction with apoptotic protease-activating factor-1 (APAF-1) and procaspase-9, forms the apoptosome, which activates caspase-9. Activated caspase-9 then activates downstream executioner caspases, such as caspase-3 and caspase-7, leading to the execution phase of apoptosis, marked by cellular degradation (Singh *et al*., 2019).

Conversely, the extrinsic pathway is triggered by external death signals binding to cell surface death receptors. Key receptors in this pathway include tumour necrosis factor (TNF) receptor 1 and the Fas receptor. Ligand binding recruits adaptor proteins and initiator caspases, such as caspase-8, forming the death-inducing

signalling complex (DISC). This complex facilitates the activation of caspase-8, which subsequently activates executioner caspases. The extrinsic pathway also intersects with the intrinsic pathway, as activated caspase-8 can cleave and activate pro-apoptotic BH3-only proteins, linking the two pathways (Kalkavan and Green, 2017).

Additionally, the endoplasmic reticulum (ER) stress pathway contributes to apoptosis, though it is less well-characterized. This pathway is activated by ER stress conditions, such as hypoxia or nutrient deprivation, and involves caspase-12. This pathway is distinct from the mitochondrial and death receptor pathways and adds complexity to the apoptotic network (Yoneda *et al*., 2001; Wong, 2011).

4.1.8.1.2. Morphological and biochemical changes

Apoptosis is distinguished by specific morphological changes, including cell shrinkage, chromatin condensation, nuclear fragmentation, and membrane blebbing. Biochemical markers of apoptosis include caspase activation, DNA fragmentation, and phosphatidylserine (PS) externalization. The translocation of PS to the outer leaflet of the plasma membrane serves as an "eat-me" signal for phagocytes, facilitating the clearance of apoptotic cells and preventing the release of pro-inflammatory cellular contents (Elmore, 2007; Chaurio *et al*., 2009).

DNA fragmentation during apoptosis is executed by endonucleases, resulting in a characteristic laddering pattern of DNA fragmentation. The activation of caspases plays a crucial role in apoptosis, leading to the cleavage of a range of cellular proteins involved in maintaining structural integrity and cellular function (Kari *et al*., 2022).

4.1.8.2. Apoptosis in cancer

Cancer cells frequently develop resistance to apoptosis, allowing them to survive and proliferate despite accumulated genetic damage. This resistance is often mediated by overexpression of anti-apoptotic proteins, such as Bcl-2, or loss of pro-apoptotic factors. For instance, overexpression of Bcl-2 is commonly observed in

various cancers and contributes to resistance against intrinsic apoptotic stimuli and certain chemotherapeutic agents. The equilibrium between pro-apoptotic and anti-apoptotic Bcl-2 family proteins is a critical determinant of cellular fate (Qian *et al*., 2022).

The evasion of apoptosis by cancer cells facilitates tumour progression and contributes to therapeutic resistance. Consequently, strategies aimed at restoring apoptotic sensitivity in cancer cells are under active investigation. These strategies involve targeting specific components of the apoptotic machinery to enhance the efficacy of existing therapies or to overcome resistance mechanisms.

4.1.8.3. Targeting apoptosis in cancer therapy

Given the pivotal role of apoptosis in cancer, therapeutic strategies targeting apoptosis are a major focus of cancer treatment research. These strategies can be broadly classified into two categories: pro-apoptotic agents and anti-apoptotic inhibitors.

Pro-apoptotic agents aim to promote apoptosis by mimicking or enhancing the effects of pro-apoptotic proteins. BH3 mimetics are one such class of drugs that inhibit anti-apoptotic Bcl-2 family proteins, thereby allowing pro-apoptotic factors to induce cell death. Other pro-apoptotic strategies include the use of death receptor agonists or ligands that activate the extrinsic apoptotic pathway directly (Chonghaile and Letai, 2008).

Conversely, anti-apoptotic inhibitors are designed to counteract the effects of anti-apoptotic proteins. Inhibitors targeting Bcl-2, Bcl-XL, and XIAP are in development to neutralize these proteins' protective effects, thereby promoting apoptosis in cancer cells. These inhibitors are being tested in various preclinical and clinical settings to evaluate their efficacy and safety (D'Aguanno and Del Bufalo, 2020).

Despite the potential of these therapeutic approaches, challenges remain, including issues related to specificity, resistance, and potential off-target effects. The development of combination therapies that target multiple apoptotic pathways may offer improved efficacy and reduced resistance.

Apoptosis is a fundamental cellular process with significant implications for cancer biology and therapy. A comprehensive understanding of the intrinsic, extrinsic, and ER stress pathways provides valuable insights into how cells regulate survival and death. The dysregulation of apoptosis in cancer underscores the need for targeted therapeutic strategies aimed at restoring apoptotic sensitivity in tumour cells. Ongoing research into novel targets and therapeutic agents continues to advance the field, offering hope for more effective and personalized cancer treatments.

4.1.8.4. Apoptosis and carcinogenesis

Cancer can be conceptualized as a result of a series of genetic alterations that transform normal cells into malignant ones. A critical aspect of this transformation is the evasion of apoptosis, which is a fundamental change that contributes to malignant progression. Since the 1970s, research has increasingly linked apoptosis to the elimination of potentially malignant cells, hyperplasia, and tumour progression. Consequently, reduced apoptosis or resistance to it plays a pivotal role in carcinogenesis. This resistance can manifest through several mechanisms, broadly categorized into: 1) disrupted balance of pro-apoptotic and anti-apoptotic proteins, 2) reduced caspase function, and 3) impaired death receptor signalling (Wong, 2011).

4.1.8.5. Disrupted balance of pro-apoptotic and anti-apoptotic proteins

A crucial determinant of apoptosis regulation is the balance between pro-apoptotic and anti-apoptotic proteins. This balance, rather than the absolute quantities of these proteins, plays a significant role in controlling cell death. Alterations in the expression levels of these proteins can contribute to carcinogenesis by influencing apoptosis rates in cancer cells (Wong, 2011).

4.1.8.5.1. Bcl-2 family of proteins

The Bcl-2 family of proteins is integral to the regulation of apoptosis, particularly through the intrinsic pathway. These proteins located on the outer mitochondrial membrane influence mitochondrial membrane permeability and apoptosis. The family is classified into three main groups (Dewson and Kluck, 2010) based on their Bcl-2 homology (BH) domains:

- **Anti-apoptotic Proteins**: These include Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1/Bfl-1, and Bcl-B/Bcl2L10. They possess all four BH domains and protect cells from apoptotic stimuli by preventing mitochondrial outer membrane permeabilization.

- **BH3-only Proteins**: This group includes Bid, Bim, Puma, Noxa, Bad, Bmf, Hrk, and Bik. They are characterized by the presence of only the BH3 domain and are typically pro-apoptotic. They are activated in response to cellular stresses such as DNA damage and growth factor deprivation.

- **Pro-apoptotic Proteins**: Bax, Bak, and Bok/Mtd fall into this category. They contain all four BH domains and promote apoptosis by facilitating mitochondrial membrane permeabilization.

Disruption in the balance between these proteins can lead to dysregulated apoptosis. Overexpression of anti-apoptotic proteins or underexpression of pro-apoptotic proteins can contribute to cancer development. For instance, Raffo *et al.* (1995) demonstrated that Bcl-2 overexpression protects prostate cancer cells from apoptosis, while Fulda *et al.* (2002) reported that Bcl-2 overexpression inhibits TRAIL-induced apoptosis in neuroblastoma, glioblastoma, and breast carcinoma cells. Similarly, Bcl-xL overexpression has been associated with multi-drug resistance in tumour cells (Minn *et al*., 1995). In contrast, mutations in the bax gene, such as those found in colorectal cancers with microsatellite instability, impair apoptosis and contribute to treatment resistance (Miquel *et al*., 2005). Chronic lymphocytic leukemia (CLL) cells exhibit high levels of anti-apoptotic Bcl-2 and low levels of pro-apoptotic Bax, leading to reduced apoptosis and leukaemogenesis (Goolsby *et al*., 2005; Wong, 2011).

4.1.8.5.2. P53 tumour suppressor protein

The P53 protein, encoded by the *TP53* gene located on chromosome 17 (17p13.1), is one of the most well-characterized tumour suppressors. Initially identified as a transformation-related protein, P53 was later recognized for its role in various

cellular processes including apoptosis, cell cycle regulation, development, differentiation, and DNA repair (Wong, 2011). It is often referred to as the "guardian of the genome" due to its crucial role in maintaining genomic stability (Lane, 1992).

(Wong, 2011). It is often referred to as the "guardian of the genome" due to its crucial role in maintaining genomic stability (Lane, 1992).

Mutations in the *TP53* gene are found in over 50% of human cancers (Bai and Zhu, 2006). Avery-Kiejda *et al.* reported aberrant expression of *p53* target genes in melanoma cells, contributing to abnormal *p53* activity and cell proliferation (Avery-Kiejda *et al.,* 2011*)*. Mouse models with specific *p53* mutations, such as the Δ122*p53* mutant, display decreased survival, altered tumour spectra, increased cell proliferation, and reduced apoptosis (Slatter *et al*., 2011). Silencing mutant *p53* has been shown to decrease colony growth in human cancer cells by inducing apoptosis, further emphasizing its role in tumour progression (Vikhanskaya *et al.*, 2007).

4.1.8.5.3. Inhibitor of apoptosis proteins (IAPs)

Inhibitor of Apoptosis Proteins (IAPs) regulate apoptosis, cytokinesis, and signal transduction. Characterized by the presence of baculovirus IAP repeat (BIR) domains, IAPs inhibit caspase activity and are crucial for apoptosis regulation (LaCasse *et al.,* 2008). The eight identified IAPs include NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7), and IAP-like protein 2 (BIRC8) (Vucic and Fairbrother, 2007). IAPs inhibit caspase activity by binding to their active sites, promoting degradation of active caspases, or preventing their interaction with substrates (Wong, 2011).

The mechanisms through which apoptosis evasion contributes to carcinogenesis are multifaceted. Disruption in the balance of pro-apoptotic and anti-apoptotic proteins, p53 mutations, and the action of IAPs all play critical roles in cancer development and progression. Understanding these mechanisms provides valuable insights for

developing targeted therapeutic strategies aimed at restoring apoptosis in cancer cells.

4.1.8.6. Targeting apoptosis in cancer treatment

4.1.8.6.1. Agents targeting the Bcl-2 family of proteins

One notable agent in this category is oblimersen sodium, an antisense oligonucleotide targeting Bcl-2. As the first Bcl-2-targeting agent to enter clinical trials, oblimersen has demonstrated chemosensitizing effects when combined with conventional anticancer drugs in chronic myeloid leukaemia, leading to improved survival rates (Rai *et al*., 2008; Abou-Nasser and Brown, 2010). Other small molecule inhibitors of the Bcl-2 family include those affecting gene or protein expression, such as sodium butyrate, depsipeptide, fenretinide, and flavipirodol. Additionally, direct protein inhibitors include gossypol, ABT-737, ABT-263, GX15-070, and HA14-1 (Kang and Reynolds, 2009; Wong, 2011). BH3 mimetics, which mimic the BH3-only proteins binding to anti-apoptotic Bcl-2 proteins, also show promise. ABT-737, for example, inhibits Bcl-2, Bcl-xL, and Bcl-W, demonstrating cytotoxicity in lymphoma and lung carcinoma cell lines, and causing regression of established tumours in animal models (Oltersdorf *et al.*, 2005). Other BH3 mimetics, such as ATF4, ATF3, and NOXA, have been reported to inhibit Mcl-1 (Albershardt *et al*., 2011).

4.1.8.6.2. Silencing anti-apoptotic proteins/genes

Silencing genes encoding anti-apoptotic Bcl-2 proteins has also been explored. Bcl-2 specific siRNA can inhibit Bcl-2 expression *in vitro* and *in vivo*, leading to increased apoptosis in pancreatic carcinoma cells (Ocker *et al*., 2005). Similarly, silencing *Bmi-1* in MCF breast cancer cells downregulates pAkt and Bcl-2, making cells more sensitive to doxorubicin and increasing apoptosis (Wu *et al*., 2011).

4.1.8.6.3. Targeting *p53*

4.1.8.6.3.1. *p53*-based gene therapy

Gene therapy involving *p53* has been investigated extensively. Initial studies used retroviral vectors to deliver wild-type *p53* genes to tumour cells, showing feasibility

in non-small cell lung carcinoma (Roth *et al.*, 1996). Subsequent research combined *p53* gene therapy with other anticancer strategies, such as ionizing radiation, to sensitize tumours (Chène, 2001). Although some studies advanced to phase III clinical trials, FDA approval has not yet been achieved (Suzuki and Matsubara, 2011). Oncolytic viruses like ONYX-015, which selectively replicate in p53-deficient cells, also show promise (Nemunaitis *et al*., 2000).

4.3.2 *p53*-Based Drug Therapy

Drug therapies targeting *p53* include small molecules that restore mutant *p53* to wild-type functions. Phikan083, a carbazole derivative, and CP-31398, which alters DNA-*p53* interactions, have shown effectiveness in restoring *p53* function (Boeckler *et al.,* 2008; Rippin *et al*., 2002). Nutlins, MI-219, and tenovins are other drugs that stabilize p53 or disrupt its interaction with MDM2, enhancing tumour sensitivity to treatments and reducing tumour growth (Wong, 2011).

4.3.3 P53-Based Immunotherapy

P53 vaccines have been tested in clinical trials, with mixed results. For instance, a recombinant adenoviral vector vaccine showed stable disease in a subset of patients with advanced cancer (Kuball *et al.,* 2002). Dendritic-cell based vaccines, including P53 peptide-pulsed dendritic cells, also demonstrated some clinical responses (Svane *et al*., 2004). Various peptide-based vaccines are under investigation (Vermeij *et al*., 2011).

4.1.8.6.3.4. Targeting inhibitors of apoptosis proteins (IAPs)

4.1.8.7.1. Targeting XIAP

XIAP, a potent IAP, inhibits apoptosis by binding and inhibiting caspases-9, -3, and -7. Strategies targeting XIAP include antisense oligonucleotides and siRNA. Antisense oligonucleotides have shown improved tumour control with radiotherapy and enhanced chemotherapeutic activity (Cao *et al*., 2004; Ohnishi *et al*., 2006).

SiRNA targeting XIAP has increased radiation sensitivity and enhanced chemotherapeutic responses.

4.1.8.7.2. Targeting survivins

Survivin-targeting approaches include antisense oligonucleotides and siRNAs. Antisense Survivin has induced apoptosis in melanoma and other cancer types (Grossman *et al*., 1999; Sharma *et al.*, 2005; Du *et al*., 2006). SiRNAs targeting Survivin have been effective in reducing radioresistance and inducing apoptosis across various cancer cells, including pancreatic, lung, and ovarian cancers (Kami *et al*., 2005, Zhang *et al*., 2009; Yang *et al.*, 2010). Small molecule antagonists and gene therapies are also being explored (Wong, 2011).

4.1.8.7.3. Other IAP antagonists

Other IAP antagonists include Smac mimetics and non-peptidic small molecules. Cyclopeptidic Smac mimetics, such as compounds 2 and 3, restore caspase activity inhibited by XIAP and cIAPs (Sun *et al*., 2010). Non-peptidic inhibitors like SM-164 enhance TRAIL activity by targeting XIAP and cIAP1 (Lu *et al*., 2011).

4.1.8.8. Targeting caspases

4.1.8.8.1. Caspase-based drug therapy

Drugs that activate caspases include Apoptin, which selectively induces apoptosis in malignant cells (Rohn and Noteborn, 2004), and small molecule caspase activators that lower activation thresholds or directly activate caspase-3 (Philchenkov *et al*., 2004).

4.1.8.8.2. Caspase-based gene therapy

Gene therapy approaches involving caspases have shown promise. For instance, human *caspase-3* gene therapy combined with etoposide reduced tumour volume and induced apoptosis (Yamabe *et al.*, 1999). Gene transfer of active *caspase-3* and

recombinant adenoviruses carrying immunocaspase 3 have demonstrated anti-cancer effects in various cancer models (Cam *et al*., 2005; Li *et al.*, 2007).

4.1.8.9. Molecules targeting apoptosis in clinical trials

A range of new molecules targeting apoptosis are currently in various stages of clinical trials. These trials investigate the efficacy of these agents in targeting specific proteins involved in apoptosis, particularly focusing on IAPs (Inhibitors of Apoptosis Proteins) and the Bcl-2 family of proteins. Below is a summary of ongoing or recently completed clinical trials involving these molecules:

The literature indicates that defects in apoptotic pathways are integral to carcinogenesis, and targeting these pathways represents a promising strategy for cancer treatment. The molecules in clinical trials offer hope for new treatment modalities, potentially improving outcomes for various cancer types. Many of these agents are being tested in combination with conventional anticancer drugs to enhance treatment efficacy. However, challenges remain, including the risk of inducing resistance in tumours and the potential for substantial normal cell death. It is crucial to address these issues to minimize side effects and maximize the effectiveness of these new treatments. Ongoing research must continue to refine these strategies, ensuring they selectively induce apoptosis in cancer cells without harming normal tissues (Wong, 2011).

4.1.8.10. Apoptotic changes in cancer

4.1.8.10.1. Mechanisms of apoptotic evasion in cancer cells

Cancer cells employ various mechanisms to evade apoptosis, which can result in either prosurvival or proapoptotic regulation. Prosurvival genes, although not always classified as oncogenic, can acquire mutations that increase their expression, thereby contributing to tumourigenesis. Conversely, proapoptotic genes may function as tumour suppressors. Disruptions in the normal expression levels of these genes are commonly observed in cancer cell lines. For example, elevated levels of BCL-2 are found in nearly half of all human cancers (Kaloni *et al.*, 2023).

Traditional anticancer drugs predominantly target apoptosis through BCL-2/BAX-dependent mechanisms. Disruption of this pathway can lead to drug resistance and intrinsic chemoresistance (Pfeffer and Singh, 2018). Additionally, defects in apoptosis increase the threshold for chemotherapy or radiotherapy, further contributing to resistance. Altered apoptotic signalling pathways not only hinder the effectiveness of these therapies but also promote resistance to the immune system, which relies on intact apoptosis pathways for its function.

4.1.8.10.2. Proapoptotic regulation in tumour cells

Cancer cells, despite their typical evasion of apoptosis, can respond to various signals that push them towards apoptosis. These cells are often described as 'primed for death,' meaning they are more predisposed to initiating the apoptotic pathway compared to normal cells (Elkholi *et al*., 2014). This heightened sensitivity to apoptotic signals is due to the dual upregulation of both proapoptotic and antiapoptotic proteins (Lopez and Tait, 2015). When the upregulation of antiapoptotic proteins is disrupted, proapoptotic proteins can trigger apoptosis, leading to the potential death of tumour cells. Targeting these primed cells with inhibitors of antiapoptotic proteins may thus be an effective strategy for inducing apoptosis (Pfeffer and Singh, 2018).

Cancer cells also exhibit increased sensitivity to apoptosis due to environmental stressors such as nutrient deprivation or hypoxia. Generally, tumour cells are more responsive to the extrinsic apoptotic pathway than the intrinsic one, suggesting that targeting the extrinsic pathway could be beneficial for cancer therapy. Furthermore, oncogenes and tumour suppressors can mediate or influence apoptosis. For instance, the tumour suppressor *p53* activates the transcription of proapoptotic proteins from the BCL-2 family. If mutations in tumour suppressors are responsible for apoptotic evasion, alternative methods to activate the apoptotic pathway must be explored to effectively target cancer cells (Danial and Korsmeyer, 2004; Elkholi *et al*., 2014; Lopez and Tait, 2015).

4.1.8.10.3. Prosurvival regulation in tumour cells

Cancer cells often overcome apoptosis by overexpressing inhibitors of apoptotic pathways. The increased expression of antiapoptotic proteins, such as BCL-2, and the downregulation of proapoptotic proteins, such as BAX, contribute to this evasion. These defects in apoptosis mechanisms allow tumour cells to resist traditional therapies, including chemotherapy and radiotherapy, by raising the threshold needed for cell death. Additionally, this resistance extends to the immune system, which relies on intact apoptotic pathways for effective function (Liu *et al*., 2017; Hassan *et al*., 2014).

Prosurvival proteins involved in the apoptotic pathway include BCL-2, BCL-xl, BCL-w, MCL-1, A1, NR-13, BHRF1, LMW5-HL, ORF16, KS-BCL-2, and E1b-19K (Adams and Cory, 1998). Many of these proteins are overexpressed in various cancers. For example, BCL-2 is upregulated in over half of all cancers, and XIAP is frequently overexpressed in multiple tumour types (Yip and Reed, 2008; Elkholi *et al*., 2014). This overexpression inhibits apoptosis triggered by signals such as hypoxia, growth factor deprivation, and oxidative stress (Yip and Reed, 2008; Pfeffer and Singh, 2018)

In contrast, proapoptotic proteins are often underexpressed or inhibited in cancer cells. Executioner caspases, for example, are frequently found at reduced levels in tumour cells. Additionally, neuroglobin (NGB), which associates with cytochrome c (Burmester and Hankeln, 2009), impairs its release into the cytosol, thereby interfering with the intrinsic apoptotic pathway. Cancer cells can also avoid complete apoptosis by preventing mitochondrial outer membrane permeabilization (MOMP) before the critical threshold is reached (Elkholi *et al*., 2014).

4.1.8.10.5. Blebbishield formation

Cancer stem cells employ a mechanism known as blebbishield formation to evade apoptosis. This process activates an emergency program to protect apoptotic cancer stem cells (Jinesh and Kamat, 2016a). During blebbishield formation, apoptotic

blebs merge to form a structured sphere called a blebbishield (Jinesh *et al*., 2013). This mechanism has been linked to immune evasion, tumourigenesis, enhanced glycolysis, chromosomal instability, drug resistance, and metastasis. Although cells undergoing blebbishield formation display visual signs of apoptosis, this response is halted, allowing the cell to survive (Pfeffer and Singh, 2018).

Blebbishields resemble mitotic cells rather than apoptotic ones, utilizing endocytosis and serpentine filopodia formation to prevent complete apoptosis (Jinesh and Kamat, 2016b). These structures avoid secondary necrosis, typically caused by a lack of ATP, through enhanced glycolysis (Jinesh *et al*., 2016). Caspases, BAD activation, and K-ras signalling all play roles in blebbishield formation. K-ras signalling regulates BAD phosphorylation, which in turn regulates glycolysis, ensuring that enough ATP is produced to prevent secondary necrosis. BAX and BAK also contribute to glycolysis during this process (Jinesh *et al*., 2013; Jinesh and Kamat, 2016a; Jinesh *et al*., 2016).

To ensure therapeutic success, strategies must target both the survival of cancer stem cells and the apoptotic pathways. Potential candidates include caspase inhibitors, Smac mimetics, and internal ribosome entry site (IRES) translation inhibitors. IRES translation controls antiapoptotic proteins like cIAP-2 and XIAP, promoting survival by shifting the balance towards antiapoptotic mechanisms. Inhibiting IRES translation can prevent blebbishield formation, with N-Myc being a targeted IRES-translational candidate to disrupt this process (Jinesh and Kamat, 2016a).

4.1.8.10.5. Apoptotic pathways

4.1.8.10.5.1. Extrinsic death receptor pathway

The extrinsic death receptor pathway is characterized by its initiation outside the cell. This pathway involves death receptors, intracellular death domain and formation of death-inducing signalling complex (DISC). Death receptors are transmembrane proteins that can activate the apoptotic program when bound by death ligands. Key death receptors include FasR (CD95)**:** Activated by FasL,

TNFR1**:** Activated by TNF-α, DR3**:** Activated by Apo3L, DR4 and DR5: Activated by Apo2L. Intracellular Death Domain**:** These receptors have an intracellular death domain that recruits adapter proteins and cysteine proteases. Important adapter proteins include TRADD (TNF Receptor-Associated Death Domain), FADD (Fas-Associated Death Domain). The binding of death ligands to receptors results in the formation of DISC, which facilitates the activation of pro-caspase 8. Caspase 8, as an initiator caspase, then cleaves downstream or executioner caspases to propagate apoptosis (Elmore et al., 2007; Wong et al., 2011; Weinberg *et al.*, 2014).

4.1.8.10.5.2. The intrinsic mitochondrial pathway

The intrinsic mitochondrial pathway of apoptosis is triggered by internal cellular stressors such as irreparable genetic damage, hypoxia, high cytosolic Ca^{2+} concentrations, and severe oxidative stress (Pristritto *et al*., 2016). These conditions lead to increased mitochondrial permeability and the release of pro-apoptotic factors, including cytochrome c, Smac/DIABLO, AIF, and Omi/HtrA2, from the mitochondria into the cytoplasm. Cytochrome c then aggregates with Apaf-1 to form the apoptosome, which activates procaspase 9. Activated caspase 9 cleaves and activates procaspases 3, 6, and 7, driving the execution phase of apoptosis (Wang and Youle, 2009; Wong, 2011). The intrinsic pathway converges with the extrinsic pathway at caspase 3, which initiates the cleavage of various proteins involved in cell cycle regulation, DNA repair, and other cellular functions, leading to the characteristic morphological changes of apoptosis.

4.1.8.10.5.3. The intrinsic endoplasmic reticulum pathway

The intrinsic endoplasmic reticulum pathway, though less well-characterized, is activated by cellular stressors such as hypoxia, free radicals, and glucose starvation, leading to endoplasmic reticulum injury. This injury causes the dissociation of the adaptor protein TNF receptor-associated factor 2 (TRAF2) from procaspase 12, resulting in the activation of procaspase 12 (Wong, 2011). This pathway operates independently of mitochondria and contributes to the overall apoptotic process.

4.1.8.10.6. Inhibitors of apoptosis (IAPs)

Inhibitors of apoptosis (IAPs), also known as BIR domain-containing proteins, are crucial regulators of apoptosis that act predominantly on the intrinsic pathway by inhibiting caspases 3, 7, and 9. The eight known mammalian IAPs include BIRC1 (NAIP), BIRC2 (cIAP1), BIRC3 (cIAP2), BIRC4 (XIAP), BIRC5 (Survivin), BIRC6 (BRUCE), BIRC7 (Livin), and BIRC8 (IAP-like protein 2) (Vucic and Fairbrother, 2007). These proteins not only inhibit apoptosis but also modulate cell cycle progression, cell division, and signal transduction pathways (Wong, 2011). XIAP is particularly well-characterized for its ability to inhibit caspases and contribute to chemoresistance in cancer cells (Cao *et al*., 2004). Survivin, another small IAP, is highly expressed in many cancers and is associated with poor clinical outcomes. Targeting IAPs presents a promising strategy for cancer treatment by restoring normal apoptotic signalling and overcoming drug resistance.

4.1.9. The Epithelial-to-mesenchymal transition (EMT) process

The Epithelial-to-Mesenchymal Transition (EMT) is a pivotal biological process wherein epithelial cells, distinguished by their tight junctions and apical-basal polarity, undergo a transformation to acquire mesenchymal characteristics (Kalluri and Weinberg, 2009). This transition is integral to various physiological processes, including embryonic development, wound healing, and tissue fibrosis. During EMT, cells undergo profound morphological and functional changes, becoming more motile and invasive, hallmarks of mesenchymal cells. Key changes include the loss of epithelial markers such as E-cadherin and the gain of mesenchymal markers like N-cadherin and vimentin (Loh *et al*., 2019; Ribatti *et al*., 2020). Importantly, EMT is not a static event but a dynamic, reversible process, with cells displaying a spectrum of epithelial and mesenchymal traits, including partial and transient forms of the transition.

4.1.9.1. EMT in cancer

In the context of cancer, EMT is frequently exploited by tumour cells to enhance their invasive and metastatic potential. Cancer cells undergoing EMT acquire traits that facilitate migration and invasion into surrounding tissues and distant organs.

This ability is further compounded by increased resistance to apoptosis, making these cells more challenging to target with conventional therapies. The complexity of EMT in cancer is underscored by its dynamic nature, where cells can transition between epithelial and mesenchymal states depending on the cancer stage and microenvironmental conditions (Ribatti *et al*., 2020).

4.1.9.2. Transcription factors in EMT

The regulation of EMT is orchestrated by a variety of factors, including transcription factors, epigenetic modifications, microRNAs, and long non-coding RNAs. Transcription factors play a central role in driving EMT by modulating the expression of key genes. Notable EMT transcription factors include SNAIL1, SNAIL2, TWIST1, TWIST2, ZEB1, and ZEB2. SNAIL1 and SNAIL2, zinc-finger transcription factors, contribute to EMT by repressing epithelial markers like E-cadherin and promoting mesenchymal traits. Similarly, TWIST1 and TWIST2, members of the basic helix-loop-helix (bHLH) family, drive EMT through analogous mechanisms. ZEB1 and ZEB2, another pair of zinc-finger transcription factors, also repress epithelial markers and enhance mesenchymal characteristics (Debnath *et al*., 2022). The expression of these transcription factors is regulated by various signalling pathways and environmental cues.

4.1.9.3. Role of EMT in cancer metastasis

EMT plays a crucial role in cancer metastasis by enabling cancer cells to migrate and invade other tissues. This process necessitates substantial metabolic reprogramming to meet the increased demands for nutrients and energy. The role of EMT in metastasis varies depending on cancer type and stage. Evidence suggests that mesenchymal cells can revert to an epithelial state (MET) at metastatic sites, facilitating tumour colonization and growth (Ribatti *et al*., 2020). Understanding the balance between EMT and MET is critical for developing effective therapeutic strategies.

4.1.9.4. EMT-related signalling pathways

Epithelial-to-Mesenchymal Transition (EMT) involves a fundamental transformation where epithelial cells lose their apical-basal polarity and cell–cell

adhesion, transitioning to a more invasive mesenchymal phenotype. Conversely, Mesenchymal-to-Epithelial Transition (MET) is the reverse process, wherein mesenchymal cells return to a polarized epithelial state. EMT is crucial in various biological and pathological contexts, including embryonic development, wound healing, cancer metastasis, and drug resistance.

During EMT, epithelial cells exhibit a reduction in the expression of epithelial markers such as E-cadherin, ZO-1, and occludin, and an increase in mesenchymal markers like N-cadherin, vimentin, and fibronectin. The loss of E-cadherin, a prominent epithelial adhesion molecule, often signifies the onset of EMT. This transition results in significant phenotypic changes, including altered cell morphology, decreased adhesion, and the acquisition of stem cell-like properties (Gonzalez and Medici, 2014; Heerboth *et al.*, 2015; Ribatti *et al*., 2020).

4.1.9.4. EMT-related signalling pathways

Several critical signalling pathways regulate EMT, each contributing to the process through distinct mechanisms:

4.1.9.4.1. Transforming Growth Factor Beta (TGFβ) Pathway: The TGFβ pathway is a major regulator of EMT. TGFβ family ligands, including TGFβs, bone morphogenetic proteins (BMPs), and Nodal, bind to their specific TGFβ receptors, initiating signalling cascades (Du and Shim, 2016). This pathway can be divided into SMAD-dependent and SMAD-independent pathways. In the SMAD-dependent pathway, activation of TGFβ receptors leads to the formation of a SMAD complex that promotes the expression of mesenchymal genes, such as vimentin. In the SMAD-independent pathway, TGFβ signaling can activate EMT through GTPases, phosphoinositide 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways (Vignais and Fafet, 2013; Gonzalez and Medici, 2014).

4.1.9.4.2. Wnt/β-Catenin Pathway: The Wnt signalling pathway plays a crucial role in EMT by promoting the stabilization and nuclear translocation of β-catenin.

This process induces the expression of mesenchymal transcription factors such as ZEB1, contributing to the acquisition of mesenchymal traits (Yook *et al*. 2005).

4.1.9.4.3. Notch Pathway: Notch signalling influences EMT by modulating the expression of various transcription factors involved in the transition. The pathway affects cellular differentiation and maintains a balance between epithelial and mesenchymal states (Timmerman *et al*., 2004).

4.1.9.4.4. Hedgehog Pathway: The Hedgehog signalling pathway impacts EMT by regulating the expression of transcription factors and modulating cellular interactions within the tumour microenvironment (Yoo *et al*., 2011).

4.1.9.4.5. AKT-mTOR Pathway: The AKT-mTOR signalling axis plays a role in regulating cell growth, survival, and metabolism, impacting EMT by influencing the expression of key transcription factors involved in the process (Lamouille *et al.*, 2012).

4.1.9.4.6. MAPK/ERK Pathway: The MAPK/ERK pathway contributes to EMT by regulating various transcription factors and signalling molecules that drive the transition from epithelial to mesenchymal states (Grotegut *et al*., 2006).

4.1.9.4.7. NF-κB Pathway: NF-κB signalling influences EMT by regulating the expression of genes involved in inflammation, cell survival, and metastasis. This pathway plays a role in modulating cellular responses and promoting EMT in cancer cells (Kim *et al*., 2006).

4.1.9.5. EMT transcription factors and their targets

EMT is orchestrated by several key transcription factors that act as master regulators of the process (Debnath *et al*., 2022). These include:

- **SNAIL Factors**: SNAIL1 (Snail) and SNAIL2 (Slug) are critical in repressing epithelial markers such as E-cadherin and promoting mesenchymal gene expression. They bind to specific DNA sequences, such as E-boxes, to regulate EMT-target genes.

- **bHLH Factors**: Basic Helix-Loop-Helix (bHLH) factors like TWIST1 and TWIST2, along with E12 and E47, play a role in driving EMT by influencing gene expression patterns that support mesenchymal traits.
- **ZEB Factors**: ZEB1 and ZEB2 are zinc-finger transcription factors that repress epithelial genes and enhance mesenchymal characteristics. They contribute to the EMT process by modulating cellular adhesion and morphology.
- **SOX and FOX Transcription Factors**: Recently identified EMT-TFs, such as SOX and FOX factors, also play significant roles in regulating EMT by interacting with DNA and influencing gene expression.

4.1.9.6. Targets of EMT transcription factors

EMT transcription factors target a range of proteins involved in epithelial cell–cell junctions and cytoskeletal organization (Debnath *et al*., 2022). Notable targets include:

- **Epithelial Cell–Cell Junction Proteins**: Proteins such as occludin, claudins, desmoplakin, and E-cadherin are repressed by EMT-TFs, leading to loss of cell adhesion and junction integrity (Du and Shim, 2016).
- **Cytoskeletal and Polarity Complex Proteins**: Proteins involved in cell morphology and motility, including fibronectin, vitronectin, vimentin, N-cadherin, and matrix metalloproteinases (MMPs), are activated by EMT-TFs, facilitating cell migration and invasion (Du and Shim, 2016).

4.1.9.7. EMT and cancer drug resistance

The connection between epithelial-to-mesenchymal transition (EMT) and cancer cell drug resistance was first suggested in the early 1990s. Research by Sommers *et al*. (1992) demonstrated that adriamycin-resistant MCF-7 cell lines and vinblastine-resistant ZR-75-B cell lines exhibited EMT characteristics, such as increased vimentin expression and reduced formation of desmosomes and tight junctions, which are typical of EMT. However, not all drug-resistant MCF-7 cells showed

EMT phenotypes, indicating that within the heterogeneous cancer cell population; EMT cells may possess a selective growth advantage in the presence of drugs. This association has been increasingly observed across various cancers, including pancreatic (Arumugam *et al.*, 2009), bladder (McConkey *et al*., 2009), and breast cancers (Huang *et al.*, 2015).

Recent studies using genetically engineered mouse models have provided further evidence of a causal relationship between EMT and drug resistance (Fischer *et al*., 2015; Zheng *et al*., 2015). Fischer *et al*. (2015) developed an EMT lineage-tracing system to monitor reversible EMT in mice. Their system utilized an Fsp1 (fibroblast-specific protein 1) promoter-driven Cre recombinase to activate GFP expression, allowing for the tracking of EMT cells even after they reverted to an epithelial phenotype (MET). In this model, chemotherapy with cyclophosphamide significantly reduced primary tumor growth by 60%. However, GFP-positive EMT cells in the primary tumor were resistant to apoptosis and did not show a significant reduction in number under chemotherapy, whereas non-EMT cells were more susceptible. Additionally, the control group showed fewer EMT cells in lung metastases compared to the chemotherapy-treated mice, suggesting that EMT contributes to drug resistance and metastasis after chemotherapy (Du and Shim, 2016).

Signaling pathways known to promote EMT also contribute to drug resistance. For example, TGFβ, a well-known EMT-related cytokine, was associated with drug resistance in the 1990s. Teicher *et al.* (1996) found that TGFβ-neutralizing antibodies could restore drug sensitivity in alkylating agent-resistant tumors. Further studies showed that TGFβ induces EMT, which subsequently leads to drug resistance. Doxorubicin, a common chemotherapy drug, was observed to induce TGFβ expression in animal models, leading to EMT. Inhibition of the TGFβ/Smad4 signaling pathway reversed doxorubicin-induced EMT (Lindner, 2014). Similarly, Wnt and Hedgehog pathways are implicated in drug resistance. Overexpression of Wnt3 activates the Wnt/β-catenin signaling pathway, promoting EMT and leading to trastuzumab resistance in HER2-overexpressing breast cancer cells (Wu *et al*.,

2012). Hedgehog pathway activation mediates resistance to EGF receptor tyrosine kinase inhibitors (EGFR-TKIs) by inducing EMT in lung cancer cells (Della *et al*., 2015). EMT transcription factors (EMT-TFs) such as Twist, FOXC2, Snail, Slug, and ZEB have also been linked to drug resistance (Haslehurst *et al*., 2012; Siebzehnrubl *et al*., 2013; Cai *et al*., 2015). Twist, for instance, induces EMT and promotes oxaliplatin resistance in colorectal cancer by increasing multidrug resistance protein 1 (MDR1) (Deng *et al*., 2016).

4.1.9.7.1. Mechanism of EMT-induced drug resistance

Understanding the mechanisms behind EMT-induced drug resistance has gained clarity with the advancement of cancer stem cell (CSC) research. CSCs, a subset of tumor cells responsible for tumorigenesis (Singh *et al*., 2004), share similarities in signaling pathways with those activated during EMT, such as Wnt, Hedgehog, and Notch pathways. These pathways are critical for CSC self-renewal and maintenance (Huber *et al*., 2005). Cells undergoing EMT often exhibit stem cell-like properties, sharing key signaling pathways and drug resistance characteristics with CSCs. However, EMT is not a prerequisite for stemness, as there are heterogeneous CSC populations, including both mesenchymal-like (EMT CSCs) and epithelial-like (non-EMT CSCs) CSCs (Du and Shim, 2016).

One primary mechanism of drug resistance in CSCs is the overexpression of ATP-binding cassette (ABC) transporter family proteins, which actively efflux drugs from the cell (Saxena *et al*., 2011). Key ABC transporters involved in drug resistance include MDR1 (P-glycoprotein), MDR-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP). EMT cells often overexpress these ABC transporters, contributing to a drug-resistant phenotype similar to CSCs. Saxena et al. demonstrated that EMT-TFs like Twist, Snail, and FOXC2 upregulate ABC transporter expression, leading to increased drug resistance.

Another crucial mechanism of EMT-driven drug resistance is the acquisition of resistance to drug-induced apoptosis. EGFR-TKIs, such as erlotinib and gefitinib, are used to treat non-small cell lung cancer (NSCLC) by inhibiting EGFR and

inducing cell apoptosis (Uramoto *et al*., 2010; Du and Shim, 2016). However, EMT has been shown to confer resistance to these drugs. For example, Slug inhibits apoptosis induced by gefitinib by suppressing Bim expression and enhancing caspase-9 activity (Chang *et al*., 2011). Notch-1 overexpression is associated with EMT and resistance to gefitinib in EGFR-mutant cells (Xie *et al*., 2013). Nevertheless, some studies, such as those by Soucheray *et al*. and Yoshida *et al*. have shown that targeting EMT alone may not be sufficient to overcome drug resistance in NSCLC, suggesting that intratumoral heterogeneity and multiple resistance mechanisms complicate treatment (Soucheray *et al*., 2015; Yoshida *et al*., 2016).

The tumor microenvironment also plays a role in EMT-driven drug resistance. Cancer-associated fibroblasts (CAF), which are part of the tumor stroma, support cancer cell proliferation and invasion through cell interactions and cytokine secretion. CAFs facilitate EMT in cancer cells through the secretion of cytokines like IL-6 and TCF21. Hypoxia, another component of the tumor microenvironment, promotes EMT and drug resistance by activating HIF-1α, which increases MDR1 expression. Knockdown of HIF-1α has been shown to reverse EMT and restore drug sensitivity under hypoxic conditions (Du and Shim, 2016).

4.1.9.7.2. Overcoming drug resistance by targeting EMT

In the 1990s, the calcium channel blocker verapamil was tested as a chemosensitizer to reverse drug resistance by inhibiting MDR1 (Bellamy, 1996). However, its clinical efficacy was limited due to dose-limiting toxicity. Despite this, extensive research has focused on overcoming drug resistance by targeting EMT directly. Gupta et al. identified salinomycin, an antibiotic, as a selective inhibitor of breast CSCs through high-throughput screening (Gupta et al., 2009). Salinomycin was found to inhibit EMT induced by doxorubicin and enhance doxorubicin sensitivity in hepatocellular carcinoma (HCC) cells by reducing drug efflux pump expression and activity (Zhou *et al*., 2015).

Several other small molecule inhibitors targeting EMT have been identified and tested. Curcumin, an active ingredient in curry, sensitizes colorectal cancer cells resistant to 5-fluorouracil by suppressing EMT through miRNA regulation (Toden *et al*., 2015). Mocetinostat, a histone deacetylase (HDAC) inhibitor, reverses EMT in drug-resistant pancreatic cancer cells by restoring miR-203 and enhancing chemotherapy sensitivity (Meidhof *et al*., 2015). Zidovudine, an antiviral drug, inhibits the Akt/GSK3β/Snail1 pathway, reversing gemcitabine resistance in pancreatic cancer cells (Namba *et al*., 2015). Metformin, originally used for type-2 diabetes, has shown potential in targeting CSCs and inhibiting EMT in various cancers (Hirsch *et al*., 2009; Jiralerspong *et al*., 2009; Du and Shim, 2016). It reduces EMT by decreasing key EMT-TFs, such as ZEB1, Twist1, and SNAI2 (Vazquez-Martin *et al*., 2010), and inhibits the IL-6/STAT3 axis in lung adenocarcinoma (Zhao *et al.*, 2014). Metformin's potential in cancer treatment is under investigation in numerous clinical trials.

Recent advances in drug screening technologies, such as high-content screening platforms and microfluidic systems that mimic the tumour microenvironment, have facilitated the identification of novel EMT inhibitors (Mi *et al*., 2016). For instance, Chua *et al.* developed an EMT spot migration detection system for high-throughput screening of small molecule EMT inhibitors (Chua *et al.*, 2012). Aref *et al.* (2013) created a microfluidic system with tumor cell spheroids and an endothelial monolayer to identify effective EMT drugs in complex in vivo environments.

Table 4.1.1. summarizes recent advances in EMT-targeting small molecule drugs, highlighting their functions and cancer types (Du and Shim, 2016).

Table 4.1.1. Recent advances in EMT-targeting small molecule drugs, highlighting their functions and cancer types.

Drugs	Target Genes	Function	Cancer
Curcumin	*BMI1, SUZ12* and *EZH2*	Inhibits EMT and reverses 5-fluorouracil resistance	Colorectal cancer
Mocetinostat	*HDAC*	Induces sensitivity against chemotherapy	Pancreatic cancer
Zidovudine	*Akt-GSK3 beta-Snail* pathway	Inhibits EMT and reverses gemcitabine resistance	Pancreatic cancer
Evodiamine	*WNT* pathway	Inhibits EMT and reverses oxaliplatin resistance	Gastric cancer
Pyrvinium pamoate	*WNT* pathway	Inhibits EMT	Breast cancer
Moscatilin	*Vimentin, Slug*, and *Snail*	Inhibits EMT and sensitizes anoikis	Lung cancer
Metformin	*ZEB1, Slug, Twist* and *Vimentin*	Inhibits EMT	Breast cancer Ovarian cancer
Palbociclib	*c-Jun/COX-2*	Inhibits EMT	Breast cancer
Icaritin	*PTEN/Akt/HIF-1α* pathway	Inhibits EMT	Glioblastoma
Disulfiram	*ERK/NF-kappa B/Snail* pathway	Inhibits EMT and stem cell-like features	Breast cancer
Zerumbone	*TGFβ* pathway	Inhibits EMT	Non-small cell lung cancer
Bufalin	*TGFβ* pathway	Inhibits EMT	Lung cancer

4.1.9.8. The role of EMT in cancer stem cells

EMT is closely linked to the generation of cancer stem cells (CSCs), which exhibit both epithelial and mesenchymal traits. CSCs contribute to tumour heterogeneity, therapy resistance, and metastasis. EMT can enhance the stemness properties of CSCs, making them more resistant to conventional therapies. Targeting EMT-related pathways may provide novel approaches to eliminate CSCs and improve treatment outcomes (Wang *et al*., 2015; Du and Shim, 2016).

EMT is a multifaceted process with significant implications for cancer progression and metastasis. Advances in understanding the regulatory mechanisms of EMT,

including transcription factors, epigenetic modifications, and metabolic pathways, offer potential therapeutic targets. Continued research into the role of EMT in cancer stem cells and hybrid states will be crucial for developing effective strategies to combat cancer metastasis and resistance to treatment.

4.1.10. Plant-derived compounds exhibiting anti-cancerous activity

With the rise in resistance to conventional chemotherapy and radiotherapy, along with the toxicity of these treatments to both cancerous and normal cells, there is a growing need for non-toxic, alternative anticancer therapies. Plant-based compounds, which are often less toxic to healthy cells, offer a promising solution. Plants have been utilized for medicinal purposes for over 5,000 years, and approximately a quarter of modern medicines are derived from them (Fridlender *et al*., 2015). The major anticancer compounds from plants are graviola from *Annona muricata* **(**Graviola promotes apoptosis by inhibiting BCL-2 proteins and enhancing BAX levels, with non-toxic effects on healthy cells (Ioannis *et al*., 2015; Ko *et al*., 2011), Aloe-Emodin from *Rheum palmatum* (Aloe-emodin induces apoptosis in cancer cells by triggering cytochrome c release (Dalimi *et al*., 2015), Black Cohosh from *Actaea racemosa* **(**Black cohosh activates caspases, leading to apoptosis (Grant and Ramasamy, 2012), Curcumin from Turmeric (Yallapu *et al*., 2015)**,** **e**pigallocatechin-3-Gallate (EGCG) EGCG, found in green tea, that activates cell death receptors and contributes to apoptosis (Levitsky and Dembitsky, 2014), Genistein from Soybeans (Genistein induces cell cycle arrest, inhibiting cancer cell proliferation (Zhang *et al*., 2013), Juglone found in *Juglans mandshurica* (Juglone promotes apoptosis by enhancing caspase-9 cleavage (Wu *et al*., 2017) and Quercetin found in apples and red onions, quercetin modulates cell cycle regulators, contributing to its anticancer effects (Ranganathan *et al*., 2015). Curcumin, from *Curcuma longa*, (has anti-inflammatory, antioxidant, and anti-carcinogenic effects), It enhances other anticancer drugs and interacts with multiple cancer pathways. Curcumin-ND improves efficacy by increasing reactive oxygen species, activating caspase-3, and inducing cell cycle arrest (Ravindran *et al*., 2009; Singh, 2011; Kanai, 2014; Shanmugham *et al*., 2015).

Table 4.1.2. Summary of plant-derived compounds and their mechanisms.

Compound	Found in	Mechanism
Aloe-emodin	*Rheum palmatum*	Induces cytochrome c release (Dalimi *et al*., 2015)
Black cohosh	*Actaea racemosa*	Activates caspases (Grant and Ramasamy, 2012)
Curcumin	Turmeric	Inhibits BCL-2 and XIAP, enhances BAX and BAK expression (Ravindran *et al*., 2009)
Epigallocatechin-3-gallate (EGCG)	Green tea component	Activates cell death receptors (Irimie *et al*., 2015)
Genistein	Soybeans	Activates cell cycle arrest (Zhang *et al*., 2013)
Graviola	*Annona muricata*	Inhibits BCL-2 and activates BAX (Ioannis *et al*., 2015)
Juglone	*Juglans mandshurica*	Increases caspase-9 cleavage (Wu *et al*., 2017)
Quercetin	Various plants	Modulates cell cycle regulators (Ranganathan *et al*., 2015)

Plant-derived compounds such as graviola, curcumin, and others demonstrate significant anticancer activity through various apoptotic pathways (Table 4.1.2) (Pfeffer and Singh, 2018). Their non-toxic nature towards healthy cells, combined with their ability to induce apoptosis in cancer cells, makes them promising candidates for future cancer therapies. The exploration of these compounds could offer new avenues for treating cancer with fewer side effects compared to traditional methods.

4.1.10. Research gap

To date, the literature lacks extensive studies on the anticancer properties of *Anamirta cocculus* and *Melia dubia*, and their mechanisms of action remain to be fully explored. While their antimicrobial, antioxidant, and anti-inflammatory activities have been investigated, research on their anticancer potential is limited. In this chapter, we evaluated the efficacy of the ethyl acetate extract from *A. cocculus* and the purified fraction of chloroform extract from *M. dubia* in inhibiting cancer progression. This investigation aimed to assess the anti-cancer potential of these plant-derived natural extracts, focusing on their ability to disrupt cancerous processes and contribute to the understanding of natural compounds as potential cancer agents.

MATERIALS AND METHODS

4.2.1. Chemicals

All chemicals used in cell culture-based assays were of tissue culture grade. Trypan blue, acridine orange, and ethidium bromide were sourced from HiMedia, India. Propidium iodide was obtained from Sigma-Aldrich, USA, and the Annexin-V apoptosis detection kit came from Invitrogen, USA. JC-1 dye, FITC conjugated mouse anti-P53, PE conjugated mouse anti-BCL2 antibodies, and their isotype controls were procured from BD Biosciences, USA. Tryzol reagent was supplied by Originlab, India. The Verso cDNA synthesis kit was acquired from Thermo Fisher Scientific, USA, while SYBR green master mix was purchased from Genes2me, India. Primers were synthesized and procured from ILS, India, and both primary and secondary antibodies were obtained from Cell Signalling Technology, USA.

4.2.2. Cytotoxicity assay and IC_{50} calculation

The assessment of cytotoxicity was conducted using the MTT assay, a versatile and widely utilized method for evaluating cell viability and proliferation. At its core, the MTT assay relies on the enzymatic reduction of a yellow tetrazolium salt, MTT, by mitochondrial enzymes within living cells. Succinate dehydrogenase, a key enzyme in cellular respiration, primarily drives this reduction process. As MTT is reduced, it forms purple formazan crystals, the accumulation of which provides a measure of cell viability. The rate of formazan is directly proportional to the metabolic activity of the cells, making it a reliable indicator of cellular health.

In our study, the MTT assay was employed to assess the cytotoxic effects of extracts derived from two plants species, *A. cocculus* and *M. dubia*. Additionally, the study investigated the impact of active fractions isolated from *M. dubia* leaves on various cell lines, including HT29, MCF7, PA1, UM-SCC-83B and HaCaT.

Experimental procedures involved seeding the respective cell lines at a density of 5000 cells/ well into 96 well tissue culture plates. Following overnight incubation to allow for cell adhesion, the cells were exposed to the extracts from *A. cocculus* and *M. dubia* as well as varying concentrations of active fractions of *M. dubia* prepared in DMSO. Control wells, consisting of growth medium alone and DMSO vehicle controls, were included to establish baseline measurements.

After exposure to the test substances, the MTT reagent was added at a final concentration of 500µg/mL in each well, and the plates were incubated incubation at 37°C inside the incubator for 3-4 hours to facilitate formazan crystal formation. Subsequent solubilization of the formazan crystals using lysis buffer containing 20% (w/v) sodium dodecyl sulphate in 50% dimethyl formamide allowed for the measurement of absorbance spectrophotometrically at 570nm on an Elisa plate reader (Shimadzu). This provides quantitative data on cellular metabolic activity, reflecting cell viability and proliferation rates. The cell mortality was determined from the difference of absorbance of the treated samples from that of the untreated control samples using the formula:

$$\text{Cell mortality rate (in \%)} = 100 - \left\{ \frac{(\text{Absorbance}_{\text{Sample}} - \text{Absorbance}_{\text{Blank}})}{(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Blank}})} \right\}$$

4.2.3. Trypan blue dye exclusion method

Aside from assessing cytotoxicity by MTT assay, the viability of cells treated with the selected extracts or its purified fractions were also determined using trypan blue dye exclusion method. This method, founded upon the fundamental principle of cellular membrane integrity, serves as a pivotal tool in discerning the physiological impact of treatment on cellular health.

Initially, a standardized density of 1 x 10^5 cells/mL of each cell line was seeded in to 12 well plates, allowing for optimal adherence and proliferation during an overnight incubation period. Subsequently, the cells were subjected to a 48 hour treatment

regime with the respective extracts to elicit cellular responses. Cells were trypsinized and collected by centrifugation. The cells were then washed with PBS (pH 7.4) to eliminate extraneous media components or residual treatment agents. The cells were subjected to staining with 0.4% trypan blue solution, a vital stain known for its ability to permeate cells with compromised membrane integrity. This facilitates the discrimination between viable cells, characterized by intact cellular membranes that exclude the dye, and non-viable cells, characterized by damaged membranes that permit dye intake and subsequent staining. The cells were loaded and counted using a haemocytometer (Strober *et al.*, 1997)

4.2.4. Clonogenic assay

The clonogenic assay, also referred to as the colony formation assay, constitutes an essential in vitro quantitative method utilized to ascertain the proliferative potential of individual cells and their capability to generate colonies, with a colony typically defined as comprising no fewer than 50 cells. This assay stands as a pivotal tool for gauging the presence of undifferentiated cancer stem cells due to its sensitivity to aberrant cell division (Rajendran and Jain, 2018).

In this assay, an initial population of 100-200 cells is meticulously seeded into individual wells of 6 well tissue culture plates and were treated with varying concentrations of *A. cocculus* ethyl acetate extract and active fraction of *M. dubia* and incubated for duration from 7 to 14 days. Concurrently, untreated control samples were also included to provide a baseline for comparison. Upon completion of the designated incubation period, the colonies were stained with 0.25 % (w/v) Giemsa stain. Subsequent to staining, colonies harbouring more than 50 cells were systematically enumerated.

4.2.5. Scratch wound healing assay

The *in vitro* scratch assay is a simple, low cost and popular method grounded on the principle of cell migration and wound closure. It also offers insights into various

cellular processes involved in wound healing, including cell migration, proliferation, and interactions with the extracellular matrix (Liang *et al*., 2007).

In this experimental setup, the cell lines under investigation were seeded into 12 well tissue culture plates to establish a confluent monolayer. Subsequently, a controlled disruption was introduced in the form of a scratch at the midpoint of the monolayer using a sterile 10 µL micropipette tip, thus creating an artificial gap. Any dislodged floating cells were gently washed away with growth media to ensure consistency. The cells positioned at the edges of the newly formed gap exhibited a natural propensity to migrate and close the void.

Following the scratch induction, the cells were exposed to varying concentrations of the extract under study. The migratory capacity of the cells, manifested through the closure of the gap, was then observed and documented at intervals of 0, 24 and 48 hours. This observation involved capturing a series of photographs detailing the progression of cell movement within the scratched area (Liang et al, 2007; Grada *et al.,* 2017)

4.2.6. Cell migration assay/ Boyden chamber/ Trans–well migration assay

Exploring the intricacies of cell migration in cancer development, trans-well inserts are employed as they are renowned for their ability to exploit chemotaxis. In this method, cells were seeded into the upper compartment of the trans-well insert, where they were encouraged to traverse the porous membrane into the lower compartment, stimulating the migratory behaviour observed in vivo. In the lower chamber, varying concentrations of the active fraction of *M. dubia* containing media was added, and in the control, media alone was added. After a precisely controlled incubation period lasting 16 hours, indicative of a critical window for migratory events, the membrane demarcating the two compartments was washed in 1XPBS, fixed in ice cold methanol and subsequently, stained with 0.25 % (w/v) Giemsa stain, facilitating the detailed examination of the migrated cells, thereby elucidating key aspects of cancer cell motility and invasion.

4.2.7. Apoptosis analysis

The apoptotic reaction serves to eliminate cells undergoing abnormal proliferation or those experiencing DNA damage due to issues like checkpoint malfunction or repair deficiencies. Directing focus towards apoptotic pathways within cancerous cells represents a robust approach in anticancer therapy.

4.2.7.1. Cytomorphological analysis of apoptosis

After a 48 hour treatment period, cytomorphological changes induced by the extract(s) or its purified fraction (SC3-F8) were assessed using light (phase contrast), fluorescence, and electron microscopy techniques. Both control and treated cells, maintained at a concentration of $1x10^5$ cells/mL, and processed as per the specific requirements of the microscopic studies as detailed below.

4.2.7.1.1. Phase contrast microscopy

Control and treated cells were collected, rinsed with ice-cold PBS, and subsequently examined using an inverted microscope equipped with phase-contrast optics for visualization.

4.2.7.1.2. Field-emission scanning electron microscopy

The control and treated cells were harvested and then washed with ice-cold PBS. They were fixed in a solution containing 2.5% glutaraldehyde prepared in PBS, followed by centrifugation and two additional washes with ice-cold PBS. The cells were then dehydrated using a graded ascending series of acetone (75-85-100%) before being observed under CARL-Zeiss Gemini 300 scanning electron microscope.

4.2.7.1.3. Acridine orange-Ethidium bromide (AO-EB) dual staining

To assess morphological changes associated with apoptosis induction, dual staining with acridine orange and ethidium bromide was performed. AO is a vital stain capable of staining live and dead cells, while EB selectively stains cells with

compromised membrane integrity. Live cells exhibit a consistent green fluorescence, whereas cells undergoing apoptosis gradually shift towards yellow to orange coloration (Raju *et al.,* 2004; Liu *et al*., 2015).

Control and treated cells were collected, centrifuged at 800xg, and washed with cold PBS (pH 7.4). They were then adjusted to a cell density of $1x10^5$ cells/mL using PBS. Following this, the cell suspension was treated with an AO-EB solution (1:1, v/v) at a final concentration of 100µg/mL before examination under a fluorescence microscope. Enumeration of live (green) and apoptotic (yellow to orange) cells enabled the calculation of the percentage of apoptotic cells using the formula:

Percentage of apoptotic cells = Total number of apoptotic cells/ Total number of live and apoptotic cells X 100

4.2.7.1.4. Assessment of mitochondrial membrane potential

The mitochondrial membrane potential in untreated and treated cells was determined using the lipophilic dye rhodamine-123 (5 µg/mL), where cells were incubated for 30 minutes at 37°C and the fluorescence was observed under a fluorescence microscope. To further assess apoptosis, DAPI staining was performed, with apoptotic cells displaying higher membrane permeability, enabling more DAPI to enter, which intensified the blue fluorescence.

JC-1 dye was also utilized for staining, and the number of cells in different apoptotic phases was quantitatively assessed through flow cytometry.

4.2.7.2. Annexin-V assay

During apoptosis, phosphatidileserine (PS), typically situated on the inner leaflet of the plasma membrane bilayer, relocates to the cell surface (Martin *et al.,* 1995). To assess this externalization of PS in treated cells, staining was performed using the Annexin-V Apoptosis detection kit (Invitrogen, USA) following the manufacturers' protocol.

Control and treated cells were harvested and washed with ice-cold PBS. They were then resuspended in 1x annexin V binding buffer, followed by staining with 5 µL of propidium iodide (PI) solution. After incubation for 10 minutes at room temperature in the dark, cellular fluorescence intensity was assessed using flow cytometry on a BD FACS ARIA III machine. Data analysis was conducted using BD FACS software.

4.2.7.3. Genotoxicity evaluation by comet assay

The comet assay was employed for the identification of DNA strand breaks in individual cells, with gel electrophoresis conducted under alkaline conditions. The treated cells were layered over 1% agarose precoated slides and immersed in cold fresh lysis solution for membrane lysis. Later place in cold electrode buffer for unwinding DNA. Conducted electrophoresis for 30 min, stained with ethidium bromide and observed under fluorescence microscope.

4.2.7.4. DNA fragmentation

DNA fragmentation is widely recognized as a hallmark of apoptosis, indicating the final stages of programmed cell death. To assess DNA fragmentation in treated and untreated cells, both the treated and untreated cells were washed with PBS to remove any residual media and other contaminants. The cells were then resuspended in a lysis buffer containing 20 mM Tris (pH8.0), 10 mM EDTA, and 0.2% Triton X-100. This specific composition of the lysis buffer is designated to disrupt the cell membrane and nuclear envelop, releasing the cellular contents, including fragmented DNA. The cell suspension was incubated at room temperature for 15 minutes to allow complete lysis.

Following lysis, the cell lysates were centrifuged to separate the soluble components from the cell debris. The supernatant, containing the DNA, was treated with proteainase K at a concentration of 200µg/mL. Proteinase K digestion at 37ºC for 90 minutes is a critical step that degrades proteins ,including histones, which are tightly bound to DNA is free from protein contamination, allowing for accurate analysis.

After protein digestion, RNase A was added to the solution at a concentration of 0.5 mg/mL. The mixture was incubated for an additional 2 hours at 37ºC to degrade any RNA present, which could otherwise interfere with the subsequent steps of DNA purification and analysis.

The DNA was then extracted using a phenol:chloroform:isoamyl alcohol mixture in a ratio of 25:24:1. This step separates the DNA into the aqueous phase, while proteins and lipids partition in to organic phase. Following extraction, DNA was precipitated by adding three volumes of ice-cold ethanol containing 0.3 M sodium acetate and incubated at -20ºC overnight.

The DNA pellet obtained after centrifugation was washed with 70 % ethanol to remove any remaining salts and impurities .After drying, the DNA was dissolved in Tris-EDTA buffer (pH 8.0) to stabilize the DNA and prevent degradation. The purified DNA was then subjected to electrophoresis on a 1.8% agarose gel at 50 V. Agarose gel electrophoresis is a standard technique for analysing DNA fragments , where smaller fragments migrates faster than larger ones .

The DNA fragments were stained with ethidium bromide, a fluorescent dye that intercalate between DNA bases, and visualized using a Biorad gel documentation system. This system allows for the detection of DNA fragmentation patterns, which are indicative of apoptosis.

4.2.8. Gene expression studies

4.2.8.1. Total RNA isolation

The isolation and characterization of messenger RNA (mRNA) are crucial for studying gene expression in organisms (Farrell, 1993). While genomic DNA can be extracted from both active and inactive cells, as well as from cell-free DNA in a sample, RNA isolation specifically reveals the genes being actively transcribed. This process is essential for various experiments, including reverse transcription for complementary DNA (cDNA) synthesis, cloning, RNA sequencing (RNA-seq), and

gene expression analyses. While DNA analyses determine the presence of genes, RNA analyses measure gene expression and can indicate gene function or role.

Total RNA was extracted using ORIonX TRI reagent according to the manufacturer's protocol (Chomczynski and Sacchi, 1987). Cells, both treated and untreated, were lysed in TRI reagent. To ensure complete dissociation of nucleoprotein complexes, the samples were allowed to stand for 5 minutes at room temperature. For phase separation, 0.2 mL of chloroform per mL of TRI reagent was added; the samples were vigorously shaken for 15 seconds, and then left to stand for 2-15 minutes at room temperature. The mixture was then centrifuged at 12,000xg for 15 minutes at 4°C, resulting in three distinct phases. The aqueous phase containing RNA was transferred to a fresh tube, where 0.5 mL of isopropanol per mL of TRI reagent was added and allowed to stand for 5-10 minutes at room temperature. The mixture was centrifuged again at 12,000xg for 10 minutes. The resulting RNA pellet was washed with 1 mL of 75% ethanol, dried, and eluted in RNase-free water. The isolated RNA was then visualized on an agarose gel.

4.2.8.2. cDNA synthesis

mRNA is more fragile than DNA, making it challenging to handle and analyze. To address this issue, researchers often convert mRNA into complementary DNA (cDNA) through a process called reverse transcription. Unlike the natural cellular process that synthesizes mRNA from DNA; reverse transcription uses an enzyme called reverse transcriptase, not DNA or RNA polymerase. This enzyme creates cDNA sequences complementary to each mRNA transcript, converting them into a more stable form. The resulting single-stranded cDNA is then converted into double-stranded cDNA.

In this study, cDNA was synthesized from isolated mRNA using the Verso cDNA synthesis kit, following the manufacturer's protocol. A master mix was prepared with the provided reagents and enzyme, and 2000 ng of the template. Reverse transcription was performed in a thermal cycler. The cycling condition was:

Step 1: 25ºC for 10 minutes

Step 2: 37ºC for 120 minutes

Step 3: 85ºC for 5 minutes

Step 4: hold 4ºC

4.2.8.3. RT– qPCR analysis

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is widely regarded as the gold standard for the precise, sensitive, rapid, reliable and reproducible quantification of gene expression changes in response to treatment, providing insights into the molecular mechanisms of cell survival and apoptosis. This technique enables the detection and quantification of specific mRNAs within various cellular RNA samples.

The target genes for this transcription profiling play critical roles in pathways regulating cell survival and apoptosis. The housekeeping gene *GAPDH* was used as an internal reference to normalize the expression levels of the genes under investigation. The relative expression levels of the selected genes were calculated using the normalized $\Delta\Delta C_T$ method. The gene primers required were synthesized and are enlisted in the table 4.2.1. This method involves several steps:

Normalization: The C_T values of the target genes were normalized to the C_T values of the housekeeping gene (*GAPDH*) for each sample, generating ΔC_T values.

$\Delta\Delta C_T$ Calculation: The ΔC_T values of the treated samples were then normalized to the ΔC_T values of the control samples, yielding $\Delta\Delta C_T$ values.

Relative Quantification: The relative expression levels were determined using the formula $2^{-\Delta\Delta CT}$, providing the fold change in gene expression relative to the control.

$\Delta C_{T(Control)} = C_{T(Target\ gene,\ Control)} - C_{T(GAPDH,\ Control)}$

$\Delta C_{T(Treated)} = C_{T(Target\ gene,\ Treated)} - C_{T(GAPDH,\ Treated)}$

$\Delta\Delta C_T = \Delta C_{T(Control)} - \Delta C_{T(Treated)}$

Relative gene expression $= 2^{-\Delta\Delta CT}$

Wherein, C_T represents threshold cycle, the fractional cycle number at which the amount of amplified gene expression reaches a fixed threshold and 2 is the exponential constant of the formula.

Table 4.2.1. List of the primer pairs used in RT-qPCR.

Sl. No.	Target gene	Orientation	Primer sequence (5'-3')	Reference
1	*Caspase 3*	F	TGGCATACTCCACAGCACCTGGTTA	Kuppusamy *et al.,* 2016
		R	CATGGCACACAAAGCGACTGGATGAA	
2	*Caspase 8*	F	CATCCAGTCACTTTGCCAGA	
		R	GCATCTGTTTCCCCATGTTT	
3	*Caspase 9*	F	TTCCCAGGTTTTGTTTCCTG	
		R	CCTTTCACCGAAACAGCATT	
4	*BAX*	F	GCCACCAGCCTGTTTGAG	Ramar *et al.,* 2014
		R	CTGCCACCCAGCCACCC	
5	*Bcl-2*	F	TATAAGCTGTCGCAGAGGGGCTA	
		R	GTACTCAGTCATCCACAGGGCGAT	Choi *et al.,* 2017
6	*PTEN*	F	CAAGATGATGTTTGAAACTATTCCAATG	
		R	CTTTAGCTGGCAGACCACAA	
7	*c-Myc*	F	AAAGGCCCCCAAGGTAGTTA	
		R	GCACAAGAGTTCGTTAGCTG	
8	*COX-2*	F	TTCAAATGAGATTGTGGGAAAAT	Goel *et al.,* 2001
		R	AGATCATCTCTGCCTGAGTATCTT	
9	*Angiostatin*	F	CCCAACATGGACCATAAGGAAGT	Yang *et al.,* 2006
		R	TGTGGGCAATTCCACAACACTC	
10	*Vegf*	F	GGAGATCCTTCGAGGAGCACTT	
		R	GGCGATTTAGCAGCAGATATAAGAA	
11	*MMP-2*	F	GGCCCTGTCACTCCTGAGAT	Chao *et al.,* 2017
		R	GGCATCCAGGTTATCGGGGA	
12	*NF-κB*	F	CACCTCAATGGCTACACAGGACCA	Guan *et al.,* 2015
		R	ATCTTGAGCTCGGCAGTGTT	
13	*GAPDH*	F	TCCCTGAGCTGAACGGGAAG	Chen *et al.,* 2015
		R	GGAGGAGTGGGTGTCGCTGT	

4.2.8.4. Study of expression of P53 ant Bcl2 using flow cytometry

The expression of two major genes in apoptosis, the tumour suppressor gene –P53 and anti-apoptotic gene –Bcl2, was quantitatively measured using flow cytometry. The treated and control cells were harvested and incubated with FITC-conjugated mouse anti-P53 and PE-conjugated mouse ant-Bcl2 antibodies according to the manufacture's protocol. The cells were resuspended in propidium iodide buffer to stain the DNA and after incubation the cell samples were run on a flow cytometer.

4.2.9. Cell cycle

The cell cycle distribution was analysed via flow cytometry using propidium iodide (PI), a DNA-intercalating dye. Based on DNA content, the percentage of cells in the

sub-G0/G1, G0/G1, S, and G2/M phases was quantified using a BD FACS-ARIA III cytometer and BD FACS Diva software (version 6.1.3). In brief, cells treated with the extract(s) or purified fraction, along with untreated controls, were harvested separately, washed twice with PBS, fixed in 70% ice-cold ethanol, and stored at -20°C until further use. Cells were then stained with a 20 µg/mL PI solution containing 200 µg/mL RNase A (DNase-free) for 30 minutes before data acquisition and analysis (Astuti *et al.,* 2012).

4.2.10. Western blotting

The treated cells were lysed using radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitor cocktail. The protein quantification was performed using BCA kit (Sigma Aldrich, USA). For western blotting, the proteins (35-50 µg) separated on a (10-12%) SDS-polyacrylamide gel by electrophoresis and transferred onto nitrocellulose membrane (GE Healthcare, USA). The protein transferred blots were blocked with 5% non-fat dry milk or 5% Bovine Serum Albumin (BSA) and incubated with primary antibody (CDH1, SNAI2, c-MYC, β-actin (1:3000, Cell Signalling Technologies, USA), SNAII, CDH2, VIM (1:3000, Cloud Clone, USA) and COX2 (ThermoFisher Scientific, USA) in shaker overnight at 4°C. The primary antibody incubated membranes were wasted trice with TBST (10 minutes each) and subsequently secondary antibodies either anti-mouse IgG-HRP or anti-rabbit IgG-HRP (1:5000, Cell Signalling, USA) were incubated for 2 hrs at room temperature. The secondary antibody incubated membranes were wasted twice with TBST and once with TBS (10 minutes each). Proteins were visualized by SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Scientific, USA), and imaging was performed with Image Quant LAS 4000 (GE Healthcare, USA).

4.2.11. Statistical analysis

The IC_{50} was determined through Probit analysis using IBM SPSS Statistics 24. Data visualization was carried out using Origin (Version 9) and Graphpad Prism 10.2.2 (397). Statistical significance of the differences between means were assessed using ANOVA with Dunnett's multiple comparison test, performed with GraphPad Prism 10.2.2(397), and significance was accepted at $p<0.05$.

RESULTS

4.3.1. Effect of leaf extract from *Anamirta cocculus* on cancer cell lines

4.3.1.1. *Anamirta cocculus* extracts exhibit differential cytotoxicity

The cytotoxic effects of *A. cocculus* leaf extracts, prepared using petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water, were assessed using the MTT assay. The extracts were tested on a range of cancer cell lines, including HT29, HCT116, A431, A498, HeLa, MCF7, MG63, PA1, PANC1, UM-SCC-83B, and the non-cancerous human keratinocyte cell line HaCaT.

Among the extracts, ethyl acetate extract demonstrated the highest cytotoxicity across the cancer cell lines, with the PA1 cell line exhibiting the highest mortality rate at 98.64±0.40%. This was followed by significant cytotoxic effects on HT29, MG63, PANC1, HCT116, A498, MCF7, UM-SCC-83B, and A431 cell lines. The acetone extract also showed notable cytotoxicity, particularly against the PA1 and MG63 cell lines. In contrast, all six extracts exhibited minimal cytotoxicity toward the HaCaT cell line, indicating a selective toxicity towards cancer cells (Chapter 2, Table 2.3.8). The ethyl acetate extract was the most cytotoxic across several cancer cell lines, especially PA1, which had the highest mortality rate (98.64±0.40%). All six extracts exhibited minimal cytotoxicity toward the HaCaT cell line, indicating a preferential toxicity towards cancer cells.

4.3.1.2. IC_{50} of ethyl acetate extract and acetone extract on different cell lines

A concentration-dependent cytotoxicity was observed for both the ethyl acetate extract and acetone extract when tested on cell lines HT29, HCT116, A431, A498, HeLa, MCF7, MG63, PA1, PANC1, UM-SCC-83B, and HaCaT. The ethyl acetate extract exhibited the lowest IC_{50} value towards the PA1 cell line, with an IC_{50} of 8.30±0.38 µg/mL (Table 4.3.1), suggesting the presence of potent anticancer compounds. The HT29 cell line also showed high sensitivity to the etyl acetate

extract, with an IC_{50} of 17.97±0.63 µg/mL. Both PA1 and HT29 cell lines displayed increased mortality with longer incubation times. Therefore, it is worthwhile to purify and identify the anticancer molecules present in the ethyl acetate extract of *A. cocculus* (Figure 4.3.1).

Table 4.3.1. IC_{50} values of ethyl acetate and acetone extracts of *A. cocculus* on different cell lines after 48 hours incubation.

Sl. No	Cell Line	IC_{50} Values (µg/mL)-48 hours	
		Ethyl acetate extract	Acetone extract
1	PA1	8.30±0.38	47.75±0.61
2	HT29	17.97±0.63	>100
3	MG63	48.68±3.83	51.98±0.86
4	PANC1	63.45±0.87	>100
5	MCF7	85.60±2.15	>100
6	UM-SCC-83B	88.92±2.89	>100
7	A498	89.83±0.40	92.16±2.45
8	HCT116	90.7±0.81	>100
9	HeLa	90.17±1.31	>100
10	A431	95.33±1.71	92.21±1.45
11	HaCaT	>100	>100

Experiments were done three times in duplicates. Data are expressed as mean±SEM.

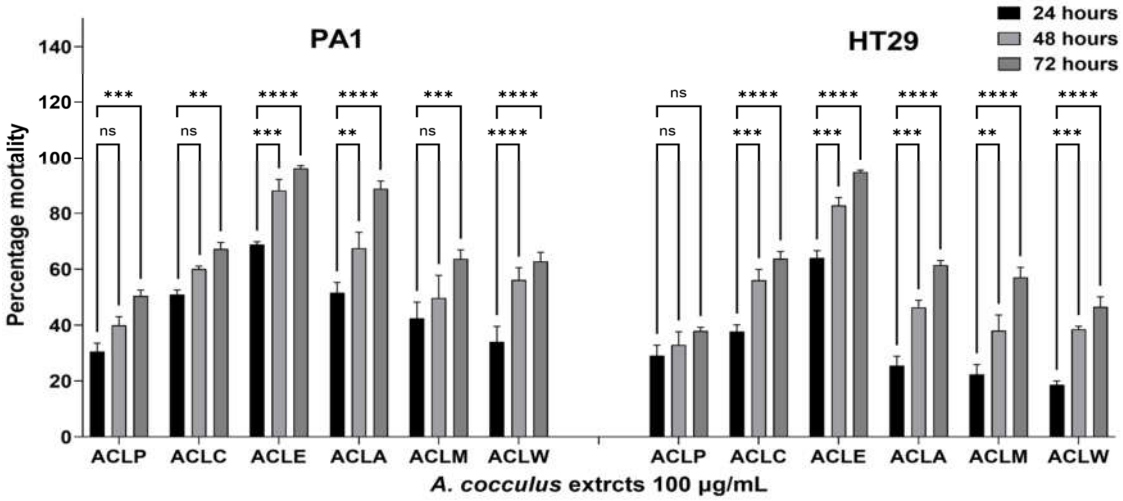


Figure 4.3.1. The percentage mortality of the different solvent extracts of *A. cocculus* at a concentration of 100µg/mL on PA1 and HT29 after exposure of 24, 48 and 72 hours. ACLP: petroleum ether extract; ACLC: chloroform extract; ACLE: ethyl acetate extract; ACLA: acetone extract; ACLM: methanol extract; ACLW: water extract. Data are expressed as mean±SEM. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

4.3.1.3. Trypan blue dye exclusion assay

In addition to the MTT assay, cell viability was further evaluated using the trypan blue dye exclusion method. This method was applied to the PA1 and HT29 cell lines, which exhibited higher sensitivity to the ethyl acetate extract.

Both cell lines were treated with increasing concentrations of the ethyl acetate extract and then subjected to trypan blue staining. The results showed a decrease in cell viability as the concentration of the ethyl acetate extract increased, consistent with the findings from the MTT assay (Figure 4.3.2). This correlation between the trypan blue staining and MTT assay results confirms the cytotoxic effects of the ethyl acetate extract on these cell lines.

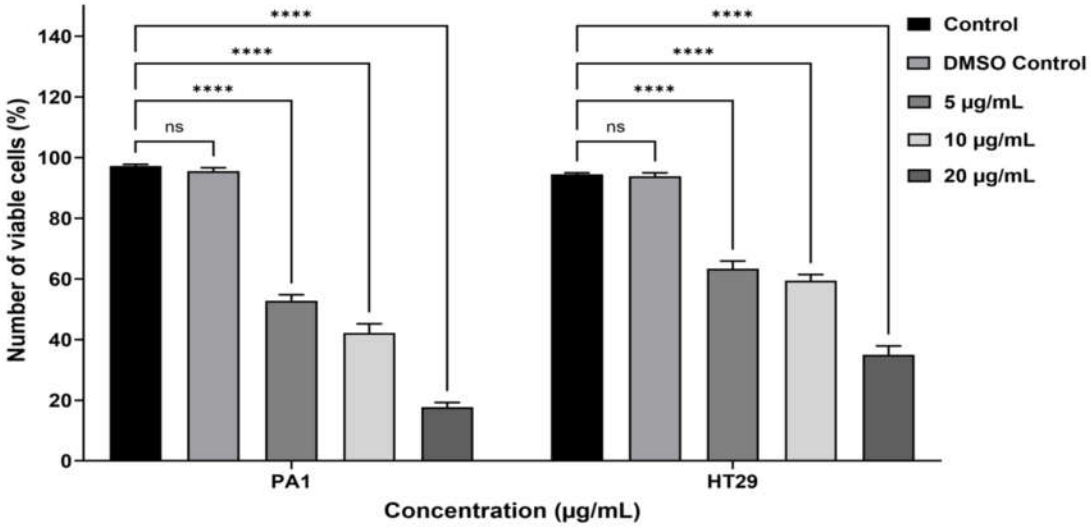


Figure. 4.3.2. The percentage of viable cells as assessed by trypan blue dye exclusion method on treatment with ethyl acetate extract at different concentrations on PA1 and HT29 cell lines incubated for 48 hours. Data are expressed as mean±SEM. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

The trypan blue dye exclusion method validated the cytotoxicity results obtained from the MTT assay. Both assays demonstrated that the ethyl acetate extract significantly reduces cell viability in PA1 and HT29 cell lines in a dose-dependent manner. This dual-method approach strengthens the evidence for the cytotoxic potential of the ethyl acetate extract against these cancer cell lines.

4.3.1.4. *Anamirta cocculus* ethyl acetate extract inhibits colony formation in cancer cell lines

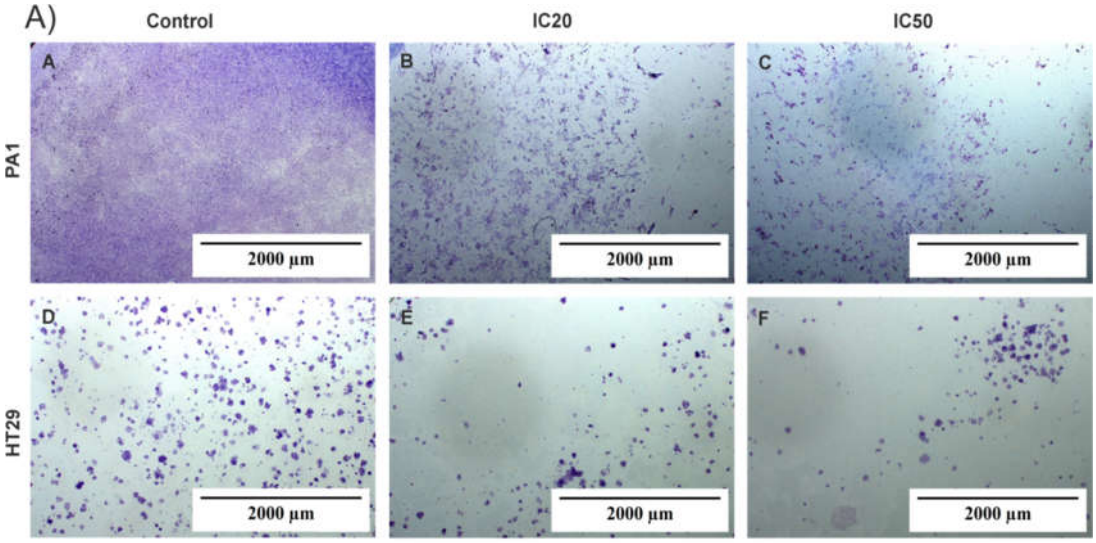


Figure. 4.3.3. Clonogenic survival assay following treatment with *A. cocculus* ethyl acetate extract. The number of colonies decreased when the cells were exposed to the extracts at different concentrations.

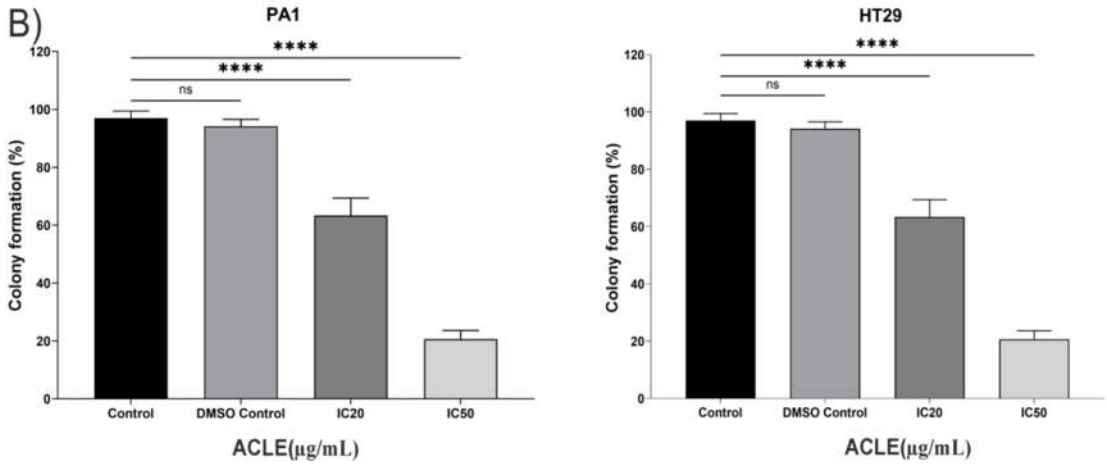


Figure. 4.3.4. Quantitative representation of the clonogenic survival assay following treatment with *A. cocculus* ethyl acetate extract. The number of colonies decreased when the cells were exposed to the extracts at different concentrations. Data are expressed as mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

Exposure of PA1 and HT29 cells to the ethyl acetate extract resulted in a concentration-dependent suppression of their colony-forming ability. At the IC_{50} concentration of the *A. cocculus* ethyl acetate extract, colony formation was inhibited by 85.2±1.72% in the PA1 cell line. Similarly, HT29 cells exhibited a

colony formation inhibition of 79.33±1.70% at IC_{50} concentration of the ethyl acetate extract. This indicates that the ethyl acetate extract significantly impairs the colony-forming potential of both cell lines, highlighting its potent anti-cancer effects (Figure. 4.3.3 and 4.3.4).

4.3.1.5. Scratch wound healing assay to assess cell migration

Treatment of PA1 cells with the *A. cocculus* ethyl acetate extract caused a concentration-dependent reduction in wound closure. At the IC_{20} concentration, there was a noticeable decrease in wound closure, while at the IC_{50} concentration, wound closure was completely inhibited. Similarly, exposure of HT29 cells to the ethyl acetate extract resulted in decreased wound closure at the IC_{20} concentration and complete cessation of wound closure at the IC_{50} concentration. These findings suggest that the ethyl acetate extract impairs cell migration in a concentration-dependent manner, which can be attributed to its effect on reducing the migratory ability of the treated cells (Figure 4.3.5 and 4.3.6).

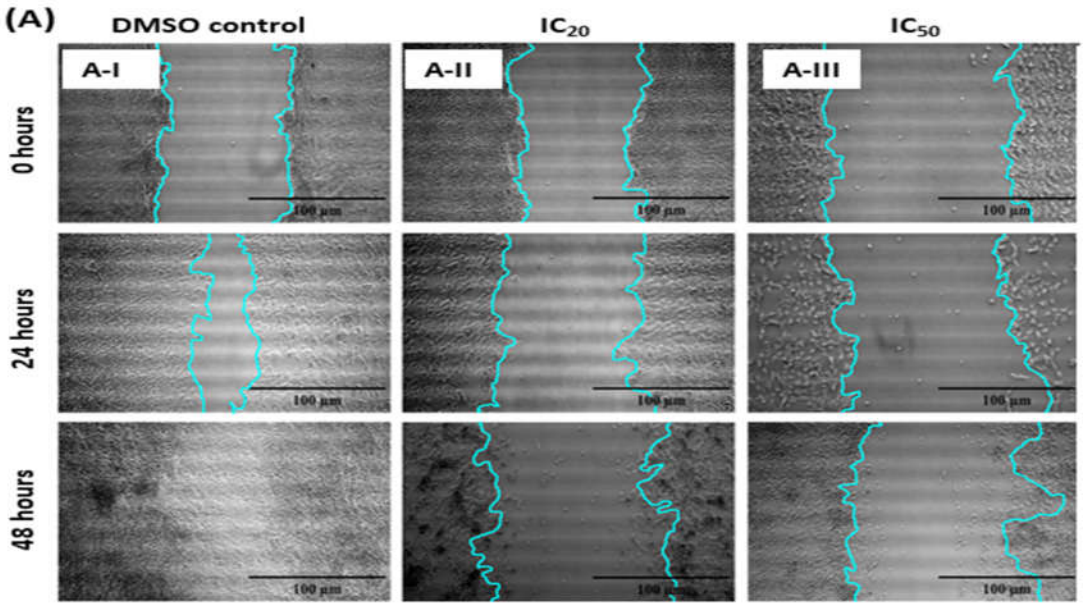


Figure 4.3.5. Scratch wound healing assay on PA1, following treatment with *A. cocculus* ethyl acetate extract at IC_{20} (A-II) and IC_{50} (A-III) concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound got completely closed in the control (A-I) after 48 hours.

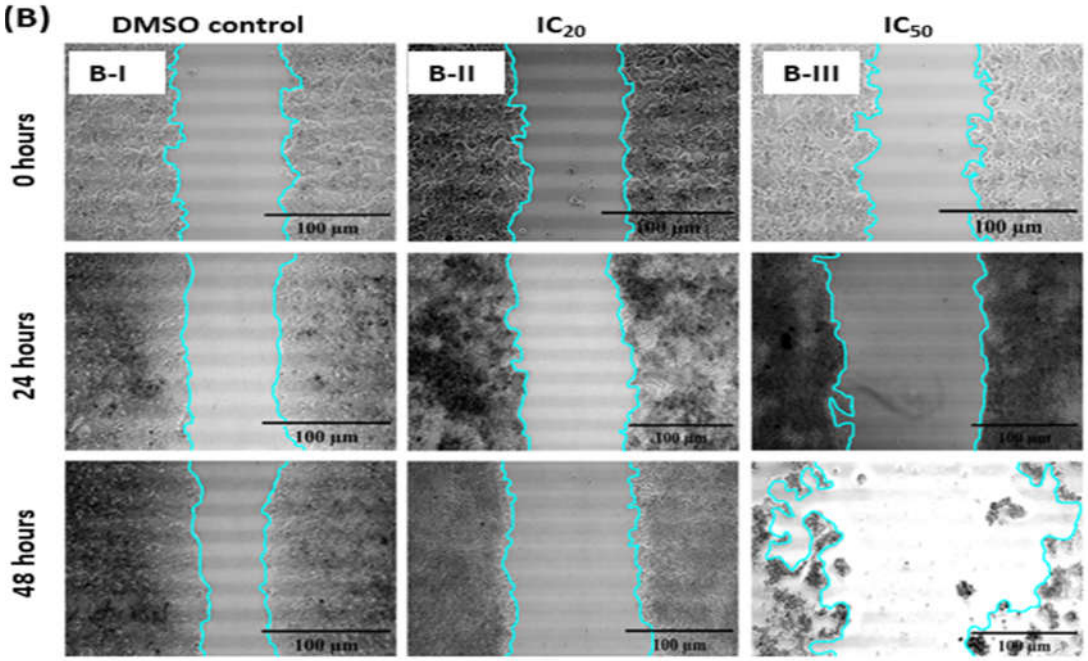


Figure 4.3.6. Scratch wound healing assay on HT29, following treatment with *A. cocculus* ethyl acetate extract at IC_{20} (B-II) and IC_{50} (B-III) concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound area reduced as the cell migrated to the wound area (B-I) after 48 hours.

4.3.1.6. Morphological changes in cancer cell lines on treatment with *A. cocculus* ethyl acetate extract

Both PA1 and HT29 cells showed dose-dependent cyto-morphological changes indicative of apoptosis when treated with the *A. cocculus* ethyl acetate extract, as observed through phase contrast (Figure 4.3.7) and scanning electron microscopy (Figure 4.3.8).

In the untreated control group, PA1 cells exhibited a polygonal shape and formed confluent aggregates. After 48 hours of treatment with the *A. cocculus* ethyl acetate extract, these cells underwent significant morphological changes, including a transition to a rounded shape, noticeable cell shrinkage, and the presence of floating dead cells and fragmented cellular debris.

Similarly, untreated HT29 cells displayed colonies of polygonal cells. Following treatment with the *A. cocculus* ethyl acetate extract, HT29 cells also exhibited rounded and shrunken morphologies, with many cells appearing detached and

floating. These observations suggest that *A. cocculus* ethyl acetate extract may be inducing apoptosis in both PA1 and HT29 cells, as evidenced by the marked morphological alterations.

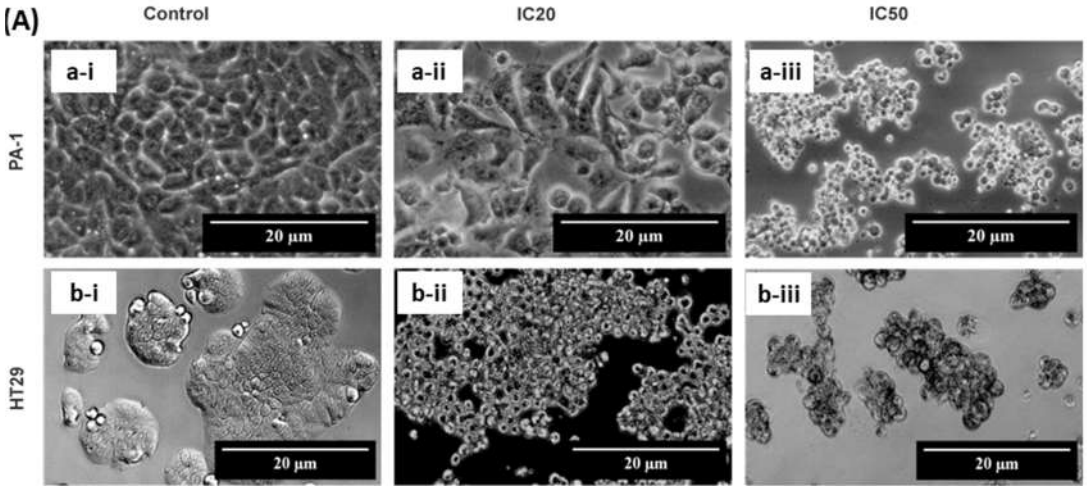


Figure 4.3.7. Phase contrast microscopic images of cytomorphological changes in PA1 and HT29 cancer cell lines on treatment of *A. cocculus* ethyl acetate extract after 48 hours (A). (a-i) PA1 Control; (a-ii) PA1 treated at IC_{20}; (a-iii) PA1 treated at IC_{50}; (b-i) HT29 Control; (b-ii) HT29 treated at IC_{20}; (b-iii) HT29 treated at IC_{50}.

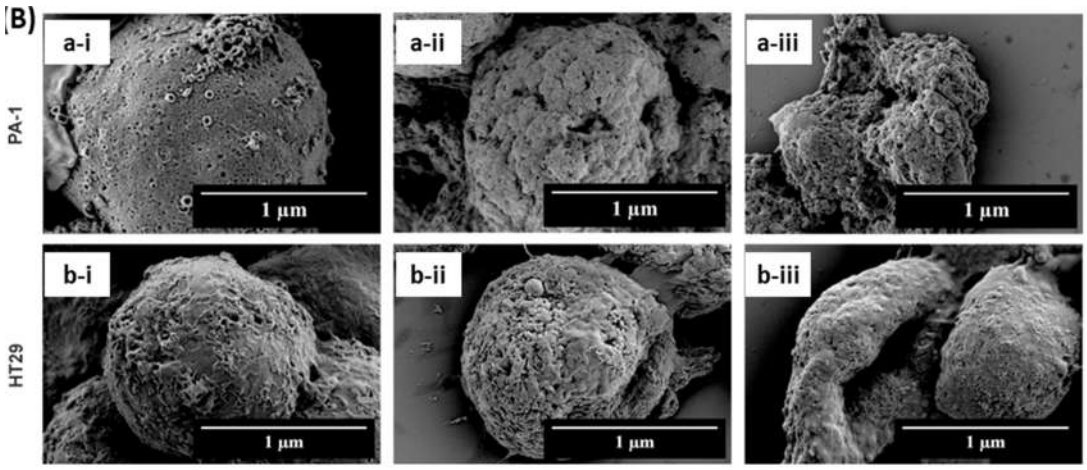


Figure 4.3.8. Scanning electron microscopic images of cytomorphological changes in PA1 and HT29 cancer cell lines on treatment with *A. cocculus* ethyl acetate extract after 48 hours (B). (a-i) PA1 Control; (a-ii) PA1 treated at IC_{20}; (a-iii) PA1 treated at IC_{50}; (b-i) HT29 Control; (b-ii) HT29 treated at IC_{20}; (b-iii) HT29 treated at IC_{50}.

4.3.1.7. Assays for apoptosis

The apoptotic characteristics of the cell line in association with the *A. cocculus* ethyl acetate extract were studied. Aspects like morphological changes, DNA fragmentation and mitochondrial membrane potential change were studied.

4.3.1.7.1. DNA fragmentation assay

DNA strand breaks, a key indicator of apoptosis, were observed in the DNA extracted from cells treated with the *A. cocculus* ethyl acetate extract. This was evident when the DNA was analyzed on 1.2% agarose gels. Compared to the control group, which showed intact DNA, the DNA from *A. cocculus* ethyl acetate extract treated cells displayed significant fragmentation, indicative of apoptosis-induced DNA damage. This result indicates that *A. cocculus* ethyl acetate extract effectively induces DNA strand breaks, a hallmark of apoptotic processes in the treated cells (Figure. 4.3.9).

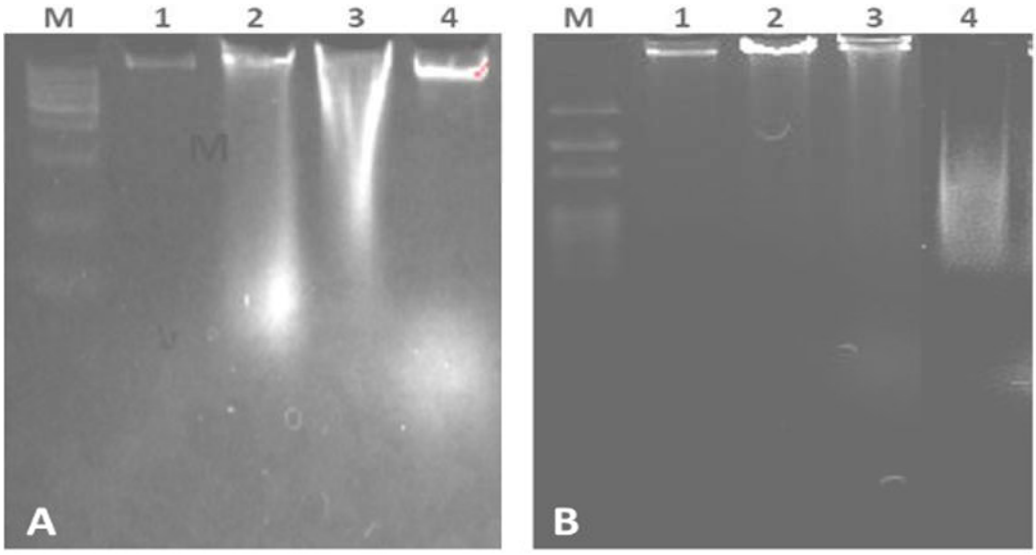


Figure 4.3.9. DNA fragmentation induced by *A. cocculus* ethyl acetate extract in (A) PA1 and (B) HT29 cell lines: (A) PA1- M: 100 bps DNA ladder, 1: DNA isolated from untreated control, 2: DNA isolated from *A. cocculus* ethyl acetate IC_{20} treated cells, 3: DNA isolated from *A. cocculus* ethyl acetate IC_{50} treated cells, 4: DNA isolated from DMSO treated control. (B) HT29- M: 100 bps DNA ladder, 1: DNA isolated from untreated control, 2: DNA isolated from DMSO treated control, 3: DNA isolated from *A. cocculus* ethyl acetate IC_{20} treated cells, 4: DNA isolated from *A. cocculus* ethyl acetate IC_{50} treated cells. DNA was isolated after 48 hours of treatment.

4.3.1.7.2. Genotoxicity evaluation by alkaline single cell electrophoresis (comet assay)

The presence of DNA strand breaks in cancer cells was assessed using the comet assay (single-cell gel electrophoresis), a technique used to evaluate genotoxicity. Upon treatment with the *A. cocculus* ethyl acetate extract, both PA1 and HT29 cells exhibited longer comet tail lengths in a concentration-dependent manner. This increase in comet tail length is indicative of DNA damage, confirming that the *A. cocculus* ethyl acetate extract induces significant genotoxic effects in the treated cells (Figure.4.3.10).

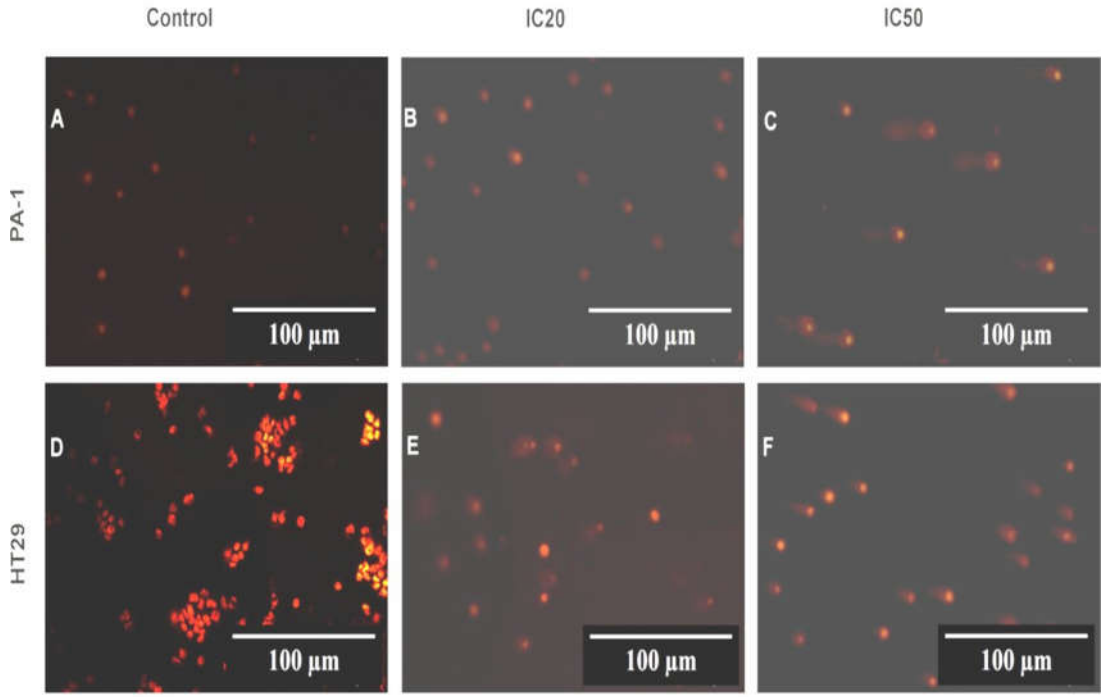


Figure 4.3.10. Genotoxicity assessment by alkaline comet assay in PA1 and HT29 cancer cell lines treated with *A. cocculus* ethyl acetate extract after 48 hours. (A) PA1 Control; (B) PA1 treated at IC_{20}; (C) PA1 treated at IC_{50}; (D) HT29 Control; (E) HT29 treated at IC_{20}; (F) HT29 treated at IC_{50}.

4.3.1.7.3. Acridine orange/Ethidium bromide dual staining

Fluorescence microscopy of PA1 and HT29 cells treated with the *A. cocculus* ethyl acetate extract, followed by dual staining with acridine orange (AO) and ethidium bromide (EB), revealed a distinct staining pattern that confirms the induction of apoptosis in a concentration-dependent manner (Figure 4.3.11. and 4.3.12).

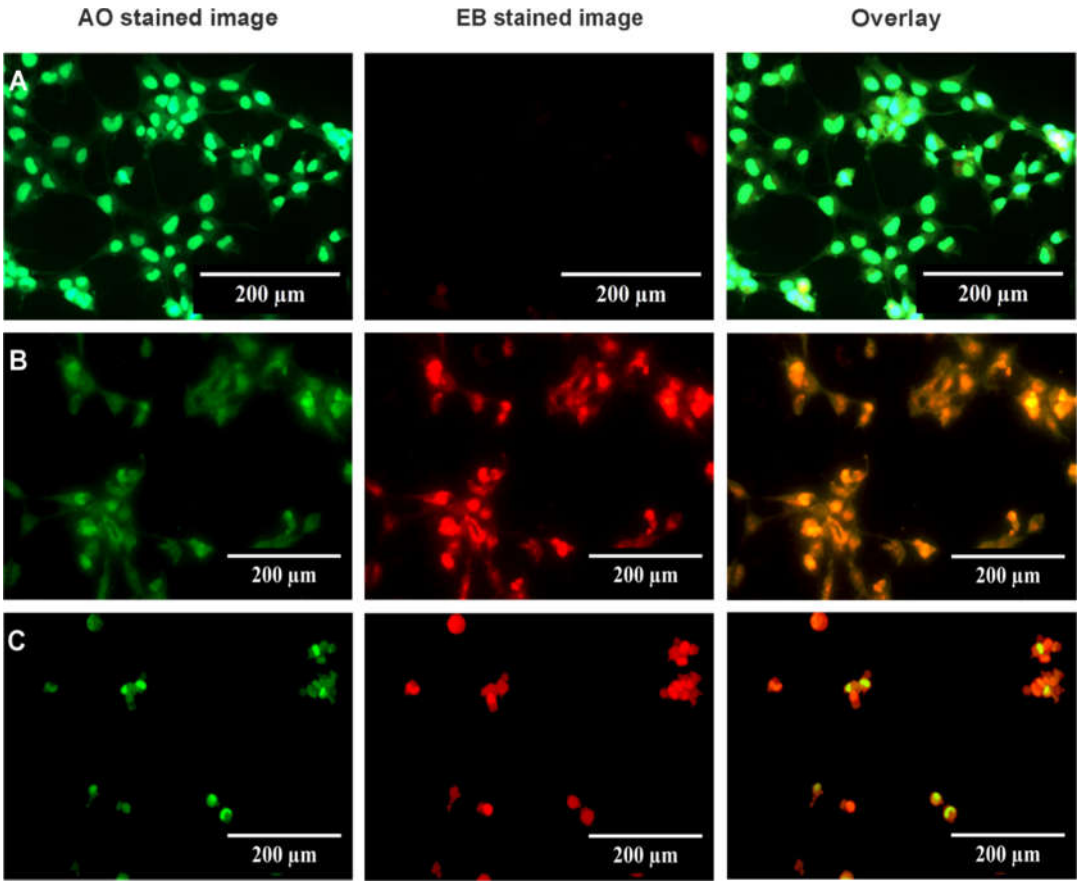


Figure 4.3.11. Effect of *A. cocculus* ethyl acetate extract on PA1 cells after 48 hours of treatment, analyzed using dual staining with acridine orange (AO) and ethidium bromide (EB). AO stains the cytoplasm of live cells (green fluorescence), while EB selectively stains apoptotic cells (orange/red fluorescence) due to compromised membrane integrity, differentiating live and apoptotic cells in treated and untreated groups. (A) DMSO control, (B) PA1 treated at 10µg/mL; (C) PA1 treated at 20µg/mL.

Acridine orange, which permeates intact cell membranes, stained the cells green. In contrast, ethidium bromide selectively entered cells with compromised membrane integrity, interacting with DNA to produce fluorescence in shades of yellow to orange. This dual staining approach highlighted the various stages of apoptosis, with the observed fluorescence patterns providing clear evidence of apoptotic cell death in response to the *A. cocculus* ethyl acetate extract (Figure 4.3.13).

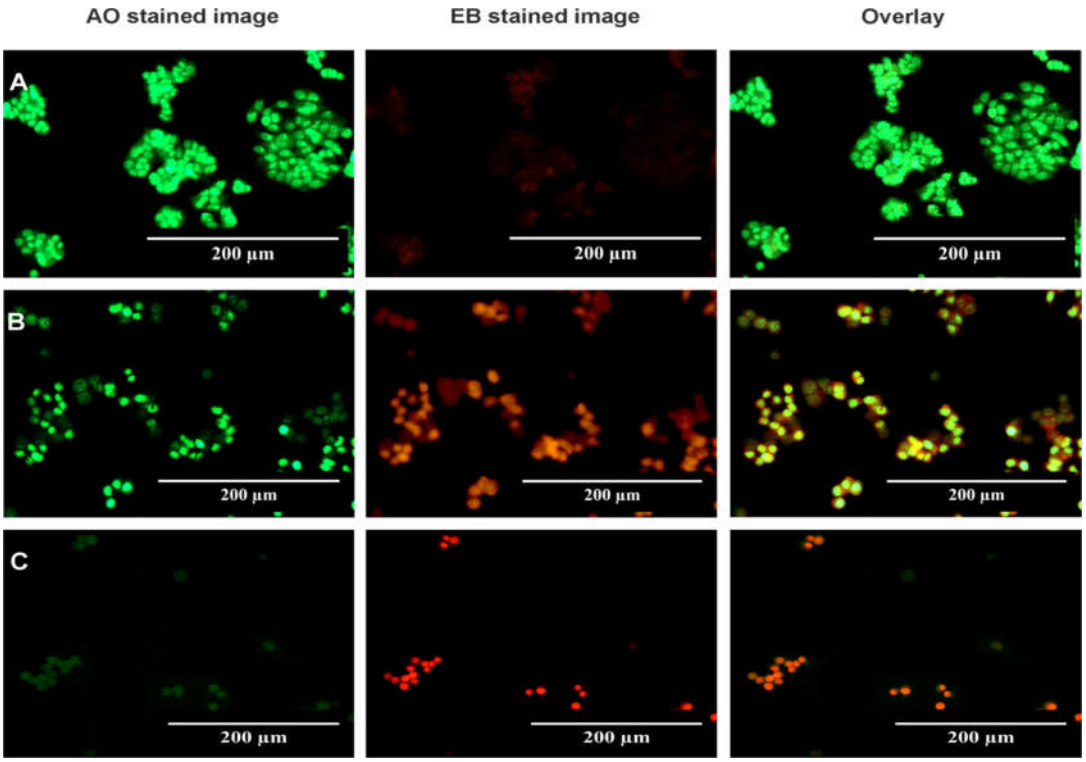


Figure 4.3.12. Effect of *A. cocculus* ethyl acetate extract on HT29 cells after 48 hours of treatment, analyzed using dual staining with acridine orange (AO) and ethidium bromide (EB). AO stains the cytoplasm of live cells (green fluorescence), while EB selectively stains apoptotic cells (orange/red fluorescence) due to compromised membrane integrity, differentiating live and apoptotic cells in treated and untreated groups. (A) DMSO control, (B) HT29 treated at 10µg/mL; (C) HT29 treated at 20µg/mL.

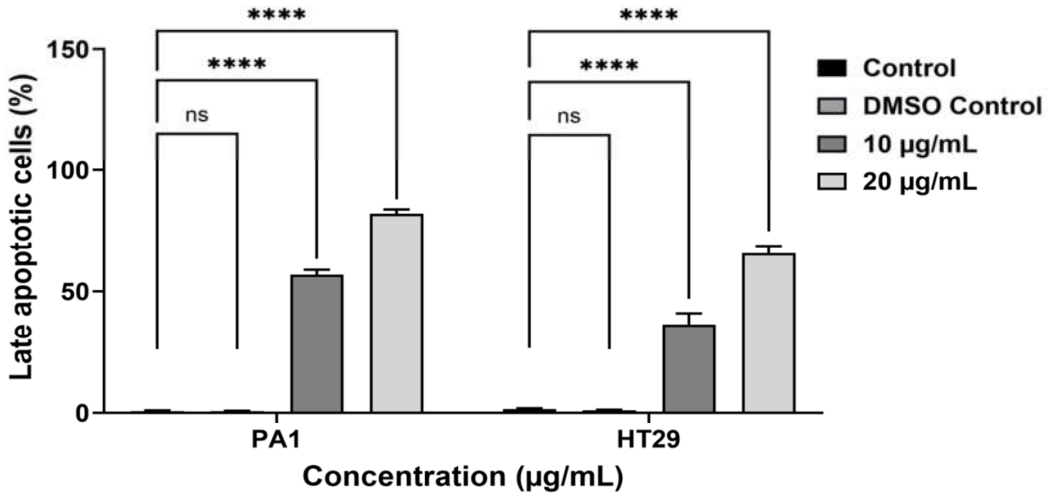


Figure 4.3.13. Effect of *A. cocculus* ethyl acetate extract on PA1 and HT29 cell lines following 48 hours treatment; Quantitative analysis of late apoptotic cells; Data are expressed as mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

4.3.1.7.4. Assessment of mitochondrial membrane potential (ΔΨM) and apoptosis

In the intrinsic apoptotic pathway, mitochondrial membrane depolarization is a key event, leading to increased permeability of the outer mitochondrial membrane due to pore formation and the subsequent release of pro-apoptotic factors like cytochrome c. The alteration in mitochondrial membrane potential (ΔΨM), a hallmark of this pathway, was assessed using the voltage-dependent fluorescent probe Rhodamine-123. Treatment with the *A. cocculus* ethyl acetate extract resulted in a dose-dependent decrease in ΔΨM after 48 hours, as observed through fluorescence microscopy (Figure 4.3.14 and 4.3.15). In contrast, untreated cells showed minimal disruption of ΔΨM.

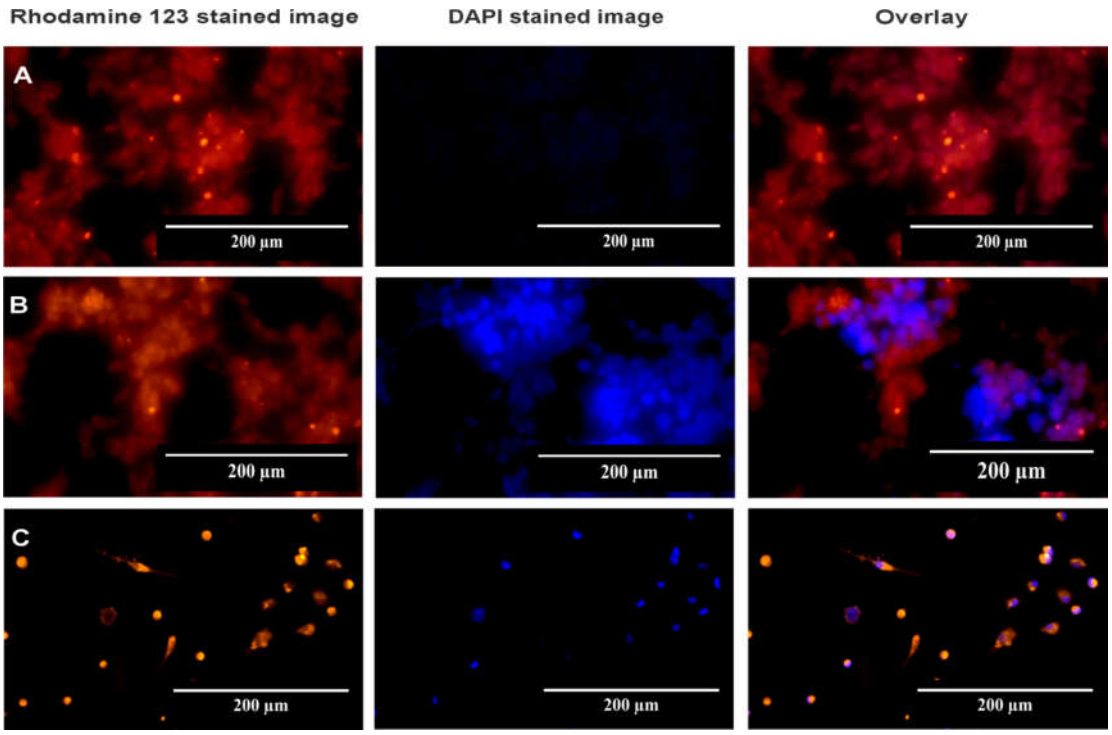


Figure 4.3.14. Effect of *A. cocculus* ethyl acetate extract on mitochondrial membrane potential in PA1 cell line after 48 hours of treatment. (A) DMSO-treated control cells showing intact mitochondrial membrane potential; (B) PA1 cell line treated with 10 µg/mL of extract displaying partial loss of mitochondrial membrane potential; (C) PA1 cell line treated with 20 µg/mL of extract exhibiting a significant reduction in mitochondrial membrane potential. Depolarization was assessed using the fluorescent dye Rhodamine-123, where changes in fluorescence intensity reflected alterations in membrane potential. Nuclei were counterstained with DAPI to visualize cell morphology.

Additionally, nuclear changes during apoptosis were analyzed using DAPI staining, which highlights chromatin condensation and fragmentation. The figures (Figures 4.3.14 and 4.3.15) depict the extent of nuclear alterations in both control and treated cells. Treatment with the *A. cocculus* ethyl acetate extract induced significant apoptosis in PA1 and HT29 cells characterized by increased permeability to DAPI, the presence of nuclear apoptotic bodies, and pronounced chromatin condensation.

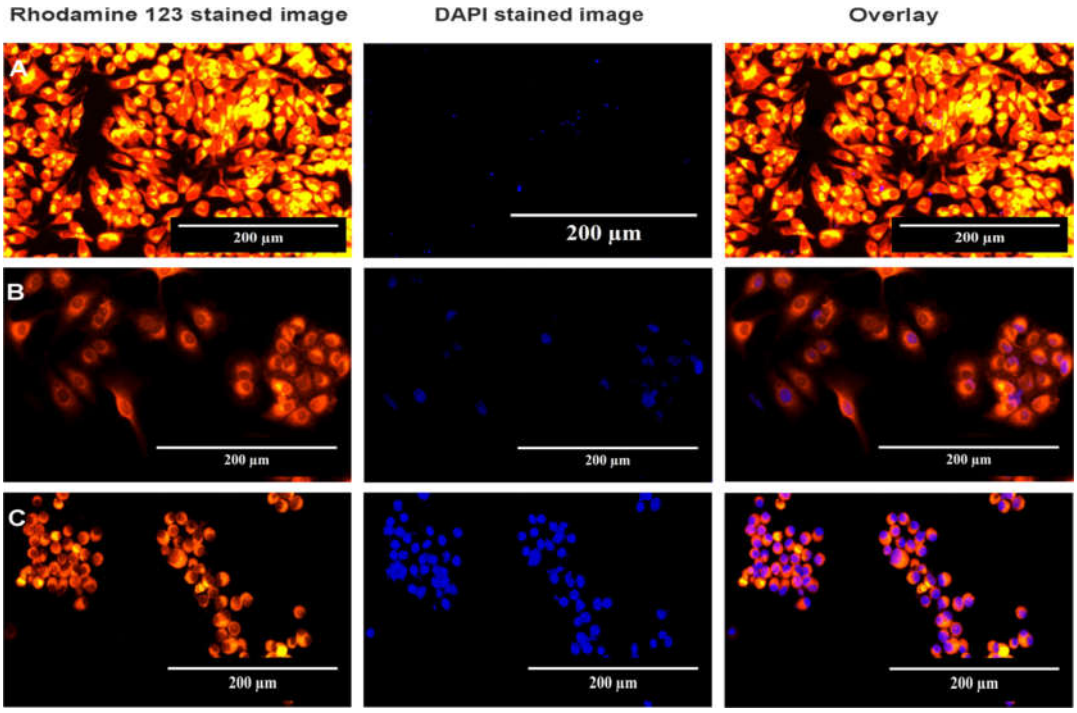


Figure 4.3.15. Effect of *A. cocculus* ethyl acetate extract on mitochondrial membrane potential in HT29 cell line after 48 hours of treatment. (A) DMSO-treated control cells showing intact mitochondrial membrane potential; (B) HT29 cell line treated with 10 µg/mL of extract displaying partial loss of mitochondrial membrane potential; (C) HT29 cell line treated with 20 µg/mL of extract exhibiting a significant reduction in mitochondrial membrane potential. Depolarization was assessed using the fluorescent dye Rhodamine-123, where changes in fluorescence intensity reflected alterations in membrane potential. Nuclei were counterstained with DAPI to visualize cell morphology.

4.3.2. Effect of active fraction from *Melia dubia* (SC3-F8) on cancer cell lines

4.3.2.1. Cytotoxicity of various extracts of *Melia dubia* leaf on different cancer cell lines and normal cell line

The cytotoxic effects of various *Melia dubia* leaf extracts-petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water-were evaluated on different

cancer cell lines and a normal cell line (HaCaT) over a 48-hour period. The chloroform and ethyl acetate extracts generally demonstrated higher cytotoxicity across most cancer cell lines, particularly HT29 (Chloroform extract: 94.23±0.35%), PA1 (chloroform extract: 92.51±0.88%), MCF7 (Chloroform extract: 88.92±1.25%, Ethyl acetate extract: 81.44±1.10%), and UM-SCC-83B (Chloroform extract: 89.22±0.69). In contrast, these extracts showed minimal cytotoxicity across most of the other cell lines, especially the HaCaT (Chloroform extract: 28.70±1.21%, Ethyl acetate extract: 22.38±1.98%) (Chapter 2; Table 2.3.10).

4.3.2.2. IC_{50} calculation of the *Melia dubia* chloroform extract and purified fractions against cancer cell lines

The IC_{50} values of the crude extract and its purified fractions from *Melia dubia* leaf were evaluated for their cytotoxic potency across various cancer cell lines (HT29, MCF7, PA1, UM-SCC-83B) (Table 4.3.1). TLC-B3 demonstrated the lowest IC_{50} values across all tested cell lines, indicating its highest potency among the fractions. With each step in purification, the IC_{50} value decreased from crude to TLC-B3.

Table 4.3.1. IC_{50} of *M. dubia* chloroform extract and the active fractions obtained from each step of purification.

Sl. No.	Sample	IC_{50} Concentration (µg/mL)			
		HT29	MCF7	PA1	UMSCC83B
1	Crude	61.26±2.55	75.54±1.16	29.77±0.86	31.28±1.77
2	SC1-F8	31.07±4.46	60.26±2.21	18.93±1.37	23.30±1.90
3	SC2-F8	17.86±1.29	42.21±3.20	12.65±1.35	11.86±1.31
4	SC3-F8	10.27±0.79	19.62±0.26	8.01±1.54	10.84±1.46
5	TLC-B3	7.87±1.32	12.91±1.72	5.37±1.62	8.01±0.95

Experiments were done three times in duplicates. Data are expressed as mean±SEM.

4.3.2.3. Trypan blue dye exclusion method

The Trypan Blue dye exclusion method is a widely-used assay to evaluate cell viability, based on the principle that live cells possess intact cell membranes that exclude the Trypan Blue dye, while dead cells have compromised membranes that

allow the dye to enter, staining them blue. This method was employed to assess the effects of an active fraction on the viability of various cancer cell lines, including HT29 (colon cancer), MCF7 (breast cancer), PA1 (ovarian cancer), and UM-SCC-83B (head and neck cancer). The study measured cell viability of untreated cells and at different concentrations of the active fraction (SC3-F8): IC_{20}, IC_{50}, and IC_{80}. The results, illustrated in the bar graph (Figure 4.3.16) demonstrate a clear trend across all cell lines: the number of viable cells is highest in the control group and progressively decreases with increasing concentrations of the active fraction. This indicates that the active fraction is effective in reducing cell viability in a dose-dependent manner.

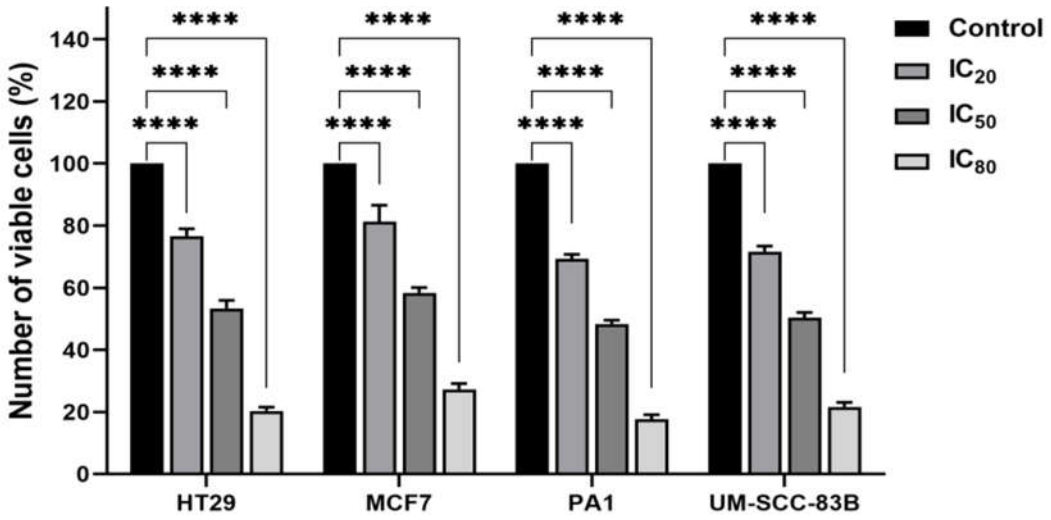


Figure 4.3.16. The percentage of viable cells as assessed by trypan blue dye exclusion method on treatment with the active fraction, SC3-F8 at different concentrations on HT29, MCF7, PA1 and UM-SCC-83B cell lines incubated for 48 hours. Data are expressed as mean±SEM. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

For all the cell lines, the control group showed 100% viability. Upon treatment with the purified fractions, in HT29, cell viability decreased to 20.33±1.20% at IC□□. Similarly, MCF7 cell line exhibited a substantial reduction in viability upon treatment at IC_{80} concentration (27.33±1.86%). The other cell lines, both PA1 and UM-SCC-83B also showed the same trend in the viability (at IC_{80}, cell viability: 17.67±1.45% and 21.67±1.45% respectively). This significant drop in cell viability across these concentrations suggests that the active fraction is highly effective against the four cancer cell lines. The differences in cell viability between the

control and treated groups were statistically significant, with p-values less than 0.0001 (denoted by ****). This high level of significance underscores the reliability of the results and the potent effect of the active fraction.

4.3.2.4. Clonogenic assay

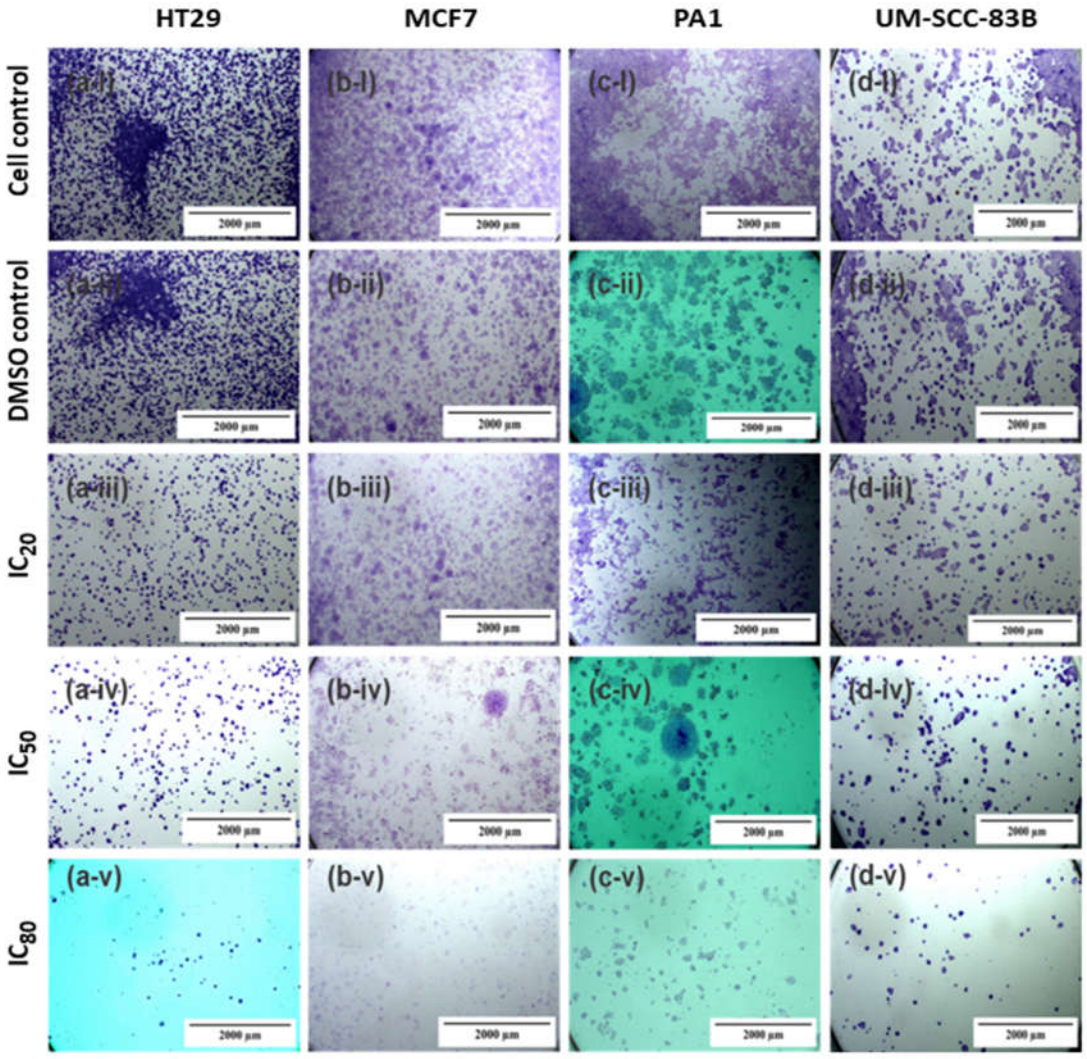


Figure 4.3.17. Clonogenic survival assay following treatment with the active fraction, SC3-F8 from *M. dubia* chloroform extract. The number of colonies decreased when the cells were exposed to the extracts at different concentrations.

The clonogenic assay, also known as the colony formation assay, is a critical technique in cancer research that evaluates the ability of a single cell to grow into a colony, thereby indicating its reproductive viability and potential for proliferation. In the context of the study, this assay was performed on four cancer cell lines: HT29 (colon cancer), MCF7 (breast cancer), PA1 (ovarian cancer), and UM-SCC-83B (head and neck cancer) to assess the effects of an active fraction, SC3-F8 at various inhibitory concentrations: IC_{20}, IC_{50}, and IC_{80}, with untreated cells serving as the control group. The results, visually represented in the provided image (Figure 4.3.17), revealed a dose-dependent reduction in colony formation across all cell lines, demonstrating the active fraction's efficacy in inhibiting clonogenic survival. In the control groups, a dense population of colonies was observed, indicating high clonogenic potential. The DMSO control groups showed a slight reduction in colony density which was not significant ($p>0.05$) compared to the untreated control, but still exhibited substantial colony formation. Upon treatment with the SC3-F8, there was a noticeable reduction in the number of colonies formed. For HT29 cells, the DMSO control group showed high viability with dense colonies (98.47±1.03%), while treatment with IC_{20} (64.44±3.36%), IC_{50} (48.62±1.16%), and IC_{80} (5.91±0.83%) resulted in a progressive decrease in colony formation, culminating in almost complete inhibition at IC_{80}. Similarly, MCF7 cells exhibited robust colony formation in the control group (98.76±3.78%), with substantial reductions observed at IC_{20} (70.76±5.64%), IC_{50} (44.33±0.81%), and IC_{80} (20.32±1.75%), demonstrating a strong inhibitory effect of the active fraction. PA1 and UM-SCC-83B cell lines followed the same trend (reduced from 89.36±0.94% to 8.92±2.15% and from 93.38±1.63% to 14.29±3.73% respectively), with significant reductions in colony numbers as the concentration of the active fraction increased, indicating effective inhibition of clonogenic potential across all concentrations tested (Figure 4.3.18).

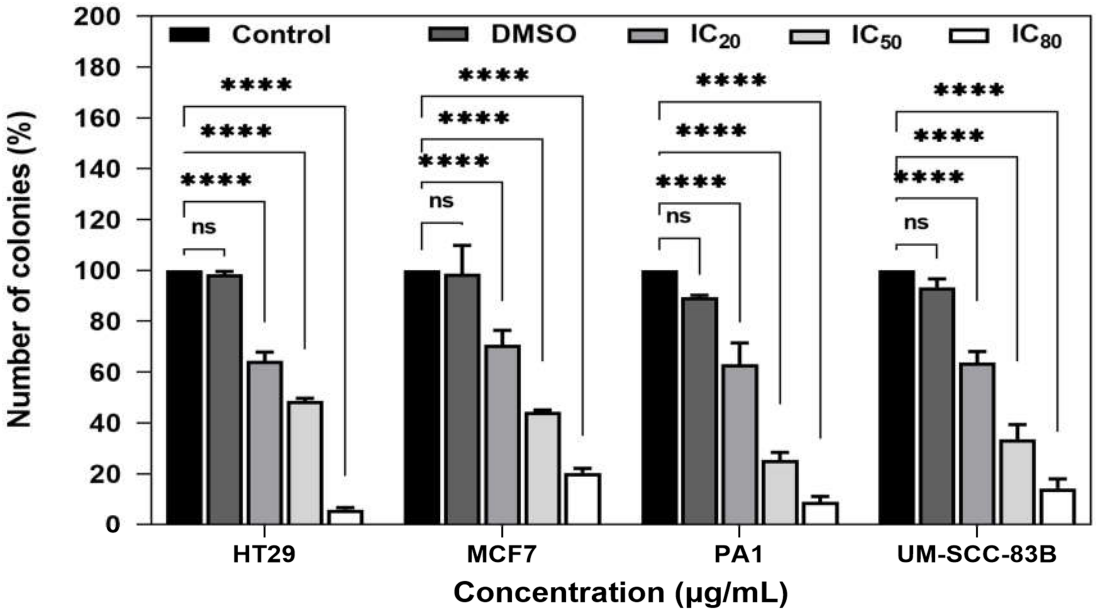


Figure 4.3.18. Quantitative representation of the clonogenic survival assay following treatment with the active fraction, SC3-F8 from *M. dubia* chloroform extract. The number of colonies decreased when the cells were exposed to the extracts at different concentrations. Data are expressed as mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns - not significant.

4.3.2.5. Apoptosis analysis

The analysis of apoptosis was done by studying various aspects/changes in cells upon undergoing apoptosis. The major aspects under the study were cytomorphological changes, reduction in mitochondrial membrane potential, phosphatidyl serine externalization, DNA fragmentation, and the mRNA level gene expression of the genes involved in apoptosis using real time PCR and protein level expression using fluorescent dye conjugated anti-antibody in flow cytometry.

4.3.2.5.1. Cytomorphological analysis of apoptosis

The cytomorphological changes associated with apoptosis on treatment with the extracts were analysed using phase contrast microscopy and scanning electron microscopy.

4.3.2.5.1.1. Phase contrast microscopy

Phase contrast microscopy observing apoptosis reveals several key morphological changes associated with programmed cell death. Early stages of apoptosis are characterized by cell shrinkage, which is evident as cells in phase contrast images

appear smaller compared to healthy, untreated cells that are typically more spread out and expanded. Additionally, apoptotic cells often exhibit rounding and become more spherical, as shown by their deviation from the usual elongated or spread-out appearance. As the process progresses, cells may lose their adherence to the surface, leading to an increased number of detached or floating cells in the culture medium, particularly in treated groups (Figure 4.3.19).

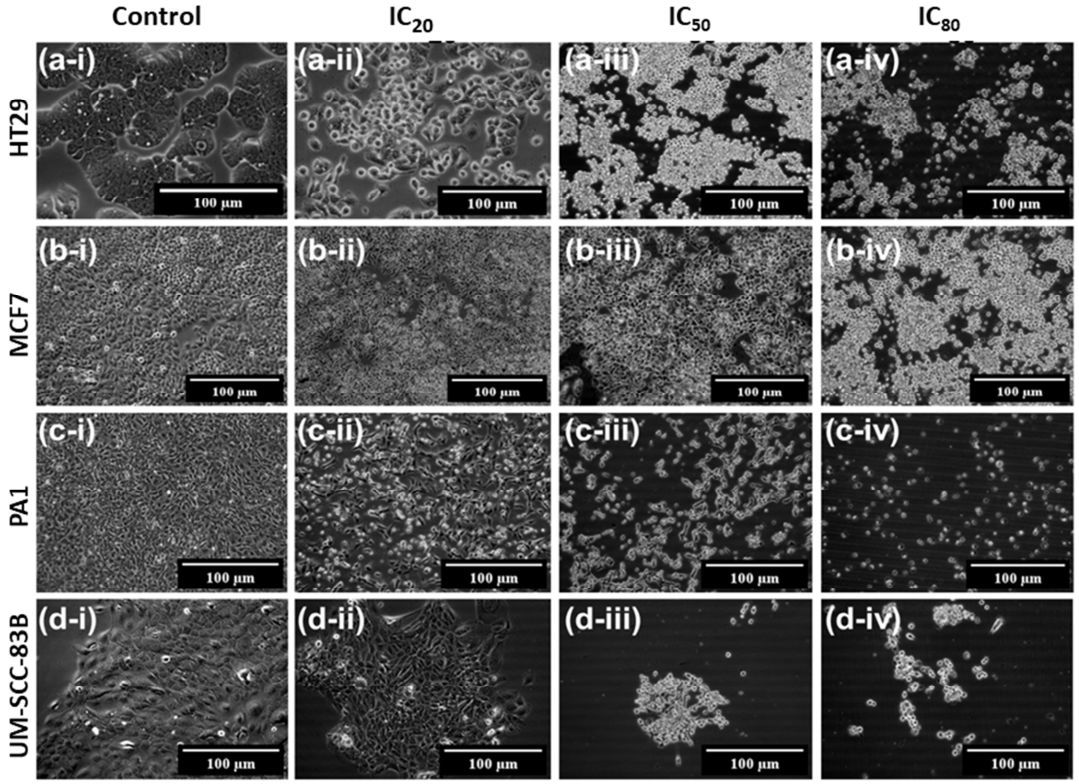


Figure 4.3.19. Phase contrast microscopic images of cytomorphological changes in HT29, MCF7, PA1 and UM-SCC-83B cancer cell lines on treatment of the active fraction, SC3-F8 from *M. dubia* chloroform extract after 48 hours. (a-i) HT29 - DMSO Control; (a-ii) HT29 treated at IC_{20}; (a-iii) HT29 treated at IC_{50}; (a-iv) HT29 treated at IC_{80}; (b-i) MCF7 - DMSO Control; (b-ii) MCF7 treated at IC_{20}; (b-iii) MCF7 treated at IC_{50}; (b-iv) MCF7 treated at IC_{80}; (c-i) PA1 - DMSO Control; (c-ii) PA1 treated at IC_{20}; (c-iii) PA1 treated at IC_{50}; (c-iv) PA1 treated at IC_{80}; (d-i) UM-SCC-83B - DMSO Control; (d-ii) UM-SCC-83B treated at IC_{20}; (d-iii) UM-SCC-83B treated at IC_{50}; (d-iv) UM-SCC-83B treated at IC_{80}.

Further indications of apoptosis include membrane blebbing, where small bulges form on the cell surface, visible in phase contrast images as protruding membrane blebs not typically observed in healthy cells. Although nuclear changes such as condensation and fragmentation are not directly visible with phase contrast microscopy, alterations in cell morphology, such as increased fragmentation or irregularity, indirectly suggest these nuclear changes. Overall, the phase contrast microscopy images show a decreased density of viable cells in treated conditions compared to controls, reflecting increased cell death and confirming the presence and extent of apoptosis in the cell lines under study.

4.3.2.5.1.2. Field-emission scanning electron microscopy

Scanning electron microscopy (SEM) provided a detailed view of the morphological changes associated with apoptosis, revealing key features of programmed cell death. Early signs of apoptosis observed with SEM included cell shrinkage, where cells appeared reduced in size compared to healthy, untreated cells, which typically had a more expanded and rounded appearance. Additionally, membrane blebbing, characterized by the formation of small bulges on the cell surface, was a prominent feature that SEM clearly visualized. The presence of these blebs indicated underlying cellular damage. Although nuclear changes such as condensation and fragmentation were not directly visible with SEM, alterations in cell morphology, such as increased fragmentation or irregularity, suggested these nuclear changes. Overall, SEM images showed a reduced density of viable cells in treated samples compared to controls, highlighting increased cell death and confirming the presence and extent of apoptosis in the observed cell lines (Figure 4.3.20).

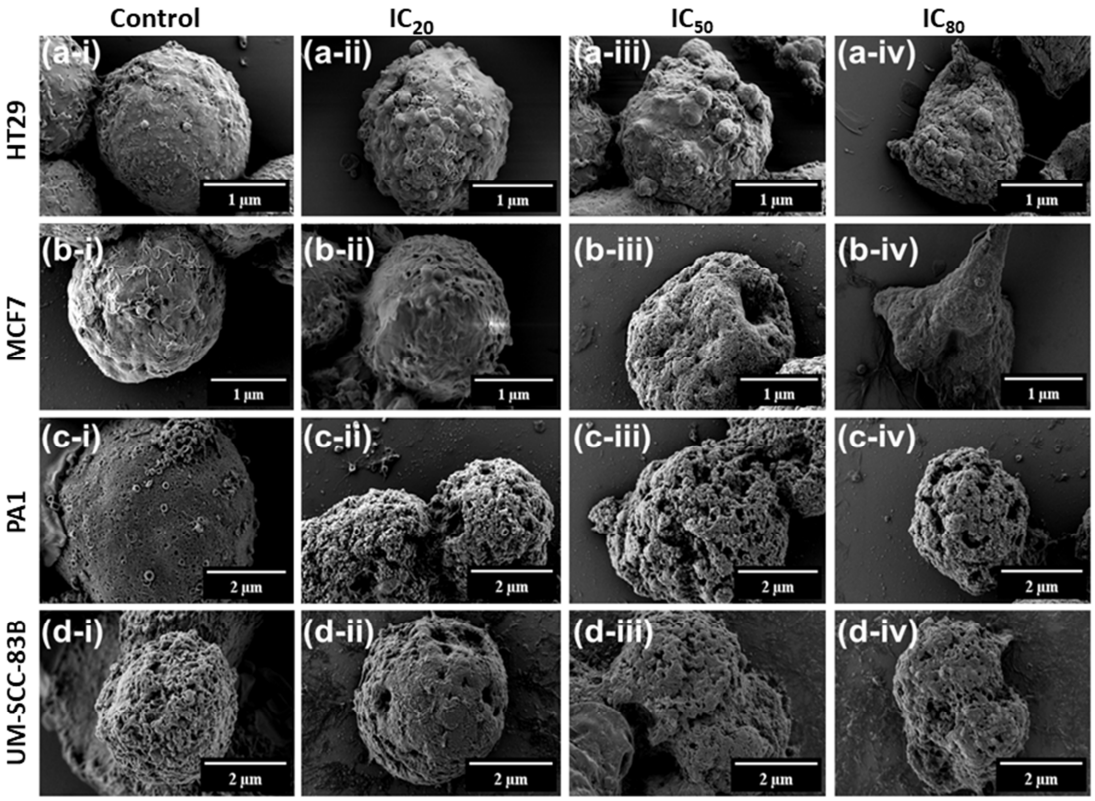


Figure 4.3.20. Scanning electron microscopic images of cytomorphological changes in HT29, MCF7, PA1 and UM-SCC-83B cancer cell lines on treatment of the active fraction, SC3-F8 from *M. dubia* chloroform extract after 48 hours. (a-i) HT29 - DMSO Control; (a-ii) HT29 treated at IC_{20}; (a-iii) HT29 treated at IC_{50}; (a-iv) HT29 treated at IC_{80}; (b-i) MCF7 - DMSO Control; (b-ii) MCF7 treated at IC_{20}; (b-iii) MCF7 treated at IC_{50}; (b-iv) MCF7 treated at IC_{80}; (c-i) PA1 - DMSO Control; (c-ii) PA1 treated at IC_{20}; (c-iii) PA1 treated at IC_{50}; (c-iv) PA1 treated at IC_{80}; (d-i) UM-SCC-83B - DMSO Control; (d-ii) UM-SCC-83B treated at IC_{20}; (d-iii) UM-SCC-83B treated at IC_{50}; (d-iv) UM-SCC-83B treated at IC_{80}.

4.3.2.5.1.3. Acridine orange-Ethidium bromide (AO-EB) dual staining

Dual staining with acridine orange (AO) and ethidium bromide (EB) is an effective technique for assessing apoptosis qualitatively and quantitatively. AO, being permeable to intact cell membranes, stains the nuclei green, while EB, which only enters cells with compromised membrane integrity, binds to DNA and emits yellow to orange fluorescence depending on the stage of apoptosis. In this assay, normal cells exhibit uniformly bright green nuclei, early apoptotic cells show green nuclei with bright patches indicative of condensed or fragmented DNA, and late apoptotic cells display orange to red nuclei with highly fragmented and condensed chromatin (Liu *et al.,*2015).

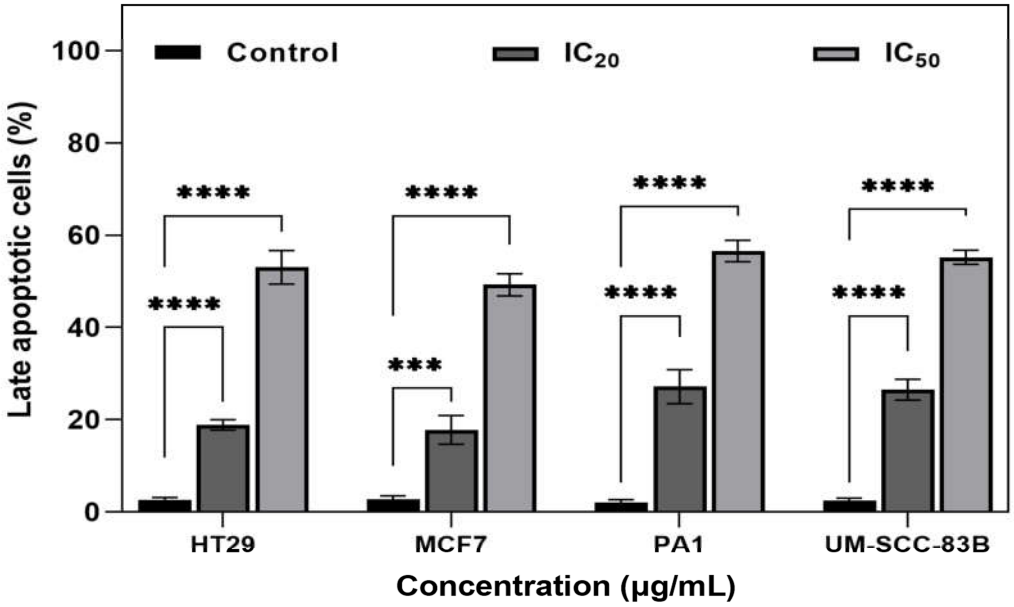


Figure 4.3.21. Effect of the active fraction, SC3-F8 from *M. dubia* chloroform extract on HT29, MCF7, PA1 and UM-SCC-83B cell lines following 48 hours treatment; Quantitative analysis of late apoptotic cells; Data are expressed as mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and ns-not significant.

In the SC3-F8-treated cell lines, an increase in cells with orange fluorescence, indicative of apoptosis, was observed in a concentration-dependent manner compared to the control cells, which primarily exhibited bright green nuclei and normal morphology. Specifically, at the highest concentration of the active fraction, SC3-F8 (IC_{50}), there was a significant increase in the apoptotic cell population: 53.06±3.65% in HT29 cells, 49.24±2.39% in MCF7 cells, 56.60±2.33% in PA1 cells, and 55.24±2.61% in UM-SCC-83B cells (Figure 4.3.21). This data suggests that the active fraction induces apoptosis in these cell lines, with the effect being more mmd at higher concentrations.

4.3.2.5.3. Mitochondrial membrane potential

JC-1 staining was analysed using flow cytometry to assess mitochondrial membrane potential and apoptosis in HT29, MCF7, PA1, and UMSCC83B cell lines under various treatment conditions (DMSO Control, IC_{50}, and IC_{80}).

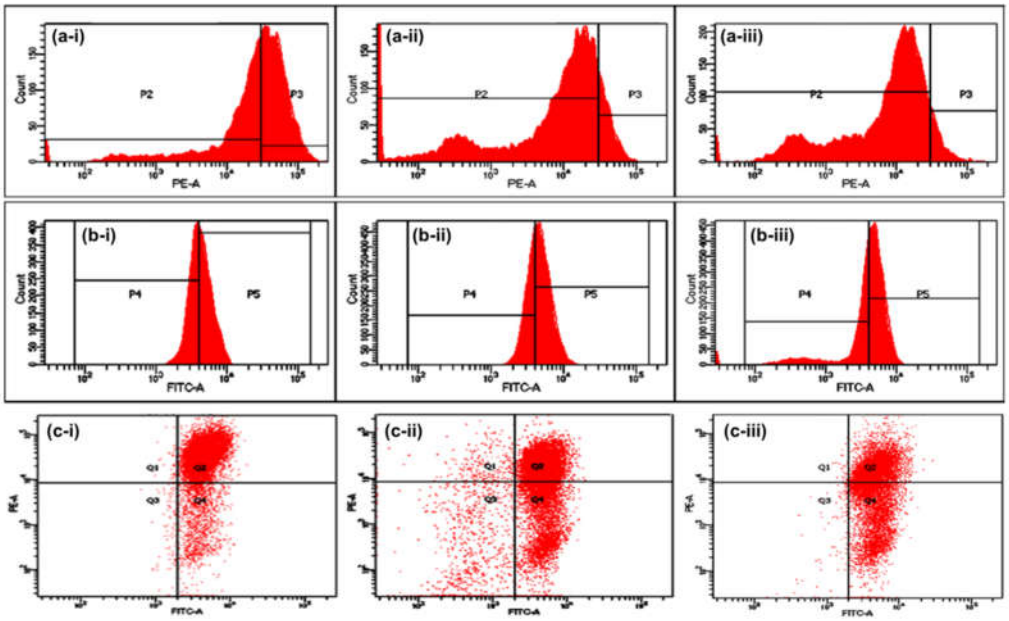


Figure 4.3.22. Effect of the active fraction SC3-F8 from *M. dubia* chloroform extract on mitochondrial membrane potential in HT29 cells after 48 hours of treatment assessed using flow cytometry with JC-1 as the fluorochrome. (a) Cell population gated in the PE channel: (a-i) DMSO-treated control cells showing intact mitochondrial membrane potential; (a-ii) HT29 cells treated with the IC_{50} concentration of extract displaying partial loss of mitochondrial membrane potential; (a-iii) HT29 cells treated with the IC_{80} concentration of extract showing significant reduction in mitochondrial membrane potential. (b) Cell population gated in the FITC channel: (b-i) DMSO-treated control cells; (b-ii) HT29 cells treated with the IC_{50} concentration; (b-iii) HT29 cells treated with the IC_{80} concentration. (c) Population shift from the PE channel to the FITC channel, indicative of apoptosis. Mitochondrial membrane depolarization was evaluated by the fluorescence shift of JC-1, where changes in fluorescence intensity corresponded to alterations in membrane potential.

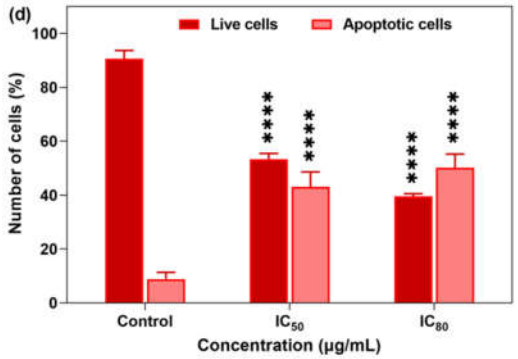


Figure 4.3.23. Quantitative representation of live and apoptotic cells in the control and treated HT29 cell line, generated using flow cytometry with JC-1 as the fluorochrome. The bar graph depicts the proportion of live cells (intact mitochondrial membrane potential, represented by red fluorescence in the PE channel) and apoptotic cells (depolarized mitochondrial membrane, represented by green fluorescence in the FITC channel) in the DMSO-treated control group, and in HT29 cells treated with IC_{50} and IC_{80} concentrations of the SC3-F8 fraction from *M. dubia* chloroform extract. A shift from red to green fluorescence indicates mitochondrial membrane depolarization and apoptosis, with higher extract concentrations leading to an increased apoptotic cell population. Data are represented as mean±SEM. Level of significance: *=p<0.05, **=p<0.01, ***=p<0.001 and ****=p<0.0001.

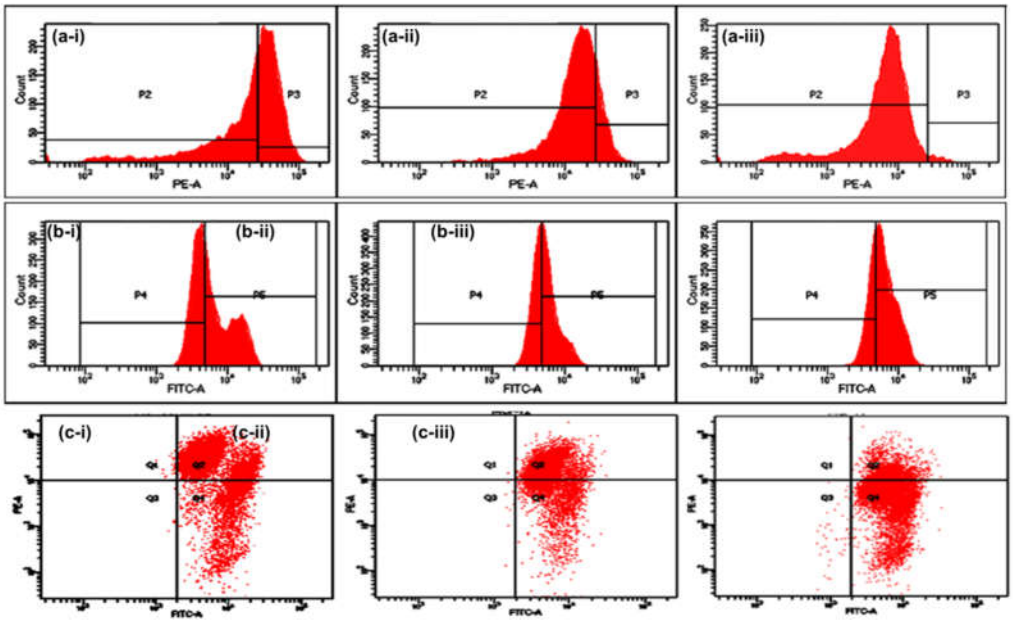


Figure 4.3.24. Effect of the active fraction SC3-F8 from *M. dubia* chloroform extract on mitochondrial membrane potential in MCF7 cells after 48 hours of treatment assessed using flow cytometry with JC-1 as the fluorochrome. (a) Cell population gated in the PE channel: (a-i) DMSO-treated control cells showing intact mitochondrial membrane potential; (a-ii) MCF7 treated with the IC_{50} concentration of extract displaying partial loss of mitochondrial membrane potential; (a-iii) MCF7 cells treated with the IC_{80} concentration of extract showing significant reduction in mitochondrial membrane potential. (b) Cell population gated in the FITC channel: (b-i) DMSO-treated control cells; (b-ii) MCF7 cells treated with the IC_{50} concentration; (b-iii) MCF7 cells treated with the IC_{80} concentration. (c) Population shift from the PE channel to the FITC channel, indicative of apoptosis. Mitochondrial membrane depolarization was evaluated by the fluorescence shift of JC-1, where changes in fluorescence intensity corresponded to alterations in membrane potential.

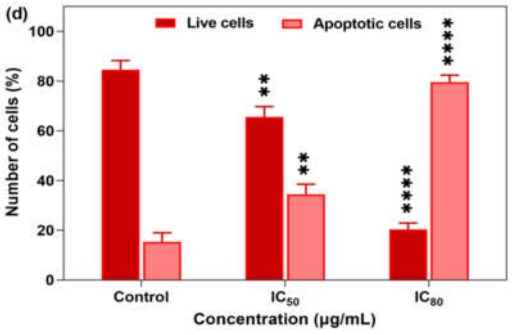


Figure 4.3.25. Quantitative representation of live and apoptotic cells in the control and treated MCF7 cell line, generated using flow cytometry with JC-1 as the fluorochrome. The bar graph depicts the proportion of live cells (intact mitochondrial membrane potential, represented by red fluorescence in the PE channel) and apoptotic cells (depolarized mitochondrial membrane, represented by green fluorescence in the FITC channel) in the DMSO-treated control group, and in MCF7 cells treated with IC_{50} and IC_{80} concentrations of the SC3-F8 fraction from *M. dubia* chloroform extract. A shift from red to green fluorescence indicates mitochondrial membrane depolarization and apoptosis, with higher extract concentrations leading to an increased apoptotic cell population. Data are represented as mean±SEM. Level of significance: *=p<0.05, **=p<0.01, ***=p<0.001 and ****=p<0.0001.

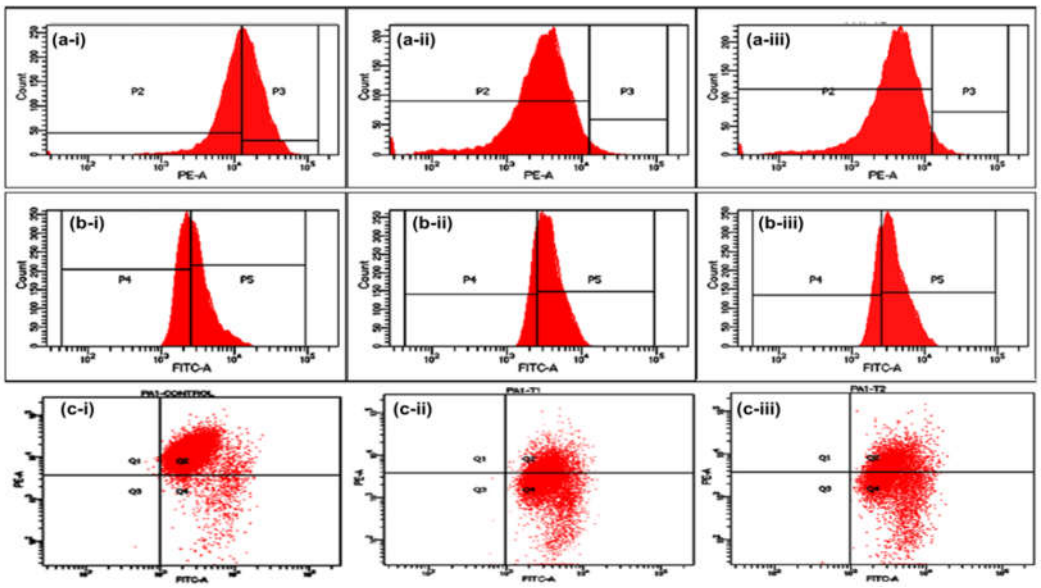


Figure 4.3.26. Effect of the active fraction SC3-F8 from *M. dubia* chloroform extract on mitochondrial membrane potential in PA1 cell line after 48 hours of treatment assessed using flow cytometry with JC-1 as the fluorochrome. (a) Cell population gated in the PE channel: (a-i) DMSO-treated control cells showing intact mitochondrial membrane potential; (a-ii) PA1 treated with the IC_{50} concentration of extract displaying partial loss of mitochondrial membrane potential; (a-iii) PA1 cells treated with the IC_{80} concentration of extract showing significant reduction in mitochondrial membrane potential. (b) Cell population gated in the FITC channel: (b-i) DMSO-treated control cells; (b-ii) PA1 cells treated with the IC_{50} concentration; (b-iii) PA1 cells treated with the IC_{80} concentration. (c) Population shift from the PE channel to the FITC channel, indicative of apoptosis. Mitochondrial membrane depolarization was evaluated by the fluorescence shift of JC-1, where changes in fluorescence intensity corresponded to alterations in membrane potential.

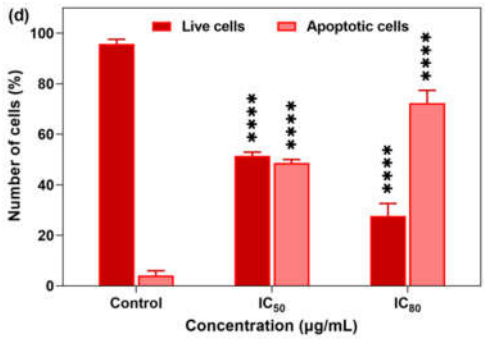


Figure 4.3.27. Quantitative representation of live and apoptotic cells in the control and treated PA1 cell line generated using flow cytometry with JC-1 as the fluorochrome. The bar graph depicts the proportion of live cells (intact mitochondrial membrane potential, represented by red fluorescence in the PE channel) and apoptotic cells (depolarized mitochondrial membrane, represented by green fluorescence in the FITC channel) in the DMSO-treated control group, and in PA1 cells treated with IC_{50} and IC_{80} concentrations of the SC3-F8 fraction from *M. dubia* chloroform extract. A shift from red to green fluorescence indicates mitochondrial membrane depolarization and apoptosis, with higher extract concentrations leading to an increased apoptotic cell population. Data are represented as mean±SEM. Level of significance: *=p<0.05, **=p<0.01, ***=p<0.001 and ****=p<0.0001.

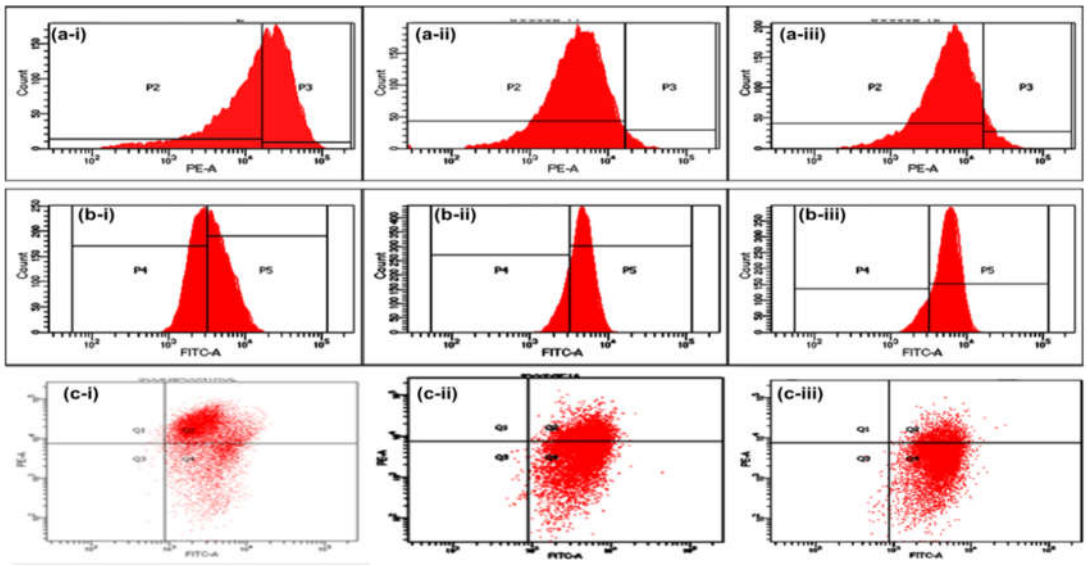


Figure 4.3.28. Effect of the active fraction SC3-F8 from *M. dubia* chloroform extract on mitochondrial membrane potential in UM-SCC-83B cell line after 48 hours of treatment assessed using flow cytometry with JC-1 as the fluorochrome. (a) Cell population gated in the PE channel: (a-i) DMSO-treated control cells showing intact mitochondrial membrane potential; (a-ii) UM-SCC-83B treated with the IC_{50} concentration of extract displaying partial loss of mitochondrial membrane potential; (a-iii) UM-SCC-83B cells treated with the IC_{80} concentration of extract showing significant reduction in mitochondrial membrane potential. (b) Cell population gated in the FITC channel: (b-i) DMSO-treated control cells; (b-ii) UM-SCC-83B cells treated with the IC_{50} concentration; (b-iii) UM-SCC-83B cells treated with the IC_{80} concentration. (c) Population shift from the PE channel to the FITC channel, indicative of apoptosis. Mitochondrial membrane depolarization was evaluated by the fluorescence shift of JC-1, where changes in fluorescence intensity corresponded to alterations in membrane potential.

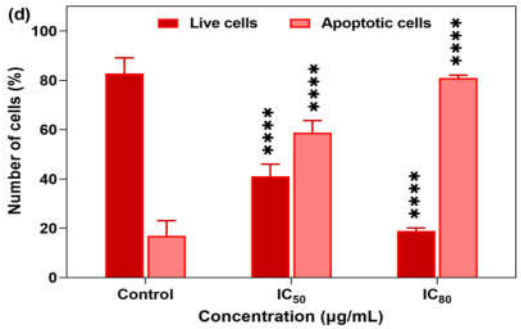


Figure 4.3.29. Quantitative representation of live and apoptotic cells in the control and treated UM-SCC-83B cell line, generated using flow cytometry with JC-1 as the fluorochrome. The bar graph depicts the proportion of live cells (intact mitochondrial membrane potential, represented by red fluorescence in the PE channel) and apoptotic cells (depolarized mitochondrial membrane, represented by green fluorescence in the FITC channel) in the DMSO-treated control group, and in UM-SCC-83B cells treated with IC_{50} and IC_{80} concentrations of the SC3-F8 fraction from *M. dubia* chloroform extract. A shift from red to green fluorescence indicates mitochondrial membrane depolarization and apoptosis, with higher extract concentrations leading to an increased apoptotic cell population. Data are represented as mean±SEM. Level of significance: *=p<0.05, **=p<0.01, ***=p<0.001 and ****=p<0.0001.

For all cell lines, the control showed high red fluorescence (90.63±3.02% live cells) and low green fluorescence (8.83±2.50% apoptotic cells), indicating stable mitochondrial membrane potential. As the concentration increased, red fluorescence decreased with increase in green fluorescence reflecting reduced mitochondrial membrane potential and increased apoptosis. At IC_{80} concentration, in HT29, the apoptotic cells were 43.03±5.53% (Figures 4.3.22, 4.3.23). In the IC_{80} treated populations, the percentage of apoptotic cells were 79.57±2.80%, 72.37±4.97% and 80.93±1.13% in MCF7, PA1 and UM-SCC-83B respectively (Figures 4.3.24-4.3.29).

4.3.2.5.4. Annexin-V assay

Apoptosis analysis using Annexin V staining, which detects the appearance of phosphatidylserine (PS) on the cell surface—a hallmark of apoptosis—was performed on HT29, PA1, and UM-SCC-83B cell lines under different treatment conditions (control, IC_{50}, and IC_{80}). Phosphatidylserine, normally restricted to the inner membrane layer, can be detected by Annexin V tagged with a fluorochrome using fluorescence microscopy and flow cytometry. Annexin V-PI co-staining further differentiates late apoptotic/necrotic cells from early apoptotic cells, as propidium iodide (PI) only enters cells with compromised membrane integrity and interacts with DNA (Rieger *et al.,* 2011; Koc *et al.,* 2018).

For HT29 cells, the control group exhibited a high percentage of healthy cells (98.00±0.26%) and minimal early (1.23±0.49%) and late apoptotic (0.37±0.12%) cells, with only a small fraction of necrotic cells (0.43±0.15%). Following IC_{50} treatment, there was a significant increase in early apoptotic cells to 42.67±1.50% and a rise in late apoptotic cells to 14.03±1.75%, while healthy cells dropped to 33.57±2.28%. At IC_{80}, the proportion of healthy cells further decreased to 11.73±0.55%, with late apoptotic cells increasing substantially to 65.03±2.56%, and early apoptotic cells reduced to 19.13±1.27%. Necrotic cells were slightly lower at 4.07±2.27% (Figure 4.3.30).

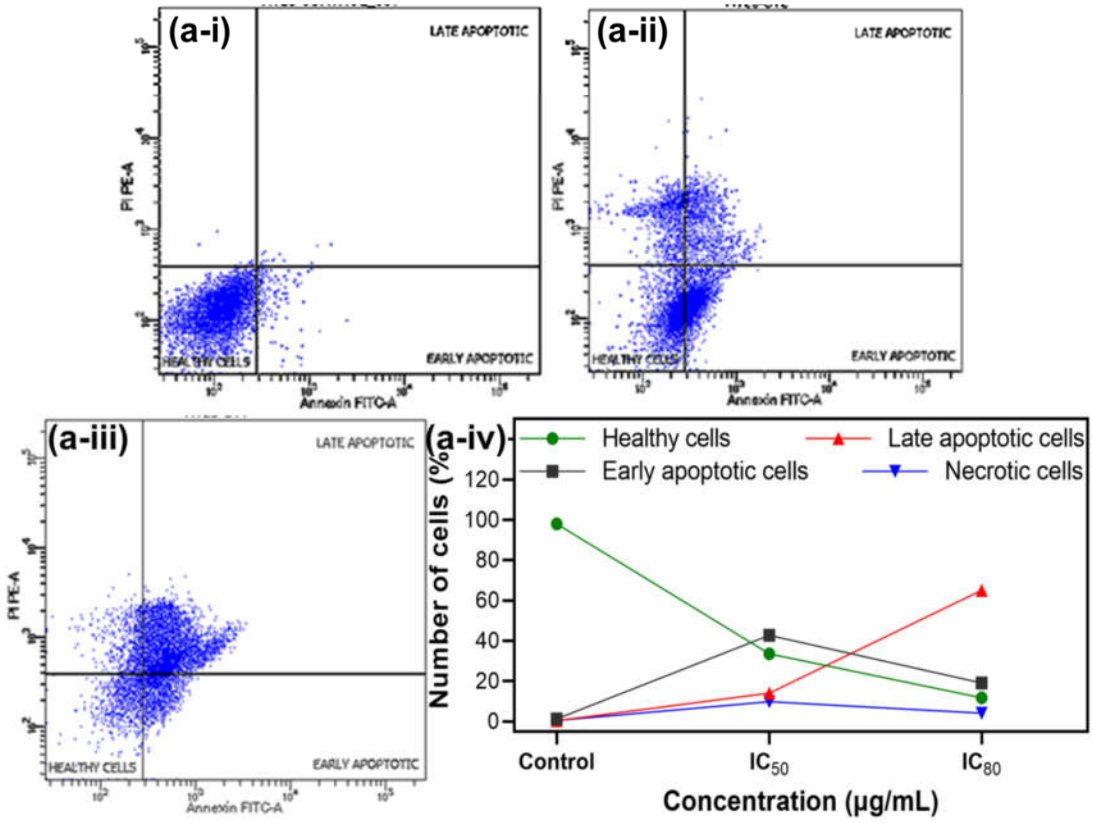


Figure 4.3.30. The SC3-F8 fraction from *M. dubia* chloroform extract induces apoptosis in HT29 cells. (a-i) Control cell population; (a-ii) Cells treated with IC_{50} of the extract for 48 hours; (a-iii) Cells treated with IC_{80} of the extract for 48 hours, followed by staining with annexin-V/PI. The Q3 quadrant (annexin V−/PI−) represents the percentage of normal cells; the Q4 quadrant (annexin V+/PI−) shows the percentage of cells in early apoptosis; and the Q2 quadrant (annexin V+/PI+) indicates cells in late apoptosis. (a-iv) Line diagram illustrating the percentage of cells in each stage of apoptosis (normal, early, and late) for each treatment condition.

In PA1 cells, the control group had a high percentage of healthy cells (96.20±0.61%). IC_{50} treatment resulted in a decrease in healthy cells to 53.03±2.14% and a moderate rise in late apoptotic cells to 28.7±5.02%. At IC_{80}, the percentage of healthy cells dropped to 34.77±2.62%, early apoptotic cells rose significantly to 45.33±1.92%, and late apoptotic cells increased to 18.40±2.66%. Necrotic cells were relatively low at 1.2% (Figure 4.3.31).

For UM-SCC-83B cells, the control group showed 85.23±2.77% healthy cells and low early and late apoptotic cells and necrotic cells. IC_{50} treatment caused a reduction in healthy cells to 32.30±3.74% and a significant increase in early apoptotic cells to 43.93±1.88%, with late apoptotic cells rising to 20.37±2.26%. At

IC_{80}, healthy cells were further reduced to 11.17±1.84%, early apoptotic cells were 13.03±2.86%, and late apoptotic cells increased dramatically to 71.23±2.64%. Necrotic cells remained relatively stable at 4.5±2.11% (Figure 4.3.32).

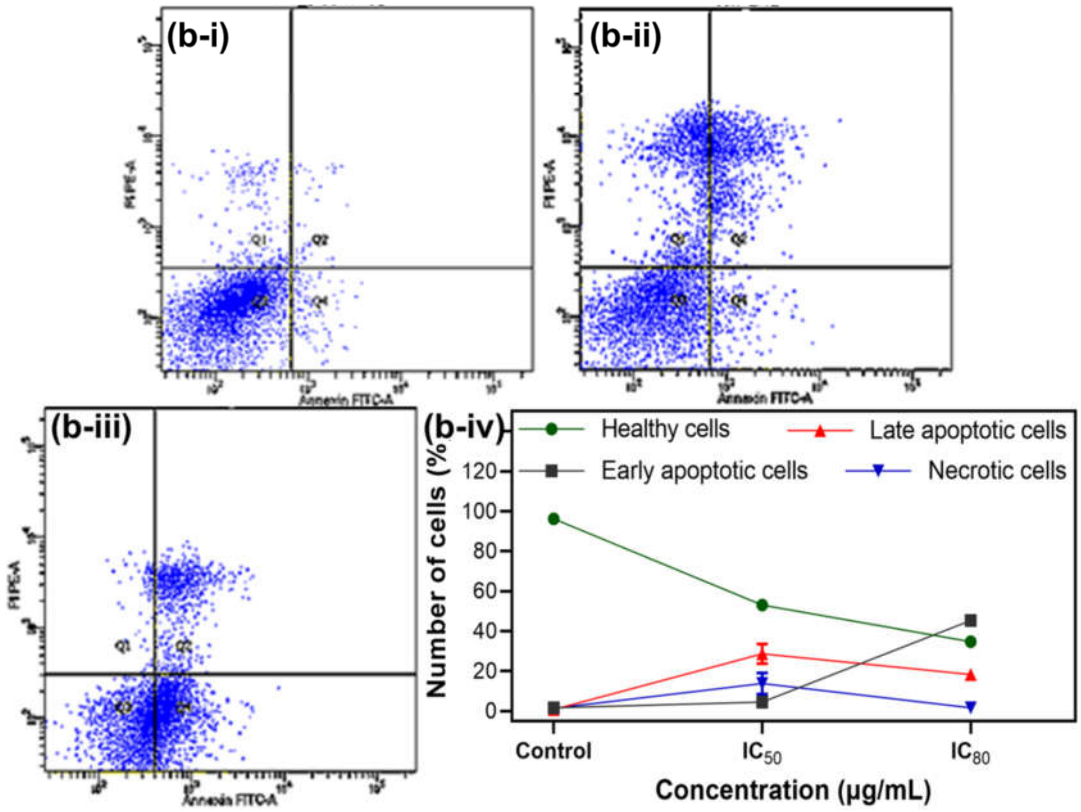


Figure 4.3.31. The SC3-F8 fraction from *M. dubia* chloroform extract induces apoptosis in PA1 cells. (a-i) Control cell population; (a-ii) Cells treated with IC_{50} of the extract for 48 hours; (a-iii) Cells treated with IC_{80} of the extract for 48 hours, followed by staining with annexin-V/PI. The Q3 quadrant (annexin V−/PI−) represents the percentage of normal cells; the Q4 quadrant (annexin V+/PI−) shows the percentage of cells in early apoptosis; and the Q2 quadrant (annexin V+/PI+) indicates cells in late apoptosis. (a-iv) Line diagram illustrating the percentage of cells in each stage of apoptosis (normal, early, and late) for each treatment condition.

These results demonstrate the impact of increasing treatment concentrations on apoptosis, highlighting significant shifts from healthy to early and late apoptotic states and changes in necrosis across the different cell lines.

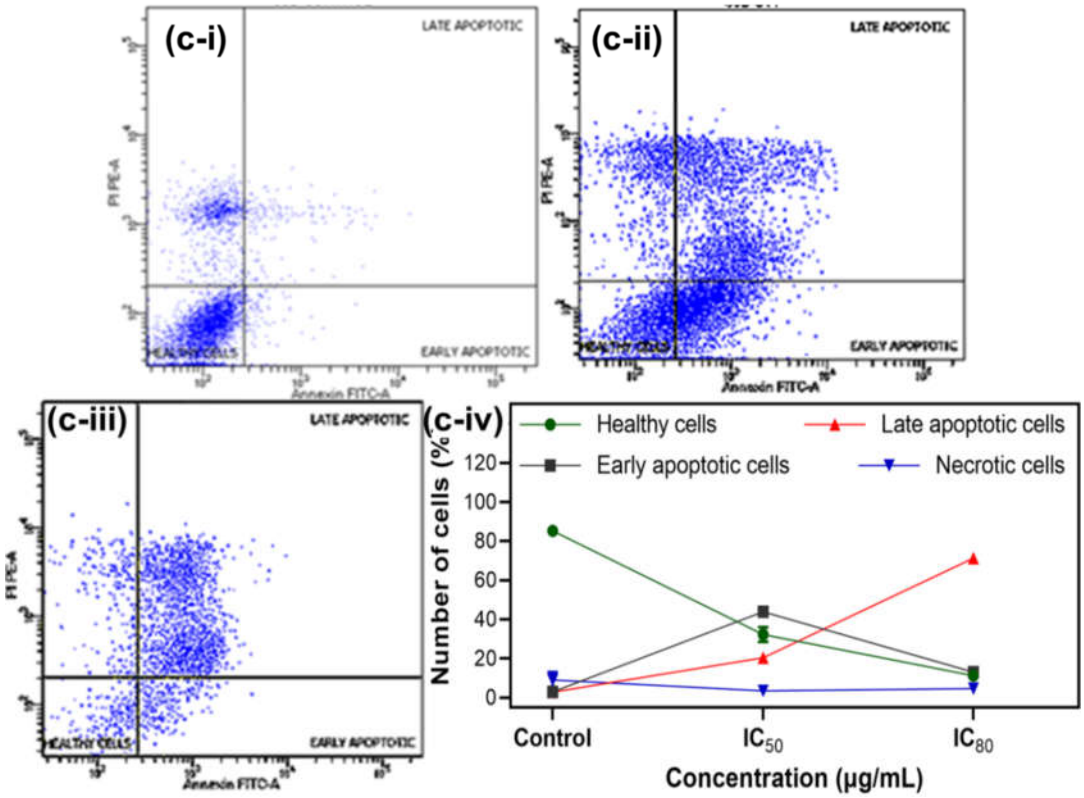


Figure 4.3.32. The SC3-F8 fraction from *M. dubia* chloroform extract induces apoptosis in UM-SCC-83B cells. (a-i) Control cell population; (a-ii) Cells treated with IC_{50} of the extract for 48 hours; (a-iii) Cells treated with IC_{80} of the extract for 48 hours, followed by staining with annexin-V/PI. The Q3 quadrant (annexin V−/PI−) represents the percentage of normal cells; the Q4 quadrant (annexin V+/PI−) shows the percentage of cells in early apoptosis; and the Q2 quadrant (annexin V+/PI+) indicates cells in late apoptosis. (a-iv) Line diagram illustrating the percentage of cells in each stage of apoptosis (normal, early, and late) for each treatment condition.

4.3.2.5.5. Genotoxicity evaluation by comet assay

The comet assay, or single-cell electrophoresis, was done to detect DNA strand breaks in individual cells. The cells were embedded in agarose on a microscope slide, then lysed, electrophoresed under alkaline conditions, and stained with ethidium bromide. DNA with strand breaks migrates out of the nuclei, forming a comet shape where the intact DNA appears as the 'head' and the damaged DNA forms the 'tail' (Collins, 2004). This assay was employed to evaluate the genotoxicity of the active fraction in HT29, MCF7, PA1, and UMSCC83B cell lines, as shown in Figure 4.3.33.

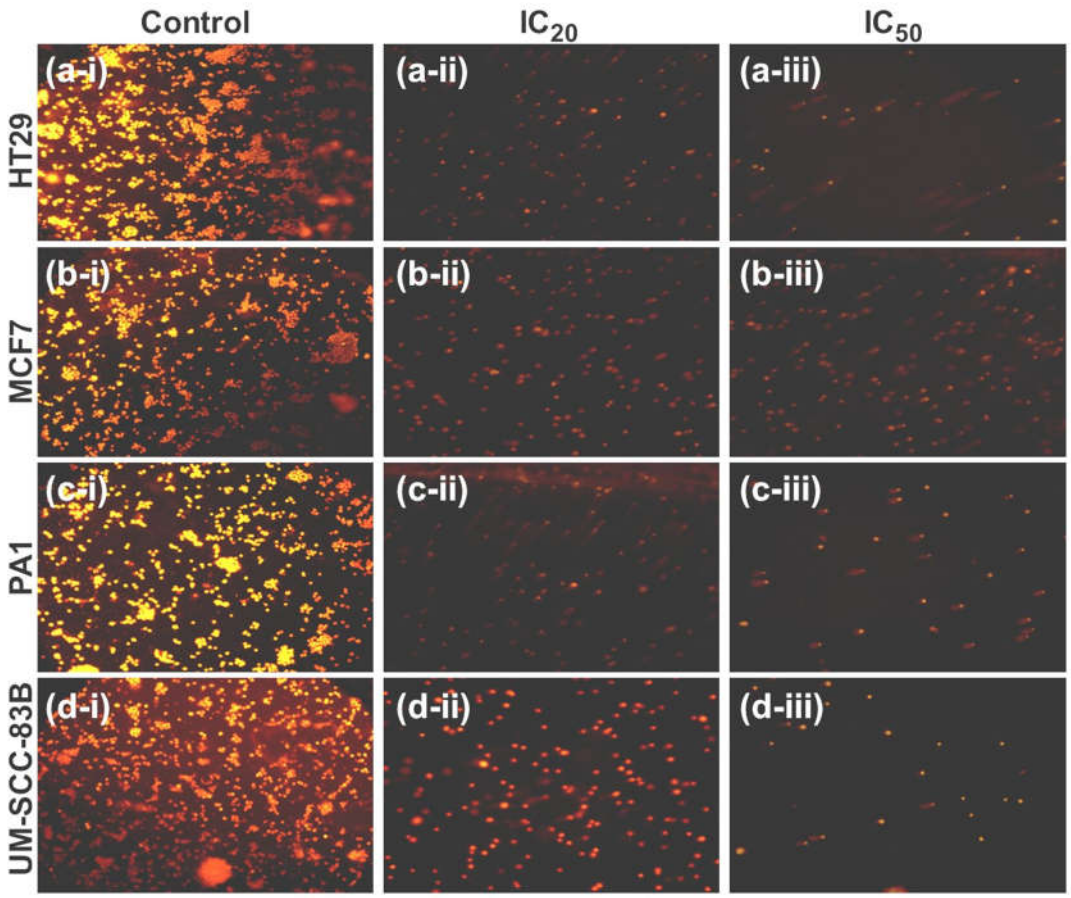


Figure 4.3.33. Genotoxicity assessment by alkaline comet assay in HT29, MCF7, PA1 and UM-SCC-83B cancer cell lines treated with the active fraction, SC3-F8 from *M. dubia* chloroform extract after 48 hours. (a-i) HT29 Control; (a-ii) HT29 treated at IC_{20}; (a-iii) HT29 treated at IC_{50}; (b-i) MCF7 Control; (b-ii) MCF7 treated at IC_{20}; (b-iii) MCF7 treated at IC_{50}; (c-i) PA1 Control; (c-ii) PA1 treated at IC_{20}; (c-iii) PA1 treated at IC_{50}; (d-i) UM-SCC-83B Control; (d-ii) UM-SCC-83B treated at IC_{20}; (d-iii) UM-SCC-83B treated at IC_{50}.

4.3.2.5.6. DNA fragmentation

DNA fragmentation is a critical biochemical hallmark of apoptosis, characterized by the cleavage of chromosomal DNA into internucleosomal 180-bp oligonucleotides by caspase-activated endonuclease (CAD). This process can be detected as a distinct DNA ladder pattern on agarose gels (Hua and Xu, 2000; Majtnerova and Rousar, 2018). In this study, we assessed the induction of apoptosis by evaluating DNA fragmentation in HT29, MCF7, PA1, and UMSCC83B cell lines treated with the active fraction. DNA was extracted from untreated and vehicle-treated control cells, as well as from cells exposed to varying concentrations of the active fraction, SC3-F8.

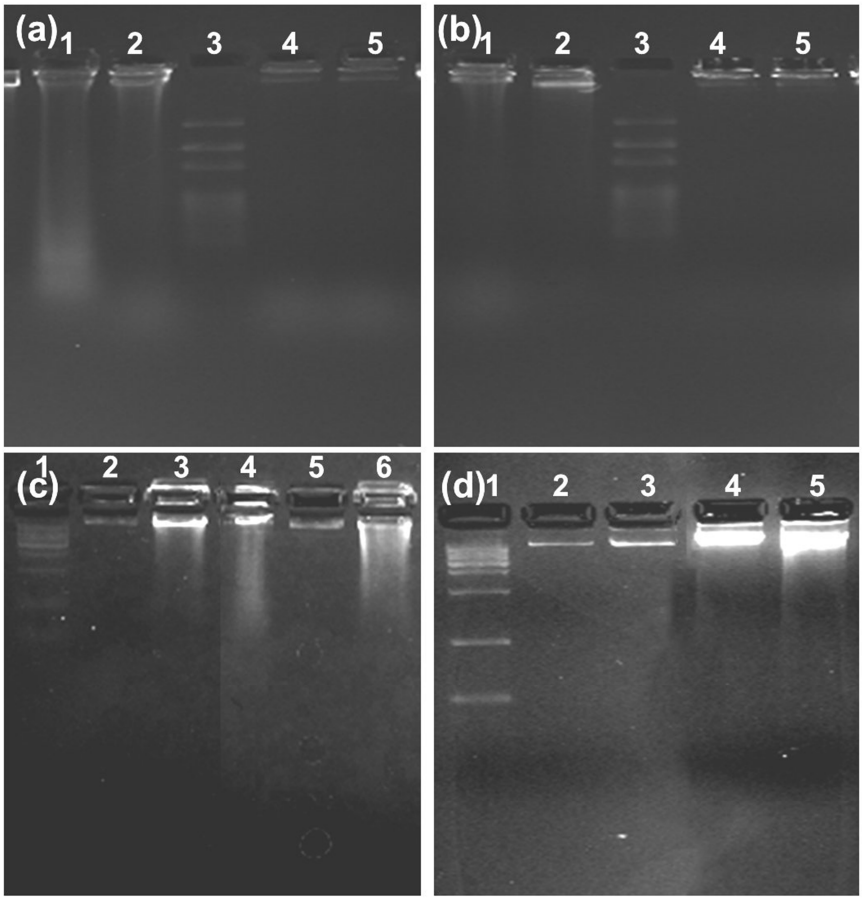


Figure 4.3.34. DNA fragmentation induced by active fraction, SC3-F8 from *M. dubia* chloroform extract in (a) HT29, (b) MCF7 (c) PA1 and (d) UM-SCC-83B cell lines: (a) lane 1- DNA isolated from IC_{50} treated cells; lane 2- DNA isolated from IC_{20} treated cells; lane 3-100 bps DNA ladder; lane 4- DNA isolated from DMSO treated control; lane 5- DNA isolated from untreated control. (b) lane 1- DNA isolated from IC_{50} treated cells; lane 2- IC_{20} treated; lane 3-100 bps DNA ladder; lane 4- DNA isolated from DMSO treated control; lane 5- Cell control. (c) lane 1- 100 bps DNA ladder, lane 2- DNA isolated from untreated control, lane 3- DNA isolated from IC_{20} treated cells,; lane 3- DNA isolated from IC_{50} treated cells; lane 4- DNA isolated from DMSO treated control; lane 6- DNA isolated from IC_{80} treated cells. (d) lane 1- 100 bps DNA ladder; lane 2- DNA isolated from untreated control; lane 3- DNA isolated from DMSO treated control; lane 4- 3: DNA isolated from IC_{20} treated cells; lane 5- DNA isolated from IC_{50} treated cells. DNA was isolated after 48 hours of treatment.

The extracted DNA samples were electrophoresed on a 1.8% agarose gel, where a clear DNA laddering pattern was observed in the active fraction-treated samples, in contrast to the control groups (Figure 4.3.34). This laddering pattern indicates the occurrence of internucleosomal DNA fragmentation, which is a definitive sign of apoptosis. Specifically, as the concentration of the active fraction increased, the intensity of the DNA bands also increased in a dose-dependent manner for HT29,

MCF7, PA1, and UMSCC83B cell lines. This suggests that higher concentrations of the active fraction, SC3-F8 lead to more extensive DNA fragmentation.

The absence of a DNA laddering pattern in the untreated and vehicle-treated control cells further supports the specificity of the observed effects, confirming that the DNA fragmentation was induced by the the active fraction extract rather than occurring spontaneously. These results collectively demonstrate that the active fraction treatment effectively induces apoptosis in HT29, MCF7, PA1, and UMSCC83B cell lines through the activation of apoptotic pathways leading to DNA fragmentation. This confirms the apoptotic potential of the active fraction and highlights its utility in inducing programmed cell death.

4.3.2.6. Gene expression studies

4.3.2.6.1. Total RNA isolation

The isolation of total RNA is a critical step in many molecular biology experiments, ensuring that high-quality RNA is available for downstream applications such as reverse transcription, quantitative PCR, and RNA sequencing. In this study, total RNA was successfully isolated from treated and untreated cancer cell. The quantity and quality of the isolated RNA were assessed using spectrophotometric analysis and agarose gel electrophoresis. The spectrophotometric readings indicated high RNA purity, with A260/A280 ratios consistently between 1.8 and 2.0, which is within the accepted range for pure RNA. Agarose gel electrophoresis further confirmed the integrity of the RNA, as evidenced by the presence of distinct 28S and 18S rRNA bands without significant smearing, indicating minimal degradation (Figure 4.3.35).

Using the isolated RNA as template, corresponding first strand cDNA were synthesised in thermal cycler under appropriate cycling conditions using the proper primer sequences of gene of interest.

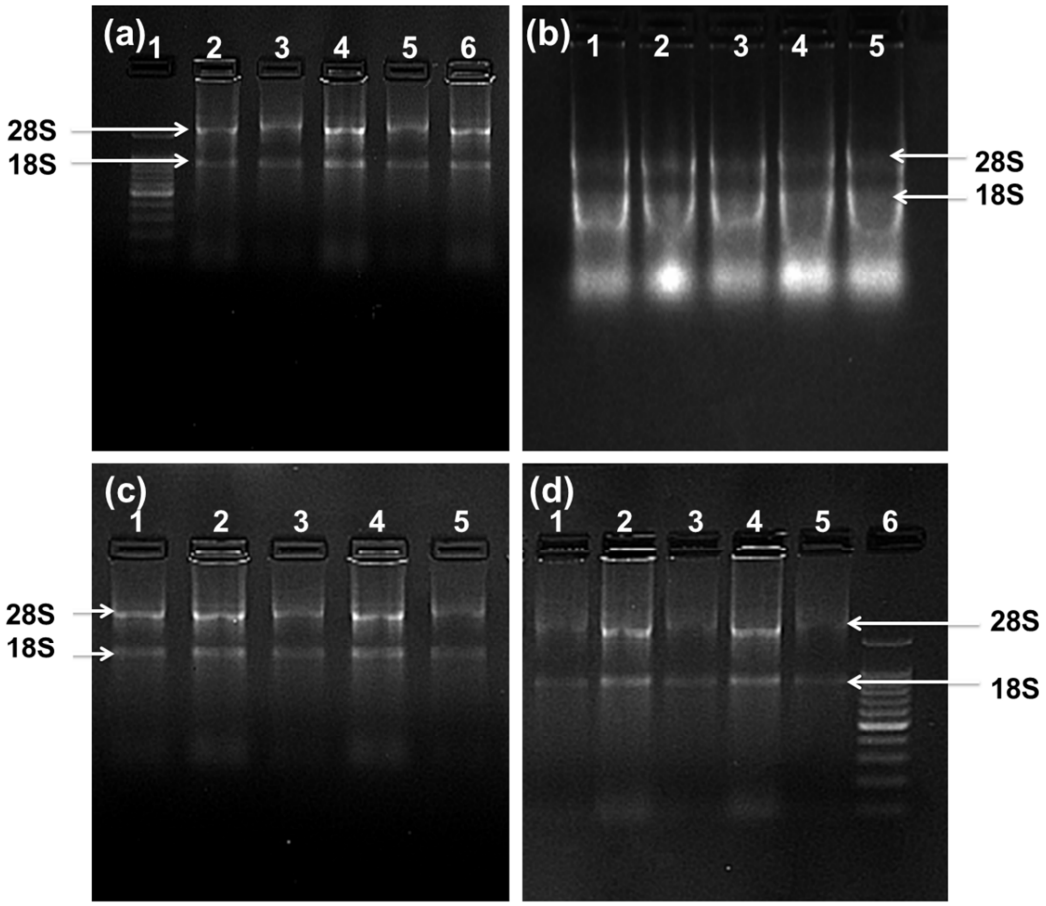


Figure 4.3.35. Agarose gel image showing the two distinct bands (bands of 28S rRNA and 18S rRNA) in the treated and untreated cell groups. (a) Total RNA isolated from MCF7 cell line; (b) Total RNA isolated from PA1 cell line (c) Total RNA isolated from HT29 cell line (d) Total RNA isolated from UM-SCC-83B cell line.

4.3.2.6.2. RT– qPCR analysis

mRNA expression analysis in the HT29 cell line revealed a significant upregulation in pro-apoptotic genes, including *caspase 3* (4.32±0.17-fold increase), *caspase 8* (7.81±0.05-fold increase), *caspase 9* (4.27±0.22-fold increase), *PTEN* (8.73±0.05-fold increase), and *BAX* (9.68±0.06-fold increase) ($p<0.05$). Conversely, a notable downregulation was observed in the expression of *c-Myc* (0.74±0.23-fold decrease), *COX-2* (0.07±0.09-fold decrease), *Bcl-2* (0.25±0.23-fold decrease), and *NF-κB* (0.11±0.13-fold decrease) (Figure 4.3.36).

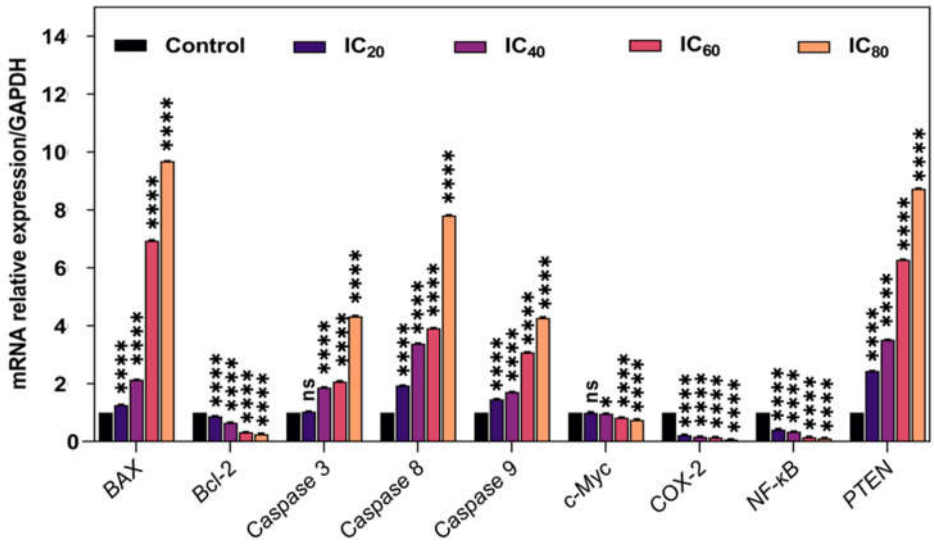


Figure 4.3.36. Changes in gene expression level in HT29 cell line when treated with SC3-F8.

In the MCF7 cell line, a significant upregulation was observed in the expression of pro-apoptotic genes, including *caspase 3* (10.99±0.02-fold increase), *caspase 8* (5.23±0.94-fold increase), *caspase 9* (4.95±0.16-fold increase), *PTEN* (4.14±0.02-fold increase), and *BAX* (3.10±0.37-fold increase) ($p<0.05$). Concurrently, a downregulation was noted in the expression of *c-Myc* (0.4±0.05-fold decrease), *COX-2* (0.10±0.30-fold decrease), *Bcl-2* (0.33±1.35-fold decrease), and *NF-κB* (0.68±0.03-fold decrease) (Figure 4.3.37).

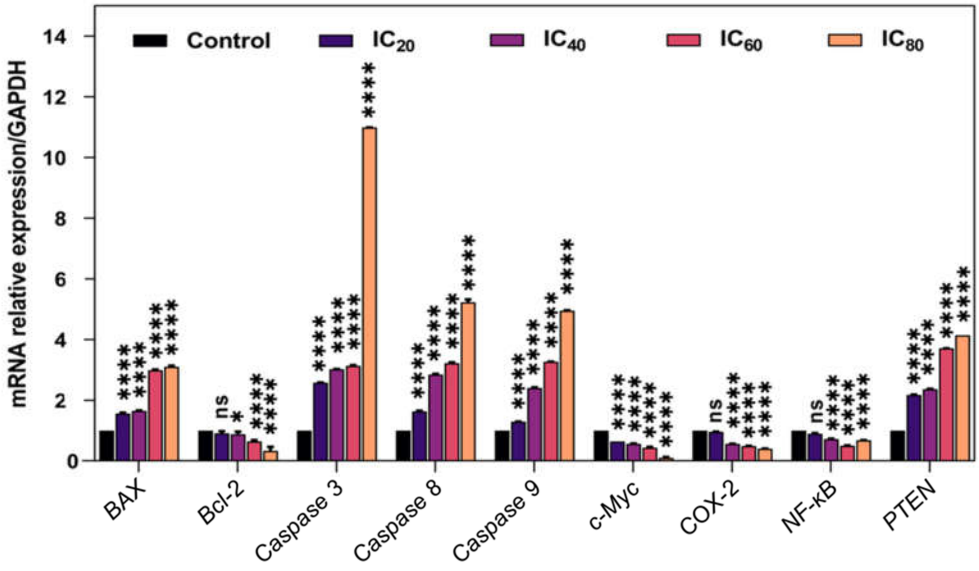


Figure 4.3.37. Changes in gene expression level in MCF7 cell line when treated with SC3-F8.

In the PA1 cell line, a significant upregulation was observed in the expression of pro-apoptotic genes, including *caspase 3* (1.93±0.14-fold increase), *caspase 8* (3.26±0.08-fold increase), *caspase 9* (1.83±0.07-fold increase), *PTEN* (2.52±0.05-fold increase), and *BAX* (3.40±0.20-fold increase) ($p<0.05$). Meanwhile, a notable downregulation was recorded in the expression of *c-Myc* (0.22±0.02-fold decrease), *COX-2* (0.01±1.23-fold decrease), and *Bcl-2* (0.20±0.11-fold decrease) (Figure 4.3.38).

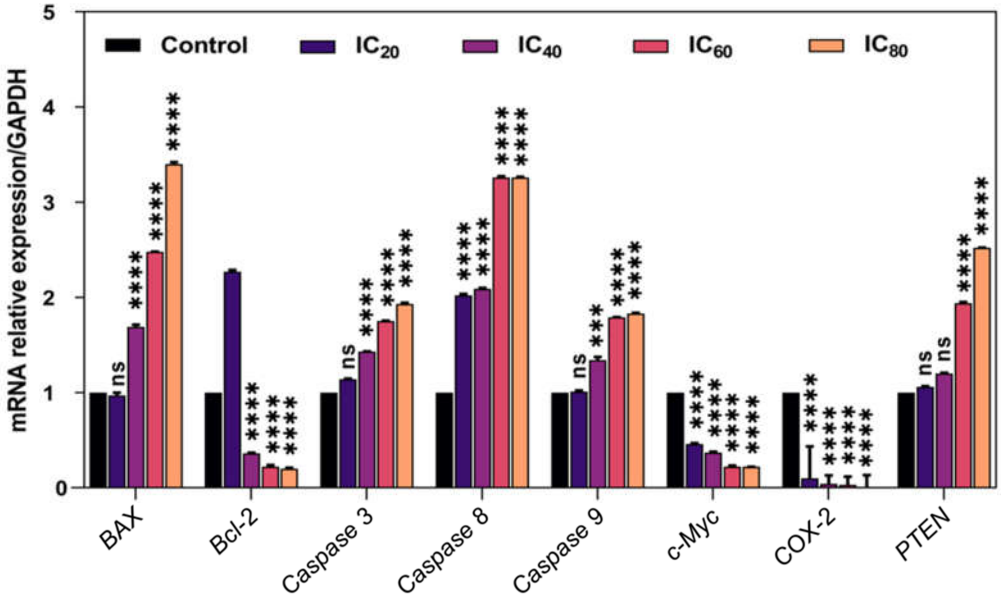


Figure 4.3.38. Changes in gene expression level in PA1 cell line when treated with SC3-F8.

In the UM-SCC-83B cell line, a significant upregulation was observed in the expression of pro-apoptotic genes, including *caspase 3* (7.67±0.05-fold increase), *caspase 8* (1.7±0.22-fold increase), *caspase 9* (1.9±0.11-fold increase), *PTEN* (5.8±0.23-fold increase), and *BAX* (2.44±0.07-fold increase) ($p<0.05$). Concurrently, a marked downregulation was seen in the expression of genes associated with cell survival and inflammation, such as c-Myc (0.5±0.14-fold decrease), *COX-2* (0.12±0.04-fold decrease), *Bcl-2* (0.22±0.19-fold decrease), and *NF-κB* (0.04±0.38-fold decrease) (Figure 4.3.39).

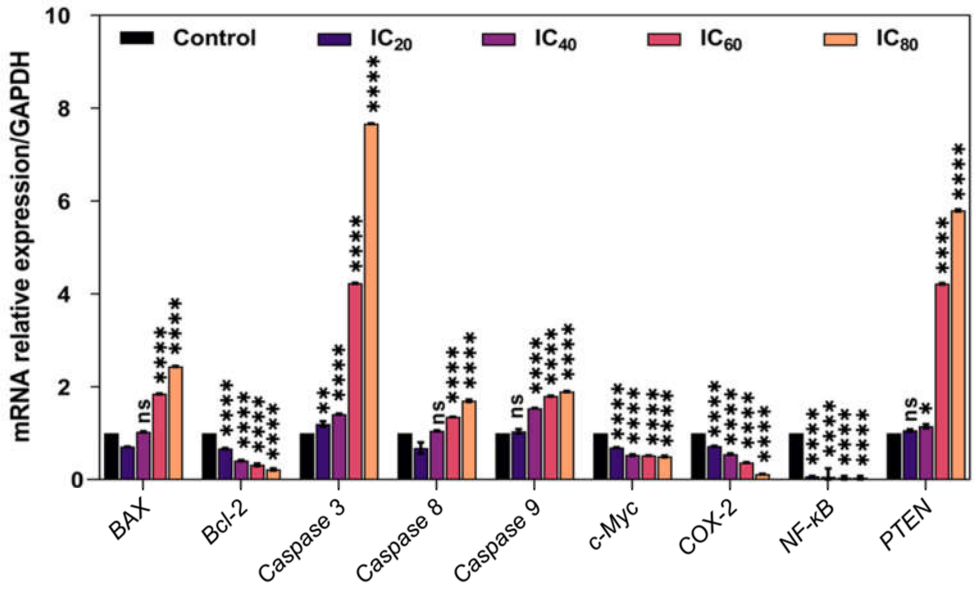


Figure 4.3.39. Changes in gene expression level in UM-SCC-83B cell line when treated with SC3-F8.

The treatment with SC3-F8 caused change in the expression of genes associated with apoptosis at mRNA level. The proapototic genes upregulated and antiapoptotic proteins downregulated, indicating potential involvement in apoptosis.

4.3.2.6.3. Study of P53 and Bcl2 expression using flow cytometry

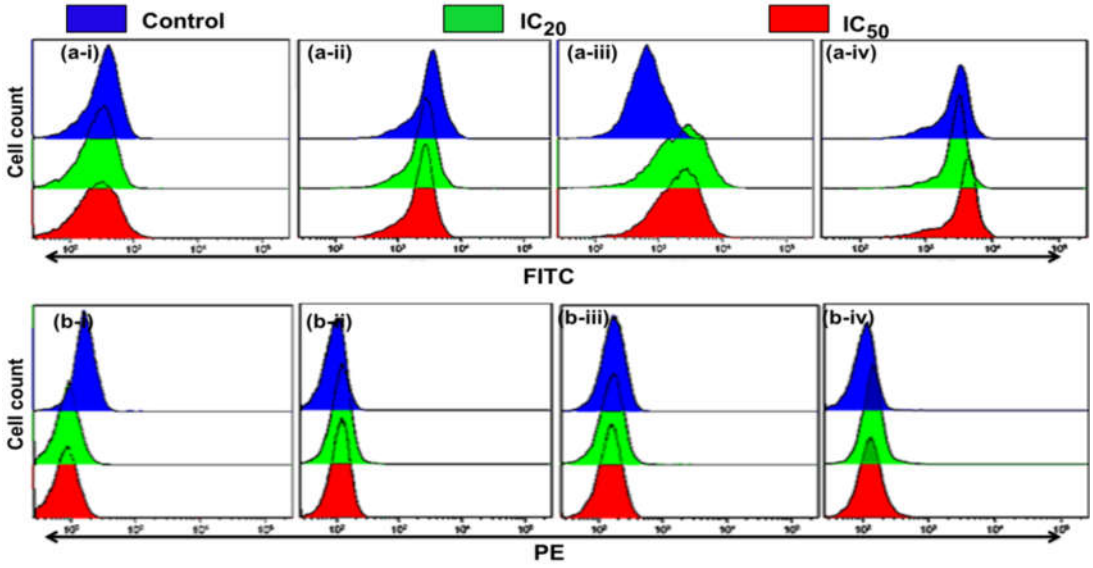


Figure 4.3.40. Flowcytometric analysis of P53 and Bcl2 in HT29, MCF7, PA1 and UM-SCC-83B after treatment with SC3-F8. (a) Expression of P53 I: HT29, ii: MCF7, iii: PA1, iv: UM-SCC-83B. (b) Expression of BCL2 i: HT29, ii: MCF7, iii: PA1, iv: UM-SCC-83B.

P53 and Bcl-2 were studied at the protein level using flow cytometry on permeabilized cells (Figure 4.3.40). A shift in the peak position of the cell population in the treated samples compared to the control indicated an increased expression of P53 and a decreased expression of Bcl-2.

4.3.2.7. Cell cycle Analysis

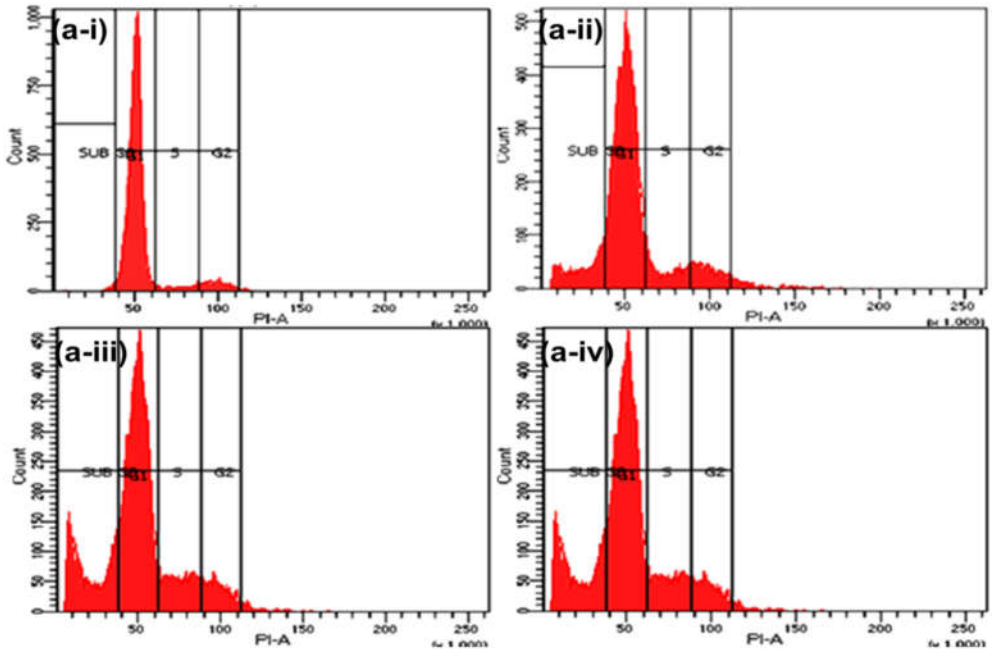


Figure 4.3.41. Histogram showing the cell distribution in various cell cycle stages in HT29 cell line. (a-i) DMSO control; (a-ii) IC_{20} treated cells; (a-iii) IC_{40} treated cells (a-ivi) IC_{60} treated cells.

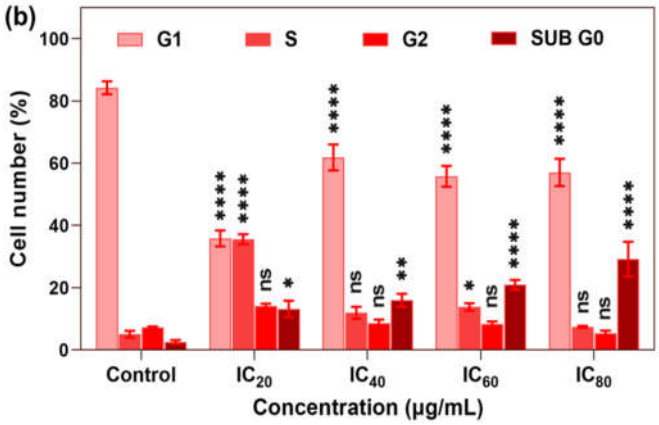


Figure 4.3.42. Bar diagram showing the cell distribution in different stages of cell cycle in DMSO control and treated groups in HT29 cell line.

In evaluating the cell cycle phase-specific distribution of the active fraction, SC3-F8-treated cells, flow cytometric analysis was conducted using propidium iodide (PI) to stain DNA. This analysis reveals the distribution of cells across the G1, S, and G2/M phases of the cell cycle, as well as the sub-G1 phase, which represents apoptotic cells with fractional DNA content (Kajstura *et al.,* 2007).

In HT29 cell line, treated with the active fraction, SC3-F8, notable changes were observed across different concentrations. The treatment led to an increase in the proportion of cells in the subG0 phase, rising from 5.73±1.58% to 12.53±7.08%, indicative of apoptosis. Additionally, a higher number of cells accumulated in the G1 phase, while a reduction in cell numbers was noted in the S and G2 phases, suggesting a cell cycle arrest at the G1 phase (Figures 4.3.41, 4.3.42).

In the MCF7 cell line treated with the active fraction SC3-F8, significant changes were observed at various concentrations. The treatment resulted in an increase in the proportion of cells in the subG0 phase, rising from 6.83±1.05% to 15.47±2.05%, indicating apoptosis. Furthermore, a relatively greater number of cells accumulated in the G1 phase, while a decrease in cell numbers was noted in the S and G2 phases, suggesting a cell cycle arrest in the G1 phase (Figures 4.3.43, 4.3.44).

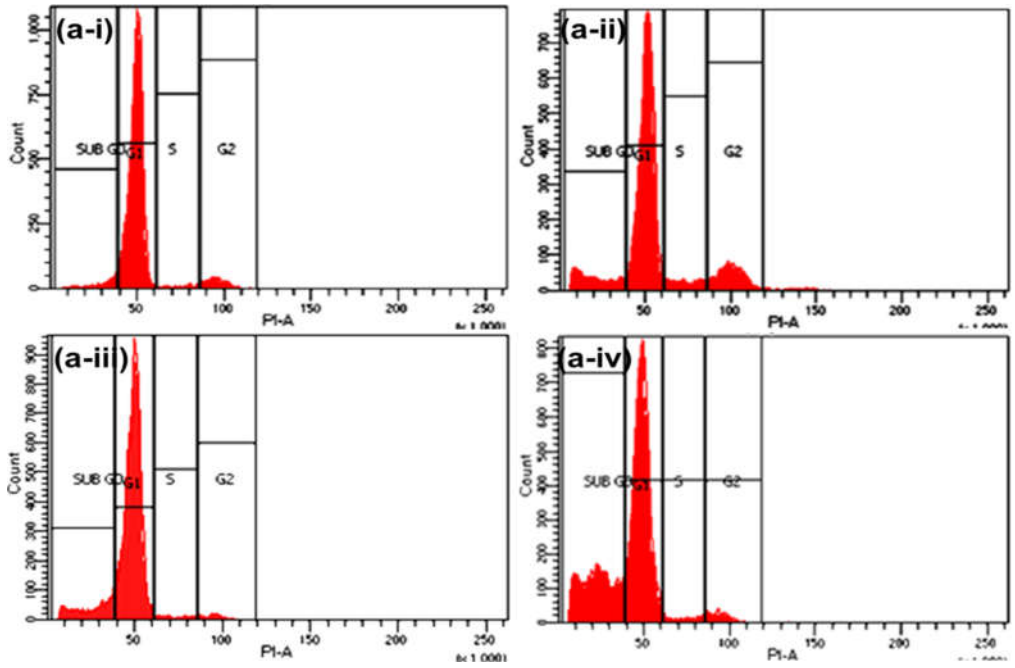


Figure 4.3.43. Histogram showing the cell distribution in various cell cycle stages in MCF7 cell line. (a-i) DMSO control; (a-ii) IC_{20} treated cells; (a-iii) IC_{40} treated cells (a-iv) IC_{60} treated cells.

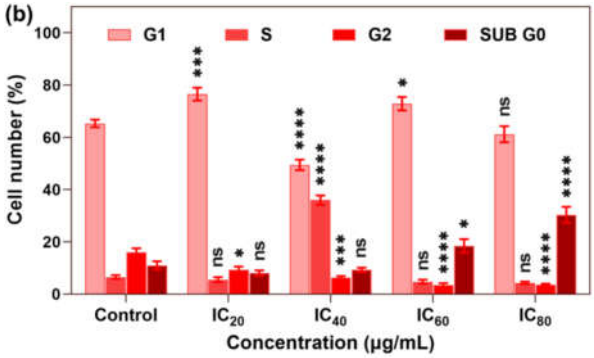


Figure 4.3.44. Bar diagram showing the cell distribution in different stages of cell cycle in DMSO control and treated groups in MCF7 cell line.

In the PA1 cell line treated with the active fraction SC3-F8, significant changes were observed at various concentrations. The treatment led to an increase in the proportion of cells in the subG0 phase, rising from 10.00±0.44% to 31.60±2.91%, indicating apoptosis. Additionally, a relatively larger number of cells accumulated in the G1 phase, while a decrease in cell numbers was noted in the S and G2 phases, suggesting a cell cycle arrest at the G1 phase (Figures 4.3.45, 4.3.46).

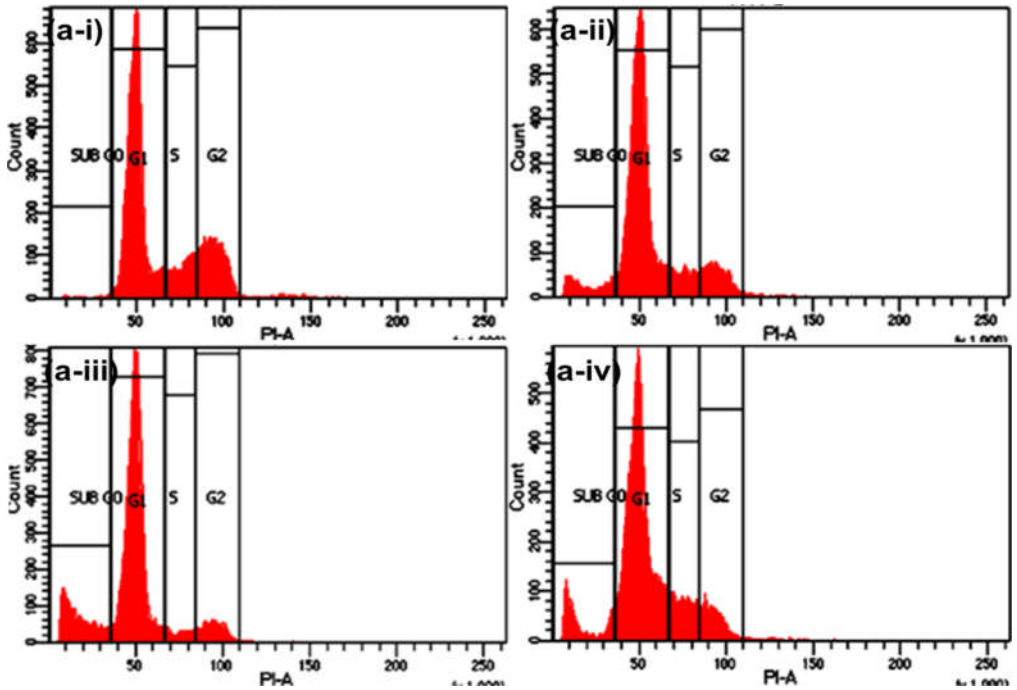


Figure 4.3.45. Histogram showing the cell distribution in various cell cycle stages in PA1 cell line. (a-i) DMSO control; (a-ii) IC_{20} treated cells; (a-iii) IC_{40} treated cells (a-iv) IC_{60} treated cells.

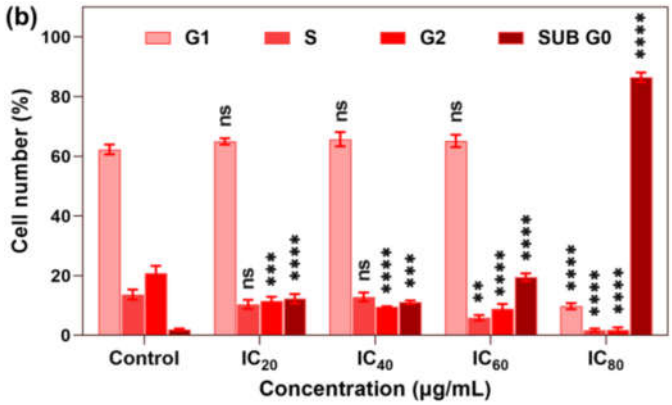


Figure 4.3.46. Bar diagram showing the cell distribution in different stages of cell cycle in DMSO control and treated groups in PA1 cell line.

In the UM-SCC-83B cell line treated with the active fraction SC3-F8, significant changes were observed at various concentrations. The treatment resulted in an increase in the proportion of cells in the subG0 phase, rising from 9.43±2.01% to 12.63±1.09%, indicating apoptosis. Furthermore, a relatively larger number of cells accumulated in the G1 phase, while a decrease in cell numbers was noted in the S

and G2 phases, suggesting a cell cycle arrest at the G1 phase (Figures 4.3.47, 4.3.48).

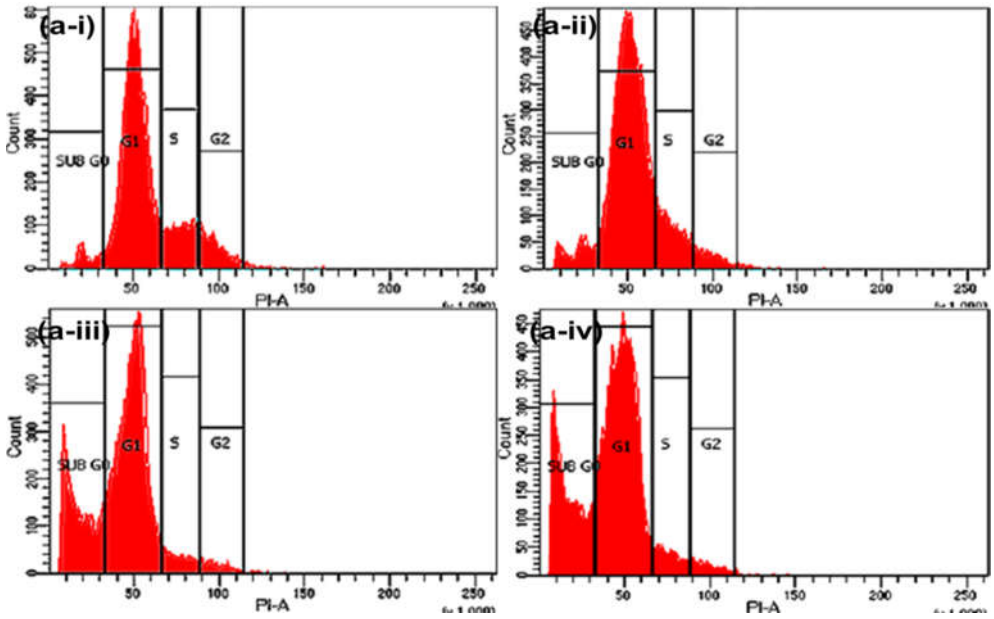


Figure 4.3.47. Histogram showing the cell distribution in various cell cycle stages in PA1 cell line. (a-i) DMSO control; (a-ii) IC_{20} treated cells; (a-iii) IC_{40} treated cells (a-vi) IC_{60} treated cells.

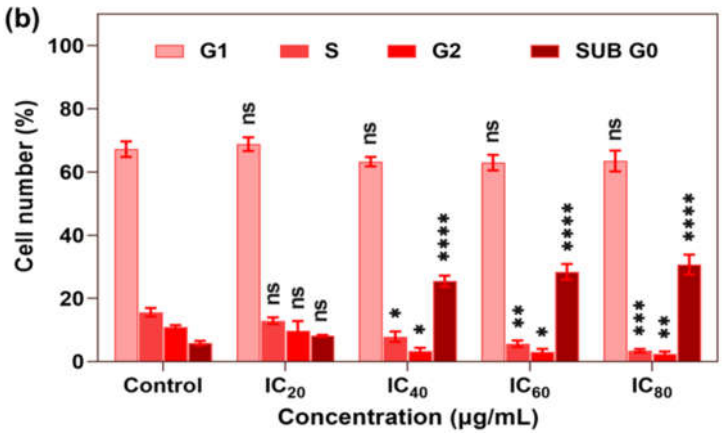


Figure 4.3.48. Bar diagram showing the cell distribution in different stages of cell cycle in DMSO control and treated groups in PA1 cell line.

This study highlights the significant impact of the active fraction treatment on cell cycle distribution and apoptosis in various cancer cell lines, including HT29, MCF7, PA1, and UM-SCC-83B. Through flow cytometric analysis using propidium iodide (PI) staining, it was observed that the active fraction induces notable changes in cell cycle phases, particularly at higher concentrations. In HT29, MCF7, and UM-SCC-

83B cells, the active fraction treatment resulted in a marked reduction in the G1 phase population, with a concomitant increase in the sub-G0 phase, indicative of apoptosis. PA1 cells showed the most dramatic response, with nearly 90% of cells in the sub-G1 phase at the highest concentration, reflecting extensive apoptosis.

These findings suggest that the active fraction exerts its anti-cancer effects by inducing cell cycle arrest and promoting apoptosis in a dose-dependent manner. The differential sensitivity among the cell lines indicates the potential specificity of the active fraction's action, which could be influenced by the unique molecular characteristics of each cell type. Overall, the active fraction shows promise as a potential anti-cancer agent, with its ability to disrupt the cell cycle and induce apoptosis, warranting further research to explore its therapeutic potential and underlying mechanisms in cancer treatment.

4.3.2.8. The active fraction, SC3-F8 prevents cell migration and inhibits EMT pathway in the cancer cells.

4.3.2.8.1. Scratch wound healing assay

At the initial time point (0 hours), a clear scratch was observed in both control and treated groups. Over time, the wound area in the control group progressively decreased, with significant cell migration observed. In contrast, treated groups showed a concentration-dependent reduction in migration. At lower concentrations of the treatment, some cell migration was observed, but cells remained largely at the wound edges at higher concentrations, indicating a more pronounced inhibitory effect on cell migration. The active fraction's effect on wound healing highlights it's potential to hinder cellular processes essential for tissue repair and cancer metastasis.

In the control group, the cells demonstrated natural wound healing abilities. Over a span of hours, cells from the edges of the scratch migrated into the wound area, gradually closing the gap. This migration is a critical aspect of wound healing, showcasing the cells' ability to move and proliferate to repair the tissue. In contrast,

the treated groups exhibited a stark difference in behaviour. At lower concentrations of the active fraction, there was still some degree of cell migration, though noticeably less than in the control group. Cells began to move towards the centre of the wound but did not cover as much area. As the concentration of the active fraction increased, the inhibitory effects became more pronounced. At higher concentrations, cell migration was significantly impaired, with cells remaining predominantly at the wound edges and showing minimal movement into the wound area. This concentration-dependent reduction in migration suggests that the active fraction disrupts key mechanisms involved in cell motility and proliferation (Figures 4.3.49-4.3.52).

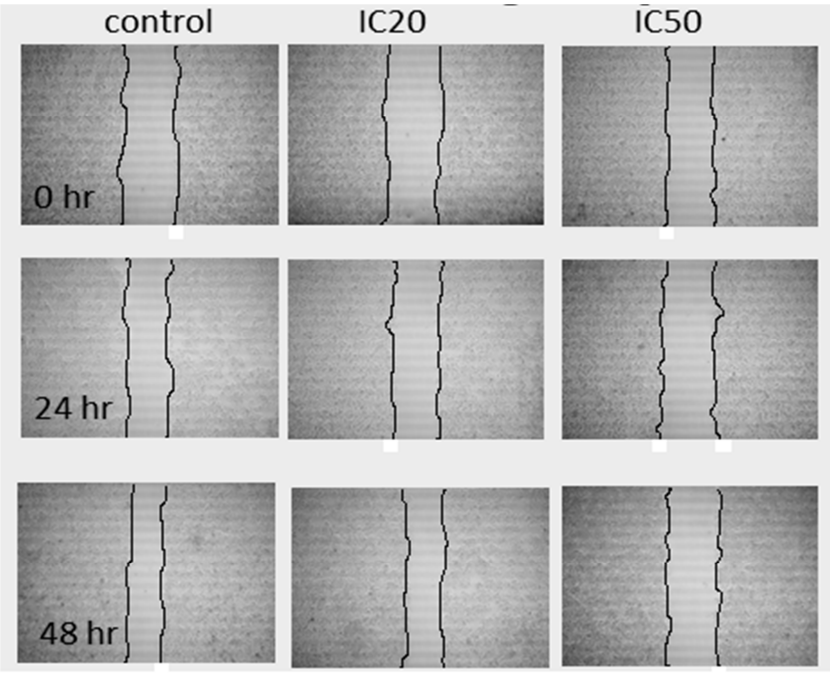


Figure 4.3.49. Scratch wound healing assay in HT29, following treatment with SC3-F8 from *M. dubia* chloroform extract at IC_{20} and IC_{50} concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound comparatively got closed or cells were migrated and grown into the wound area in the control after 48 hours.

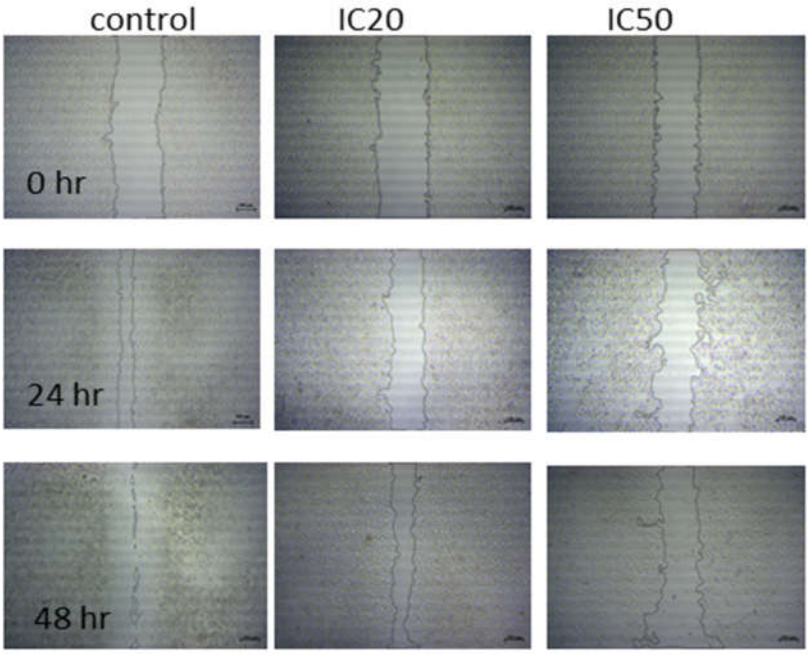


Figure 4.3.50. Scratch wound healing assay in MCF7, following treatment with SC3-F8 from *M. dubia* chloroform extract at IC_{20} and IC_{50} concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound comparatively got closed or cells were migrated and grown into the wound area in the control after 48 hours.

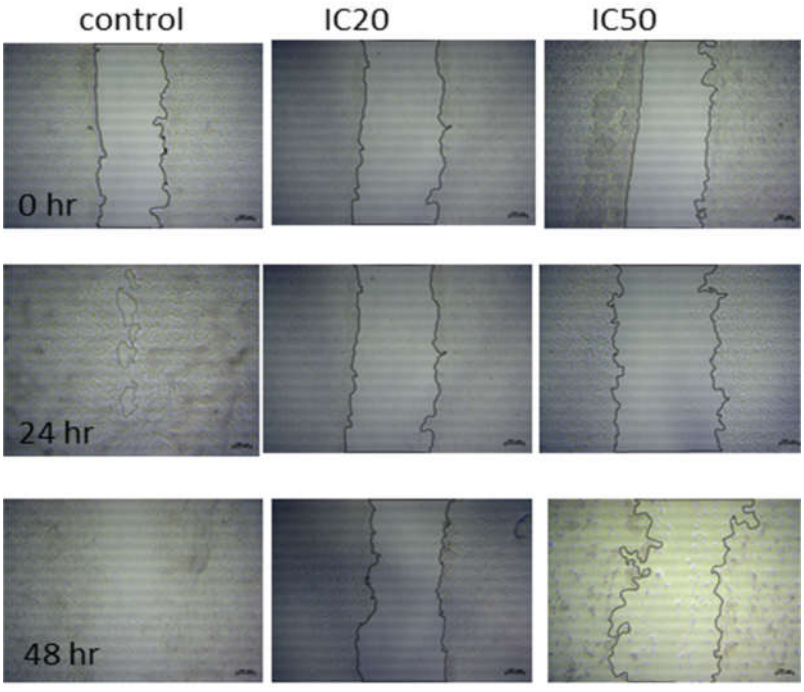


Figure 4.3.51. Scratch wound healing assay in PA1, following treatment with SC3-F8 from *M. dubia* chloroform extract at IC_{20} and IC_{50} concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound comparatively got closed or cells were migrated and grown into the wound area in the control after 48 hours.

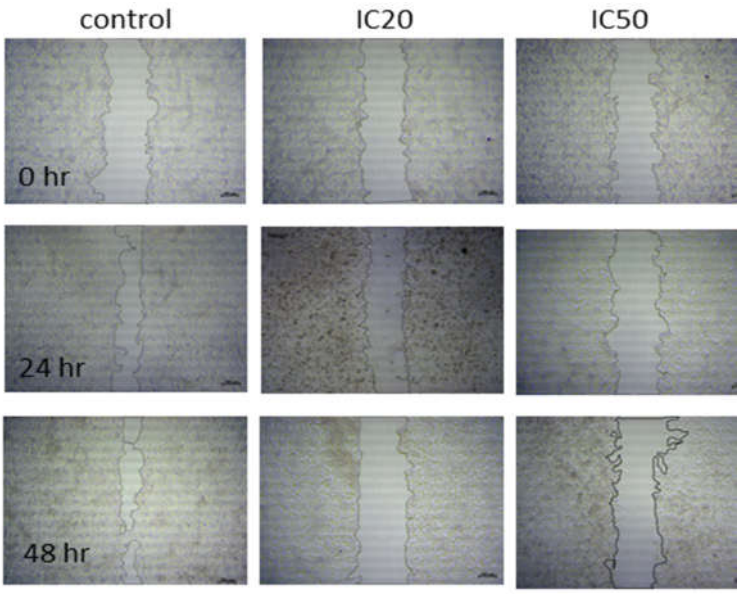


Figure 4.3.52. Scratch wound healing assay in UM-SCC-83B, following treatment with SC3-F8 from *M. dubia* chloroform extract at IC_{20} and IC_{50} concentrations. The exposure of the cells to the extract considerably inhibited or reduced the wound closure while the wound comparatively got closed or cells were migrated and grown into the wound area in the control after 48 hours.

The implications of these findings are significant, especially in the context of cancer treatment. The ability of cancer cells to migrate and invade new tissues is a fundamental characteristic of metastasis, the primary cause of cancer-related mortality.

4.3.2.8.2. Cell migration assay/ Boyden chamber/ Trans–well migration assay

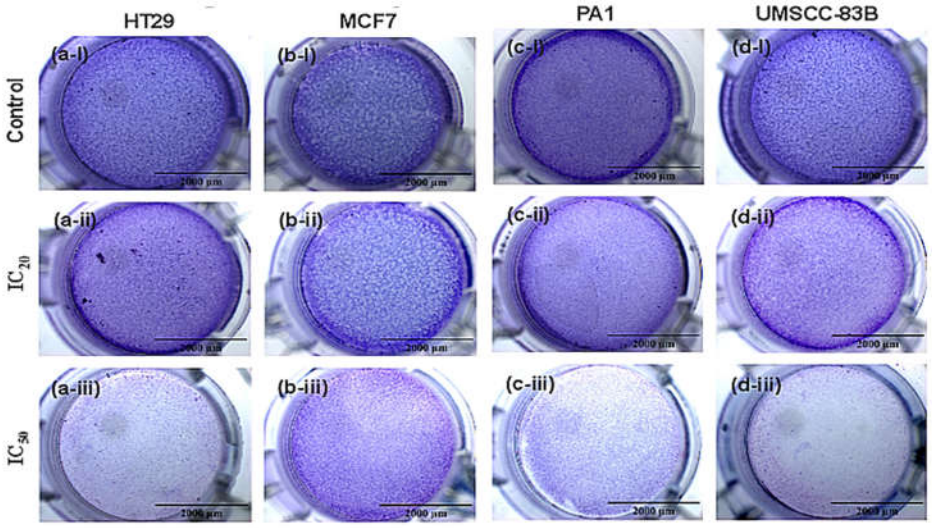


Figure 4.3.53. Effect of SC3-F8 on migration of cancer cells. The fraction caused a dose dependent decrease in the cell migration in HT29 (a-i, a-ii, a-iii), MCF7 (b-i, b-ii, b-iii), PA1 (c-i, c-ii, c-iii) and UM-SCC-83B (d-i, d-ii, d-iii).

In the transwell migration assay, the initial results show a clear trend regarding the effect of the treatment on cell migration. At the 0-hour mark, cells were placed in the upper chambers, establishing a baseline for their movement. Over the course of the experiment, the control group, which did not receive any treatment, demonstrated robust cell migration through the membrane into the lower chamber. This indicates that, in the absence of treatment, the cells have a strong tendency to migrate.

In contrast, the treated groups exhibited a concentration-dependent decrease in cell migration. At lower concentrations of the treatment, cells still managed to migrate through the membrane, but the migration was noticeably reduced compared to the control group. This suggests that even at lower doses, the treatment exerts some inhibitory effect on cell movement, although it is not complete (Figure 4.3.53).

As the concentration of the treatment increased, a more pronounced inhibition of cell migration was observed. At the highest concentrations, migration was severely impeded, with a significant reduction in the number of cells reaching the lower chamber. This result underscores a dose-dependent response to the treatment, where higher concentrations are more effective in blocking cell migration.

Overall, the data indicate that the treatment effectively inhibits cell migration in a concentration-dependent manner. This suggests that the treatment has potential as an anti-migratory agent, which could be of interest in contexts where controlling cell movement is desirable, such as in cancer metastasis or inflammatory responses.

4.3.2.8.3. SC3-F8 induces changes in angiogenesis related gene expression at mRNA level

Angiogenesis, the formation of new blood vessels, plays a crucial role in tumour growth and metastasis, with vascular endothelial growth factor A (VEGF-A) being a key driver of this process. However, due to the heterogeneity of cancer, predicting angiogenesis across various tumour types and tissues using a single mRNA marker is challenging. Consequently, a panel of potential markers was evaluated to identify

those that correlate with VEGF-A expression and contribute to the angiogenic process.

In this study, the relative expression of genes involved in angiogenesis was assessed. A significant decrease in the expression of *Vegf-A* and *MMP2* was observed in the HT29, MCF7, PA1, and UM-SCC-83B cell lines (Figure 4.3.54). Specifically, *Vegf-A* expression decreased by 0.54±0.75, 0.21±0.23, 0.46±0.25, and 0.02±0.63 folds in HT29, MCF7, PA1, and UM-SCC-83B, respectively. Similarly, *MMP2* levels also decreased in these cell lines by 0.25±0.75, 0.57±0.50, 0.13±0.35, and 0.16±0.12 folds, respectively. In contrast, the level of *Angiostatin* increased significantly by 3.55±0.20, 3.13±0.89, 4.35±0.22, and 7.41±0.43 folds in the respective cell lines.

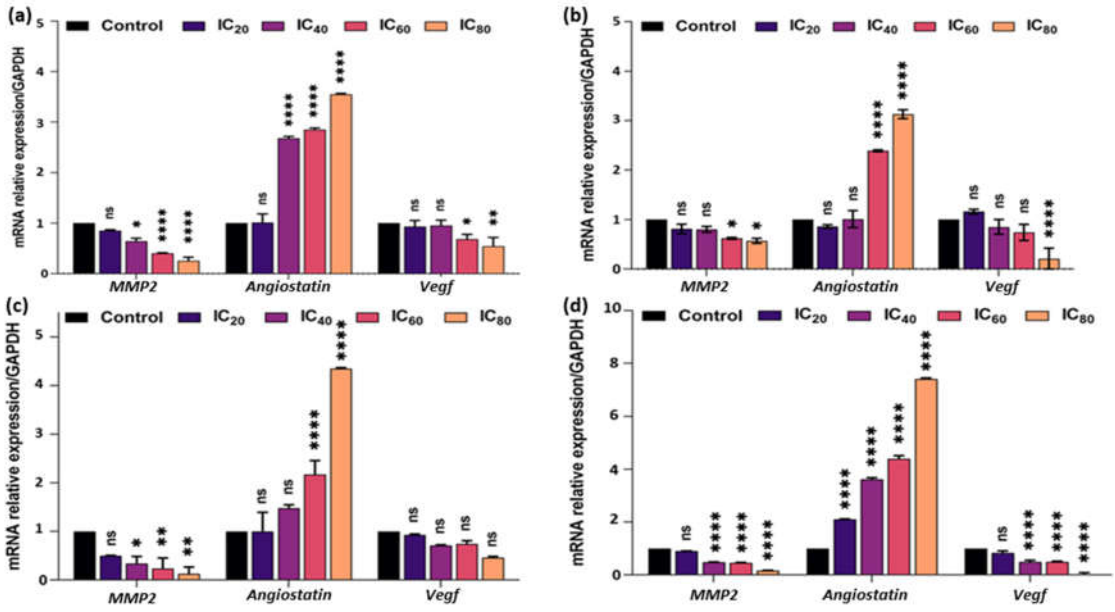


Figure 4.3.54. The gene expression changes at mRNA level in four cell types. The genes under study are angiogenesis regulating genes. (a) HT29, (b) MCF7, (c) PA1, (d) UM-SCC-83B.

4.3.2.8.4. Western blotting

To investigate the effect of SC3-F8 on cell metastasis, our study analysed the expression of various epithelial-to-mesenchymal transition (EMT) markers. The study focused on several key markers; Snail, a transcriptional repressor that regulates E-cadherin, promoting EMT, Slug and Twist are transcriptional repressors that regulates the expression of tumour suppressor E-cadherin and N-cadherin and vimentin, mesenchymal markers indicative of EMT.

The results revealed that SC3-F8 treatment led to a concentration-dependent downregulation of several EMT-related proteins; Snail, Slug, Vimentin, C-Myc and COX-2. Conversely, the expression of E-cadherin (CDH1) increased with treatment. This suggests that SC3-F8 effectively suppresses key EMT-associated proteins, potentially reversing EMT and thereby reducing cell migration and metastasis. The increase in CDH12 expression could be indicative of an attempt to re-establish epithelial characteristics or may reflect complex regulatory interactions within the EMT process. These findings provide insight into how SC3-F8 may reduce metastasis by modulating the EMT pathway, suggesting its potential as a therapeutic agent in preventing cancer progression (Figure 4.3.55, 4.3.56).

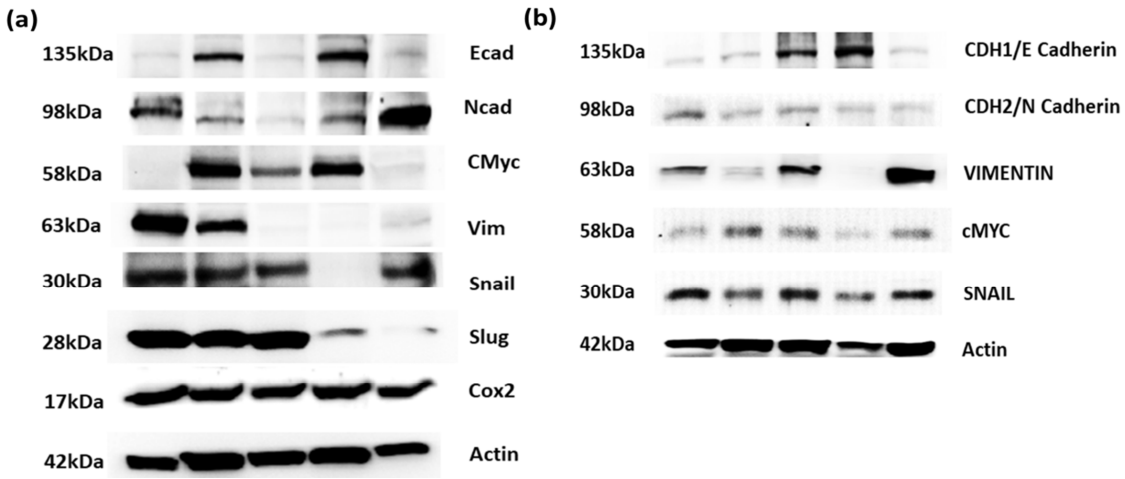


Figure 4.3.55. SC3-F8 inhibits the expression of EMT markers. The SC3-F8 downregulates the mesenchymal markers such as CDH2, and VIM, and EMT key transcription factors such as SNAI1, c-myc and slug. (a) Western blot analysis of HT29 lysate for EMT associated proteins. Lane 1: Cell control, 2: DMSO control, 3: IC_{20} concentration, 4: IC_{50} concentration, 5: 5-Fluorouracil treated (b) Western blot analysis of MCF7 lysate for EMT associated proteins. Lane 1: DMSO control, 2: IC_{20} concentration, 3: IC_{50} concentration, 4: 5-Fluorouracil.

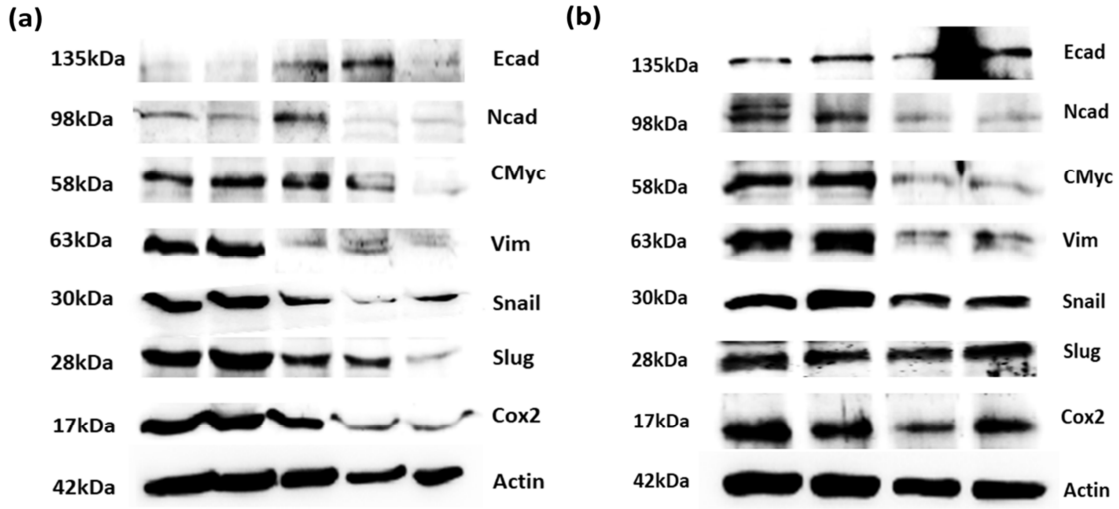


Figure 4.3.56. SC3-F8 inhibits the expression of EMT markers. (a) Western blot analysis of PA1 lysate for EMT associated proteins. Lane 1: DMSO control, 2: IC_{20}, 3: IC_{40}, IC_{60}, IC_{80} (b) Western blot analysis of UM-SCC-83B lysate for EMT associated proteins. Lane 1: DMSO control, 2: IC_{20}, 3: IC_{40}, IC_{60}, IC_{80}. The SC3-F8 downregulates mesenchymal markers (CDH2, VIM,), and EMT transcription factors (SNAI1) c-myc and slug.

DISCUSSION

Plants are rich sources known for the presence of anticancer molecules. *Anamirta cocculus* and *Melia dubia,* the two plants were selected for further base on their very high cytotoxicity against cancer cell lines (chapter 2)

4.4.1. The cytotoxic analysis of ethyl acetate extract from *Anamirta cocculus* and purified fraction from *Melia dubia* on cancer cell lines.

Ethyl acetate extract from *A. cocculus* exhibited remarkable potency, particularly against PA1 ovarian cancer cells, achieving a 98.64±0.40% mortality rate. This level of efficacy is noteworthy, especially considering that it also significantly affected HT29 colon cancer cell line. The IC_{50} obtained for the extract on HT29 and PA1 were 17.97±0.63 µg/mL and 8.30±0.38 µg/mL respectively which were under the NCI limit of 30 µg/mL and the toxicity was significantly less on normal cell line, HaCaT (>100 µg/mL). This suggests the presence of robust anticancer potential for the extract. The high degree of cell death induced by this extract suggests a robust anticancer potential that warrants further investigation. Similarly, the chloroform extract from *M. dubia* demonstrated substantial cytotoxic effects, with 94.23±0.35% cell death observed in HT29 cells. The IC_{50} values for this extract varied across different cancer cell lines, reflecting the differential sensitivity of these cells. For instance, the IC_{50} was 61.26±2.55 µg/mL for HT29 cell line, 75.54±1.16 µg/mL for MCF7, 29.77±0.86 µg/mL for PA1 cell line and 31.28±1.77 µg/mL, for UM-SCC-83B illustrating the high anticancer potential of the extract. The varying IC_{50} values among different cancer types—ranging from 29.77±0.86 µg/mL to 75.54±1.16 µg/mL on the cell lines under study—underscore the need for tailored therapeutic strategies based on cell-specific responses. The lower toxicity observed in HaCaT, normal skin cell line, further supports the selective action of these extracts on cancer cells, which is crucial for minimizing collateral damage and enhancing safety in potential therapeutic applications.

The exploration of extracts from *Anamirta cocculus* and *Melia dubia* has yielded promising results in the search for effective anticancer agents. The ethyl acetate extract from *A. cocculus* and the chloroform extract from *M. dubia* demonstrated significant cytotoxicity across various cancer cell lines, highlighting their cell type specificity and strong potential as anticancer agents. Soumya *et al.,* (2021) demonstrated that the petroleum ether extract of *Curcuma mutabilis* rhizome exhibited cytotoxicity against four cancer cell lines. The low IC_{50} values observed for *Zingiber anamalayanum* rhizome extracts on HCT116 (<5 - 25 µg/mL) and HL60 (7.9 - 24 µg/mL) cell lines suggest a high level of cytotoxicity. Furthermore, the essential oil from *Z. anamalayanum* rhizome showed anticancer activity against DLA cells in an in vivo model, as reported by Salim *et al.,* (2015). Similarly, Elufioye *et al.,* (2017) found that the ethyl acetate fraction of *Pseudocedrela kotschyi* exhibited potent anticancer activity, with IC_{50} values of 21.53 µg/mL for HeLa cells and 38.46 µg/mL for RD cells. Nelson *et al.,* (2020) also reported that the methanolic extract of *Eclipta alba* displayed significant specificity ($p < 0.005$) against HCT-116 cells compared to other cancer cell lines, while showing minimal or no toxicity to WI-38 cells. These findings are consistent with our results, underscoring the anticancer potential of plant extracts even at low concentrations.

The cytotoxicity of the extracts from *A. cocculus* and *M. dubia* indicated the presence of highly active anticancer molecules in the extracts. Further purification and characterisation of the bioactive molecules is required. Additionally, exploring the molecular targets and pathways influenced by these extracts could provide insights into their therapeutic potential and help identify optimal treatment regimens.

The Trypan Blue dye exclusion method results indicate a notable reduction in the viability of HT29 and PA1 cancer cell line when treated with *A. cocculus* ethyl acetate extract. In the same way, four cancer cell lines (HT29, MCF7, PA1, and UM-SCC-83B) exhibited reduced viability in response to the purified active fraction (SC3-F8), with effects observed in a dose-dependent manner. As the concentration of the active fraction, SC3-F8 increased (IC_{20}, IC_{50}, and IC_{80}), cell viability consistently decreased across all tested cell lines. The statistical analysis ($p <$

0.0001) supports the significance of these results, highlighting the active fraction's substantial potential as an anticancer agent. These results also align with the MTT assay results, confirming the cytotoxic potentials of the extracts.

In addition, the pronounced decrease in colony formation on treatment with *A. cocculus* ethyl acetate extract and SC3-F8 fraction from *M. dubia* indicated the presence of molecules which prevents the colony forming capacity, a characteristic feature of cancer cells. The clonogenic assay revealed a clear dose-dependent reduction in colony formation, indicating that the *A. cocculus* ethyl acetate extract and SC3-F8 fraction from *M. dubia* impair the multiplication capability of cancer cells, which inhibit tumour growth and spread. Previous studies conducted by Guo *et al.* also showed a similar dose-dependent reduction in clonogenic growth when treated with the n-butyl alcohol extract of Litchi seed (NLS) (Guo *et al.,* 2021). The methanolic extract of *Eclipta alba* in an experiment conducted by Nelson *et al.* also showed reduced migration as well as clonogenic ability of the cancer cell lines, confirming the anticancer potential of the extract against HCT-116 cells (Nelson *et al.,* 2020). MDA-MB-231 and HCT-8 cancer cell lines also exhibited about 70-90% reduction in colony formation when treated with leaf, bark and seed extracts obtained from *Moringa oleifera*. These results were in agreement with those obtained by Syed Najmuddin *et al.,* on breast cancer cell line on *Annona muricata* leaf crude extract treatment, Chaves *et al.,* on prostate cancer cell line on pomegranate juice and peel extracts treatment, Li *et al.,* on human lung adenocarcinoma, A549, and human breast cancer, MCF7 on *Rheum officinale* water extract treatment, etc. All these results emphasize the importance of anticancer property of plant extracts (Najmuddin *et al.,* 2016; Chaves *et al.,* 2020; Li *et al.,* 2009).

4.4.2. The effect of *Anamirta cocculus* ethyl acetate extract and the active fraction, SC3-F8 from *Melia dubia* on apoptosis on cancer cell lines

Apoptosis-related morphological changes have been instrumental in identifying apoptosis-inducing stimuli and guiding the study of apoptosis biochemistry and

signalling pathways. The morphological alterations observed in treated cancer cell lines, including structural distortions, surface shrinkage, and DNA fragmentation, serve as clear indicators of apoptosis, or programmed cell death. These apoptosis-specific features were particularly evident in cells treated with the ethyl acetate extract of *Anamirta cocculus* and the active fraction SC3-F8 from *Melia dubia*, suggesting their strong potential to effectively induce apoptosis in cancer cells.

Furthermore, the comet assay conducted on cancer cell lines treated with *A. cocculus* and the active fraction SC3-F8 from *M. dubia* revealed a dose-dependent rise in DNA damage. Higher extract concentrations resulted in longer comet tails, indicating increased DNA damage. In contrast, no comet tails were observed in untreated or vehicle-treated cells, confirming that the DNA damage was specifically induced by the extracts. These findings are consistent with earlier studies that used the comet assay to demonstrate the genotoxic effects of plant extracts (Mattana *et al.,*2014; Kahaliw *et al.,* 2018), highlighting the genotoxic potential of the ethyl acetate extract of *A. cocculus* and SC3-F8 from *M. dubia*. Similar results were reported by Li *et al.*, who observed DNA fragmentation in A549 and MCF7 cell lines following treatment with *Rheum officinale* water extract (Li *et al.,* 2009). Koňariková *et al.* also found comparable effects, including DNA strand breaks and oxidative damage upon administering black tea extract, leading to apoptotic cell death in HT-29 and MCF7 cells (Koňariková *et al.,* 2015). Efenberger-Szmechtyk *et al.* demonstrated that *Cornus mas* extract caused morphological changes in Caco-2 cells, such as partial cell detachment, necrosis, chromatin condensation, cytoplasmic vacuolization, and nuclear fragmentation (Efenberger-Szmechtyk *et al.,* 2020). DNA damage in Caco-2 cells after exposure to the leaf extracts was assessed using the alkaline comet assay, showing a concentration-dependent increase in DNA damage.

A decrease in mitochondrial membrane potential (MMP), a key event in apoptosis, was observed following treatment with *Anamirta cocculus* extract and the SC3-F8 fraction from *Melia dubia*. This was detected using fluorescence microscopy and flow cytometry, where changes in fluorescence indicated alterations in membrane potential. Increased apoptosis, as evidenced by chromatin condensation, further

supported these observations. The loss of mitochondrial membrane potential increased with higher extract concentrations. Similarly, Kocyigit and Guler investigated the cytotoxic, genotoxic, apoptotic effects, ROS generation, and MMP alterations of curcumin on mouse melanoma cancer cells (B16-F10) and normal fibroblastic cells (L-929) (Kocyigit and Guler, 2017). Their results showed that curcumin reduced cell viability and MMP while increasing DNA damage, apoptosis, and ROS levels in both cell types in a dose-dependent manner, with stronger effects in melanoma cells. They found a strong positive correlation between DNA damage, apoptosis, cytotoxicity, ROS generation, and MMP in curcumin-treated melanoma and normal cells. In summary, their study demonstrated that curcumin induces DNA damage, apoptosis, and cytotoxicity via its pro-oxidant activity, with these effects being more pronounced in cancer cells than in normal cells. The observed increase in MMP depolarization in treated cancer cell lines, as measured by JC-1 flow cytometry, was also described by Vijayarathna *et al.* on treating HeLa cells with *Polyalthia longifolia* methanol extract (Vijayarathna *et al.,* 2017). Comparable findings were also reported by Lakshmipriya *et al.* using *Wrightia arborea* leaf extract on the K562 cell line (Lakshmipriya *et al.,* 2018).

Annexin V staining, which detects the translocation of phosphatidylserine (PS) to the cell surface (Lecoeur *et al.,* 2001), showed a significant rise in both early and late apoptotic cells following IC_{50} treatment of the active fraction, SC3-F8. In the control group, the majority of cells were healthy, with few early or late apoptotic cells and minimal necrotic cells. At IC_{80} concentrations, there was a notable decrease in healthy cells and a significant increase in late apoptotic cells. This pattern was consistent across all four cancer cell lines (HT29, MCF7, PA1, and UM-SCC-83B), indicating that SC3-F8 induces apoptosis rather than necrosis. These results indicate that the active fraction from *M. dubia,* SC3-F8 promotes a concentration-dependent apoptosis in treated cancer cells, with a transition from healthy to apoptotic states as treatment doses increase. This suggests that SC3-F8 has potential as an anticancer agent by selectively inducing apoptosis while reducing necrosis. These findings align with the research of Soumya *et al.* and Rezadoost *et*

al. which demonstrated that the cell death induced by the petroleum ether extract of *Curcuma mutabilis* and the methanolic extracts of *Catharanthus roseus* and *Calystegia sepium* leaves were apoptosis-driven, as confirmed by annexin V binding assay (Soumya *et al.,* 2021; Rezadoost *et al.,* 2019). Further studies are necessary to isolate the anticancer molecule and to elucidate the molecular mechanisms behind these effects.

The level of expression of mRNA in the HT29 cell line, when treated with the active fraction, SC3-F8 at different concentrations, demonstrated a substantial increase in pro-apoptotic genes, including *caspase 3, caspase 8, caspase 9, PTEN*, and *BAX*. At the same time, a pronounced reduction was observed in the expression of *c-Myc*, *COX-2, Bcl-2*, and *NF-κB*.

In the MCF7 cell line, there was a significant rise in pro-apoptotic gene expression, with caspase 3 showing a 10.99±0.02-fold increase, along with *caspase 8, caspase 9*, *PTEN*, and *BAX*. This was accompanied by a decrease in the expression of *c-Myc*, *COX-2, Bcl-2*, and *NF-κB*.

Similarly, in the PA1 cell line, pro-apoptotic genes, including *caspase 3, caspase 8*, *caspase 9, PTEN,* and *BAX*, were significantly upregulated, while the expression of *c-Myc, COX-2*, and *Bcl-2* was markedly downregulated.

In the UM-SCC-83B cell line, there was a notable increase in pro-apoptotic gene expression, including *caspases 3, 8,* and *9, PTEN*, and *BAX*, coupled with a downregulation of genes involved in cell survival and inflammation, such as *c-Myc*, *COX-2, Bcl-2*, and *NF-κB*.

Gene expression analysis revealed that SC3-F8 induces apoptosis by regulating the *Bcl-2* family of genes, which influences mitochondrial membrane integrity. Specifically, the anti-apoptotic gene *Bcl-2* was downregulated, while the pro-apoptotic *BAX* gene was upregulated. The increased expression of *caspases 3, 8*, and *9* suggests activation of both the intrinsic and extrinsic apoptotic pathways, with caspase-3 playing a critical role in cleaving proteins like PARP during apoptosis. A

study conducted in 2020 demonstrated that in breast (MCF-7), liver (HepG2), and colon (HCT116) cancer cells treated with *Saussurea costus* extract, pro-apoptotic proteins were elevated while anti-apoptotic proteins were suppressed, indicating the involvement of the mitochondrial pathway in apoptosis. These findings suggest that *Saussurea costus* may be a promising anticancer agent (Shati *et al.,* 2020).

In a study by Esmaeili and Farimani, the expression of *PTEN* mRNA was detected by RT-PCR following treatment with *Salvia sahendica*, which induced apoptosis in MCF-7 breast cancer cells by upregulating *PTEN* and inhibiting the PI3K/Akt pathway (Esmaeili and Farimani, 2014). Similarly, ethanolic neem leaf extract treatment increased *caspase-3* expression and modulated *Bcl-2* family gene expression, leading to apoptosis in PC-3 and LNCaP prostate cancer cells (Gunadharini *et al.,* 2011). Additionally, *Phaleria macrocarpa* induced the expression of pro-apoptotic genes such as *BAX, BAD*, and *PUMA*, leading to *caspase-9* activation, PARP cleavage, and DNA fragmentation, which targeted both cell survival and apoptotic genes in MDA-MB-231 breast cancer cells (Tandrasasmita *et al.,* 2010).

The nuclear protein P53 is known to be involved in cell transformation and proliferation, making the study of its expression in human malignancies clinically significant. A significant shift in P53 expression was observed, with increased levels correlating with higher SC3-F8 treatment concentrations, indicating a concentration-dependent impact on apoptosis. A reduction in Bcl-2 expression, particularly in HT29 cells, confirmed the downregulation of this key anti-apoptotic protein in response to SC3-F8 treatment. In a previous study, P53 levels in stimulated lymphocytes were assessed using flow cytometry (Danova *et al.,* 1990), highlighting the potential importance of P53 regulation in cancer treatment.

Inhibiting the progression of the cell cycle is a crucial strategy in managing cancer growth (Qui *et al.,* 2011). Both DNA flow cytometry and fluorescent microscopy demonstrated that the active fraction SC3-F8 from *M. dubia* effectively arrested the cell cycle at the G1 phase in all the treated cell lines, including HT29, MCF7, PA1,

and UM-SCC-83B, while also inducing apoptosis. Propidium iodide staining revealed distinct differences between control and SC3-F8-treated cells, showing a significant increase in the number of cells in the sub-G0 phase, which is indicative of apoptosis due to DNA fragmentation (Kajstura *et al.,* 2007). This evidence suggests that SC3-F8 leads to DNA damage, which subsequently triggers apoptosis. Similarly, earlier research demonstrated G1 phase cell cycle arrest in MCF7 cells following treatment with extracts from *Glehnia littoralis* root extract (de la Cruz *et al.,* 2015) and *Dorema glabrum* root extract (Jafari *et al.,* 2016). The increase in the number of cells arrested in the G1 phase suggests that they are unable to progress further through the cell cycle, ultimately resulting in cell death.

The study further demonstrated that fraction, SC3-F8 treatment resulted in elevated mRNA levels of pro-apoptotic genes like *BAX*, while genes associated with cell proliferation and survival, including *Vegf-A, c-Myc*, and *COX-2*, were significantly downregulated. Notably, SC3-F8 led to a marked decrease in the expression of *c-Myc*, a gene frequently overexpressed in various cancers and linked to increased cell proliferation. This reduction in *c-Myc*, along with the downregulation of *COX-2*, highlights SC3-F8's potential to inhibit cancer cell growth. Similar apoptotic effects have been observed with other compounds, such as toosendanin from *Melia toosendan*, which also induces apoptosis through the inhibition of this pathway. The suppression of *Vegf-A* and *COX-2* corresponds with the inhibition of cancer cell growth and survival pathways. Likewise, morusin, a flavonoid, has been shown to downregulate *COX-2* and *Vegf-A* expression levels (Yin *et al.,* 2018). Additionally, Kong *et al.,* reported similar findings, noting the downregulation of *Vegf-A* and *COX-2* in the A549 cancer cell line following paclitaxel treatment (Kong *et al.,* 2017).

4.4.3. Cell migration and EMT pathway

The study investigated the effects of *Anamirta cocculus* extract and SC3-F8 from *Melia dubia* on the migratory properties of cancer cells, an essential process in metastasis. Both treatments significantly reduced cell migration in a concentration-

dependent manner. The wound healing assay, conducted at IC_{20} of *Anamirta cocculus* extract and SC3-F8 from *Melia dubia*, revealed a marked delay in wound closure, with a complete inhibition of migration observed at IC_{50}. This impairment in wound healing highlights the treatments' effectiveness in suppressing cell motility. In addition to the observed delay, cell migration assays confirmed that SC3-F8 inhibited migration even in the presence of chemo-attractants, demonstrating its potent anti-migratory effects. These results suggest that both extracts have the ability to impede cancer cell migration, potentially due to their influence on diminishing cell motility, supporting their role as both anti-proliferative and anti-migratory agents.

Additionally, the research explored the expression levels of key angiogenesis-related genes, including *Vegf-A*, angiostatin, and matrix metalloproteinase 2 (*MMP2*), in four different cancer cell lines. *Vegf-A* is a major pro-angiogenic factor, while *MMP2* is involved in extracellular matrix degradation, both processes being essential for tumour angiogenesis. The treatment with the active fraction, SC3-F8 revealed a notable reduction in the mRNA levels of *Vegf-A* and *MMP2*, while *Angiostatin*, an endogenous angiogenesis inhibitor, exhibited a significant concentration-dependent increase. The inverse relationship observed between *Vegf-A* and *Angiostatin* act together leading to inhibition of angiogenic signals required for tumour growth and metastasis. As an inhibitor of neovascularization, the increase in angiostatin levels could reflect an attempt by cancer cells to regulate their microenvironment by suppressing excessive angiogenesis, which could destabilize tumour growth if left uncontrolled.

The observed reduction in *MMP2* expression, combined with the decreased levels of *Vegf-A*, supports the hypothesis of a linked regulatory mechanism. *MMP2* plays a crucial role in extracellular matrix remodelling, facilitating endothelial cell migration, a key process in angiogenesis. The correlation between the downregulation of *Vegf-A* and *MMP2* and the increase in angiostatin emphasizes a coordinated network of gene expression changes that collectively modulate angiogenesis in cancer cells.

In terms of metastasis, the study analyzed the effects of SC3-F8 on the epithelial-to-mesenchymal transition (EMT), a process where epithelial cells acquire mesenchymal characteristics, enhancing their migratory and invasive abilities, which is a key factor in cancer metastasis. The research focused on several EMT markers, including Snail, Slug, Twist, Vimentin, N-cadherin (CDH2), and the pro-inflammatory marker COX-2. The results indicated a significant downregulation of EMT-related proteins Snail, Slug, Twist, Vimentin, and COX-2 following SC3-F8 treatment. Conversely, N-cadherin expression increased, suggesting that SC3-F8 may not only suppress EMT but also potentially reverse it, thereby reducing the invasive properties of cancer cells. The increase in N-cadherin expression could indicate an attempt to re-establish epithelial traits, further supporting the role of SC3-F8 in mitigating the EMT process.

These findings suggest that SC3-F8 may act as an effective therapeutic agent by reducing cancer cell migration and metastasis through its regulation of the EMT pathway. Similar findings have been reported in supporting studies. For example, the n-butyl alcohol extract of *Litchi* seed has been shown to reverse EMT by increasing E-cadherin and β-catenin expression while decreasing Vimentin and Snail levels, partially through inhibition of the Akt/GSK-3β signalling pathway (Guo *et al.,* 2021). In another study, nanoemulsions containing *Ferula assa-foetida* seed essential oil significantly decreased the expression of VEGF and VEGFR in MCF7 cells, corroborating the findings from SC3-F8 treatment (Azani *et al.,* 2022). Furthermore, *Pinus massoniana* bark extract significantly inhibited the migration of lung cancer cells by downregulating MMP-9 expression, further highlighting the potential of traditional plant extracts as cancer therapeutics (Mao *et al.,* 2017).

In conclusion, the analysis of fraction, SC3-F8 from *M. dubia* and its effects on cancer cell proliferation, migration, angiogenesis, and EMT strongly suggests that this bioactive fraction holds promise as a potential anticancer agent. By modulating key pathways involved in tumour growth, angiogenesis, and metastasis, SC3-F8 may offer a multi-faceted approach to inhibiting cancer progression.

CONCLUSIONS

- The cytotoxicity analysis indicated that the extracts from *Anamirta cocculus* and *Melia dubia* purified fraction, SC3-F8 revealed significant mortality in cancer cell lines, HT29, MCF7, PA1 and UM–SCC–83B cell lines.
- The ethyl acetate extract of *A. cocculus* and the active fraction from *M. dubia*, SC3-F8 could prevent colony formation and migration in HT29 and PA1 and HT29, MCF7, PA1 and UM–SCC–83B cell lines respectively.
- The number of cells in apoptotic stages, both early apoptotic and late apoptotic stages were increased upon treatment with SC3-F8. Cytomorphology was also affected in all the above cell lines.
- SC3-F8 (the active fraction from *M. dubia*) induced a dose dependent reduction in $\Delta\Psi M$ after 48 hours, as evidenced by fluorescence microscopy.
- Assessment of PS externalization in cells treated with SC3-F8 for 48 hours using Annexin–V/propidium iodide (PI) co-staining showed an increase in the percentage of apoptotic cells in a dose dependent manner.
- On treatment with SC3-F8, the active fraction from *M. dubia,* HT29, MCF7, PA1 and UM–SCC–83B cell lines exhibited longer comet tail lengths in a concentration dependent manner, demonstrating the occurrence of DNA damage.
- Treatment of the cell lines, HT29, MCF7, PA1 and UM–SCC–83B with the active fraction, SC3-F8 caused cell cycle arrest at G1 phase.
- SC3-F8 induced the upregulation of proapoptotic genes and downregulation of anti-apoptotic genes, leading to apoptosis.
- The active fraction, SC3-F8 also reduced migration of cancer cells as revealed by cell migration assay and inhibited components of EMT pathway which may affect the angiogenesis.

Chapter 5

Liposome incorporation of active fraction from *Melia dubia*: Characterization and cytotoxicity analysis

- *To prepare liposomes incorporated with silica gel column fraction (SC1-F8) from Melia dubia.*
- *To characterize the liposomes prepared.*
- *To study the effect of liposome incorporated active fraction on cancer cell line, HT29.*

REVIEW OF LITERATURE

Liposomes are self-assembled vesicles made of phospholipids that can form either a single bilayer (unilamellar) or multiple concentric bilayers (multilamellar) surrounding an aqueous core (Figure 5.1.1) (Nsairat *et al.,* 2022). They vary in size from 30 nm to several micrometers, with the bilayer thickness being around 4–5 nm. The study of liposomes began in the mid-1960s, notably with Alec Bangham and his team at Babraham, Cambridge, who first described liposome structure in 1964, setting the stage for extensive research into their potential as drug delivery systems (Bangham, 2005). Over the years, liposomes have been explored for the delivery of small molecule drugs, proteins, nucleic acids, and imaging agents through various routes such as parenteral, pulmonary, oral, transdermal, ophthalmic, and nasal applications to enhance both therapeutic efficacy and patient compliance. Additionally, liposomes are utilized in the food and cosmetics industries.

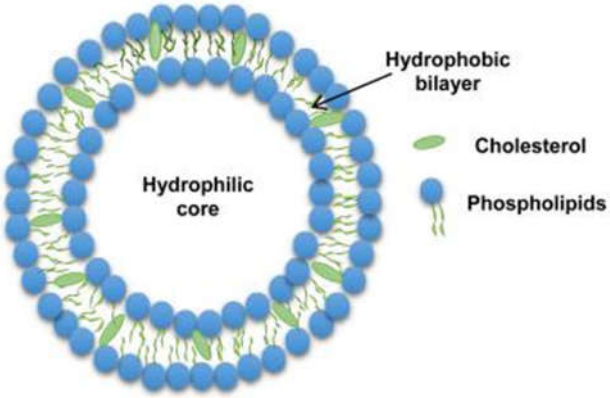


Figure 5.1.1. Structure of lipososme (Nsairat *et al.,* 2022)

As drug delivery systems, liposomes offer several benefits, including protection of encapsulated drugs from degradation, prolonged drug half-life, and controlled release. Their biocompatibility and safety are also advantageous. Liposomes can target drugs to specific diseased areas via passive and/or active targeting, which minimize systemic side effects, allows for higher tolerated doses, and improves therapeutic outcomes. A key mechanism is the enhanced permeability and retention (EPR) effect, where liposomes accumulate in tumour tissues due to the leaky nature

of their blood vessels, unlike normal tissues with tighter junctions. This property specifically enhances drug delivery to tumors (Liu *et al.,* 2022).

Active targeting involves modifying the surface of liposomes with ligands that bind to specific receptors on tumour cells, such as VEGF, EGF, folic acid, integrins, and CD44, which enhances the precision and efficacy of drug delivery (Kesharwani *et al.,* 2022). Despite their potential, only 14 liposomal products are currently available on the market, indicating that their full advantages have not yet been realized.

5.1.1. Marketed liposomal products

Table 5.1.1. Marketed liposomal products.

Product Name	API	Approved Year/Area	Dosage Form	Administration Route	Indication
Doxil / Caelyx	Doxorubicin hydrochloride (DOX•HCl)	1995, US / 1996, EU	Suspension	IV	Ovarian cancer, Kaposi's sarcoma, myeloid melanoma
DaunoXome	Daunorubicin	1996, US	Suspension	IV	Kaposi's sarcoma
AmBisome	Amphotericin B (AmpB)	1997, US	Lyophilized powder	IV	Systemic fungal infection
DepoCyt / DepoCyte	Cytarabine	1999, US / 2001, EU	Suspension	IT	Lymphomatous meningitis
Myocet	DOX•HCl	2000, EU	3 vials	IV	Breast cancer
Visudyne	Verteporfin	2000, US / 2000, EU	Lyophilized powder	IV	Wet AMD
DepoDur	Morphine	2004, US	Suspension	Epidural	Postoperative pain
Mepact	MTP-PE	2009, EU	Lyophilized powder	IV	Osteosarcoma
Exparel	Bupivacaine	2011, US / 2020, EU	Suspension	Local infiltration	Post-surgical analgesia
Marqibo	Vincristine Sulfate	2012, US	3 vials	IV	Leukemia
Onivyde	Irinotecan hydrochloride trihydrate	2015, US / 2016, EU	Suspension	IV	Pancreatic adenocarcinoma
Vyxeos	Daunorubicin, cytarabine	2017, US / 2018, EU	Lyophilized powder	IV	Leukemia
Shingrix	Recombinant varicella-zoster virus glycoprotein E	2018, EU	2 vials (powder and suspension)	IM	Against shingles and post-herpetic neuralgia
Arikayce	Amikacin sulfate	2018, US / 2020, EU	Suspension	Oral inhalation	Lung disease

Source: (Bulbake *et al.,* 2017, Liu *et al.,* 2022).

As per FDA and EMA drug databases, there are 14 approved liposomal products, excluding generics, lipid complexes, and nationally authorized European products. Doxil (doxorubicin HCl liposome injection) was the first liposomal product approved by the FDA in 1995. Among the marketed products, 43% were approved before 2000, and 57% before 2010. Most are used in cancer therapy, but they also cover areas such as infections, anesthesia, vaccines, lung diseases, and photodynamic therapy. Dosage forms include sterile suspensions and lyophilized powders, with administration routes covering intravenous infusion, intramuscular, intrathecal, epidural, local infiltration, and oral inhalation (Liu *et al.,* 2022). The marketed liposomal products are enlisted in the table 5.1.1.

5.1.2. Structures and main components of liposomes

Liposomes are categorized based on their compartment structure and lamellarity into unilamellar vesicles (ULVs), oligolamellar vesicles (OLVs), multilamellar vesicles (MLVs), and multivesicular liposomes (MVLs). OLVs and MLVs have concentric, onion-like structures with 2–5 and more than 5 lipid bilayers, respectively. MVLs contain numerous non-concentric aqueous chambers separated by a single bilayer, resembling a honeycomb. ULVs include small unilamellar vesicles (SUVs, 30–100 nm), large unilamellar vesicles (LUVs, >100 nm), and giant unilamellar vesicles (GUVs, >1000 nm). Commercial products mainly feature SUVs due to their longer circulation times and effective passive targeting. LUVs, such as Arikayce, and bilamellar systems like Vyxeos, offer distinct advantages based on size and structure. MVLs, used in products like Mepact and Exparel, provide substantial space for drug encapsulation (Nsairat *et al.,* 2022).

5.1.2.1. Main components of liposomes

Marketed liposomal products typically contain glycerolphospholipids (GP), sphingomyelin (SM), and cholesterol (Chol). Glycerolphospholipids have hydrophobic fatty acid chains and a hydrophilic head group. Depending on their composition, liposomes can be negatively charged (e.g., DSPG) or neutral (e.g., DSPC, EPC). Negatively charged DSPG, found in AmBisome and Vyxeos, helps

prevent aggregation, while neutral DSPC contributes to high purity and thermal stability. Cholesterol is commonly used to enhance membrane fluidity and stability, with its concentration affecting liposome properties. For example, in Shingrix, Chol prevents hydrolysis, and in AmBisome, it reduces formulation toxicity. Other sterols can also influence membrane rigidity (Giordani *et al.,* 2023).

5.1.3. Drug-loading methods for liposomes

Liposomes have become effective carriers for therapeutic agents, with various strategies developed to direct them specifically to tumour sites. These strategies include passive targeting, which relies on the natural accumulation of liposomes in tumors, and active targeting through surface modifications. Additionally, different stimuli can be employed to initiate the release of drugs from liposomes.

5.1.3.1. Passive drug-loading involves incorporating drugs during liposome preparation, either within the aqueous core or embedded in the lipid bilayer. While straightforward, it often results in lower encapsulation efficiency, requiring additional steps to remove unencapsulated drugs. Examples include Visudyne, where verteporfin is encapsulated due to its lipophilic nature, and Arikayce, which uses ethanol infusion for high-efficiency encapsulation of amikacin sulphate (Fulton and Najahi-Missaoui, 2023).

5.1.3.2. Active drug-loading method uses external pH or ion gradients to drive drugs into pre-formed liposomes, achieving high encapsulation efficiency. Examples include Doxil, which uses an ammonium sulfate gradient to load doxorubicin, and Onivyde, which employs an electrochemical gradient for loading irinotecan (Fulton and Najahi-Missaoui, 2023).

5.1.3.3. Covalent drug-lipid conjugation: This approach chemically attaches drugs to lipids, ensuring high entrapment efficiency and reduced drug leakage. Mepact, which conjugates muramyl dipeptide to phosphatidyl ethanolamine, is an example.

5.1.3.4. Combination method

Integrates both passive and active loading techniques, allowing for the encapsulation of multiple drugs within a single liposome. Vyxeos is an example, where cytarabine is passively encapsulated, and daunorubicin is actively loaded (Liu *et al.,* 2022).

5.1.3.5. Nanoparticles and liposome-mediated drug delivery

Nanoparticles, including liposomes, offer advantages such as improved tissue penetration, enhanced drug stability, and prolonged circulation time. Liposomes leverage the EPR effect to preferentially accumulate in tumors, reducing systemic toxicity (Chehelgerdi *et al.,* 2023). Despite their benefits, challenges such as potential toxicity and large-scale preparation difficulties persist. Techniques like lyophilization enhance stability, but achieving consistent quality across batches remains a challenge.

5.1.4. Critical Quality Attributes (CQAs)

CQAs for liposomes include particle size and distribution, morphology, lamellar structure, surface properties (e.g., zeta potential, PEGylation), phase transition temperature of the lipid membrane, drug loading efficiency, and release rate. These attributes impact drug release rate, circulation time, and overall therapeutic efficacy (Alshaer *et al.,* 2022).

5.1.5. Future prospects of liposome mediated drug delivery

Biodegradable Liposomes: Focuses on developing liposomes that break down into non-toxic metabolites in the body.

Targeted Liposomes: Advances in receptor-mediated targeting aim to deliver drugs directly to specific cells or tissues.

Multifunctional Liposomes: Combining drug delivery with imaging or therapeutic agents for enhanced treatment options.

Improved Manufacturing Techniques: Streamlining production methods to ensure consistency, quality, and scalability of liposome products.

Personalized Medicine: Tailoring liposome formulations to individual patient needs for more effective and safer treatments (Park, 2004).

5.1.6. Local stimuli to trigger drug release from liposomes

Local Stimuli-Based Drug Release strategies enhance the release of drugs from liposomes once they reach the tumour site, leveraging the specific pathological features of the tumour microenvironment. These strategies exploit factors such as altered pH, increased temperature, and the presence of overexpressed enzymes.

5.1.6.1. pH-responsive liposomes

Tumour microenvironments often exhibit acidic conditions compared to normal tissues. To exploit this, pH-sensitive copolymers can be incorporated into liposomal formulations. These polymers remain stable at physiological pH but undergo hydrolysis in acidic conditions (pH $\leq$ 6), common in tumour environments. Examples include poly (acrylic acid) and poly (methacrylic acid). This approach facilitates drug release when the liposomes encounter the acidic microenvironment of the tumour (Chu *et al.,* 2022, Lee and Thompson, 2017).

5.1.6.2. Temperature-triggered drug delivery

Another method involves local heating of the tumour site to increase the permeability of tumour blood vessels. This technique expands vascular pore size, enhancing liposomal extravasation and accumulation. Temperature-sensitive liposomes are designed with thermosensitive lipids that transition from a gel to a liquid phase at specific temperatures. For instance, dipalmitoylphosphatidylcholine (DPPC) is used in temperature-sensitive liposomes to release over 80% of encapsulated methotrexate when the temperature is raised from 37°C to 41°C. ThermoDox® is an example of such a formulation, which has shown improved doxorubicin delivery and efficacy in preclinical studies and is undergoing Phase III

clinical trials for treating hepatocellular carcinoma and breast cancer (May and Li, 2013, Bulbake *et al.,* 2017).

5.1.6.3. Enzyme-responsive liposomes

Tumour tissues often have elevated levels of proteolytic enzymes like matrix metalloproteinases (MMPs) and secretory phospholipases. Liposomes can be engineered to be sensitive to these enzymes, leading to drug release upon enzyme-mediated cleavage. This approach targets the specific enzymatic activity present in the tumour microenvironment, enhancing drug delivery and efficacy (Fouladi *et al.,* 2017, Mock *et al.,* 2013).

Despite these advancements, liposomes face limitations in achieving optimal therapeutic efficacy. For example, Doxil®, although a successful nanomedicine, still provides only modest benefits (Barenholz, 2012). The PEG layer in stealth liposomes, while providing advantages, also poses challenges for drug delivery and cell uptake, necessitating further development and optimization.

5.1.7. PEGylation of liposomes

PEGylation involves attaching polyethylene glycol (PEG) to the surface of liposomes to enhance their stability and circulation time while evading the mononuclear phagocytic system (MPS). This modification reduces protein adsorption, phagocytosis, and cellular adhesion of liposomes, extending their presence in the bloodstream (Vllasaliu, *et al.,* 2014).

5.1.7.1. Accelerated blood clearance

A significant issue with PEGylated liposomes is the accelerated blood clearance phenomenon**.** In animal models, a second injection of PEGylated liposomes within a short interval can lead to increased clearance and accumulation in the liver and spleen, along with elevated IgM production. This phenomenon is not commonly observed with Doxil® in clinical practice but remains a topic of debate. Strategies to address this issue include using alternative PEG-lipid derivatives or cleavable PEG

to mitigate accelerated clearance while improving drug delivery (Mohamed *et al.,* 2019).

5.1.7.2. Cell uptake and cargo delivery

PEGylation can influence cell uptake, tumor-targeting efficiency, and endosomal escape of nanoparticles. For example, increased PEG length and density can inhibit liposome fusion and reduce cellular uptake. Studies have shown that longer PEG chains or hydrophobic anchors can impair gene transfection and drug release, affecting therapeutic efficacy. Balancing PEG length and density is crucial for optimizing cargo delivery and therapeutic outcomes (Nosova *et al.,* 2019).

5.1.7.3. Cleavable PEG coatings

To retain the benefits of PEGylation while addressing its limitations, researchers have developed cleavable PEG coatings. These coatings use acid-labile linkages (e.g., acetal, hydrazine, hydrazone, or vinyl ether) to allow PEG to be shed in response to specific stimuli like the acidic tumour microenvironment. For example, Kanamala *et al.,* demonstrated that cleavable PEG coatings improved drug release and therapeutic efficacy compared to non-cleavable PEG coatings in preclinical models (Kanamala *et al.,* 2018). Similarly, strategies such as near-infrared (NIR) light-triggered PEG shedding have shown promise in enhancing tumour targeting and drug delivery (Fang *et al.,* 2017).

Despite advances, challenges remain in ensuring that acid-labile linkages do not prematurely release drug payloads at physiological pH, while still enabling effective release at lower pH levels found in tumors. Innovations in linkage stability, such as the PhosAm technology, show potential in aligning with the extended circulation times of stealth liposomes while achieving controlled drug release (Immordino *et al.,* 2006, Fulton and Najahi-Missaoui, 2023).

Liposomes represent a versatile drug delivery system with potential applications in targeted therapy across various diseases. Continued research and development are

essential to overcoming current limitations and optimizing the therapeutic benefits of liposomal formulations.

5.1.8. Research gap in liposomes mediated drug delivery

Despite progress, significant gaps remain in using liposomes for cancer treatment. One major need is improving drug loading and release. Current liposomal methods often struggle with limited drug amounts and inefficient release at tumour sites. Research is needed to develop new liposomal designs that can carry more drugs and release them more effectively in tumors.

Another challenge is related to PEGylation, a technique used to extend liposome circulation in the bloodstream. PEGylation can sometimes lead to quicker clearance of liposomes from the body and reduced targeting to tumors over time. There is a need to better understand and address this issue, perhaps by finding new ways to use PEGylation or exploring other methods to improve liposome performance.

Targeting tumors more effectively is also a crucial area for improvement. While current targeting strategies show promise, they often lack precision and can affect healthy tissues. More research is needed to develop better targeting methods that increase the concentration of liposomes in tumors while minimizing side effects.

Additionally, the long-term safety and distribution of liposomes in the body need more investigation. Ensuring that liposomes are safe for patients and have minimal environmental impact is vital for their successful clinical use.

Finally, personalizing liposome treatments to fit individual patient needs and tumour types could greatly enhance their effectiveness. Research into tailored liposomal therapies could lead to more successful cancer treatments.

MATERIALS AND METHODS

5.2.1. Chemicals

Soy lecithin was purchased from SRL. Cholesterol was obtained from Merck and other solvents used were of HPLC grade. Other chemicals used were of analytical grade and those used for cell line culture were of animal tissue culture grade.

5.2.2. Preparation of liposome

Control liposome without incorporating plant extracts was prepared by dissolving soylecithin (150 mg) and cholesterol (40 mg) in chloroform:methanol (2:1 ratio v/v) in a 50 mL round bottom flask. The solvents were evaporated in a rotory flash evaporator to get a thin film of lipid mix inside the flask. It was redissolved in 1XPBS and sonicated for 2 hours at 4°C. Liposomes were centrifuged to separate liposomes from unincorporated lipids/ plant extract components. It was stored at 4°C under N_2 (Ref). For incorporating crude/ active fraction (30 mg) was dissolved in chloroform:methanol along with soy lecithin and cholesterol and processed as above.

5.2.3. Characterization of liposome

5.2.3.1. Dynamic Light Scattering (DLS)

Hydrodynamic diameters of the liposome were determined using a Zetasizer Nano S90 Red badge DLS system, featuring a He-Ne laser operating at 632.8 nm. The particle sizes provided are intensity averages, calculated through the non-negative least squares analysis technique. For each sample, a single measurement was taken with a run time of 10 seconds, and a detection angle of 90° was applied. This DLS analysis was used to evaluate the size of the liposomes that were prepared.

5.2.3.2. UV-Visible spectrophotometry

The liposome molecules were centrifuged down, washed in PBS and scanned for absorption in the range of 200–700 nm. The buffer was taken as the base line.

5.2.3.3. LC-MS analysis

LC-MS analysis of the components of the liposomes, liposomes were dissolved in methanol and run in LC-MS analysis system (name) using a C18 column, with a mobile phase consisting of a gradient of water and acetonitrile containing 0.1% formic acid. The analysis was run in a gradient method over 30 minutes at a flow rate of 150 µL per minute. A sample injection volume of 10 µL was used, and detection was performed at a wavelength of 300 nm.

5.2.4. Cytotoxicity analysis of the liposomes

MTT assay was performed on human colorectal carcinoma cell line, HT29, to evaluate the cytotoxicity (Mosmann, 1983) of liposome incorporated fractions (chloroform extract and purified fraction from *Melia dubia* chloroform extract, SC1-F8). The cytotoxic effects of these liposome incorporated extracts were compared against the chloroform extract and SC1-F8 fraction at a concentration of 100 µg/mL (without incorporating in liposomes). A control liposome without the plant extract was also run to study the effect of liposome alone in inducing cytotoxicity. The liposomes were also tested at varying concentrations to determine their IC_{50} values, which were compared to the IC_{50} values of the chloroform extract and SC1-F8 alone to assess the toxicity of liposome incorporated extracts compared to unincorporated. The IC_{50} values were calculated using probit analysis in SPSS.

5.2.5. Statistical analysis

IC_{50} of the liposomes were calculated using probit analysis and significance by paired T test using SPSS.

RESULTS

Liposomes are a commonly used tool for encapsulating biological materials. These self-assembled structures are composed of lipid bilayers that encircle the *M. dubia* leaf chloroform extract or SC1-F8, which may deliver of the *M. dubia* chloroform extract or SC1-F8 into the cancer cell line.

5.3.1. DLS- size distribution

The mean size, size distribution and z-potential values are the physicochemical parameters those describe the liposomal system. The control liposome, which was not incorporated with the extract or the purified fraction, was analysed for size distribution. The average particle size was 70.22±1.40 d.nm and Z- average was 65.22 d.nm at 20ºC. The average particle size of the crude chloroform extract incorporated liposome was 93.89±5.94 d.nm at 20ºC. The average particle size of the SC1-F8 incorporated liposome was 83.21±3.32 d.nm and the Z-average was 299.6 d.nm at 20ºC (Figures 5.3.1-5.3.3).

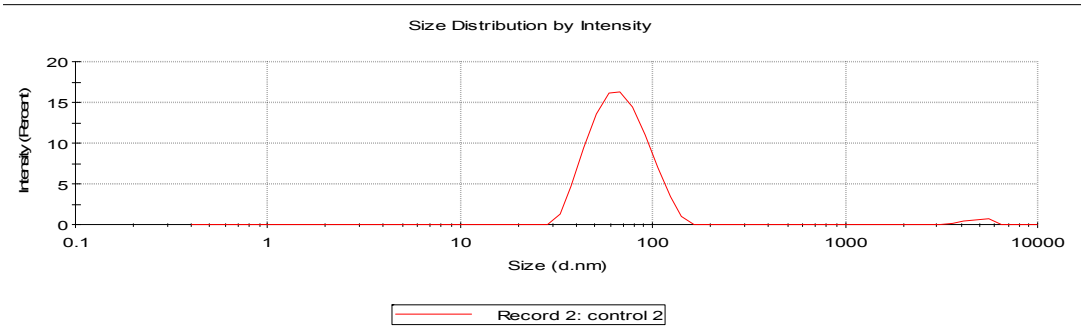


Figure 5.3.1.: Diagram showing the size distribution of control liposome (without incorporating plant extract).

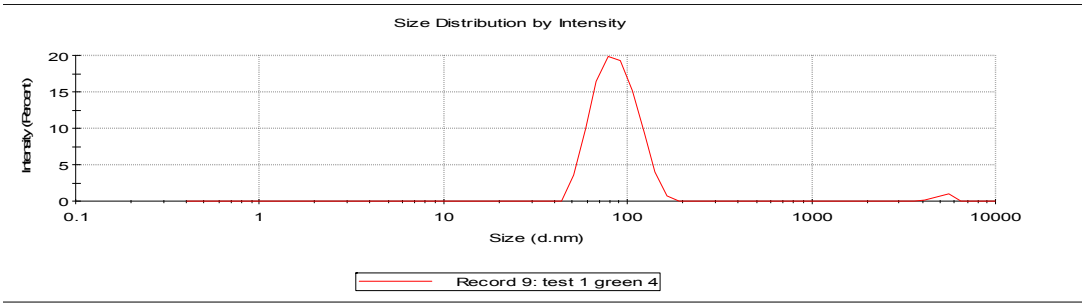


Figure 5.3.2. Diagram showing the size distribution of *Melia dubia* chloroform extract incorporated liposome.

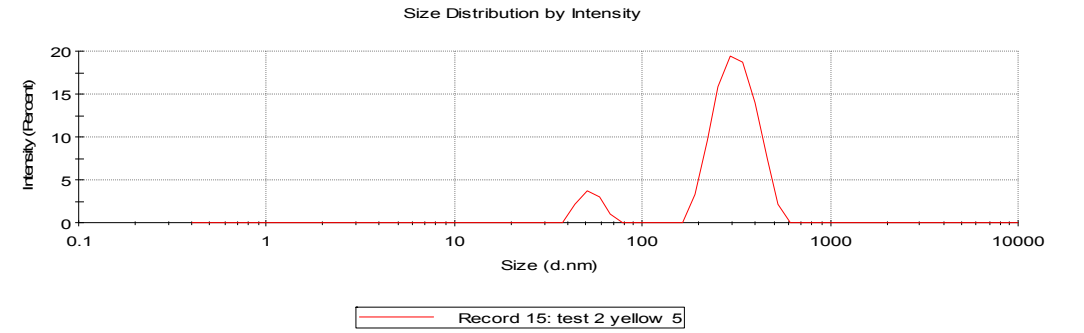


Figure 5.3.3. Diagram showing the size distribution of purified fraction (SC1-F8) from *Melia dubia* incorporated liposome.

5.3.2. UV- Visible spectrum

Further the extract/purified fraction incorporation was confirmed by UV-visible absorption method. The 1XPBS was taken as base line. The scan between 200-700 nm was recorded and the crude extract and the SC1-F8 loaded liposomes were containing multiple peaks than the control liposome indicating the incorporation into the liposomes.

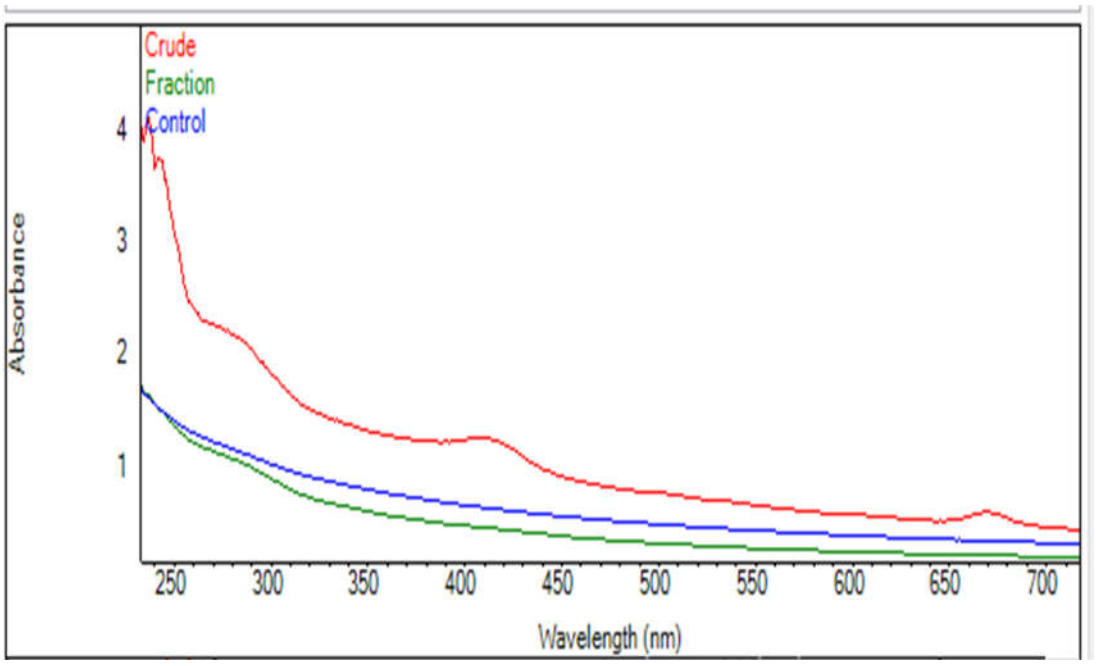


Figure 5.3.4. UV-Visible absorption spectrum of liposomes. The samples were scanned between 200-700nm. The blue line indicates the absorption spectrum of control liposome, red line indicates the absorption spectrum of crude extract containing liposome and green line indicates the spectrum of SC1-F8 incorporated liposome.

5.3.3. LC-MS analysis

The incorporation of the chloroform extract and purified fraction SC1-F8 was further confirmed by comparing the mass spectrum of the crude extract/SC1-F8 incorporated liposomes with that of unincorporated crude extract and SC1-F8 fraction (Figures 5.3.5-5.3.9).

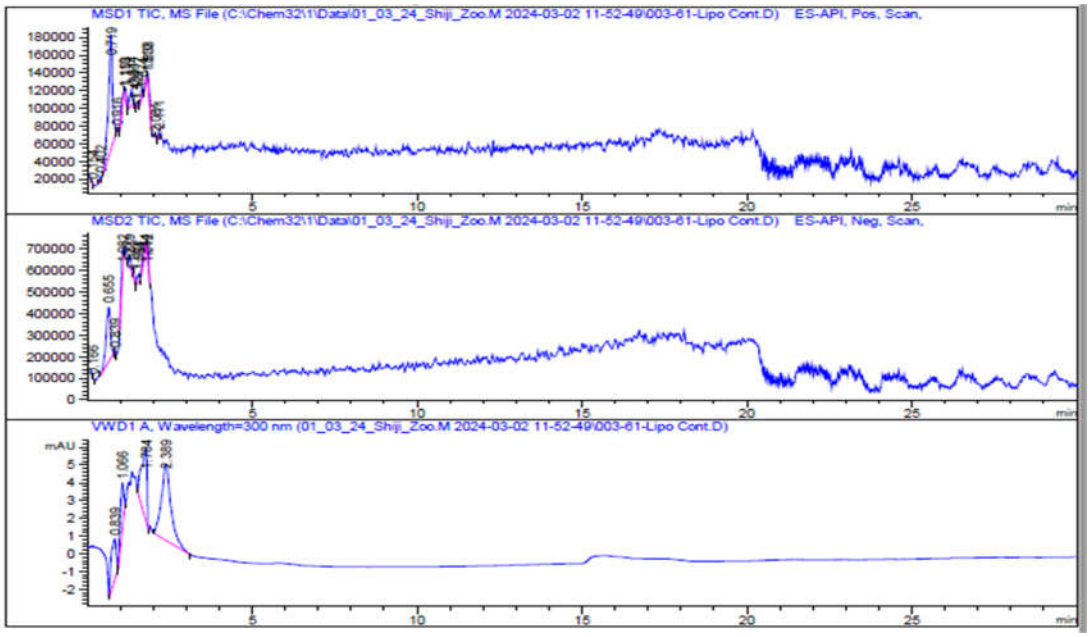


Figure 5.3.5. LC-MS chromatogram of control liposome

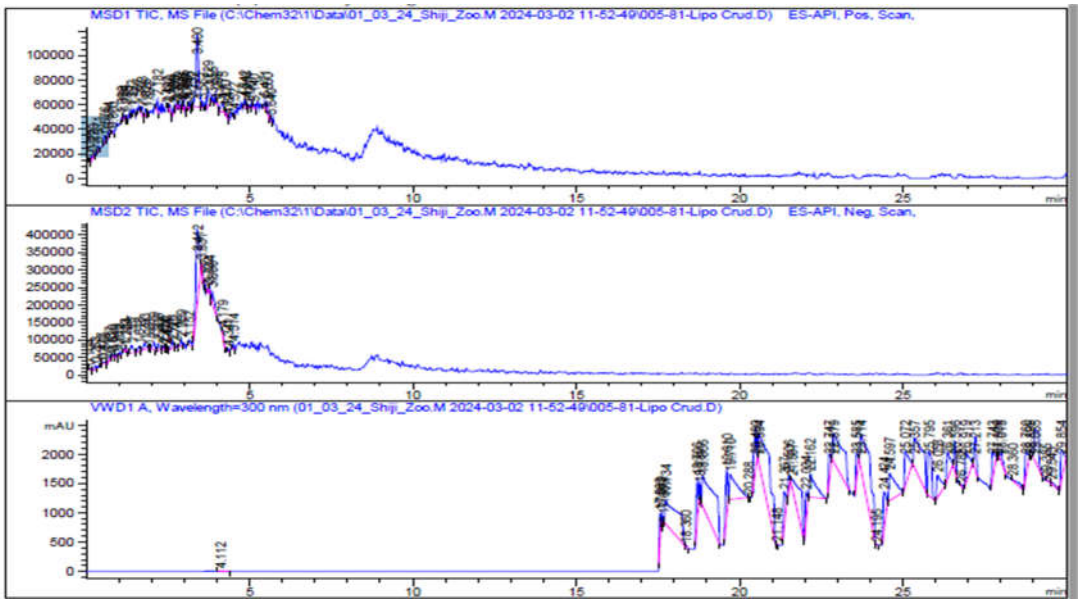


Figure 5.3.6.: LC-MS chromatogram of crude extract containing liposome.

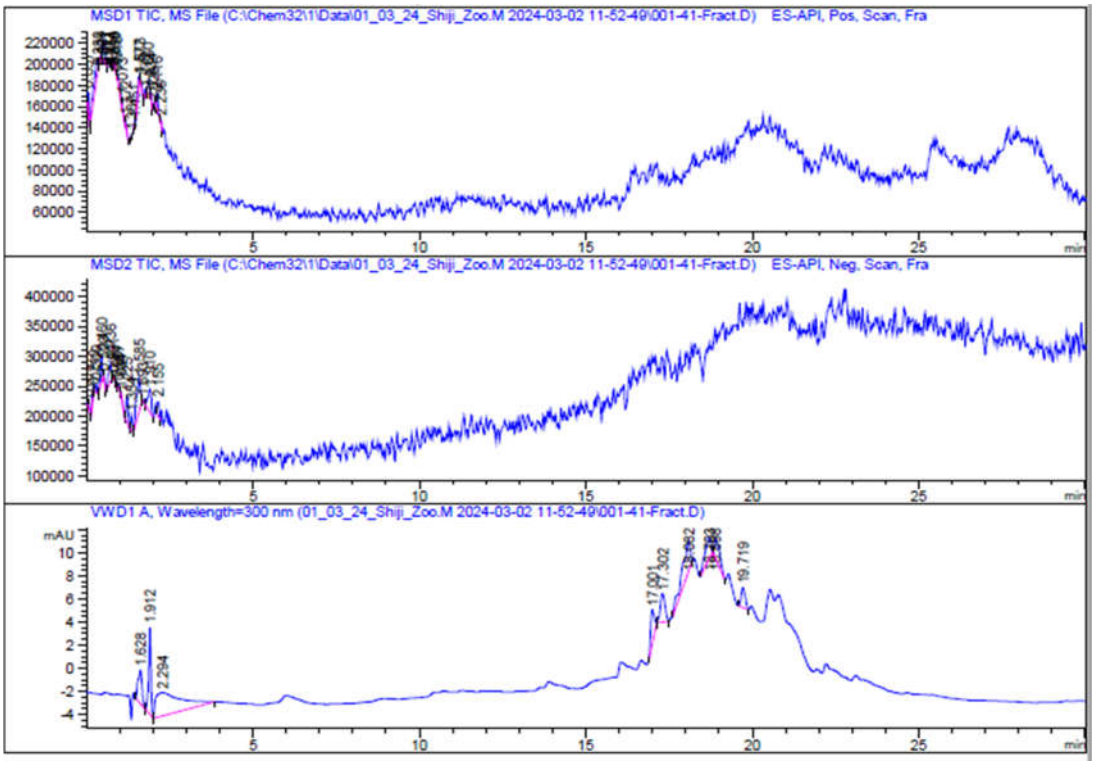


Figure 5.3.7. LC-MS chromatogram of unincorporated crude extract.

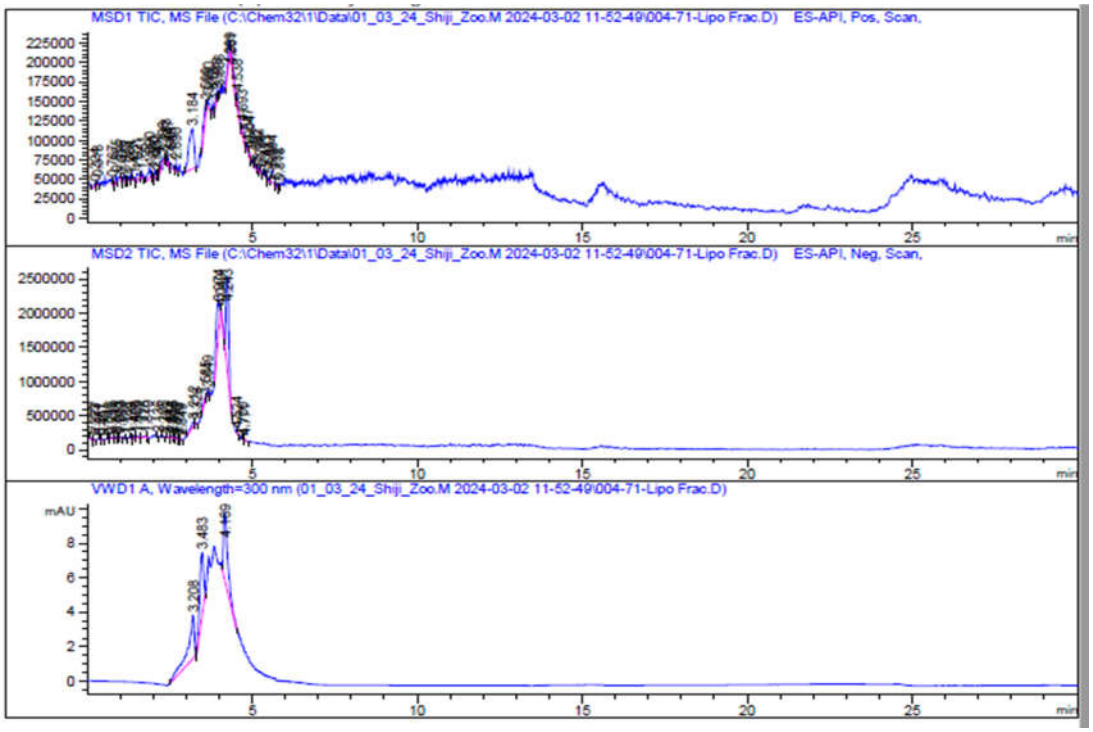


Figure 5.3.8. LC-MS chromatogram of purified fraction, SC1-F8 containing liposome.

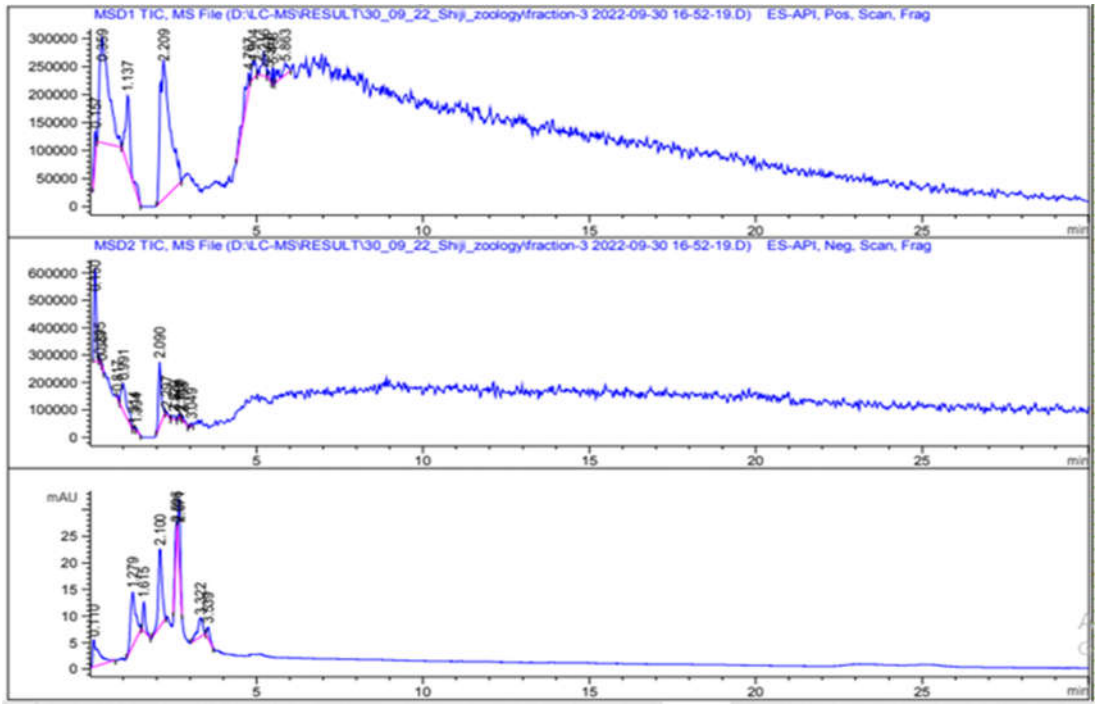


Figure 5.3.9.: LC-MS chromatogram of unincorporated purified fraction SC1-F8.

5.3.4. IC_{50} analysis of the liposomes

To evaluate the liposome mediated delivery of the crude extract of *M. dubia* and the bioactive fraction from the first column chromatography, the mortality percentage of HT29 cells was calculated and compared to their plant extract/SC1-F8 (without incorporating into liposomes). Treatments were administered at varying concentrations, and the IC_{50} values were determined for each. The unincorporated crude extract had an IC_{50} of 61.26±2.55 µg/mL, whereas the liposome-incorporated crude extract exhibited a significantly reduced IC_{50} of 36.66±0.87 µg/mL, with a highly significant p-value of less than 0.001. This marked reduction indicates enhanced cytotoxic efficacy when the crude extract is encapsulated in liposomes.

Similarly, the bioactive fraction from the first chromatography column (SC1-F8) showed an IC_{50} of 31.07±4.46 µg/mL in its unincorporated form. When SC1-F8 was incorporated into liposomes, its IC_{50} significantly decreased to 16.73±0.77 µg/mL, with a p-value of 0.003. These findings highlight the increased effect of the crude extract and the bioactive fraction when delivered via liposomal encapsulation,

reducing the amount needed to achieve cytotoxic effects on the HT29 cell line. This enhancement in cytotoxicity suggests improved bioavailability due to liposomal incorporation (p=0.003) (Figure 5.3.10).

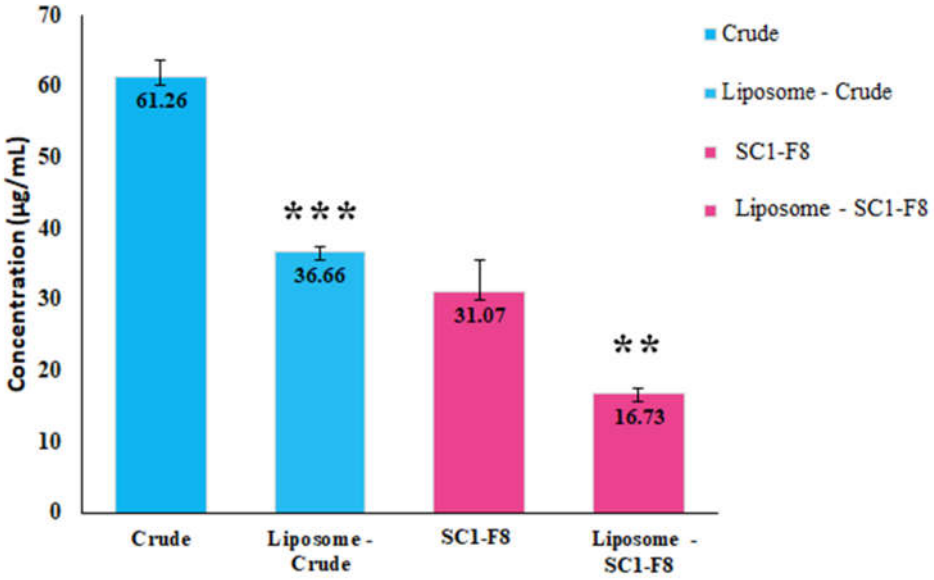


Figure 5.3.10.: Graph showing the comparison between the IC_{50} concentrations of liposome incorporated crude extract and bioactive fraction SC1-F8 with that of unincorporated crude extract/SC1-F8. The level of significance is indicated as ***=p<0.001 and **=p<0.01 when compared to unincorporated is indicated.

DISCUSSION

Liposomes serve as an effective drug delivery system by enhancing the therapeutic efficacy and safety of medications, primarily through targeted delivery and sustained release at the site of action (Gregoriadis and Florence, 1993). They also safeguard bioactive compounds from degradation in the stomach, allowing for increased absorption in the gastrointestinal tract, which improves both bioactivity and bioavailability (Takahashi *et al.,* 2007). Colorectal cancer (CRC) ranks among the most prevalent cancers in both men and women. With around one-third of patients not surviving beyond five years post-diagnosis, there is a pressing need to enhance treatment options, including innovative drug delivery methods. One promising approach is the use of liposomal formulations, which enable the delivery of both hydrophobic and hydrophilic drugs with improved biocompatibility and fewer side effects. Compared to free drugs, liposomal formulations have demonstrated superior antitumour efficacy, prolonged drug retention, and minimal toxicity to healthy cells (Krajewska *et al.,* 2019).

The dynamic light scattering (DLS) analysis of the liposomal formulations provided critical insights into their size distribution and Z-average values, which are directly linked to the performance and stability of the liposomal system. The control liposome, without any incorporated extract, exhibited an average particle size of 69.40±22.47 nm and a Z-average of 65.22 nm at 20ºC. This size falls within the optimal range for stability and potential cellular uptake. Upon incorporation of the crude chloroform extract, the average particle size increased to 79.71±9.52 nm, indicating a slight enlargement, but still within a size range considered favourable for drug delivery. This moderate size increase suggests that the extract slightly alters the liposomal structure without causing significant aggregation or instability.

In contrast, the SC1-F8 incorporated liposome demonstrated a decrease in average particle size to 53.57±7.35 nm, which may enhance cellular uptake, as smaller particles typically penetrate cells more efficiently. However, the Z-average dramatically increased to 299.6 nm, suggesting a broader size distribution or

potential aggregation. This larger Z-average could indicate some instability or heterogeneity in the liposomal formulation, potentially affecting its uniformity and delivery efficiency. The size differences observed highlights the relevance of particle size in influencing factors like encapsulation efficiency, bio-distribution, and overall therapeutic effectiveness. Smaller liposomes, as seen with SC1-F8, may have better internalization but could be prone to aggregation, while larger liposomes, like the crude extract-incorporated ones, may provide more stability but could face challenges with cellular uptake.

The findings of this study on the incorporation of crude extracts and the bioactive fraction SC1-F8 into liposomes align with broader trends in enhancing the bioavailability of poorly soluble natural compounds, as demonstrated with curcumin. Curcumin, a well-known natural anticancer agent, suffers from poor absorption in the gastrointestinal tract due to its low aqueous solubility, which limits its clinical efficacy. However, its bioavailability was significantly improved through liposomal encapsulation, as highlighted by Hasan *et al.,* (Hasan *et al.,* 2013). Their study demonstrated that liposomal curcumin significantly increased cytotoxicity in MCF7 cancer cells compared to free curcumin, which had a lower impact on cancer cell proliferation.

Similarly, in our current study, the liposomal encapsulation of the crude extract and SC1-F8 bioactive fraction resulted in a significant reduction in the IC_{50} values when tested on HT29 cancer cells. The IC_{50} of the crude extract decreased from 61.26±2.55 µg/mL to 36.66±0.87 µg/mL, and the IC_{50} of the SC1-F8 fraction dropped from 31.07±4.46 µg/mL to 16.73±0.77 µg/mL after liposome incorporation. These findings highlight the parallels between the improved cytotoxicity of liposomal curcumin and our liposome-encapsulated formulations, supporting the notion that liposomal delivery systems can greatly enhance the bioavailability and therapeutic potential of poorly soluble compounds.

The enhancement of cytotoxic effects upon liposomal incorporation is likely due to the liposomes' ability to protect the encapsulated compounds from degradation,

improve cellular uptake, and allow for controlled and sustained release. This results in higher intracellular concentrations of the active compounds, leading to greater efficacy at lower concentrations. The reduction in IC_{50} values seen in our study supports the conclusion drawn by Hasan *et al.* that liposome encapsulation increases bioavailability and enhances the anticancer effects of natural compounds (Hasan *et al.,* 2013). Thus, liposomal delivery systems represent a promising strategy for improving the clinical potential of plant-derived and other bioactive compounds in cancer treatment.

Chen *et al.* highlighted the benefits of using liposome-based drug delivery systems for β-elemene, which offered significant advantages compared to traditional formulations. As a promising anti-lung cancer agent, β-elemene faces limitations in clinical use due to its hydrophobic nature, instability, and low bioavailability (Chen *et al.,* 2012).

Similarly, the most effective treatments for breast cancer currently include anthracyclines and taxanes, notably paclitaxel and docetaxel. There is ongoing research aimed at developing improved delivery strategies for taxanes to increase tumour targeting while minimizing side effects such as neurotoxicity, edema, fatigue, and alopecia. The inherent challenges associated with taxanes further underscore the need for advanced delivery systems. Both paclitaxel and docetaxel are poorly soluble in aqueous environments, which have led to formulations utilizing Cremaphor EL and polysorbate 80 (TWEEN). However, these formulations are highly allergenic, require extensive premedication, and are largely responsible for the acute toxicities observed during taxane therapy, rather than the active agents themselves (Park, 2002; Park *et al*., 2004). Current clinical trials are exploring innovative delivery methods, including liposome-encapsulated paclitaxel and the polymer conjugate poly (L-glutamic acid)-paclitaxel (Cabanes *et al.,* 1998; Li *et al.,* 1999).

In addition, delivery systems are being employed for other chemotherapy drugs approved for breast cancer treatment, including vinca alkaloids, platinum

compounds, and camptothecins. In these cases, utilizing delivery systems like liposomes or polymers can improve pharmacokinetics, enhance tumour accumulation, and reduce associated toxicities (Chavda *et al.,* 2022).

These studies collectively emphasize the effectiveness of encapsulating poorly soluble or insoluble drugs within lipid-based vesicles, particularly liposomes. This underscores the relevance of the present study, which focuses on the incorporation of bioactive fractions into liposomes for targeted cancer applications. By enhancing the bioavailability and therapeutic efficacy of these compounds, liposomal delivery systems could play a crucial role in advancing cancer treatment strategies.

CONCLUSIONS

- The crude extract and the bioactive fraction from *M. dubia* were found highly effective when incorporated into liposomes.
- The IC_{50} of the crude and active fraction, SC1-F8 were significantly reduced upon incorporated into liposomes.
- The average size of the liposomes was in between 60-90 d.nm.

Summary and Conclusions

In this study 426 extracts from 65 plants were screened for their cytotoxic effects on cancer cell lines. Among the cancer cell lines tested, the human colorectal carcinoma cell line HT29 was sensitive to extracts from 59 plants, each resulting in over 50% cell death. The colorectal carcinoma cell line HCT116 showed a similar response to 34 extracts, with more than 50% mortality. In the human skin cancer cell line A431, 48 extracts caused more than 50% cell death, while 35 extracts had the similar effect on the human renal carcinoma cell line A498. The human cervical cancer cell line HeLa was susceptible to 38 extracts, and the human breast cancer cell line MCF7 to 48 extracts, both displaying over 50% cell death. For the osteoblast cell line MG63, 51 extracts induced over 50% mortality, and 86 extracts showed more than 50% cytotoxicity in the ovarian teratocarcinoma cell line PA1. In the pancreatic carcinoma cell line PANC1, 36 extracts demonstrated over 50% cell death, while 47 extracts exhibited similar cytotoxicity in the metastatic oral cancer cell line UM-SCC-83B. These findings suggest the presence of potent anticancer compounds in these plant extracts.

In addition to their efficacy against cancer cells, the cytotoxicity of these extracts was also evaluated in the normal human cell line HaCaT. Interestingly, 294 extracts caused less than 50% cell death, indicating their reduced toxicity toward non-cancerous cells. Moreover, 50 extracts resulted in less than 20% cell mortality in this normal cell line.

Out of the 65 plants screened cytotoxicity against cancer cell lines were first report for the extracts from *Pancratium triflorum, Anamirta cocculus, Chassalia curviflora, Antigonon leptopus, Otacanthus caeruleus, Hewittia malabarica* and *Melia dubia*.

The chloroform extract of *Hewittia malabarica* aerial parts exhibited notable cytotoxicity against the human colorectal carcinoma cell line HT29, with a mortality

rate of 81.59±4.90% at100 µg/mL and an IC_{50} of 31.97±3.07 µg/mL, while sparing normal HaCaT cells. GC-MS analysis revealed 17 compounds, with tritetracontane and neodene being the most abundant. *Pancratium triflorum* extracts also displayed selective cytotoxicity, with the ethyl acetate extract showing strong effects on the human osteoblast (MG63) and cervical cancer (HeLa) cell lines. The chloroform extract, in particular, caused 92.36±0.81% mortality in HeLa cells. *Conyza canadensis* petroleum ether extract exhibited the highest cytotoxicity, with nearly complete mortality against PANC1 and HT29 cancer cell lines, while its chloroform extract was similarly effective against MG63 and PANC1. *Anamirta cocculus* extract was most potent on the PA1 ovarian cancer cell line, achieving 98.64±0.40% mortality. *Melia dubia* extracts also demonstrated selective cytotoxicity; the petroleum ether extract showed a 97.48±1.77% mortality rate in PANC1 cells, with reduced effects on normal HaCaT cells. GC-MS analysis across these plants identified a variety of bioactive compounds, including tetratetracontane, (E)-Phytol, and 3-eicosene, (E)-, which could contribute to their anticancer properties.

Anamirta cocculus, a woody climber from the Menispermaceae family, has long been used in traditional Asian medicine, primarily for the antibacterial and antimicrobial properties of its stem and root alkaloids. This study investigates the cytotoxic effects of *A. cocculus* leaf extracts on both cancer and normal cell lines. The ethyl acetate extract exhibited the most potent anticancer activity, showing high cytotoxicity against ovarian cancer (PA1) and colorectal adenocarcinoma (HT29) cell lines, with IC_{50} values of 8.30±0.38 µg/mL and 17.97±0.63 µg/mL, respectively. Importantly, the extract displayed low toxicity towards normal HaCaT cells, even at concentrations as high as100 µg/mL, indicating selective cytotoxicity. The acetone extract also demonstrated significant effects on various cancer cell lines, including A498 (renal), MG63 (osteosarcoma), PA1 (ovarian), and UM-SCC-83B (oral squamous carcinoma). The ethyl acetate extract further inhibited colony formation in HT29 and PA1 cells, induced apoptosis, which was confirmed by characteristic markers such as membrane blebbing, chromatin condensation, and

DNA fragmentation. These findings highlight the potential of *A. cocculus* leaf extracts as a promising source of anticancer agents.

The chloroform extract of *Melia dubia* leaves was subjected to fractionation through silica gel column chromatography to enhance the anticancer activity. Bioactivity-guided purification progressively enriched the extract, resulting in significant improvements in cytotoxic potency across fractions. The crude extract showed an IC_{50} of 61.26±2.55 µg/mL, while the more purified fractions SC1-F8, SC2-F8, SC3-F8, and TLC-B3 exhibited IC_{50} values of 31.07±4.46, 17.86±1.29, 10.27±0.79, and 7.87±1.32 µg/mL, respectively. The final fraction, TLC-B3, was subjected to GC-MS analysis, revealing ten compounds that are likely responsible for its potent anticancer effects. This stepwise purification resulted in enriching anticancer compounds. The *in silico* docking studies showed that the observed high cytotoxicity of the purified fraction TLC-B3 towards HT29 cancer cell line may be contributed by Petroselinic acid individually or in combination with 9-Octadecenoic acid by additive or even synergistic mode.

The cytotoxicity analysis of *Melia dubia* purified extracts revealed significant mortality in HT29, MCF7, PA1, and UM-SCC-83B cancer cell lines. The purified fraction, SC3-F8, effectively inhibited colony formation and cell migration in these lines, while also induced a notable increase in cells undergoing apoptosis, both in early and late apoptotic stages, with visible alterations in cytomorphology as revealed by scanning electron microscopy. The purified fraction SC3-F8 further demonstrated a dose-dependent reduction in mitochondrial membrane potential ($\Delta\Psi M$) after 48 hours, as confirmed by fluorescence microscopy. Annexin-V/propidium iodide co-staining showed an increase in apoptotic cells in a dose-dependent manner. DNA damage was evident as longer comet tail lengths were observed in all treated cell lines. Additionally, SC3-F8 induced G1 phase cell cycle arrest and triggered the upregulation of pro-apoptotic genes like *caspase 3, caspase 8, caspase 9, BAX,* and *PTEN* while downregulating anti-apoptotic genes such as *Bcl2, cMyc, COX2*, and *NFκB* promoting apoptosis. The fraction also inhibited cancer cell migration and suppressed components of the epithelial-mesenchymal

transition (EMT) pathway, potentially disrupting angiogenesis. Thus some of the extracts and purified fractions from *M. dubia* contain very active anticancer molecules, which will serve as lead compounds in drug discovery for cancer. Further studies are required to utilize such molecules for their potential in cancer therapy.

The liposomal encapsulation of the crude and bioactive fraction, SC1-F8, resulted in a significant reduction in the IC_{50} values when they tested on HT29 cancer cell line, indicating their enhanced cytotoxic activity.

Recommendations

Cancer is a disease which still needs better therapeutic intervention in spite of the advances in the treatment modalities currently available, as some of the cancers are hard to cure and may cancers develop resistance against the currently used drugs. Plants are a repository of molecules with anticancer activities. To explore the anticancer activity of molecules in the plant extracts we screened plant extracts and identified some of the extracts with potential anticancer compounds and purified enriched fractions with anticancer molecules.

Following a thorough screening of 426 extracts from plants, two were selected for further investigation based on their high cytotoxicity: the ethyl acetate extract of *Anamirta cocculus* and the chloroform extract of *Melia dubia*. The crude extract of *A. cocculus* has demonstrated the ability to inhibit growth of cancer cell lines by apoptosis and inhibited cell migrations and colony formation two main characteristics of cancer cells. Further purification and isolation of the active compound are recommended for developing an anticancer drug.

Partial purification of the *M. dubia* chloroform extract yielded a mixture of approximately ten compounds, identified via GC-MS. Pinpointing the compound responsible for the extract's anticancer activity would significantly contribute to drug development for cancer treatment. Molecular docking studies have identified potential molecules with anticancer properties in the extract like Petroselinic acid and 9-Octadecenoic acid. The observed anticancer activity may be contributed by Petroselinic acid individually or in combination with 9-Octadecenoic acid by additive or even synergistic mode. It is worth investigating the potential of these compounds as anticancer agents. Thus future studied should explore the potential of these compounds as anticancer agents. Additionally, chemical synthesis of the identified anticancer compounds and their incorporation into nanomaterials, is proposed to enhance delivery and absorption of anticancer agents for more effective treatment.

References

1. Abal, M., Andreu, J. M., & Barasoain, I. (2003). Taxanes: microtubule and centrosome targets, and cell cycle dependent mechanisms of action. Current cancer drug targets, 3(3), 193–203. https://doi.org/10.2174/1568009033481967

2. Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. a. R. (2016). Natural polyphenols: An overview. International Journal of Food Properties, 20(8), 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

3. Abdelkarim, A. (2023). Characterization of flavonoids from *Combretum indicum* l. growing in Egypt as antioxidant and antitumor agents. Egyptian Journal of Chemistry, 0(0), 0. https://doi.org/10.21608/ejchem.2023.210414.7966

4. Abotaleb, M., Samuel, S. M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., & Büsselberg, D. (2018). Flavonoids in cancer and apoptosis. Cancers, 11(1), 28. https://doi.org/10.3390/cancers11010028

5. Abou-Nassar, K., & Brown, J. R. (2010). Novel agents for the treatment of chronic lymphocytic leukemia. Clinical advances in hematology & oncology: H&O, 8(12), 886–895.

6. Abraham, J., & Staffurth, J. (2016). Hormonal therapy for cancer. Medicine, 44(1), 30–33. https://doi.org/10.1016/j.mpmed.2015.10.014

7. Abu-Shandi, K., Al-Soufi, H., & Sawalqa, M. (2015). A Quick GC/MS Method correlated with LC/MS/MS for the Identification of Medicinal Natural Products in Convolvulus arvensis: An Injury Healing Plant. Eurasian Journal of Analytical Chemistry, 10(3).

8. Achkar, I. W., Mraiche, F., Mohammad, R. M., & Uddin, S. (2017). Anticancer potential of sanguinarine for various human malignancies. Future medicinal chemistry, 9(9), 933–950. https://doi.org/10.4155/fmc-2017-0041

9. Adams, G. P., & Weiner, L. M. (2005). Monoclonal antibody therapy of cancer. Nature biotechnology, 23(9), 1147–1157. https://doi.org/10.1038/nbt1137

10. Adams, J. M., & Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. Science (New York, N.Y.), 281(5381), 1322–1326. https://doi.org/10.1126/science.281.5381.1322

11. Adewusi, E. A., Fouche, G., & Steenkamp, V. (2012). Cytotoxicity and acetylcholinesterase inhibitory activity of an isolated crinine alkaloid from *Boophane disticha* (Amaryllidaceae). Journal of ethnopharmacology, 143(2), 572–578. https://doi.org/10.1016/j.jep.2012.07.011

12. Ahmad, M.F., Ahmed, M.A., Thayyil, H., Zameeruddin, K. & Ibrahim, M. (2008). Antioxidative activity of *Melia azadirach* Linn leaf extract. Iranian Journal of Pharmacology and Therapeutics 7, 31-34.https://www.sid.ir/FileServer/JE/ 101020080107

13. Ahrendt, S. A., Hu, Y., Buta, M., McDermott, M. P., Benoit, N., Yang, S. C., Wu, L., & Sidransky, D. (2003). p53 mutations and survival in stage I non-small-cell lung cancer: results of a prospective study. Journal of the National Cancer Institute, 95(13), 961–970. https://doi.org/10.1093/jnci/95.13.961

14. Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors (Oxford, England), 48(3), 611–633. https://doi.org/10.1002/biof.1831

15. Albershardt, T. C., Salerni, B. L., Soderquist, R. S., Bates, D. J., Pletnev, A. A., Kisselev, A. F., & Eastman, A. (2011). Multiple BH3 mimetics antagonize antiapoptotic MCL1 protein by inducing the endoplasmic reticulum stress response and up-regulating BH3-only protein NOXA. The Journal of biological chemistry, 286(28), 24882–24895. https://doi.org/10.1074/jbc.M111.255828

16. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). An overview of the cell cycle. Molecular Biology of the Cell - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK26869/

17. Al-Khayri, J. M., Mascarenhas, R., Harish, H. M., Gowda, Y., Lakshmaiah, V. V., Nagella, P., Al-Mssallem, M. Q., Alessa, F. M., Almaghasla, M. I., & Rezk, A. A. (2023). Stilbenes, a versatile class of natural metabolites for inflammation-an overview. Molecules (Basel, Switzerland), 28(9), 3786. https://doi.org/10.3390/molecules28093786

18. Almagro, L., Fernández-Pérez, F., & Pedreño, M. A. (2015). Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. Molecules (Basel, Switzerland), 20(2), 2973–3000. https://doi.org/10.3390/molecules20022973

19. Alrumman, S. A., Mostafa, Y. S., Al-Izran, K. A., Alfaifi, M. Y., Taha, T. H., & Elbehairi, S. E. (2019). Production and anticancer activity of an L-Asparaginase from *Bacillus licheniformis* isolated from the Red Sea, Saudi Arabia. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-40512-x

20. Alshaer, W., Nsairat, H., Lafi, Z., Hourani, O. M., Al-Kadash, A., Esawi, E., & Alkilany, A. M. (2022). Quality by Design approach in liposomal formulations: Robust product development. Molecules (Basel, Switzerland), 28(1), 10. https://doi.org/10.3390/molecules28010010

21. Amani, J., Gorjizadeh, N., Younesi, S., Najafi, M., Ashrafi, A. M., Irian, S., Gorjizadeh, N., & Azizian, K. (2021). Cyclin-dependent kinase inhibitors (CDKIs) and the DNA damage response: The link between signaling pathways and cancer. DNA repair, 102, 103103. https://doi.org/10.1016/j.dnarep.2021.103103

22. American Cancer Society. (2024). Global Cancer Facts & Figures 5th Edition. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-cancer-facts-and-figures-2024.pdf

23. American Cancer Society. (2024). Global Cancer Facts & Figures 5th Edition. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-cancer-facts-and-figures-2024.pdf

24. Amin, A. R. M. R., Wang, D., Nannapaneni, S., Lamichhane, R., Chen, Z. G., & Shin, D. M. (2021). Combination of resveratrol and green tea epigallocatechin gallate induces synergistic apoptosis and inhibits tumor growth *in vivo* in head and neck cancer models. Oncology reports, 45(5), 87. https://doi.org/10.3892/or.2021.8038

25. Amjad, M. T., Chidharla, A., & Kasi, A. (2023). Cancer Chemotherapy. In StatPearls. StatPearls Publishing.https://www.ncbi.nlm.nih.gov/books/NBK564367/

26. Amna, T., Puri, S. C., Verma, V., Sharma, J. P., Khajuria, R. K., Musarrat, J., Spiteller, M., & Qazi, G. N. (2006). Bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of an anticancer alkaloid camptothecin. Canadian journal of microbiology, 52(3), 189–196. https://doi.org/10.1139/w05-122

27. Anusuya, N., Anusuya, S., Manian, R., Siddhuraju, P., & Manian, S. (2009). Antioxidant and free radical scavenging activity of certain dietary and medicinal plant extracts. Food, 2(1), 47-52.

28. Aqil, F., Munagala, R., Jeyabalan, J., & Vadhanam, M. V. (2013). Bioavailability of phytochemicals and its enhancement by drug delivery systems. Cancer letters, 334(1), 133–141. https://doi.org/10.1016/j.canlet.2013.02.032

29. Aragão, L., Fernandes, S. S. L., Mallmann, V., Facco, J. T., De Fátima Cepa Matos, M., Cabral, M. R. P., De Carvalho, J. E., Ruiz, A. L. T. G., Cardoso, C. a. L., De Lara Da Silva, R. C., Anjos, A. D., & Simionatto, E. (2019b). Chemical Composition and Evaluation of Antitumoral Activity of Leaf and Root Essential Oils of *Conyza canadensis* (Asteraceae). Orbital - the Electronic Journal of Chemistry, 11(5). https://doi.org/10.17807/orbital.v11i5.1376

30. Aref, A. R., Huang, R. Y., Yu, W., Chua, K. N., Sun, W., Tu, T. Y., Bai, J., Sim, W. J., Zervantonakis, I. K., Thiery, J. P., & Kamm, R. D. (2013). Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. Integrative biology: quantitative biosciences from nano to macro, 5(2), 381–389. https://doi.org/10.1039/c2ib20209c

31. Arley, N., & Eker, R. (1962). Mechanisms of carcinogenesis. In Advances in biological and medical physics (pp. 375–436). https://doi.org/10.1016/b978-1-4831-9927-6.50011-3

32. Arruebo, M., Vilaboa, N., Sáez-Gutierrez, B., Lambea, J., Tres, A., Valladares, M., & González-Fernández, A. (2011). Assessment of the evolution of cancer treatment therapies. Cancers, 3(3), 3279–3330. https://doi.org/10.3390/cancers3033279

33. Arumugam, T., Ramachandran, V., Fournier, K. F., Wang, H., Marquis, L., Abbruzzese, J. L., Gallick, G. E., Logsdon, C. D., McConkey, D. J., & Choi, W. (2009). Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer research, 69(14), 5820–5828. https://doi.org/10.1158/0008-5472.CAN-08-2819

34. Arvelo, F., Sojo, F., & Cotte, C. (2016). Tumour progression and metastasis. Ecancermedicalscience,10.https://doi.org/10.3332/ecancer.2016.617

35. Asadbeigi, M., Mohammadi, T., Rafieian-Kopaei, M., Saki, K., Bahmani, M., & Delfan, M. (2014). Traditional effects of medicinal plants in the treatment of respiratory diseases and disorders: an ethnobotanical study in the Urmia. Asian Pacific journal of tropical medicine, 7S1, S364–S368. https://doi.org/10.1016/S1995-7645(14)60259-5

36. Asensi, M., Ortega, A., Mena, S., Feddi, F., & Estrela, J. M. (2011). Natural polyphenols in cancer therapy. Critical reviews in clinical laboratory sciences, 48(5-6), 197–216. https://doi.org/10.3109/10408363.2011.631268

37. Astuti, P., D Utami, E., Nugrahani, A. W., & Sudjadi, S. (2012). Genistein abrogates G2 arrest induced by curcumin in p53 deficient T47D cells. Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences, 20(1), 82. https://doi.org/10.1186/2008-2231-20-82

38. Athikkavil, F. M., Aiswarya, S. U., Johny, R., Sudhesh, M., Nisthul, A. A., Lankalapalli, R. S., Anto, R. J., & Bava, S. V. (2023). A potent bioactive fraction against colon cancer from *Plectranthus vettiveroides*. Exploration of targeted anti-tumor therapy, 4(2), 227–239. https://doi.org/10.37349/etat.2023.00131

39. Augustine, B. B., Dash, S., Lahkar, M., Sarma, U., Samudrala, P. K., & Thomas, J. M. (2014). Leucas aspera inhibits the Dalton's ascitic lymphoma in Swiss albino mice: A preliminary study exploring possible mechanism of action. Pharmacognosy magazine, 10(38), 118–124. https://doi.org/10.4103/0973-1296.131022

40. Avery-Kiejda, K. A., Bowden, N. A., Croft, A. J., Scurr, L. L., Kairupan, C. F., Ashton, K. A., Talseth-Palmer, B. A., Rizos, H., Zhang, X. D., Scott, R. J., & Hersey, P. (2011). P53 in human melanoma fails to regulate target genes associated with apoptosis and the cell cycle and may contribute to proliferation. BMC cancer, 11, 203. https://doi.org/10.1186/1471-2407-11-203

41. Ayaz, F., Sarimahmut, M., Kucukboyaci, N., & Ulukaya, E. (2016). Cytotoxic Effect of Conyza canadensis (L.) Cronquist on Human Lung Cancer Cell Lines/*Conyza canadensis* (L) Cronquist'in Insan Akciger Kanser Hucre Hatlari Uzerine Sitotoksik Aktivitesi. Turkish Journal of Pharmaceutical Sciences, 13(3), 342-347.

42. Ayoola, G. A., Coker, H. A., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical journal of pharmaceutical research, 7(3), 1019-1024.

43. Azani H, Homayouni Tabrizi M, Neamati A, Khadem F& Khatamian N. The Ferula Assa-foetida Essential Oil Nanoemulsion (FAEO-NE) as the selective, apoptotic, and anti-angiogenic anticancer compound in human MCF-7 breast cancer cells and murine mammary tumor models.(2022) Nutr Cancer, 74, 2196–2206.

44. Baby, A. A., & K, R. R. (2018). Analysis of the antioxidant property, cytotoxicity and anti-tumour efficiency of *Bauhinia phoenicea*. Asian Journal of Pharmaceutical and Clinical Research, 11(12), 277. https://doi.org/10.22159/ajpcr.2018.v11i12.28136

45. Bagul, M., Kakumanu, S., & Wilson, T. A. (2015). Crude garlic extract inhibits cell proliferation and induces cell cycle arrest and apoptosis of cancer cells *in vitro*. Journal of medicinal food, 18(7), 731–737. https://doi.org/10.1089/jmf.2014.0064

46. Baharuddin, A. A., Roosli, R. A., Zakaria, Z. A., & Tohid, S. F. M. (2021). *Dicranopteris linearis*; a potential medicinal plant with anticancer properties. Boletín Latinoam. Y Del Caribe De Plantas Med. Y Aromáticas, 20, 28-37.

47. Bai, L., & Zhu, W. G. (2006). p53: structure, function and therapeutic applications. J Cancer Mol, 2(4), 141-153.

48. Baker N. E. (2020). Emerging mechanisms of cell competition. Nature reviews. Genetics, 21(11), 683–697. https://doi.org/10.1038/s41576-020-0262-8

49. Baldwin, E. L., & Osheroff, N. (2005). Etoposide, topoisomerase II and cancer. Current medicinal chemistry. Anti-cancer agents, 5(4), 363–372.https://doi.org/10.2174/1568011054222364

50. Ballout, F., Habli, Z., Monzer, A., Rahal, O. N., Fatfat, M., & Gali-Muhtasib, H. (2019). Anticancer alkaloids: molecular mechanisms and clinical manifestations. Bioactive Natural Products for the Management of Cancer: from Bench to Bedside, 1-35.

51. Baloglu, E., & Kingston, D. G. (1999). A new semisynthesis of paclitaxel from baccatin III. Journal of natural products, 62(7), 1068–1071. https://doi.org/10.1021/np990040k

52. Bama, P. & Sankaranarayanan, S. & Bhuvaneswari, N. (2012). Cytotoxicity effect of alkaloidal compound isolated from the leaf of *Alangium salvifolium* on *in vitro* MCF-7 cell line. International Journal of Pharmacy and Pharmaceutical Sciences. 4. 210-214

53. Bangham A. D. (2005). Liposomes and the physico-chemical basis of unconsciousness. FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 19(13), 1766–1768. https://doi.org/10.1096/fj.05-1103ufm

54. Barenholz Y. (2012). Doxil®--the first FDA-approved nano-drug: lessons learned. Journal of controlled release: official journal of the Controlled Release Society, 160(2), 117–134. https://doi.org/10.1016/j.jconrel.2012.03.020

55. Barhoumi, L. M., Shakya, A. K., Al-Fawares, O., & Al-Jaber, H. I. (2024). *Conyza canadensis* from Jordan: phytochemical profiling, antioxidant, and antimicrobial activity evaluation. Molecules (Basel, Switzerland), 29(10), 2403. https://doi.org/10.3390/molecules29102403

56. Basili, S., & Moro, S. (2009). Novel camptothecin derivatives as topoisomerase I inhibitors. Expert opinion on therapeutic patents, 19(5), 555–574. https://doi.org/10.1517/13543770902773437

57. Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. W. (2012). Cancer and radiation therapy: current advances and future directions. International journal of medical sciences, 9(3), 193–199. https://doi.org/10.7150/ijms.3635

58. Batova A, Altomare D, Chantarasriwong O, Ohlsen KL, Creek KE, Lin YC, Messersmith A, Yu AL, Yu J, Theodorakis EA. (2010). The synthetic caged garcinia xanthone cluvenone induces cell stress and apoptosis and has immune modulatory activity. Mol Cancer Ther. Nov;9(11):2869-78. doi: 10.1158/1535-7163.MCT-10-0517. Epub 2010 Sep 29. PMID: 20881270; PMCID: PMC2978789.

59. Baykara, O. (2015). Current therapies and latest developments in cancer treatment. Horizons in Cancer Research, 57, 105-156.

60. Beamish, H., Kedar, P., Kaneko, H., Chen, P., Fukao, T., Peng, C., Beresten, S., Gueven, N., Purdie, D., Lees-Miller, S., Ellis, N., Kondo, N., & Lavin, M. F. (2002). Functional link between BLM defective in Bloom's syndrome and the ataxia-telangiectasia-mutated protein, ATM. The Journal of biological chemistry, 277(34), 30515–30523. https://doi.org/10.1074/jbc.M203801200

61. Bell, L., Lamport, D. J., Butler, L. T., & Williams, C. M. (2015). A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. Nutrients, 7(12), 10290–10306. https://doi.org/10.3390/nu7125538

62. Bellamy W. T. (1996). P-glycoproteins and multidrug resistance. Annual review of pharmacology and toxicology, 36, 161–183. https://doi.org/10.1146/annurev.pa.36.040196.001113

63. Belluti, F., Fontana, G., Dal Bo, L., Carenini, N., Giommarelli, C., & Zunino, F. (2010). Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: Identification of novel proapoptotic agents. Bioorganic & medicinal chemistry, 18(10), 3543–3550. https://doi.org/10.1016/j.bmc.2010.03.069

64. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., Bourne, P. E. (2000). The Protein Data Bank. Nucleic acids research. 28(1), 235–242. https://doi.org/10.1093/nar/28.1.235

65. Bernhardt, P., McCoy, E., & O'Connor, S. E. (2007). Rapid identification of enzyme variants for reengineered alkaloid biosynthesis in periwinkle. Chemistry & biology, 14(8), 888–897. https://doi.org/10.1016/j.chembiol.2007.07.008

66. Bertram, J. S. (2000). The molecular biology of cancer. Molecular Aspects of Medicine, 21(6), 167–223. https://doi.org/10.1016/s0098-2997(00)00007-8

67. Bhat, G. R., Sethi, I., Sadida, H. Q., Rah, B., Mir, R., Algehainy, N., Albalawi, I. A., Masoodi, T., Subbaraj, G. K., Jamal, F., Singh, M., Kumar, R., Macha, M. A., Uddin, S., Akil, A. S. A., Haris, M., & Bhat, A. A. (2024). Cancer cell plasticity: from cellular, molecular, and genetic mechanisms to tumor heterogeneity and drug resistance. Cancer and Metastasis Reviews. https://doi.org/10.1007/s10555-024-10172-z

68. Bianco, R., Melisi, D., Ciardiello, F., & Tortora, G. (2006). Key cancer cell signal transduction pathways as therapeutic targets. European journal of cancer (Oxford, England: 1990), 42(3), 290–294. https://doi.org/10.1016/j.ejca.2005.07.034

69. Boeckler, F. M., Joerger, A. C., Jaggi, G., Rutherford, T. J., Veprintsev, D. B., & Fersht, A. R. (2008). Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. Proceedings of the National Academy of Sciences of the United

States of America, 105(30), 10360–10365. https://doi.org/10.1073/pnas.0805326105

70. Böhm, V., Lietz, G., Olmedilla-Alonso, B., Phelan, D., Reboul, E., Bánati, D., Borel, P., Corte-Real, J., de Lera, A. R., Desmarchelier, C., Dulinska-Litewka, J., Landrier, J. F., Milisav, I., Nolan, J., Porrini, M., Riso, P., Roob, J. M., Valanou, E., Wawrzyniak, A., Winklhofer-Roob, B. M., … Bohn, T. (2021). From carotenoid intake to carotenoid blood and tissue concentrations - implications for dietary intake recommendations. Nutrition reviews, 79(5), 544–573. https://doi.org/10.1093/nutrit/nuaa008

71. Boland, P. M., Yurgelun, M. B., & Boland, C. R. (2018). Recent progress in Lynch syndrome and other familial colorectal cancer syndromes. CA: a cancer journal for clinicians, 68(3), 217–231. https://doi.org/10.3322/caac.21448

72. Bouyahya, A., Chamkhi, I., Guaouguaou, F. E., Benali, T., Balahbib, A., El Omari, N., Taha, D., El-Shazly, M., & El Menyiy, N. (2020). Ethnomedicinal use, phytochemistry, pharmacology, and food benefits of *Thymus capitatus*. Journal of ethnopharmacology, 259, 112925. https://doi.org/10.1016/j.jep.2020.112925

73. Bozic, I., Antal, T., Ohtsuki, H., Carter, H., Kim, D., Chen, S., Karchin, R., Kinzler, K. W., Vogelstein, B., & Nowak, M. A. (2010). Accumulation of driver and passenger mutations during tumor progression. Proceedings of the National Academy of Sciences of the United States of America, 107(43), 18545–18550. https://doi.org/10.1073/pnas.1010978107

74. Braga, T. M., Rocha, L., Chung, T. Y., Oliveira, R. F., Pinho, C., Oliveira, A. I., Morgado, J., & Cruz, A. (2020). Biological activities of gedunin-a limonoid from the Meliaceae Family. Molecules (Basel, Switzerland), 25(3), 493. https://doi.org/10.3390/molecules25030493

75. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394–424. https://doi.org/10.3322/caac.21492

76. Brown G. (2021). Oncogenes, proto-oncogenes, and lineage restriction of cancer stem cells. International journal of molecular sciences, 22(18), 9667. https://doi.org/10.3390/ijms22189667

77. Brown, J. M., & Attardi, L. D. (2005). The role of apoptosis in cancer development and treatment response. Nature reviews. Cancer, 5(3), 231–237. https://doi.org/10.1038/nrc1560

78. Budiman, M. R., Wiraswati, H. L., & Rezano, A. (2021). Purple sweet potato Phytochemicals: potential chemo-preventive and anticancer activities. Open Access Macedonian Journal of Medical Sciences, 9(F), 288–298. https://doi.org/10.3889/oamjms.2021.6784

79. Bulbake, U., Doppalapudi, S., Kommineni, N., & Khan, W. (2017). Liposomal Formulations in Clinical Use: An Updated Review. Pharmaceutics, 9(2), 12. https://doi.org/10.3390/pharmaceutics9020012

80. Burmester, T., & Hankeln, T. (2009). What is the function of neuroglobin?. The Journal of experimental biology, 212(Pt 10), 1423–1428. https://doi.org/10.1242/jeb.000729

81. Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods--a review. International journal of food microbiology, 94(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

82. Cabanes, A. K. E. B., Briggs, K. E., Gokhale, P. C., Treat, J. A., & Rahman, A. (1998). Comparative *in vivo* studies with paclitaxel and liposome-encapsulated paclitaxel. International journal of oncology, 12(5), 1035-1075.

83. Cai, Y. S., Wang, C., Tian, C., Sun, W. T., Chen, L., Xiao, D., Zhou, S. Y., Qiu, G., Yu, J., Zhu, K., & Yang, S. P. (2019). Octahydro-protoberberine and protoemetine-type alkaloids from the stems of *Alangium salviifolium* and their cytotoxicity. Journal of natural products, 82(9), 2645–2652. https://doi.org/10.1021/acs.jnatprod.9b00670

84. Cam, L., Boucquey, A., Coulomb-L'hermine, A., Weber, A., & Horellou, P. (2005). Gene transfer of constitutively active caspase-3 induces apoptosis in a human hepatoma cell line. The journal of gene medicine, 7(1), 30–38. https://doi.org/10.1002/jgm.636

85. Canel, C., Moraes, R. M., Dayan, F. E., & Ferreira, D. (2000). Podophyllotoxin. Phytochemistry, 54(2), 115–120. https://doi.org/10.1016/s0031-9422(00)00094-7

86. Cao, C., Mu, Y., Hallahan, D. E., & Lu, B. (2004). XIAP and survivin as therapeutic targets for radiation sensitization in preclinical models of lung cancer. Oncogene, 23(42), 7047–7052. https://doi.org/10.1038/sj.onc.1207929

87. Carvalho, P. S., Napolitano, H. B., Camargo, A. J., Silva, V. H., Ellena, J. A., Rocha, W. C., & Vieira, P. C. (2011). X-ray diffraction and theoretical investigation of the gedunin crystal structure. Journal of Molecular Structure, 1008, 83–87. https://doi.org/10.1016/j.molstruc.2011.11.028

88. Chae, H. S., & Hong, S. T. (2022). Overview of cancer metabolism and signaling transduction. International journal of molecular sciences, 24(1), 12. https://doi.org/10.3390/ijms24010012

89. Chakraborty, S., & Rahman, T. (2012). The difficulties in cancer treatment. Ecancermedicalscience, 6, ed16. https://doi.org/10.3332/ecancer.2012.ed16

90. Chan, E. W. C., Wong, S. K., & Chan, H. T. (2021). An overview on the chemistry, pharmacology and anticancer properties of tetrandrine and fangchinoline (alkaloids) from *Stephania tetrandra* roots. Journal of integrative medicine, 19(4), 311–316. https://doi.org/10.1016/j.joim.2021.01.001

91. Chang J. C. (2016). Cancer stem cells: Role in tumor growth, recurrence, metastasis, and treatment resistance. Medicine, 95(1 Suppl 1), S20–S25. https://doi.org/10.1097/MD.0000000000004766

92. Chang, T. H., Tsai, M. F., Su, K. Y., Wu, S. G., Huang, C. P., Yu, S. L., Yu, Y. L., Lan, C. C., Yang, C. H., Lin, S. B., Wu, C. P., Shih, J. Y., & Yang, P. C. (2011). Slug confers resistance to the epidermal growth factor receptor tyrosine kinase

inhibitor. American journal of respiratory and critical care medicine, 183(8), 1071–1079. https://doi.org/10.1164/rccm.201009-1440OC

93. Chantarasriwong, O., Batova, A., Chavasiri, W., & Theodorakis, E. A. (2010). Chemistry and biology of the caged *Garcinia xanthones*. Chemistry (Weinheim an der Bergstrasse, Germany), 16(33), 9944–9962. https://doi.org/10.1002/chem.201000741

94. Chanthuru, A., Prabhu, M. M., Ayshaand, O., & Karthik, R. (2014). Evaluation of leaf and root extracts of *Melia dubia* L. against larvae of *Culex quinquefasciatus* and five important human pathogens. Biosciences Biotechnology Research Asia, 11(SE), 207–210. https://doi.org/10.13005/bbra/1412

95. Charde, R. M., Dhongade, H. J., Charde, M. S., & Kasture, A. V. (2010). Evaluation of antioxidant, wound healing and anti-inflammatory activity of ethanolic extract of leaves of *Ficus religiosa*. Int J Pharm Sci Res, 19(5), 73-82.http://dx.doi.org/10.13040/IJPSR.0975-8232.1(5).73-82

96. Charmsaz, S., Collins, D. M., Perry, A. S., & Prencipe, M. (2019). Novel Strategies for Cancer Treatment: Highlights from the 55th IACR Annual Conference. Cancers, 11(8), 1125. https://doi.org/10.3390/cancers11081125

97. Chaurasia B, Singh VK, Chaudhury S, Singh R (2021) *Hewittia malabarica* (Convolvulaceae): a new record for the flora of Uttar Pradesh, India. Indian Journal of Forestry. 43(4): 318-321.

98. Chaurio, R. A., Janko, C., Muñoz, L. E., Frey, B., Herrmann, M., & Gaipl, U. S. (2009). Phospholipids: key players in apoptosis and immune regulation. Molecules (Basel, Switzerland), 14(12), 4892–4914. https://doi.org/10.3390/molecules14124892

99. Chavda, V. P., Patel, A. B., Mistry, K. J., Suthar, S. F., Wu, Z. X., Chen, Z. S., & Hou, K. (2022). Nano-drug delivery systems entrapping natural bioactive compounds for cancer: Recent progress and future challenges. Frontiers in oncology, 12, 867655. https://doi.org/10.3389/fonc.2022.867655

100. Chaves, F. M., Pavan, I. C. B., Da Silva, L. G. S., De Freitas, L. B., Rostagno, M. A., Antunes, A. E. C., Bezerra, R. M. N., & Simabuco, F. M. (2019). Pomegranate juice and peel extracts are able to inhibit proliferation, migration and colony formation of prostate cancer cell lines and modulate the Akt/mTOR/S6K signaling pathway. Plant Foods for Human Nutrition, 75(1), 54–62. https://doi.org/10.1007/s11130-019-00776-0

101. Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H., & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169. https://doi.org/10.1186/s12943-023-01865-0

102. Chen, M., & Zhao, H. (2019). Next-generation sequencing in liquid biopsy: cancer screening and early detection. Human genomics, 13(1), 34. https://doi.org/10.1186/s40246-019-0220-8

103. Chen, M., Zhang, J., Yu, S., Wang, S., Zhang, Z., Chen, J., Xiao, J., & Wang, Y. (2012). Anti-Lung-Cancer Activity and Liposome-Based Delivery Systems of β-Elemene. Evidence-based complementary and alternative medicine : eCAM, 2012, 259523. https://doi.org/10.1155/2012/259523

104. Chen, W. Q., Zeng, H. M., Zheng, R. S., Zhang, S. W., & He, J. (2012). Cancer incidence and mortality in china, 2007. Chinese journal of cancer research = Chung-kuo yen cheng yen chiu, 24(1), 1–8. https://doi.org/10.1007/s11670-012-0001-6

105. Chen, W., Zheng, R., Zhang, S., Zeng, H., Zuo, T., Xia, C., Yang, Z., & He, J. (2017). Cancer incidence and mortality in China in 2013: an analysis based on urbanization level. Chinese journal of cancer research = Chung-kuo yen cheng yen chiu, 29(1), 1–10. https://doi.org/10.21147/j.issn.1000-9604.2017.01.01

106. Chène, P. (2001). p53 as a drug target in cancer therapy. Expert Opinion on Therapeutic Patents, 11(6), 923-935.

107. Cheng, H. Y., Tian, D. M., Tang, J. S., Shen, W. Z., & Yao, X. S. (2016). Cardiac glycosides from the seeds of Thevetia peruviana and their pro-apoptotic activity toward cancer cells. Journal of Asian natural products research, 18(9), 837–847. https://doi.org/10.1080/10286020.2016.1170814

108. Chidambara Murthy, K. N., Jayaprakasha, G. K., & Patil, B. S. (2012). The natural alkaloid berberine targets multiple pathways to induce cell death in cultured human colon cancer cells. European journal of pharmacology, 688(1-3), 14–21. https://doi.org/10.1016/j.ejphar.2012.05.004

109. Chik, S. C., Or, T. C., Luo, D., Yang, C. L., & Lau, A. S. (2013). Pharmacological effects of active compounds on neurodegenerative disease with gastrodia and uncaria decoction, a commonly used poststroke decoction. The Scientific World Journal, 2013, 896873. https://doi.org/10.1155/2013/896873

110. Chikara, S., Nagaprashantha, L. D., Singhal, J., Horne, D., Awasthi, S., & Singhal, S. S. (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer letters, 413, 122–134. https://doi.org/10.1016/j.canlet.2017.11.002

111. Chirumbolo, S., Bjørklund, G., Lysiuk, R., Vella, A., Lenchyk, L., & Upyr, T. (2018). targeting cancer with phytochemicals via their fine tuning of the cell survival signaling pathways. International Journal of Molecular Sciences, 19(11), 3568. https://doi.org/10.3390/ijms19113568

112. Cho, H. J., Kim, W. K., Kim, E. J., Jung, K. C., Park, S., Lee, H. S., Tyner, A. L., & Park, J. H. (2003). Conjugated linoleic acid inhibits cell proliferation and ErbB3 signaling in HT-29 human colon cell line. American journal of physiology. Gastrointestinal and liver physiology, 284(6), G996–G1005. https://doi.org/10.1152/ajpgi.00347.2002

113. Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical biochemistry, 162(1), 156–159. https://doi.org/10.1006/abio.1987.9999

114. Chonghaile, T. N., & Letai, A. (2008). Mimicking the BH3 domain to kill cancer cells. Oncogene, 27(S1), S149–S157. https://doi.org/10.1038/onc.2009.52

115. Chu, D. T., Nguyen, T. T., Tien, N. L. B., Tran, D. K., Jeong, J. H., Anh, P. G., Thanh, V. V., Truong, D. T., & Dinh, T. C. (2020). Recent progress of stem cell therapy in cancer treatment: Molecular mechanisms and potential applications. Cells, 9(3), 563. https://doi.org/10.3390/cells9030563

116. Chu, S., Shi, X., Tian, Y., & Gao, F. (2022). pH-responsive polymer nanomaterials for tumor therapy. Frontiers in oncology, 12, 855019. https://doi.org/10.3389/fonc.2022.855019

117. Chua, K. N., Sim, W. J., Racine, V., Lee, S. Y., Goh, B. C., & Thiery, J. P. (2012). A cell-based small molecule screening method for identifying inhibitors of epithelial-mesenchymal transition in carcinoma. PloS one, 7(3), e33183. https://doi.org/10.1371/journal.pone.0033183

118. Chung, K. S., Han, G., Kim, B. K., Kim, H. M., Yang, J. S., Ahn, J., Lee, K., Song, K. B., & Won, M. (2013). A novel antitumor piperazine alkyl compound causes apoptosis by inducing RhoB expression via ROS-mediated c-Abl/p38 MAPK signaling. Cancer chemotherapy and pharmacology, 72(6), 1315–1324. https://doi.org/10.1007/s00280-013-2310-y

119. Çiftçi, G. A., Temel, H. E., & Yurttaş, L. (2022). Apoptotic effect of novel benzimidazole derivatives bearing pyridyl/pyrimidinyl piperazine moiety. Anti-cancer agents in medicinal chemistry, 22(9), 1780–1792. https://doi.org/10.2174/1871520621666210708095110

120. Clark, P. I., & Slevin, M. L. (1987). The clinical pharmacology of etoposide and teniposide. Clinical pharmacokinetics, 12(4), 223–252. https://doi.org/10.2165/00003088-198712040-00001

121. Collins A. R. (2004). The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular biotechnology, 26(3), 249–261. https://doi.org/10.1385/MB:26:3:249

122. Cooper, G. M. (2000). The development and causes of cancer. The Cell - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK9963/

123. Cos, P., Maes, L., Vlietinck, A., & Pieters, L. (2008). Plant-derived leading compounds for chemotherapy of human immunodefiency virus (HIV) infection–an update (1998–2007). Planta Medica, 74(11), 1323-1337.

124. Coverley, D., Laman, H., & Laskey, R. A. (2002). Distinct roles for cyclins E and A during DNA replication complex assembly and activation. Nature cell biology, 4(7), 523–528. https://doi.org/10.1038/ncb813

125. Cowell J. K. (1992). Tumour suppressor genes. Annals of oncology: official journal of the European Society for Medical Oncology, 3(9), 693–698. https://doi.org/10.1093/oxfordjournals.annonc.a058319

126. Csupor-Löffler, B., Hajdú, Z., Zupkó, I., Molnár, J., Forgo, P., Vasas, A., Kele, Z., & Hohmann, J. (2011). Antiproliferative constituents of the roots of *Conyza canadensis*. Planta medica, 77(11), 1183–1188. https://doi.org/10.1055/s-0030-1270714

127. Cuesta, C., Arévalo-Alameda, C., & Castellano, E. (2021). The Importance of Being PI3K in the RAS Signaling Network. Genes, 12(7), 1094. https://doi.org/10.3390/genes12071094

128. D'Aguanno, S., & Del Bufalo, D. (2020). Inhibition of Anti-Apoptotic BCL-2 proteins in preclinical and clinical studies: Current overview in cancer. Cells, 9(5), 1287. https://doi.org/10.3390/cells9051287

129. da Silva, G. N. S., Pozzatti, P., Rigatti, F., Hörner, R., Alves, S. H., Mallmann, C. A., & Heinzmann, B. M. (2015). Antimicrobial evaluation of sesquiterpene α-curcumene and its synergism with imipenem. Journal of Microbiology, Biotechnology and Food Sciences, 4(5), 434-436.https://doi.org/10.15414/jmbfs.2015.4.5.434-436

130. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1). https://doi.org/10.1038/srep42717

131. Daines, A., Payne, R., Humphries, M., & Abell, A. (2003). The synthesis of naturally occurring vitamin K and vitamin K analogues. Current Organic Chemistry, 7(16), 1625–1634. https://doi.org/10.2174/1385272033486279

132. Dalimi, A., Delavari, M., Ghaffarifar, F., & Sadraei, J. (2015). *In vitro* and *in vivo* antileishmanial effects of aloe-emodin on Leishmania major. Journal of traditional and complementary medicine, 5(2), 96–99. https://doi.org/10.1016/j.jtcme.2014.11.004

133. Dallakyan, S., Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. Methods in molecular biology (Clifton, N.J.). 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

134. Dancey, J., & Eisenhauer, E. A. (1996). Current perspectives on camptothecins in cancer treatment. British journal of cancer, 74(3), 327–338. https://doi.org/10.1038/bjc.1996.362

135. Danial, N. N., & Korsmeyer, S. J. (2004). Cell death: critical control points. Cell, 116(2), 205–219. https://doi.org/10.1016/s0092-8674(04)00046-7

136. Danova, M., Giordano, M., Mazzini, G., & Riccardi, A. (1990). Expression of p53 protein during the cell cycle measured by flow cytometry in human leukemia. Leukemia research, 14(5), 417–422. https://doi.org/10.1016/0145-2126(90)90027-7

137. Das U. N. (2006). Tumoricidal and anti-angiogenic actions of gamma-linolenic acid and its derivatives. Current pharmaceutical biotechnology, 7(6), 457–466. https://doi.org/10.2174/138920106779116892

138. de Alencar, M. V. O. B., Islam, M. T., da Mata, A. M. O. F., Dos Reis, A. C., de Lima, R. M. T., de Oliveira Ferreira, J. R., de Castro E Sousa, J. M., Ferreira, P. M. P., de Carvalho Melo-Cavalcante, A. A., Rauf, A., Hemeg, H. A., Alsharif, K. F., & Khan, H. (2023). Anticancer effects of phytol against Sarcoma (S-180) and Human Leukemic (HL-60) cancer cells. Environmental science and pollution research international, 30(33), 80996–81007. https://doi.org/10.1007/s11356-023-28036-4

139. de la Cruz, J. F., Vergara, E. J., Cho, Y., Hong, H. O., Oyungerel, B., & Hwang, S. G. (2015). *Glehnia littoralis* root extract induces G0/G1 phase cell cycle arrest in the MCF-7 human breast cancer cell line. Asian Pacific journal of cancer prevention: APJCP, *16*(18), 8113–8117. https://doi.org/10.7314/apjcp.2015.16.18.8113

140. Debbarma, M., Pala, N. A., Kumar, M., & Bussmann, R. W. (2017). Traditional knowledge of medicinal plants in tribes of Tripura in northeast, India. African journal of traditional, complementary, and alternative medicines: AJTCAM, 14(4), 156–168. https://doi.org/10.21010/ajtcam.v14i4.19

141. Debela, D. T., Muzazu, S. G., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., Kitui, S. K., & Manyazewal, T. (2021). New approaches and procedures for cancer treatment: Current perspectives. SAGE open medicine, 9, 20503121211034366. https://doi.org/10.1177/20503121211034366

142. Debnath, P., Huirem, R. S., Dutta, P., & Palchaudhuri, S. (2022). Epithelial-mesenchymal transition and its transcription factors. Bioscience reports, 42(1), BSR20211754. https://doi.org/10.1042/BSR20211754

143. Degirmenci, U., Yap, J., Sim, Y. R. M., Qin, S., & Hu, J. (2021). Drug resistance in targeted cancer therapies with RAF inhibitors. Cancer drug resistance (Alhambra, Calif.), 4(3), 665–683. https://doi.org/10.20517/cdr.2021.36

144. Dehnavi, M. K., Ebrahimpour-Koujan, S., Lotfi, K., & Azadbakht, L. (2024). The association between circulating carotenoids and risk of breast cancer: A systematic review and dose-response meta-analysis of prospective studies. Advances in nutrition (Bethesda, Md.), 15(1), 100135. https://doi.org/10.1016/j.advnut.2023.10.007

145. Dejong, J. M., Liu, Y., Bollon, A. P., Long, R. M., Jennewein, S., Williams, D., & Croteau, R. B. (2006). Genetic engineering of taxol biosynthetic genes in Saccharomyces cerevisiae. Biotechnology and bioengineering, 93(2), 212–224. https://doi.org/10.1002/bit.20694

146. Della Corte, C. M., Bellevicine, C., Vicidomini, G., Vitagliano, D., Malapelle, U., Accardo, M., Fabozzi, A., Fiorelli, A., Fasano, M., Papaccio, F., Martinelli, E., Troiani, T., Troncone, G., Santini, M., Bianco, R., Ciardiello, F., & Morgillo, F. (2015). SMO Gene Amplification and Activation of the Hedgehog Pathway as Novel Mechanisms of Resistance to Anti-Epidermal Growth Factor Receptor Drugs in Human Lung Cancer. Clinical cancer research: an official journal of the American Association for Cancer Research, 21(20), 4686–4697. https://doi.org/10.1158/1078-0432.CCR-14-3319

147. Demain, A. L., & Vaishnav, P. (2011). Natural products for cancer chemotherapy. Microbial biotechnology, 4(6), 687–699. https://doi.org/10.1111/j.1751-7915.2010.00221.x

148. Deng, J. J., Zhang, W., Xu, X. M., Zhang, F., Tao, W. P., Ye, J. J., & Ge, W. (2016). Twist mediates an aggressive phenotype in human colorectal cancer cells. International journal of oncology, 48(3), 1117–1124. https://doi.org/10.3892/ijo.2016.3342

149. Deorukhkar, A., Krishnan, S., Sethi, G., & Aggarwal, B. B. (2007). Back to basics: how natural products can provide the basis for new therapeutics. Expert opinion on investigational drugs, 16(11), 1753–1773. https://doi.org/10.1517/13543784.16.11.1753

150. Desborough, M. J. R., & Keeling, D. M. (2017). The aspirin story - from willow to wonder drug. British journal of haematology, 177(5), 674–683. https://doi.org/10.1111/bjh.14520

151. Dewson, G., & Kluck, R. M. (2010). Bcl-2 family-regulated apoptosis in health and disease. Cell Health and Cytoskeleton, 2, 9–22. https://doi.org/10.2147/CHC.S6228

152. Dhyani, P., Quispe, C., Sharma, E., Bahukhandi, A., Sati, P., Attri, D. C., Szopa, A., Sharifi-Rad, J., Docea, A. O., Mardare, I., Calina, D., & Cho, W. C. (2022). Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. Cancer cell international, 22(1), 206. https://doi.org/10.1186/s12935-022-02624-9

153. Du, B., & Shim, J. S. (2016). Targeting Epithelial-Mesenchymal Transition (EMT) to overcome drug resistance in cancer. Molecules (Basel, Switzerland), 21(7), 965. https://doi.org/10.3390/molecules21070965

154. Du, Z. X., Zhang, H. Y., Gao, D. X., Wang, H. Q., Li, Y. J., & Liu, G. L. (2006). Antisurvivin oligonucleotides inhibit growth and induce apoptosis in human medullary thyroid carcinoma cells. Experimental & molecular medicine, 38(3), 230–240. https://doi.org/10.1038/emm.2006.28

155. Eddershaw, P. J., Beresford, A. P., & Bayliss, M. K. (2000). ADME/PK as part of a rational approach to drug discovery. Drug discovery today, 5(9), 409–414. https://doi.org/10.1016/s1359-6446(00)01540-3

156. Efenberger-Szmechtyk, M., Nowak, A., & Nowak, A. (2020). Cytotoxic and DNA-Damaging Effects of *Aronia melanocarpa*, *Cornus mas*, and *Chaenomeles superba* Leaf extracts on the human colon adenocarcinoma cell line Caco-2. Antioxidants (Basel, Switzerland), 9(11), 1030. https://doi.org/10.3390/antiox9111030

157. Egamberdieva, D., Shrivastava, S., & Varma, A. (2015). Plant-Growth-Promoting rhizobacteria (PGPR) and medicinal plants. In Soil biology. https://doi.org/10.1007/978-3-319-13401-7

158. El Gizawy, H. A., El Zanaty, S. A., El Ghaly, E. M., & Seif-Eldein, N. A. (2023). *Thevetia peruviana* leaves, HPLC profile, isolation, and *in vitro* cytotoxicity. RSC advances, 13(18), 12072–12079. https://doi.org/10.1039/d3ra00588g

159. Elgorashi, E. E. (2019). Phytochemistry and Pharmacology of the family Amaryllidaceae: An overview of research at RCPGD. Natural Product Communications, 14(9), 1934578X1987292. https://doi.org/10.1177/1934578x19872929

160. Elhaj, A.M.,E. Osman, E. E., Koko, W. S., Garbi, M. I., & Kabbashi, A. S., (2015). Antioxidant activity, phytochemical screening and cytotoxicity of ethanolic leaves extract of *Antigonon leptopus*. 4. 202-207.

161. El-Hussein, A., Manoto, S. L., Ombinda-Lemboumba, S., Alrowaili, Z. A., & Mthunzi-Kufa, P. (2021). A review of chemotherapy and photodynamic therapy for lung cancer treatment. Anti-cancer agents in medicinal chemistry, 21(2), 149–161. https://doi.org/10.2174/1871520620666200403144945

162. Elkholi, R., Renault, T. T., Serasinghe, M. N., & Chipuk, J. E. (2014). Putting the pieces together: How is the mitochondrial pathway of apoptosis regulated in cancer and chemotherapy?. Cancer & metabolism, 2, 16. https://doi.org/10.1186/2049-3002-2-16

163. Elmore S. (2007). Apoptosis: a review of programmed cell death. Toxicologic pathology, 35(4), 495–516. https://doi.org/10.1080/01926230701320337

164. Elufioye, T. O., Abdul, A. A., & Moody, J. O. (2017). Cytotoxicity studies of the extracts, fractions, and isolated compound of *Pseudocedrela kotschyi* on cervical cancer (HeLa), breast cancer (MCF-7) and skeletal muscle cancer (RD) cells. Pharmacognosy research, 9(1), 46–50. https://doi.org/10.4103/0974-8490.199776

165. Engwa, G. A. (2018). Free radicals and the role of plant phytochemicals as antioxidants against oxidative Stress-Related diseases. In InTech eBooks. https://doi.org/10.5772/intechopen.76719

166. Esmaeili, M. A., & Farimani, M. M. (2014). Inactivation of PI3K/Akt pathway and upregulation of PTEN gene are involved in daucosterol, isolated from *Salvia sahendica*, induced apoptosis in human breast adenocarcinoma cells. South African Journal of Botany, 93, 37-47.

167. Evans, G. A., & Farrar, W. L. (1998). Phorbol Esters. In Elsevier eBooks (pp. 1940–1942). https://doi.org/10.1006/rwei.1999.0488

168. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental health perspectives, 109(suppl 1), 69-75.

169. Fan, Y., Shen, J., Liu, Z., Xia, K., Zhu, W., & Fu, P. (2022). Methylene-bridged dimeric natural products involving one-carbon unit in biosynthesis. Natural Product Reports, 39(6), 1305–1324. https://doi.org/10.1039/d2np00022a

170. Fang, Y., Xue, J., Gao, S., Lu, A., Yang, D., Jiang, H., He, Y., & Shi, K. (2017). Cleavable PEGylation: a strategy for overcoming the "PEG dilemma" in efficient drug delivery. Drug delivery, 24(sup1), 22–32. https://doi.org/10.1080/10717544.2017.1388451

171. Farabe, A., Ovi, A. H., Rakib, M. M. R., Saha, J., & Khan, R. (2024). Evaluation of Anthelmintic and Neuropharmacological Activities of the leaves of *Chassalia*. European Journal of Medicinal Plants, 35(6), 54-62.

172. Fares, J., Fares, M. Y., Khachfe, H. H., Salhab, H. A., & Fares, Y. (2020). Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduction and Targeted Therapy, 5(1). https://doi.org/10.1038/s41392-020-0134-x

173. Farooqi, A. A., Qureshi, M. Z., Khalid, S., Attar, R., Martinelli, C., Sabitaliyevich, U. Y., Nurmurzayevich, S. B., Taverna, S., Poltronieri, P., & Xu, B. (2019).

Regulation of Cell Signaling Pathways by Berberine in Different Cancers: Searching for Missing Pieces of an Incomplete Jig-Saw Puzzle for an Effective Cancer Therapy. Cancers, 11(4), 478. https://doi.org/10.3390/cancers11040478

174. Farrell, R.E. (1993). RNA methodologies: a laboratory guide for isolation and characterization.

175. Fatima, N., Sripisut, T., Youn, U. J., Ahmed, S., Ul-Haq, I., Munoz-Acuna, U., Simmons, C. J., Qazi, M. A., Jadoon, M., Tan, G. T., de Blanco, E. J. C., & Chang, L. C. (2017). Bioactive Constituents from an Endophytic Fungus, *Penicillium polonicum* NFW9, Associated with Taxus fauna. Medicinal chemistry (Shariqah (United Arab Emirates)), 13(7), 689–697. https://doi.org/10.2174/1573406413666170216145121

176. Fatima, N., Sripisut, T., Youn, U. J., Ahmed, S., Ul-Haq, I., Munoz-Acuna, U., Simmons, C. J., Qazi, M. A., Jadoon, M., Tan, G. T., de Blanco, E. J. C., & Chang, L. C. (2017). Bioactive Constituents from an Endophytic Fungus, Penicillium polonicum NFW9, Associated with Taxus fauna. Medicinal chemistry (Shariqah (United Arab Emirates)), 13(7), 689–697. https://doi.org/10.2174/1573406413666170216145121

177. Ferrara, N., Hillan, K. J., & Novotny, W. (2005). Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochemical and biophysical research communications, 333(2), 328–335. https://doi.org/10.1016/j.bbrc.2005.05.132

178. Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. Cancer medicine, 5(10), 3007–3017. https://doi.org/10.1002/cam4.816

179. Fiorenza, S., Balshaw-Biddle, K., Lowe, D. F., Oubre, C. L., & Ward, C. H. (1997). Innovative bioremediation technologies: The DOD/AATDF contribution. In Global Environmental Biotechnology: Proceedings of the Third International Symposium on the International Society for Environmental Biotechnology (pp. 365-376). Springer Netherlands.

180. Fischer, K. R., Durrans, A., Lee, S., Sheng, J., Li, F., Wong, S. T., Choi, H., El Rayes, T., Ryu, S., Troeger, J., Schwabe, R. F., Vahdat, L. T., Altorki, N. K., Mittal, V., & Gao, D. (2015). Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature, 527(7579), 472–476. https://doi.org/10.1038/nature15748

181. Folkman J. (1971). Tumor angiogenesis: therapeutic implications. The New England journal of medicine, 285(21), 1182–1186. https://doi.org/10.1056/NEJM197111182852108

182. Fouladi, F., Steffen, K. J., & Mallik, S. (2017). Enzyme-Responsive Liposomes for the Delivery of Anticancer Drugs. Bioconjugate chemistry, 28(4), 857–868. https://doi.org/10.1021/acs.bioconjchem.6b00736

183. Fridlender, M., Kapulnik, Y., & Koltai, H. (2015). Plant derived substances with anti-cancer activity: from folklore to practice. Frontiers in plant science, 6, 799. https://doi.org/10.3389/fpls.2015.00799

184. Frye, R., Myers, M., Axelrod, K. C., Ness, E. A., Piekarz, R. L., Bates, S. E., & Booher, S. (2012). Romidepsin: a new drug for the treatment of cutaneous T-cell lymphoma. Clinical journal of oncology nursing, 16(2), 195–204. https://doi.org/10.1188/12.CJON.195-204

185. Fulda S. (2013). Modulation of mitochondrial apoptosis by PI3K inhibitors. Mitochondrion, 13(3), 195–198. https://doi.org/10.1016/j.mito.2012.05.001

186. Fulda, S., Meyer, E., & Debatin, K. M. (2002). Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. Oncogene, 21(15), 2283–2294. https://doi.org/10.1038/sj.onc.1205258

187. Fulton, M. D., & Najahi-Missaoui, W. (2023). Liposomes in Cancer Therapy: How Did We Start and Where Are We Now. International journal of molecular sciences, 24(7), 6615. https://doi.org/10.3390/ijms24076615

188. Furness, S., Roberts, H., Marjoribanks, J., & Lethaby, A. (2012). Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. The Cochrane database of systematic reviews, 2012(8), CD000402. https://doi.org/10.1002/14651858.CD000402.pub4

189. Gach, K., Długosz, A., & Janecka, A. (2015). The role of oxidative stress in anticancer activity of sesquiterpene lactones. Naunyn-Schmiedeberg's archives of pharmacology, 388(5), 477–486. https://doi.org/10.1007/s00210-015-1096-3

190. Galardi, A., Colletti, M., Palma, A., & Di Giannatale, A. (2022). An Update on Circular RNA in Pediatric Cancers. Biomedicines, 11(1), 36. https://doi.org/10.3390/biomedicines11010036

191. Gallicchio, L., Devasia, T. P., Tonorezos, E., Mollica, M. A., & Mariotto, A. (2022). Estimation of the Number of Individuals Living With Metastatic Cancer in the United States. Journal of the National Cancer Institute, 114(11), 1476–1483. https://doi.org/10.1093/jnci/djac158

192. Gallicchio, L., Devasia, T. P., Tonorezos, E., Mollica, M. A., & Mariotto, A. (2022). Estimation of the number of individuals living with metastatic cancer in the United States. JNCI: Journal of the National Cancer Institute, 114(11), 1476-1483.

193. Gano, C. A., Fatima, S., Failes, T. W., Arndt, G. M., Sajinovic, M., Mahns, D., Saedisomeolia, A., Coorssen, J. R., Bucci, J., de Souza, P., Vafaee, F., & Scott, K. F. (2023). Anti-cancer potential of synergistic phytochemical combinations is influenced by the genetic profile of prostate cancer cell lines. Frontiers in nutrition, 10, 1119274. https://doi.org/10.3389/fnut.2023.1119274

194. Gao, X., Leone, G. W., & Wang, H. (2020). Cyclin D-CDK4/6 functions in cancer. Advances in cancer research, 148, 147–169. https://doi.org/10.1016/bs.acr.2020.02.002

195. Gerstberger, S., Jiang, Q., & Ganesh, K. (2023). Metastasis. Cell, 186(8), 1564–1579. https://doi.org/10.1016/j.cell.2023.03.003

196. Gholipour, T., Healy, B., Baruch, N. F., Weiner, H. L., & Chitnis, T. (2011). Demographic and clinical characteristics of malignant multiple sclerosis. Neurology, 76(23), 1996–2001. https://doi.org/10.1212/WNL.0b013e31821e559d

197. Ghoroghi, S., Mary, B., Asokan, N., Goetz, J. G., & Hyenne, V. (2021). Tumor extracellular vesicles drive metastasis (it's a long way from home). FASEB bioAdvances, 3(11), 930–943. https://doi.org/10.1096/fba.2021-00079

198. Giglia-Mari, G., & Sarasin, A. (2003). TP53 mutations in human skin cancers. Human mutation, 21(3), 217–228. https://doi.org/10.1002/humu.10179

199. Giono, L. E., & Manfredi, J. J. (2006). The p53 tumor suppressor participates in multiple cell cycle checkpoints. Journal of cellular physiology, 209(1), 13–20. https://doi.org/10.1002/jcp.20689

200. Giordani, S., Marassi, V., Zattoni, A., Roda, B., & Reschiglian, P. (2023). Liposomes characterization for market approval as pharmaceutical products: Analytical methods, guidelines and standardized protocols. Journal of pharmaceutical and biomedical analysis, 236, 115751. https://doi.org/10.1016/j.jpba.2023.115751

201. Glaviano, A., Foo, A. S. C., Lam, H. Y., Yap, K. C. H., Jacot, W., Jones, R. H., Eng, H., Nair, M. G., Makvandi, P., Geoerger, B., Kulke, M. H., Baird, R. D., Prabhu, J. S., Carbone, D., Pecoraro, C., Teh, D. B. L., Sethi, G., Cavalieri, V., Lin, K. H., Javidi-Sharifi, N. R., … Kumar, A. P. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Molecular cancer, 22(1), 138. https://doi.org/10.1186/s12943-023-01827-6

202. Gnann, J. W., Jr, Barton, N. H., & Whitley, R. J. (1983). Acyclovir: mechanism of action, pharmacokinetics, safety and clinical applications. Pharmacotherapy, 3(5), 275–283. https://doi.org/10.1002/j.1875-9114.1983.tb03274.x

203. Goi, K., Takagi, M., Iwata, S., Delia, D., Asada, M., Donghi, R., Tsunematsu, Y., Nakazawa, S., Yamamoto, H., Yokota, J., Tamura, K., Saeki, Y., Utsunomiya, J., Takahashi, T., Ueda, R., Ishioka, C., Eguchi, M., Kamata, N., & Mizutani, S. (1997). DNA damage-associated dysregulation of the cell cycle and apoptosis control in cells with germ-line p53 mutation. Cancer research, 57(10), 1895–1902.

204. Gonzales, G. F., & Valerio, L. G., Jr (2006). Medicinal plants from Peru: a review of plants as potential agents against cancer. Anti-cancer agents in medicinal chemistry, 6(5), 429–444. https://doi.org/10.2174/187152006778226486

205. Gonzalez, D. M., & Medici, D. (2014). Signaling mechanisms of the epithelial-mesenchymal transition. Science signaling, 7(344), re8. https://doi.org/10.1126/scisignal.2005189

206. Goolsby, C., Paniagua, M., Tallman, M., & Gartenhaus, R. B. (2005). Bcl-2 regulatory pathway is functional in chronic lymphocytic leukemia. Cytometry. Part B, Clinical cytometry, 63(1), 36–46. https://doi.org/10.1002/cyto.b.20034

207. Gopa, G. R., Lakshmi, S., Jayakumaran N. A., Sudhakaran P. R., Muraleedharan D & Nair, G. M., (2020). Antiproliferative effects of total alkaloid extract of roots of *Chassalia curviflora* (Wall.) Thwaites on cancer cell lines. Indian Journal of Experimental Biology. Vol 58 (06). 389-395.

208. Goswami, M., Bhagta, S., & Sharma, D. (2020). *Melia dubia* and its Importance: A Review. International Journal of Economic Plants, 7(1), 029–033. https://doi.org/10.23910/2/2020.0351

209. Gowthami, R., Sharma, N., Pandey, R., & Agrawal, A. (2021). Status and consolidated list of threatened medicinal plants of India. Genetic resources and crop evolution, 68(6), 2235–2263. https://doi.org/10.1007/s10722-021-01199-0

210. Grada, A., Otero-Vinas, M., Prieto-Castrillo, F., Obagi, Z., & Falanga, V. (2017). Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay. The Journal of investigative dermatology, 137(2), e11–e16. https://doi.org/10.1016/j.jid.2016.11.020

211. Grant, P., & Ramasamy, S. (2012). An update on plant derived anti-androgens. International journal of endocrinology and metabolism, 10(2), 497–502. https://doi.org/10.5812/ijem.3644

212. Greer, J. B., & Whitcomb, D. C. (2007). Role of BRCA1 and BRCA2 mutations in pancreatic cancer. Gut, 56(5), 601–605. https://doi.org/10.1136/gut.2006.101220

213. Gregoriadis, G., & Florence, A. T. (1993). Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential. Drugs, 45(1), 15–28. https://doi.org/10.2165/00003495-199345010-00003

214. Gridelli, C., Peters, S., Sgambato, A., Casaluce, F., Adjei, A. A., & Ciardiello, F. (2014). ALK inhibitors in the treatment of advanced NSCLC. Cancer treatment reviews, 40(2), 300–306. https://doi.org/10.1016/j.ctrv.2013.07.002

215. Grossman, D., McNiff, J. M., Li, F., & Altieri, D. C. (1999). Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. The Journal of investigative dermatology, 113(6), 1076–1081. https://doi.org/10.1046/j.1523-1747.1999.00776.x

216. Grotegut, S., von Schweinitz, D., Christofori, G., & Lehembre, F. (2006). Hepatocyte growth factor induces cell scattering through MAPK/Egr-1-mediated upregulation of Snail. The EMBO journal, 25(15), 3534–3545. https://doi.org/10.1038/sj.emboj.7601213

217. Gunadharini, D. N., Elumalai, P., Arunkumar, R., Senthilkumar, K., & Arunakaran, J. (2011). Induction of apoptosis and inhibition of PI3K/Akt pathway in PC-3 and LNCaP prostate cancer cells by ethanolic neem leaf extract. Journal of ethnopharmacology, 134(3), 644–650. https://doi.org/10.1016/j.jep.2011.01.015

218. Guo, H., Luo, H., Yuan, H., Xia, Y., Shu, P., Huang, X., Lu, Y., Liu, X., Keller, E. T., Sun, D., Deng, J., & Zhang, J. (2021). Author Correction: Litchi seed extracts diminish prostate cancer progression via induction of apoptosis and attenuation of EMT through Akt/GSK-3β signaling. Scientific reports, 11(1), 15201. https://doi.org/10.1038/s41598-021-94623-5

219. Guo, H., Luo, H., Yuan, H., Xia, Y., Shu, P., Huang, X., Lu, Y., Liu, X., Keller, E. T., Sun, D., Deng, J., & Zhang, J. (2021). Author Correction: Litchi seed extracts diminish prostate cancer progression via induction of apoptosis and attenuation of EMT through Akt/GSK-3β signaling. Scientific reports, 11(1), 15201. https://doi.org/10.1038/s41598-021-94623-5

220. Gupta, P. B., Onder, T. T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R. A., & Lander, E. S. (2009). Identification of selective inhibitors of cancer stem cells by

high-throughput screening. Cell, 138(4), 645–659. https://doi.org/10.1016/j.cell.2009.06.034

221. Gupta, S., & Roy, A. (2021). Deciphering the role of phytoanticipins, phytoalexins, and polyphenols in plant-insect defense. Plant-Pest Interactions: From Molecular Mechanisms to Chemical Ecology: Chemical Ecology, 305-335.

222. Habli, Z., Toumieh, G., Fatfat, M., Rahal, O. N., & Gali-Muhtasib, H. (2017). Emerging Cytotoxic Alkaloids in the Battle against Cancer: Overview of Molecular Mechanisms. Molecules (Basel, Switzerland), 22(2), 250. https://doi.org/10.3390/molecules22020250.

223. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. Cell, 144(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

224. Hande K. R. (1998). Etoposide: four decades of development of a topoisomerase II inhibitor. European journal of cancer (Oxford, England: 1990), 34(10), 1514–1521. https://doi.org/10.1016/s0959-8049(98)00228-7

225. Hasan, M., Belhaj, N., Benachour, H., Barberi-Heyob, M., Kahn, C., Jabbari, E., Linder, M., & Arab-Tehrany, E. (2013). Liposome encapsulation of curcumin: Physico-chemical characterizations and effects on MCF7 cancer cell proliferation. International Journal of Pharmaceutics, 461(1–2), 519–528. https://doi.org/10.1016/j.ijpharm.2013.12.007

226. Haslehurst, A. M., Koti, M., Dharsee, M., Nuin, P., Evans, K., Geraci, J., Childs, T., Chen, J., Li, J., Weberpals, J., Davey, S., Squire, J., Park, P. C., & Feilotter, H. (2012). EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. BMC cancer, 12, 91. https://doi.org/10.1186/1471-2407-12-91

227. Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., & Sakuragi, N. (2014). Apoptosis and molecular targeting therapy in cancer. BioMed research international, 2014, 150845. https://doi.org/10.1155/2014/150845 (Retraction published Biomed Res Int. 2020 Aug 28;2020:2451249. doi: 10.1155/2020/2451249)

228. Hayet, E., Maha, M., Samia, A., Ali, M. M., Souhir, B., Abderaouf, K., Mighri, Z., & Mahjoub, A. (2008). Antibacterial, antioxidant and cytotoxic activities of extracts of *Conyza canadensis* (L.) Cronquist growing in Tunisia. Medicinal Chemistry Research, 18(6), 447–454. https://doi.org/10.1007/s00044-008-9141-0

229. Hayshi, Y., Nishikawa, Y., Mori, H., Tamura, H., Matsushita, Y., & Matsui, T. (1998). Antitumor activity of (10E, 12Z)-9-hydroxy-10,12-octadecadienoic acid from rice bran. Journal of Fermentation and Bioengineering, 86(2), 149–153. https://doi.org/10.1016/s0922-338x(98)80053-6

230. He, K., & Gan, W. J. (2023). Wnt/β-Catenin Signaling Pathway in the Development and Progression of Colorectal Cancer. Cancer management and research, 15, 435–448. https://doi.org/10.2147/CMAR.S411168

231. Hecht, S. S., & Hatsukami, D. K. (2022). Smokeless tobacco and cigarette smoking: chemical mechanisms and cancer prevention. Nature reviews. Cancer, 22(3), 143–155. https://doi.org/10.1038/s41568-021-00423-4

232. Heerboth, S., Housman, G., Leary, M., Longacre, M., Byler, S., Lapinska, K., Willbanks, A., & Sarkar, S. (2015). EMT and tumor metastasis. Clinical and translational medicine, 4, 6. https://doi.org/10.1186/s40169-015-0048-3

233. Hemmings, B. A., & Restuccia, D. F. (2012). PI3K-PKB/Akt pathway. Cold Spring Harbor perspectives in biology, 4(9), a011189. https://doi.org/10.1101/cshperspect.a011189

234. Henderson, B. E., & Feigelson, H. S. (2000). Hormonal carcinogenesis. Carcinogenesis, 21(3), 427–433. https://doi.org/10.1093/carcin/21.3.427

235. Hirsch, H. A., Iliopoulos, D., Tsichlis, P. N., & Struhl, K. (2009). Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer research, 69(19), 7507–7511. https://doi.org/10.1158/0008-5472.CAN-09-2994

236. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india

237. https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india

238. https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise

239. https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise

240. https://www.who.int/news-room/questions-and-answers/item/traditional-medicine

241. https://www.who.int/news-room/questions-and-answers/item/traditional-medicine

242. Hu, F., Liu, J., Liu, H., Li, F., Wan, M., Zhang, M., Jiang, Y., & Rao, M. (2021). Role of Exosomal Non-coding RNAs in Gastric Cancer: Biological Functions and Potential Clinical Applications. Frontiers in oncology, 11, 700168. https://doi.org/10.3389/fonc.2021.700168

243. Huang, J., Li, H., & Ren, G. (2015). Epithelial-mesenchymal transition and drug resistance in breast cancer (Review). International journal of oncology, 47(3), 840–848. https://doi.org/10.3892/ijo.2015.3084

244. Huber, M. A., Kraut, N., & Beug, H. (2005). Molecular requirements for epithelial-mesenchymal transition during tumor progression. Current opinion in cell biology, 17(5), 548–558. https://doi.org/10.1016/j.ceb.2005.08.001

245. Husseiny, S. M., Salah, T. A., & Anter, H. A. (2015). Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities. Beni-Suef University Journal of Basic and Applied Sciences, 4(3), 225–231. https://doi.org/10.1016/j.bjbas.2015.07.004

246. Immordino, M. L., Dosio, F., & Cattel, L. (2006). Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. International journal of nanomedicine, 1(3), 297–315.

247. Ince, T. A., Richardson, A. L., Bell, G. W., Saitoh, M., Godar, S., Karnoub, A. E., Iglehart, J. D., & Weinberg, R. A. (2007). Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. Cancer cell, 12(2), 160–170. https://doi.org/10.1016/j.ccr.2007.06.013

248. Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., & Folkman, J. (1990). Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature, 348(6301), 555–557. https://doi.org/10.1038/348555a0

249. Ioannis, P., Anastasis, S., & Andreas, Y. (2015). Graviola: A systematic review on its anticancer properties. Am J Cancer Prev, 3(6), 128-131.

250. Irimie, A. I., Braicu, C., Zanoaga, O., Pileczki, V., Gherman, C., Berindan-Neagoe, I., & Campian, R. S. (2015). Epigallocatechin-3-gallate suppresses cell proliferation and promotes apoptosis and autophagy in oral cancer SSC-4 cells. OncoTargets and therapy, 8, 461–470. https://doi.org/10.2147/OTT.S78358

251. Islam, F., Akter, A., Mimi, A. A., Urmee, H., Islam, M. R., Rahaman, M. S., Mamun, A. A., Rahman, M. M., Dhama, K., & Emran, T. B. (2022). Neuropharmacological effects of *Chassalia curviflora* (Rubiaceae) leaves in Swiss Albino mice model. Archives of Razi Institute, 77(2), 881–890. https://doi.org/10.22092/ARI.2021.356880.1937

252. Islam, M. T., Ali, E. S., Uddin, S. J., Islam, M. A., Shaw, S., Khan, I. N., Saravi, S. S. S., Ahmad, S., Rehman, S., Gupta, V. K., Găman, M. A., Găman, A. M., Yele, S., Das, A. K., de Castro E Sousa, J. M., de Moura Dantas, S. M. M., Rolim, H. M. L., de Carvalho Melo-Cavalcante, A. A., Mubarak, M. S., Yarla, N. S., … Kamal, M. A. (2018). Andrographolide, a diterpene lactone from *Andrographis paniculata* and its therapeutic promises in cancer. Cancer letters, 420, 129–145. https://doi.org/10.1016/j.canlet.2018.01.074

253. Itokawa, H., Morris-Natschke, S. L., Akiyama, T., & Lee, K. H. (2008). Plant-derived natural product research aimed at new drug discovery. Journal of natural medicines, 62(3), 263–280. https://doi.org/10.1007/s11418-008-0246-z

254. Itokawa, H., Shi, Q., Akiyama, T., Morris-Natschke, S. L., & Lee, K. H. (2008). Recent advances in the investigation of curcuminoids. Chinese medicine, 3, 11. https://doi.org/10.1186/1749-8546-3-11

255. Jafari, N., Zargar, S. J., Yassa, N., & Delnavazi, M. R. (2016). Induction of Apoptosis and Cell Cycle Arrest by Dorema Glabrum Root Extracts in a Gastric Adenocarcinoma (AGS) Cell Line. Asian Pacific journal of cancer prevention : APJCP, 17(12), 5189–5193. https://doi.org/10.22034/APJCP.2016.17.12.5189

256. Jaffray, D. A., & Gospodarowicz, M. K. (2015). Radiation therapy for cancer. Cancer: disease control priorities, 3, 239-248.

257. Jan, R., & Chaudhry, G. E. (2019). Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Advanced pharmaceutical bulletin, 9(2), 205–218. https://doi.org/10.15171/apb.2019.024

258. Jayanthi, M., Begum, N. S., & Ramu, R. (2020). Evaluation of anticancer activity of ethanol extract of *Leucas aspera* flower. International Journal of Green Pharmacy, 14(3). https://doi.org/10.22377/ijgp.v14i03.2939

259. Jenkin, R. D., Jeffs, R. D., Stephens, C. A., & Sonley, M. J. (1976). Wilms' tumour: adjuvant treatment with actinomycin D and vincristine. Canadian Medical Association journal, 115(2), 136–140.

260. Jiang, H., Shang, X., Wu, H., Huang, G., Wang, Y., Al-Holou, S., Gautam, S. C., & Chopp, M. (2010). Combination treatment with resveratrol and sulforaphane induces apoptosis in human U251 glioma cells. Neurochemical research, 35(1), 152–161. https://doi.org/10.1007/s11064-009-0040-7

261. Jiang, J., & Hu, C. (2009). Evodiamine: a novel anti-cancer alkaloid from *Evodia rutaecarpa*. Molecules (Basel, Switzerland), 14(5), 1852–1859. https://doi.org/10.3390/molecules14051852.

262. Jijith, U. S., Nair, C. R. S., Ajithkumar, K. C., & Pramod, K. (2016). Phytochemistry and pharmacology of *Anamirta Cocculus*Willd. Research Journal of Pharmacognosy and Phytochemistry, 8(2), 90. https://doi.org/10.5958/0975-4385.2016.00017.0

263. Jimenez, P. C., Wilke, D. V., & Costa-Lotufo, L. V. (2018). Marine drugs for cancer: surfacing biotechnological innovations from the oceans. Clinics (Sao Paulo, Brazil), 73(suppl 1), e482s. https://doi.org/10.6061/clinics/2018/e482s

264. Jinesh, G. G., & Kamat, A. M. (2016a). Blebbishield emergency program: an apoptotic route to cellular transformation. Cell death and differentiation, 23(5), 757–758. https://doi.org/10.1038/cdd.2016.26

265. Jinesh, G. G., & Kamat, A. M. (2016b). Endocytosis and serpentine filopodia drive blebbishield-mediated resurrection of apoptotic cancer stem cells. Cell death discovery, 2, 15069–. https://doi.org/10.1038/cddiscovery.2015.69

266. Jinesh, G. G., Choi, W., Shah, J. B., Lee, E. K., Willis, D. L., & Kamat, A. M. (2013). Blebbishields, the emergency program for cancer stem cells: sphere formation and tumorigenesis after apoptosis. Cell death and differentiation, 20(3), 382–395. https://doi.org/10.1038/cdd.2012.140

267. Jinesh, G. G., Molina, J. R., Huang, L., Laing, N. M., Mills, G. B., Bar-Eli, M., & Kamat, A. M. (2016). Mitochondrial oligomers boost glycolysis in cancer stem cells to facilitate blebbishield-mediated transformation after apoptosis. Cell death discovery, 2, 16003. https://doi.org/10.1038/cddiscovery.2016.3

268. Jiralerspong, S., Palla, S. L., Giordano, S. H., Meric-Bernstam, F., Liedtke, C., Barnett, C. M., Hsu, L., Hung, M. C., Hortobagyi, G. N., & Gonzalez-Angulo, A. M. (2009). Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 27(20), 3297–3302. https://doi.org/10.1200/JCO.2009.19.6410

269. Johnson, M., & Pace, R. D. (2010). Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. Nutrition reviews, 68(10), 604–615. https://doi.org/10.1111/j.1753-4887.2010.00320.x

270. Johnson, M., Maharaja, P., Janakiraman, N., Adaikala Raj, G., Menezes, I. R. A., da Costa, J. G. M., Verde, L. C. L., & Coutinho, H. D. M. (2018). *Pancratium triflorum* Roxb. (Amaryllidaceae) and *Molineria trichocarpa* (Wight) N.P. Balakr (Hypoxidaceae): Cytotoxic and antioxidant activities. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 119, 290–295. https://doi.org/10.1016/j.fct.2018.02.055

271. Jones, P. A., & Laird, P. W. (1999). Cancer-epigenetics comes of age. Nature Genetics, 21(2), 163–167. https://doi.org/10.1038/5947

272. Kaari, M., Joseph, J., Manikkam, R., Kalyanasundaram, R., Sivaraj, A., Anbalmani, S., Murthy, S., Sahu, A. K., Said, M., Dastager, S. G., & Ramasamy, B. (2023). A Novel Finding: 2,4-Di-tert-butylphenol from Streptomyces bacillaris ANS2 Effective Against Mycobacterium tuberculosis and Cancer Cell Lines. Applied Biochemistry and Biotechnology, 195(11), 6572–6585. https://doi.org/10.1007/s12010-023-04403-2

273. Kahaliw, W., Hellman, B., & Engidawork, E. (2018). Genotoxicity study of Ethiopian medicinal plant extracts on HepG2 cells. BMC complementary and alternative medicine, 18(1), 45. https://doi.org/10.1186/s12906-017-2056-x

274. Kajstura, M., Halicka, H. D., Pryjma, J., & Darzynkiewicz, Z. (2007). Discontinuous fragmentation of nuclear DNA during apoptosis revealed by discrete "sub□G1" peaks on DNA content histograms. Cytometry Part A: the journal of the International Society for Analytical Cytology, 71(3), 125-131.

275. Kalkavan, H., & Green, D. R. (2017). MOMP, cell suicide as a BCL-2 family business. Cell Death and Differentiation, 25(1), 46–55. https://doi.org/10.1038/cdd.2017.179

276. Kalluri, R., & Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. The Journal of clinical investigation, 119(6), 1420–1428. https://doi.org/10.1172/JCI39104

277. Kaloni, D., Diepstraten, S. T., Strasser, A., & Kelly, G. L. (2023). BCL-2 protein family: attractive targets for cancer therapy. Apoptosis: an international journal on programmed cell death, 28(1-2), 20–38. https://doi.org/10.1007/s10495-022-01780-7

278. Kamath, S. G., Chen, N., Xiong, Y., Wenham, R., Apte, S., Humphrey, M., Cragun, J., & Lancaster, J. M. (2009). Gedunin, a novel natural substance, inhibits ovarian cancer cell proliferation. International journal of gynecological cancer: official journal of the International Gynecological Cancer Society, 19(9), 1564–1569. https://doi.org/10.1111/IGC.0b013e3181a83135

279. Kami, K., Doi, R., Koizumi, M., Toyoda, E., Mori, T., Ito, D., Kawaguchi, Y., Fujimoto, K., Wada, M., Miyatake, S., & Imamura, M. (2005). Downregulation of survivin by siRNA diminishes radioresistance of pancreatic cancer cells. Surgery, 138(2), 299–305. https://doi.org/10.1016/j.surg.2005.05.009

280. Kanai M. (2014). Therapeutic applications of curcumin for patients with pancreatic cancer. World journal of gastroenterology, 20(28), 9384–9391. https://doi.org/10.3748/wjg.v20.i28.9384

281. Kanamala, M., Palmer, B. D., Wilson, W. R., & Wu, Z. (2018). Characterization of a smart pH-cleavable PEG polymer towards the development of dual pH-sensitive liposomes. International Journal of Pharmaceutics, 548(1), 288–296. https://doi.org/10.1016/j.ijpharm.2018.07.009

282. Kang, M. H., & Reynolds, C. P. (2009). Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. Clinical cancer research: an official journal of the American Association for Cancer Research, 15(4), 1126–1132. https://doi.org/10.1158/1078-0432.CCR-08-0144

283. Kari, S., Subramanian, K., Altomonte, I. A., Murugesan, A., Yli-Harja, O., & Kandhavelu, M. (2022). Programmed cell death detection methods: a systematic review and a categorical comparison. Apoptosis: an international journal on programmed cell death, 27(7-8), 482–508. https://doi.org/10.1007/s10495-022-01735-y

284. Karikas G. A. (2010). Anticancer and chemopreventing natural products: some biochemical and therapeutic aspects. Journal of B.U.ON. : Official journal of the Balkan Union of Oncology, 15(4), 627–638.

285. Karimi Roshan, M., Soltani, A., Soleimani, A., Rezaie Kahkhaie, K., Afshari, A. R., & Soukhtanloo, M. (2019). Role of AKT and mTOR signaling pathways in the induction of epithelial-mesenchymal transition (EMT) process. Biochimie, 165, 229–234. https://doi.org/10.1016/j.biochi.2019.08.003

286. Kaur, R. A. J. B. I. R., & Arora, S. A. R. O. J. (2015). Alkaloids-important therapeutic secondary metabolites of plant origin. J Crit Rev, 2(3), 1-8.

287. Kazazi-Hyseni, F., Beijnen, J. H., & Schellens, J. H. (2010). Bevacizumab. The oncologist, 15(8), 819–825. https://doi.org/10.1634/theoncologist.2009-0317

288. Kciuk, M., Yahya, E. B., Mohamed Ibrahim Mohamed, M., Rashid, S., Iqbal, M. O., Kontek, R., Abdulsamad, M. A., & Allaq, A. A. (2023). Recent Advances in Molecular Mechanisms of Cancer Immunotherapy. Cancers, 15(10), 2721. https://doi.org/10.3390/cancers15102721

289. Kennedy, L. B., & Salama, A. K. S. (2020). A review of cancer immunotherapy toxicity. CA: a cancer journal for clinicians, 70(2), 86–104. https://doi.org/10.3322/caac.21596

290. Kesharwani, P., Chadar, R., Sheikh, A., Rizg, W. Y., & Safhi, A. Y. (2022). CD44-Targeted Nanocarrier for Cancer Therapy. Frontiers in pharmacology, 12, 800481. https://doi.org/10.3389/fphar.2021.800481

291. Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K. (2019). Anticancer Plants: A Review of the Active Phytochemicals, Applications in Animal Models, and Regulatory Aspects. Biomolecules, 10(1), 47. https://doi.org/10.3390/biom10010047

292. Khanmohammadi, A., Aghaie, A., Vahedi, E., Qazvini, A., Ghanei, M., Afkhami, A., Hajian, A., & Bagheri, H. (2020). Electrochemical biosensors for the detection of lung cancer biomarkers: A review. Talanta, 206, 120251. https://doi.org/10.1016/j.talanta.2019.120251

293. Kim, A., & Cohen, M. S. (2016). The discovery of vemurafenib for the treatment of BRAF-mutated metastatic melanoma. Expert opinion on drug discovery, 11(9), 907–916. https://doi.org/10.1080/17460441.2016.1201057

294. Kim, E. J., Shin, H. K., Cho, J. S., Lee, S. K., Won, M. H., Kim, J. W., & Park, J. H. (2006). trans-10,cis-12 conjugated linoleic acid inhibits the G1-S cell cycle progression in DU145 human prostate carcinoma cells. Journal of medicinal food, 9(3), 293–299. https://doi.org/10.1089/jmf.2006.9.293

295. Kim, G. T., Lee, S. H., & Kim, Y. M. (2014). *Torilis japonica* extract, a new potential EMT suppressor agent by regulation of EGFR signaling pathways. International journal of oncology, 45(4), 1673–1679. https://doi.org/10.3892/ijo.2014.2546

296. Kinghorn G. R. (1993). Genital herpes: natural history and treatment of acute episodes. Journal of medical virology, Suppl 1, 33–38. https://doi.org/10.1002/jmv.1890410508

297. Ko, Y. M., Wu, T. Y., Wu, Y. C., Chang, F. R., Guh, J. Y., & Chuang, L. Y. (2011). Annonacin induces cell cycle-dependent growth arrest and apoptosis in estrogen receptor-α-related pathways in MCF-7 cells. Journal of ethnopharmacology, 137(3), 1283–1290. https://doi.org/10.1016/j.jep.2011.07.056

298. Kobayashi H. (2023). Clinicopathological characteristics, molecular features and novel diagnostic strategies for the detection of malignant transformation of endometriosis (Review). Experimental and therapeutic medicine, 25(6), 279. https://doi.org/10.3892/etm.2023.11978

299. Koç, E., Çelik-Uzuner, S., Uzuner, U., & Çakmak, R. (2018). The detailed comparison of cell death detected by Annexin V-PI counterstain using fluorescence microscope, flow cytometry and automated cell counter in mammalian and microalgae cells. Journal of Fluorescence, 28(6), 1393–1404. https://doi.org/10.1007/s10895-018-2306-4

300. Kocyigit, A., & Guler, E. M. (2017). Curcumin induce DNA damage and apoptosis through generation of reactive oxygen species and reducing mitochondrial membrane potential in melanoma cancer cells. Cellular and Molecular Biology, 63(11), 97–105. https://doi.org/10.14715/cmb/2017.63.11.17

301. Koizumi, K., Hojo, S., Akashi, T., Yasumoto, K., & Saiki, I. (2007). Chemokine receptors in cancer metastasis and cancer cell-derived chemokines in host immune response. Cancer science, 98(11), 1652–1658. https://doi.org/10.1111/j.1349-7006.2007.00606.x

302. Koňariková, K., Ježovičová, M., Keresteš, J., Gbelcová, H., Ďuračková, Z., & Žitňanová, I. (2015). Anticancer effect of black tea extract in human cancer cell lines. SpringerPlus, 4, 127. https://doi.org/10.1186/s40064-015-0871-4

303. Kong, F., Zhang, R., Zhao, X., Zheng, G., Wang, Z., & Wang, P. (2017). Resveratrol raises *in vitro* anticancer effects of paclitaxel in NSCLC cell line A549 through COX-2 expression. The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology, 21(5), 465–474. https://doi.org/10.4196/kjpp.2017.21.5.465

304. Korkina, L. G., & Afanas'ev, I. B. (1997). Antioxidant and chelating properties of flavonoids. Advances in pharmacology (San Diego, Calif.), 38, 151–163. https://doi.org/10.1016/s1054-3589(08)60983-7

305. Krajewska, J. B., Bartoszek, A., & Fichna, J. (2019). New trends in liposome-based drug delivery in colorectal cancer. mini reviews in medicinal chemistry, 19(1), 3–11. https://doi.org/10.2174/1389557518666180903150928

306. Krishnan, K., Mani, A., & Jasmine, S. (2014). Cytotoxic activity of bioactive compound 1, 2- Benzene Dicarboxylic Acid, Mono 2- Ethylhexyl Ester Extracted from a marine derived streptomyces sp. VITSJK8. International journal of molecular and cellular medicine, 3(4), 246–254.

307. Krishnan, P., Rajan, M., Kumari, S., Sakinah, S., Priya, S. P., Amira, F., Danjuma, L., Pooi Ling, M., Fakurazi, S., Arulselvan, P., Higuchi, A., Arumugam, R., Alarfaj, A. A., Munusamy, M. A., Hamat, R. A., Benelli, G., Murugan, K., & Kumar, S. S. (2017). Efficiency of newly formulated camptothecin with β-cyclodextrin-EDTA-Fe3O4 nanoparticle-conjugated nanocarriers as an anti-colon cancer (HT29) drug. Scientific reports, 7(1), 10962. https://doi.org/10.1038/s41598-017-09140-1

308. Krzyszczyk, P., Acevedo, A., Davidoff, E. J., Timmins, L. M., Marrero-Berrios, I., Patel, M., White, C., Lowe, C., Sherba, J. J., Hartmanshenn, C., O'Neill, K. M., Balter, M. L., Fritz, Z. R., Androulakis, I. P., Schloss, R. S., & Yarmush, M. L. (2018). The growing role of precision and personalized medicine for cancer treatment. Technology, 6(3-4), 79–100. https://doi.org/10.1142/S2339547818300020

309. Kuball, J., Schuler, M., Antunes Ferreira, E., Herr, W., Neumann, M., Obenauer-Kutner, L., Westreich, L., Huber, C., Wölfel, T., & Theobald, M. (2002). Generating p53-specific cytotoxic T lymphocytes by recombinant adenoviral vector-based vaccination in mice, but not man. Gene therapy, 9(13), 833–843. https://doi.org/10.1038/sj.gt.3301709

310. Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S., & Oz, F. (2023). Major phytochemicals: recent advances in health benefits and extraction method. Molecules (Basel, Switzerland), 28(2), 887. https://doi.org/10.3390/molecules28020887

311. Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology reports (Amsterdam, Netherlands), 24, e00370. https://doi.org/10.1016/j.btre.2019.e00370

312. Kumar, R. A., & Ssami, B. K. (2022). Biochemical Characterisation and in vitro anti-cancerous potential of Karpa Chenthur, an herbal formulation prepared from selective Indian Medicinal Plants. Journal of Ayurvedic and Herbal Medicine, 8(3), 183-191.

313. Kun, Z., Lin, L., & Xiang, Z. (2024). One new limonoid with cytotoxicity against glioma cell lines from *Cipadessa baccifera*. Natural product research, 38(1), 152–157. https://doi.org/10.1080/14786419.2022.2106482

314. Kurek, J. (2019). Introductory chapter: Alkaloids - their importance in nature and for human life. In IntechOpen eBooks. https://doi.org/10.5772/intechopen.85400

315. Kurniasih, N., Supriadin, A., Fajar, M., Abdulah, R., Harneti, D., Supratman, U., & Taib, M. N. a. B. M. (2019). Cytotoxic sesquterpenoid compound from the stembark of *Aglaia simplicifolia* (Meliaceae). Journal of Physics Conference Series, 1402(5), 055037. https://doi.org/10.1088/1742-6596/1402/5/055037

316. LaCasse, E. C., Mahoney, D. J., Cheung, H. H., Plenchette, S., Baird, S., & Korneluk, R. G. (2008). IAP-targeted therapies for cancer. Oncogene, 27(48), 6252–6275. https://doi.org/10.1038/onc.2008.302

317. Lakshmipriya, T., Soumya, T., Jayasree, P. R., & Kumar, P. R. M. (2017). Selective induction of DNA damage, G2 abrogation, and mitochondrial apoptosis by leaf extract of traditional medicinal plant *Wrightia arborea* in K562 cells. Protoplasma, 255(1), 203–216. https://doi.org/10.1007/s00709-017-1137-5

318. Lamouille, S., Connolly, E., Smyth, J. W., Akhurst, R. J., & Derynck, R. (2012). TGF-β-induced activation of mTOR complex 2 drives epithelial-mesenchymal transition and cell invasion. Journal of cell science, 125(Pt 5), 1259–1273. https://doi.org/10.1242/jcs.095299

319. Lane D. P. (1992). Cancer. p53, guardian of the genome. Nature, 358(6381), 15–16. https://doi.org/10.1038/358015a0

320. Laskar, Y. B., & Mazumder, P. B. (2020). Insight into the molecular evidence supporting the remarkable chemotherapeutic potential of *Hibiscus sabdariffa* L. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 127, 110153. https://doi.org/10.1016/j.biopha.2020.110153

321. Lecoeur, H., Prévost, M. C., & Gougeon, M. L. (2001). Oncosis is associated with exposure of phosphatidylserine residues on the outside layer of the plasma membrane: a reconsideration of the specificity of the annexin V/propidium iodide assay. Cytometry, 44(1), 65–72. https://doi.org/10.1002/1097-0320(20010501)44:1<65::aid-cyto1083>3.0.co;2-q

322. Lee, Y., & Thompson, D. H. (2017). Stimuli-responsive liposomes for drug delivery. Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology, 9(5), 10.1002/wnan.1450. https://doi.org/10.1002/wnan.1450.

323. Lehmann A. R. (2003). DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Biochimie, 85(11), 1101–1111. https://doi.org/10.1016/j.biochi.2003.09.010

324. Lemke, A., Kiderlen, A. F., & Kayser, O. (2005). Amphotericin B. Applied microbiology and biotechnology, 68(2), 151–162. https://doi.org/10.1007/s00253-005-1955-9

325. Levitsky, D. O., & Dembitsky, V. M. (2014). Anti-breast Cancer Agents Derived from Plants. Natural products and bioprospecting, 5(1), 1–16. Advance online publication. https://doi.org/10.1007/s13659-014-0048-9

326. Lewandowska, A. M., Rudzki, M., Rudzki, S., Lewandowski, T., & Laskowska, B. (2019). Environmental risk factors for cancer - review paper. Annals of agricultural and environmental medicine: AAEM, 26(1), 1–7. https://doi.org/10.26444/aaem/94299

327. Li, C., Price, J. E., Milas, L., Hunter, N. R., Ke, S., Yu, D. F., Charnsangavej, C., & Wallace, S. (1999). Antitumor activity of poly (L-glutamic acid)-paclitaxel on syngeneic and xenografted tumors. Clinical cancer research: an official journal of the American Association for Cancer Research, 5(4), 891–897.

328. Li, W. Y., Chan, S. W., Guo, D. J., Chung, M. K., Leung, T. Y., & Yu, P. H. (2009). Water extract of Rheum officinale Baill. induces apoptosis in human lung adenocarcinoma A549 and human breast cancer MCF-7 cell lines. Journal of ethnopharmacology, 124(2), 251–256. https://doi.org/10.1016/j.jep.2009.04.030

329. Li, W. Y., Chan, S. W., Guo, D. J., Chung, M. K., Leung, T. Y., & Yu, P. H. (2009). Water extract of *Rheum officinale* Baill. induces apoptosis in human lung adenocarcinoma A549 and human breast cancer MCF-7 cell lines. Journal of ethnopharmacology, 124(2), 251–256. https://doi.org/10.1016/j.jep.2009.04.030

330. Li, X., Fan, R., Zou, X., Gao, L., Jin, H., Du, R., Xia, L., & Fan, D. (2007). Inhibitory effect of recombinant adenovirus carrying immunocaspase-3 on hepatocellular carcinoma. Biochemical and biophysical research communications, 358(2), 489–494. https://doi.org/10.1016/j.bbrc.2007.04.134

331. Li, Y., Hermanson, D. L., Moriarity, B. S., & Kaufman, D. S. (2018). Human iPSC-Derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-tumor Activity. Cell stem cell, 23(2), 181–192.e5. https://doi.org/10.1016/j.stem.2018.06.002

332. Li, Y., Zhao, D., Zhang, W., Yang, M., Wu, Z., Shi, W., Lan, S., Guo, Z., Yu, H., & Wu, D. (2023). A novel camptothecin derivative, ZBH-01, exhibits superior antitumor efficacy than irinotecan by regulating the cell cycle. Journal of translational medicine, 21(1), 422. https://doi.org/10.1186/s12967-023-04196-2

333. Liang, C. C., Park, A. Y., & Guan, J. L. (2007). *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nature protocols, 2(2), 329–333. https://doi.org/10.1038/nprot.2007.30

334. Liang, N., Li, Y., & Chung, H. Y. (2017). Two natural eudesmane-type sesquiterpenes from *Laggera alata* inhibit angiogenesis and suppress breast cancer cell migration through VEGF- and Angiopoietin 2-mediated signaling pathways. International journal of oncology, 51(1), 213–222. https://doi.org/10.3892/ijo.2017.4004

335. Liczbiński, P., Michałowicz, J., & Bukowska, B. (2020). Molecular mechanism of curcumin action in signaling pathways: Review of the latest research. Phytotherapy research : PTR, 34(8), 1992–2005. https://doi.org/10.1002/ptr.6663

336. Lin, J. P., Lu, H. F., Lee, J. H., Lin, J. G., Hsia, T. C., Wu, L. T., & Chung, J. G. (2005). (-)-Menthol inhibits DNA topoisomerases I, II alpha and beta and promotes NF-kappaB expression in human gastric cancer SNU-5 cells. Anticancer research, 25(3B), 2069–2074.

337. Lin, W., Huang, L., Li, Y., Fang, B., Li, G., Chen, L., & Xu, L. (2019). Mesenchymal Stem Cells and Cancer: Clinical Challenges and Opportunities. BioMed research international, 2019, 2820853. https://doi.org/10.1155/2019/2820853

338. Lin, Y., Shi, R., Wang, X., & Shen, H. M. (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. Current cancer drug targets, 8(7), 634–646. https://doi.org/10.2174/156800908786241050.

339. Lindner D. (2014). Animal models and the tumor microenvironment: studies of tumor-host symbiosis. Seminars in oncology, 41(2), 146–155. https://doi.org/10.1053/j.seminoncol.2014.02.004

340. Lindqvist, A., van Zon, W., Karlsson Rosenthal, C., & Wolthuis, R. M. (2007). Cyclin B1-Cdk1 activation continues after centrosome separation to control mitotic progression. PLoS biology, 5(5), e123. https://doi.org/10.1371/journal.pbio.0050123

341. Liu, H., Su, D., Zhang, J., Ge, S., Li, Y., Wang, F., Gravel, M., Roulston, A., Song, Q., Xu, W., Liang, J. G., Shore, G., Wang, X., & Liang, P. (2017). Improvement of pharmacokinetic profile of TRAIL via trimer-tag enhances its antitumor activity *in vivo*. Scientific reports, 7(1), 8953. https://doi.org/10.1038/s41598-017-09518-1

342. Liu, K., Liu, P. C., Liu, R., & Wu, X. (2015). Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Medical science monitor basic research, 21, 15–20. https://doi.org/10.12659/MSMBR.893327

343. Liu, P., Chen, G., & Zhang, J. (2022). A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules (Basel, Switzerland), 27(4), 1372. https://doi.org/10.3390/molecules27041372

344. Loeb L. A. (2001). A mutator phenotype in cancer. Cancer research, 61(8), 3230–3239.

345. Loh, C. Y., Chai, J. Y., Tang, T. F., Wong, W. F., Sethi, G., Shanmugam, M. K., Chong, P. P., & Looi, C. Y. (2019). The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. Cells, 8(10), 1118. https://doi.org/10.3390/cells8101118

346. Long, S., GuangZhi, Y., BaoJie, G., Wei, X., YanYong, H., YingLi, W., Yang, Z., & LiHua, L. (2012). Shikonin derivatives protect immune organs from damage and promote immune responses in vivo in tumour-bearing mice. Phytotherapy research: PTR, 26(1), 26–33. https://doi.org/10.1002/ptr.3503

347. Lopez, J., & Tait, S. W. (2015). Mitochondrial apoptosis: killing cancer using the enemy within. British journal of cancer, 112(6), 957–962. https://doi.org/10.1038/bjc.2015.85

348. López-Plaza, B., Loria-Kohen, V., González-Rodríguez, L. G., & Fernández-Cruz, E. (2022). Alimentación y estilo de vida en la prevención del cáncer [Diet and lifestyle in cancer prevention]. Nutricion hospitalaria, 39(Spec No3), 74–77. https://doi.org/10.20960/nh.04317

349. Lorence, A., & Nessler, C. L. (2004). Camptothecin, over four decades of surprising findings. Phytochemistry, 65(20), 2735–2749. https://doi.org/10.1016/j.phytochem.2004.09.001

350. Lu, J. J., Bao, J. L., Chen, X. P., Huang, M., & Wang, Y. T. (2012). Alkaloids isolated from natural herbs as the anticancer agents. Evidence-based complementary and alternative medicine: eCAM, 2012, 485042. https://doi.org/10.1155/2012/485042

351. Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R., & Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. Nature, 435(7043), 834–838. https://doi.org/10.1038/nature03702

352. Lu, J., McEachern, D., Sun, H., Bai, L., Peng, Y., Qiu, S., Miller, R., Liao, J., Yi, H., Liu, M., Bellail, A., Hao, C., Sun, S. Y., Ting, A. T., & Wang, S. (2011). Therapeutic potential and molecular mechanism of a novel, potent, nonpeptide, Smac mimetic SM-164 in combination with TRAIL for cancer treatment. Molecular cancer therapeutics, 10(5), 902–914. https://doi.org/10.1158/1535-7163.MCT-10-0864

353. Lu, W., & Kang, Y. (2019). Epithelial-mesenchymal plasticity in cancer progression and metastasis. Developmental cell, 49(3), 361–374. https://doi.org/10.1016/j.devcel.2019.04.010

354. Lugassy, C., & Escande, J. P. (1997). The haematogenous theory of metastasis: Récamier did not propose it. Virchows Archiv : an international journal of pathology, 431(5), 431. https://doi.org/10.1007/s004280050113

355. Łukasik, P., Załuski, M., & Gutowska, I. (2021). Cyclin-Dependent Kinases (CDK) and their role in diseases development-Review. International journal of molecular sciences, 22(6), 2935. https://doi.org/10.3390/ijms22062935.

356. M, S., S, M., S, S., & A, N. (2022). Evaluation of the role of merromoside from *Ipomoea aquatica* Forsskal hydroalcoholic extract in the downregulation of ROS species in overcoming MDR in breast cancer. Asian Pacific journal of cancer prevention: APJCP, 23(11), 3657–3663. https://doi.org/10.31557/APJCP.2022.23.11.3657

357. Mabberley, D. J. (2010). Meliaceae. In Flowering plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae (pp. 185-211). Berlin, Heidelberg: Springer Berlin Heidelberg.

358. MacLachlan, T. K., Sang, N., & Giordano, A. (1995). Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle control and cancer. Critical reviews in eukaryotic gene expression, 5(2), 127–156. https://doi.org/10.1615/critreveukargeneexpr.v5.i2.20

359. Madhuri, K., Elango, K., & Ponnusankar, S. (2011). *Saussurea lappa* (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Oriental Pharmacy and Experimental Medicine, 12(1), 1–9. https://doi.org/10.1007/s13596-011-0043-1

360. Majtnerová, P., & Roušar, T. (2018). An overview of apoptosis assays detecting DNA fragmentation. Molecular biology reports, 45(5), 1469–1478. https://doi.org/10.1007/s11033-018-4258-9

361. Malarvannan, S., Giridharan, R., Sekar, S., Prabavathy, V. R., & Nair, S. (2009). Ovicidal activity of crude extracts of few traditional plants against *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). Journal of Biopesticides, 2(1), 64–71. http://www.jbiopest.com/users/LW8/efiles/64-71.pdf

362. Mammari, N., Albert, Q., Devocelle, M., Kenda, M., Kočevar Glavač, N., Sollner Dolenc, M., Mercolini, L., Tóth, J., Milan, N., Czigle, S., Varbanov, M., & On Behalf Of The Oemonom (2023). Natural products for the prevention and treatment of common cold and viral respiratory infections. Pharmaceuticals (Basel, Switzerland), 16(5), 662. https://doi.org/10.3390/ph16050662

363. Mamun-or-Rashid, A. N. M., Hossain, M. S., Hassan, N., Dash, B. K., Sapon, M. A., & Sen, M. K. (2014). A review on medicinal plants with antidiabetic activity. Journal of Pharmacognosy and Phytochemistry, 3(4), 149-159.https://www.phytojournal.com/vol3Issue4/Issue_nov_2014/44.1.pdf

364. Manfredi, J. J., & Horwitz, S. B. (1984). Taxol: an antimitotic agent with a new mechanism of action. Pharmacology & therapeutics, 25(1), 83–125. https://doi.org/10.1016/0163-7258(84)90025-1

365. Mao, P., Zhang, E., Chen, Y., Liu, L., Rong, D., Liu, Q., & Li, W. (2017). *Pinus massoniana* bark extract inhibits migration of the lung cancer A549 cell line. Oncology letters, 13(2), 1019–1023. https://doi.org/10.3892/ol.2016.5509

366. Martin, S. J., Reutelingsperger, C. P., McGahon, A. J., Rader, J. A., van Schie, R. C., LaFace, D. M., & Green, D. R. (1995). Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. The Journal of experimental medicine, 182(5), 1545–1556. https://doi.org/10.1084/jem.182.5.1545

367. Martin, T. A., Ye, L., Sanders, A. J., Lane, J., & Jiang, W. G. (2013). Cancer invasion and metastasis: molecular and cellular perspective. In Landes Bioscience eBooks. https://www.ncbi.nlm.nih.gov/books/NBK164700/

368. Martino, E., Della Volpe, S., Terribile, E., Benetti, E., Sakaj, M., Centamore, A., Sala, A., & Collina, S. (2017). The long story of camptothecin: From traditional medicine to drugs. Bioorganic & medicinal chemistry letters, 27(4), 701–707. https://doi.org/10.1016/j.bmcl.2016.12.085

369. Masuda, Y., Asada, K., Satoh, R., Takada, K., & Kitajima, J. (2015). Capillin, a major constituent of *Artemisia capillaris* Thunb. flower essential oil, induces apoptosis through the mitochondrial pathway in human leukemia HL-60 cells. Phytomedicine : international journal of phytotherapy and phytopharmacology, 22(5), 545–552. https://doi.org/10.1016/j.phymed.2015.03.008

370. Masuelli, L., Benvenuto, M., Mattera, R., Di Stefano, E., Zago, E., Taffera, G., Tresoldi, I., Giganti, M. G., Frajese, G. V., Berardi, G., Modesti, A., & Bei, R. (2017). *In vitro* and *in vivo* anti-tumoral effects of the flavonoid apigenin in malignant mesothelioma. Frontiers in pharmacology, 8, 373. https://doi.org/10.3389/fphar.2017.00373

371. Mathur, S., & Hoskins, C. (2017). Drug development: Lessons from nature. Biomedical reports, 6(6), 612–614. https://doi.org/10.3892/br.2017.909

372. Mattana, C. M., Cangiano, M. A., Alcaráz, L. E., Sosa, A., Escobar, F., Sabini, C., Sabini, L., & Laciar, A. L. (2014). Evaluation of cytotoxicity and genotoxicity of *Acacia aroma* leaf extracts. The Scientific World JOURNAL, 2014, 1–6. https://doi.org/10.1155/2014/380850

373. May, J. P., & Li, S. D. (2013). Hyperthermia-induced drug targeting. Expert opinion on drug delivery, 10(4), 511–527. https://doi.org/10.1517/17425247.2013.758631

374. Mbaveng, A. T., Hamm, R., & Kuete, V. (2014). Harmful and protective effects of terpenoids from African medicinal plants. In Elsevier eBooks (pp. 557–576). https://doi.org/10.1016/b978-0-12-800018-2.00019-4

375. McConkey, D. J., Choi, W., Marquis, L., Martin, F., Williams, M. B., Shah, J., Svatek, R., Das, A., Adam, L., Kamat, A., Siefker-Radtke, A., & Dinney, C. (2009). Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. Cancer metastasis reviews, 28(3-4), 335–344. https://doi.org/10.1007/s10555-009-9194-7

376. McLaughlin-Drubin, M. E., & Munger, K. (2008). Viruses associated with human cancer. Biochimica et biophysica acta, 1782(3), 127–150. https://doi.org/10.1016/j.bbadis.2007.12.00

377. Meidhof, S., Brabletz, S., Lehmann, W., Preca, B. T., Mock, K., Ruh, M., Schüler, J., Berthold, M., Weber, A., Burk, U., Lübbert, M., Puhr, M., Culig, Z., Wellner, U., Keck, T., Bronsert, P., Küsters, S., Hopt, U. T., Stemmler, M. P., & Brabletz, T. (2015). ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. EMBO molecular medicine, 7(6), 831–847. https://doi.org/10.15252/emmm.201404396

378. Mendelsohn, R., & Balick, M. J. (1995). The value of undiscovered pharmaceuticals in tropical forests. Economic botany, 49, 223-228.

379. Mendoza, M. C., Er, E. E., & Blenis, J. (2011). The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends in biochemical sciences, 36(6), 320–328. https://doi.org/10.1016/j.tibs.2011.03.006

380. Menon, V. P., & Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of curcumin. Advances in experimental medicine and biology, 595, 105–125. https://doi.org/10.1007/978-0-387-46401-5_3

381. Mi, S., Du, Z., Xu, Y., Wu, Z., Qian, X., Zhang, M., & Sun, W. (2016). Microfluidic co-culture system for cancer migratory analysis and anti-metastatic drugs screening. Scientific Reports, 6(1). https://doi.org/10.1038/srep35544

382. Minn, A. J., Rudin, C. M., Boise, L. H., & Thompson, C. B. (1995). Expression of bcl-xL can confer a multidrug resistance phenotype. Blood, 86(5), 1903–1910.

383. Miquel, C., Borrini, F., Grandjouan, S., Aupérin, A., Viguier, J., Velasco, V., Duvillard, P., Praz, F., & Sabourin, J. C. (2005). Role of bax mutations in apoptosis in colorectal cancers with microsatellite instability. American journal of clinical pathology, 123(4), 562–570. https://doi.org/10.1309/JQ2X-3RV3-L8F9-TGYW

384. Mitra, S., Simson, D. K., Khurana, H., Tandon, S., Ahlawat, P., Bansal, N., Barik, S., Sethi, J. S., Chufal, K. S., & Gairola, M. (2022). Treatment Delay during Radiotherapy of Cancer Patients due to COVID-19 Pandemic. Asian Pacific journal of cancer prevention: APJCP, 23(7), 2415–2420. https://doi.org/10.31557/APJCP.2022.23.7.2415

385. Mock, J. N., Costyn, L. J., Wilding, S. L., Arnold, R. D., & Cummings, B. S. (2013). Evidence for distinct mechanisms of uptake and antitumor activity of secretory phospholipase A2 responsive liposome in prostate cancer. Integrative biology: quantitative biosciences from nano to macro, 5(1), 172–182. https://doi.org/10.1039/c2ib20108a

386. Mohamed, M., Abu Lila, A. S., Shimizu, T., Alaaeldin, E., Hussein, A., Sarhan, H. A., Szebeni, J., & Ishida, T. (2019). PEGylated liposomes: immunological responses. Science and technology of advanced materials, 20(1), 710–724. https://doi.org/10.1080/14686996.2019.1627174

387. Mohan, A., Nair, S. V., & Lakshmanan, V. K. (2017). *Leucas aspera* nanomedicine shows superior toxicity and cell migration retarded in prostate cancer cells. Applied biochemistry and biotechnology, 181(4), 1388–1400. https://doi.org/10.1007/s12010-016-2291-5

388. Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). IMPPAT: A curated database of Indian medicinal plants, phytochemistry and therapeutics. Scientific reports, 8(1), 4329. https://doi.org/10.1038/s41598-018-22631-z

389. Mokhtari, R. B., Homayouni, T. S., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., & Yeger, H. (2017). Combination therapy in combating cancer. Oncotarget, 8(23), 38022–38043. https://doi.org/10.18632/oncotarget.16723

390. Molina, J. R., & Adjei, A. A. (2006). The Ras/Raf/MAPK pathway. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer, 1(1), 7–9

391. Momodu, I. B., Okungbowa, E. S., Agoreyo, B. O., & Maliki, M. M. (2022). Gas chromatography–mass spectrometry identification of bioactive compounds in methanol and aqueous seed extracts of azanza garckeana fruits. Nigerian Journal of Biotechnology, 38(1), 25-38.

392. Moon D. O. (2024). Curcumin in Cancer and Inflammation: An In-Depth Exploration of Molecular Interactions, Therapeutic Potentials, and the Role in Disease Management. International journal of molecular sciences, 25(5), 2911. https://doi.org/10.3390/ijms25052911

393. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1-2), 55-63.

394. Mostofa, M. G., Reza, A. S. M. A., Khan, Z., Munira, M. S., Khatoon, M. M., Kabir, S. R., Sadik, M. G., Ağagündüz, D., Capasso, R., Kazi, M., & Alam, A. K. (2023). Apoptosis-inducing anti-proliferative and quantitative phytochemical profiling with *in silico* study of antioxidant-rich *Leea aequata* L. leaves. Heliyon, 10(1), e23400. https://doi.org/10.1016/j.heliyon.2023.e23400

395. Moudi, M., Go, R., Yien, C. Y., & Nazre, M. (2013). Vinca alkaloids. International journal of preventive medicine, 4(11), 1231–1235.

396. Murray, A. (1994). Cell cycle checkpoints. Current opinion in cell biology, 6(6), 872-876.

397. Nagalakshmi, M. A. H., Thangadurai, D., & Pullaiah, T. (2003). *In vitro* antimicrobial efficacy of leaf essential oils of *Chukrasia tabularis* Adr. Juss. and *Melia dubia* Cav.(Meliaceae). Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 17(4), 414-416.

398. Nagpal, M., Nagpal, K., & Nagpal, P. N. (2007). A comparative debate on the various anti-vascular endothelial growth factor drugs: pegaptanib sodium (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin). Indian journal of ophthalmology, 55(6), 437–439. https://doi.org/10.4103/0301-4738.36478

399. Nail, A. N., Ferragut Cardoso, A. P., Montero, L. K., & States, J. C. (2023). miRNAs and arsenic-induced carcinogenesis. Advances in pharmacology (San Diego, Calif.), 96, 203–240. https://doi.org/10.1016/bs.apha.2022.10.002

400. Nair, J. J., Rárová, L., Strnad, M., Bastida, J., & van Staden, J. (2012). Apoptosis-inducing effects of distichamine and narciprimine, rare alkaloids of the plant family Amaryllidaceae. Bioorganic & medicinal chemistry letters, 22(19), 6195–6199. https://doi.org/10.1016/j.bmcl.2012.08.005

401. Nakamura, R., Ishii, H., Endo, K., Hotta, A., Fujii, E., Miyazawa, K., & Saitoh, M. (2018). Reciprocal expression of Slug and Snail in human oral cancer cells. PloS one, 13(7), e0199442. https://doi.org/10.1371/journal.pone.0199442

402. Namba, T., Kodama, R., Moritomo, S., Hoshino, T., & Mizushima, T. (2015). Zidovudine, an anti-viral drug, resensitizes gemcitabine-resistant pancreatic cancer cells to gemcitabine by inhibition of the Akt-GSK3β-Snail pathway. Cell death & disease, 6(6), e1795. https://doi.org/10.1038/cddis.2015.172

403. Nan, Y., Su, H., Zhou, B., & Liu, S. (2023). The function of natural compounds in important anticancer mechanisms. Frontiers in oncology, 12, 1049888. https://doi.org/10.3389/fonc.2022.1049888

404. Nani, A., Murtaza, B., Sayed Khan, A., Khan, N. A., & Hichami, A. (2021). Antioxidant and anti-inflammatory potential of polyphenols contained in Mediterranean diet in obesity: Molecular mechanisms. Molecules (Basel, Switzerland), 26(4), 985. https://doi.org/10.3390/molecules26040985

405. National Academies Press (US). (2000). B-Carotene and other carotenoids. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK225469/

406. Nelson, V. K., Sahoo, N. K., Sahu, M., Sudhan, H. H., Pullaiah, C. P., & Muralikrishna, K. S. (2020). *In vitro* anticancer activity of *Eclipta alba* whole plant extract on colon cancer cell HCT-116. BMC Complementary Medicine and Therapies, 20(1). https://doi.org/10.1186/s12906-020-03118-9

407. Nemunaitis, J., Ganly, I., Khuri, F., Arseneau, J., Kuhn, J., McCarty, T., Landers, S., Maples, P., Romel, L., Randlev, B., Reid, T., Kaye, S., & Kirn, D. (2000). Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. Cancer research, 60(22), 6359–6366.

408. Neophytou, C. M., Panagi, M., Stylianopoulos, T., & Papageorgis, P. (2021). The Role of Tumor Microenvironment in Cancer Metastasis: Molecular Mechanisms and Therapeutic Opportunities. Cancers, 13(9), 2053. https://doi.org/10.3390/cancers13092053

409. Netscher T. (2007). Synthesis of vitamin E. Vitamins and hormones, 76, 155–202. https://doi.org/10.1016/S0083-6729(07)76007-7

410. Newman, D. J., Cragg, G. M., & Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. Journal of natural products, 66(7), 1022–1037. https://doi.org/10.1021/np030096l

411. Newman, D.J., and Shapiro, S. (2008) Microbial prescreens for anticancer activity. SIM News 58: 132–150.https://cir.nii.ac.jp/crid/1570009750888741632

412. Nigg E. A. (1995). Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. BioEssays : news and reviews in molecular, cellular and developmental biology, 17(6), 471–480. https://doi.org/10.1002/bies.950170603

413. Ning, J., Di, Y. T., Wang, Y. Y., He, H. P., Fang, X., Li, Y., Li, S. L., & Hao, X. J. (2010). Cytotoxic activity of trijugin-type limonoids from *Cipadessa baccifera*. Planta medica, 76(16), 1907–1910. https://doi.org/10.1055/s-0030-1249979

414. Norn, S., Kruse, P. R., & Kruse, E. (2005). Opiumsvalmuen og morfin gennem tiderne [History of opium poppy and morphine]. Dansk medicinhistorisk arbog, 33, 171–184. https://pubmed.ncbi.nlm.nih.gov/17152761/

415. Nosova, A. S., Koloskova, O. O., Nikonova, A. A., Simonova, V. A., Smirnov, V. V., Kudlay, D., & Khaitov, M. R. (2019). Diversity of PEGylation methods of liposomes and their influence on RNA delivery. MedChemComm, 10(3), 369–377. https://doi.org/10.1039/c8md00515j

416. Nowell P. C. (1976). The clonal evolution of tumor cell populations. Science (New York, N.Y.), 194(4260), 23–28. https://doi.org/10.1126/science.959840

417. Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A., & Alshaer, W. (2022). Liposomes: structure, composition, types, and clinical applications. Heliyon, 8(5), e09394. https://doi.org/10.1016/j.heliyon.2022.e093934

418. Nwafor, F. I., & Orabueze, I. C. (2018). Role of phytochemistry in plant classification: Phytochemotaxonomy. In Phytochemistry (pp. 197-222). Apple Academic Press.

419. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., Hutchison, G. R., 2011. Open Babel: An open chemical toolbox. Journal of cheminformatics. 3, 33. https://doi.org/10.1186/1758-2946-3-33

420. Obrenovich, M. E., Nair, N. G., Beyaz, A., Aliev, G., & Reddy, V. P. (2010). The role of polyphenolic antioxidants in health, disease, and aging. Rejuvenation research, 13(6), 631–643. https://doi.org/10.1089/rej.2010.1043

421. Ocker, M., Neureiter, D., Lueders, M., Zopf, S., Ganslmayer, M., Hahn, E. G., Herold, C., & Schuppan, D. (2005). Variants of bcl-2 specific siRNA for silencing antiapoptotic bcl-2 in pancreatic cancer. Gut, 54(9), 1298–1308. https://doi.org/10.1136/gut.2004.056192

422. Ohnishi, K., Scuric, Z., Schiestl, R. H., Okamoto, N., Takahashi, A., & Ohnishi, T. (2006). siRNA targeting NBS1 or XIAP increases radiation sensitivity of human cancer cells independent of TP53 status. Radiation research, 166(3), 454–462. https://doi.org/10.1667/RR3606.1

423. Okon, E., Kukula-Koch, W., Halasa, M., Jarzab, A., Baran, M., Dmoszynska-Graniczka, M., Angelis, A., Kalpoutzakis, E., Guz, M., Stepulak, A., & Wawruszak, A. (2020). Magnoflorine-isolation and the anticancer potential against NCI-H1299 Lung, MDA-MB-468 Breast, T98G Glioma, and TE671 Rhabdomyosarcoma Cancer Cells. Biomolecules, 10(11), 1532. https://doi.org/10.3390/biom10111532

424. Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., Bruncko, M., Deckwerth, T. L., Dinges, J., Hajduk, P. J., Joseph, M. K., Kitada, S., Korsmeyer, S. J., Kunzer, A. R., Letai, A., Li, C., Mitten, M. J., Nettesheim, D. G., Ng, S., Nimmer, P. M., … Rosenberg, S. H. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature, 435(7042), 677–681. https://doi.org/10.1038/nature03579

425. Oršolić, N., & Jazvinšćak Jembrek, M. (2022). Molecular and cellular mechanisms of propolis and its polyphenolic compounds against cancer. International journal of molecular sciences, 23(18), 10479. https://doi.org/10.3390/ijms231810479

426. Osman, E. E. A., Shemis, M. A., Abdel-Hameed, E. S., Gouda, A. E., Hassan, H., Atef, N., & Mamdouh, S. (2024). Phytoconstituent analysis, anti-inflammatory, antimicrobial and anticancer effects of nano encapsulated Convolvulus arvensis L. extracts. BMC complementary medicine and therapies, 24(1), 122. https://doi.org/10.1186/s12906-024-04420-6

427. Ouyang, X., Telli, M. L., & Wu, J. C. (2019). induced pluripotent stem cell-based cancer vaccines. Frontiers in immunology, 10, 1510. https://doi.org/10.3389/fimmu.2019.01510

428. Paritala, V., Chiruvella, K. K., Thammineni, C., Ghanta, R. G., & Mohammed, A. (2015). Phytochemicals and antimicrobial potentials of mahogany family. Revista Brasileira de Farmacognosia, 25(1), 61-83.

429. Park J. W. (2002). Liposome-based drug delivery in breast cancer treatment. Breast cancer research: BCR, 4(3), 95–99. https://doi.org/10.1186/bcr432

430. Park, J. W., Benz, C. C., & Martin, F. J. (2004). Future directions of liposome- and immunoliposome-based cancer therapeutics. Seminars in oncology, 31(6 Suppl 13), 196–205. https://doi.org/10.1053/j.seminoncol.2004.08.009

431. Parodi, S., & Mancuso, T. (1996). A general overview of the process of carcinogenesis. Tumori, 82(4), 291–301. https://doi.org/10.1177/030089169608200401

432. Parsa N. (2012). Environmental factors inducing human cancers. Iranian journal of public health, 41(11), 1–9.

433. Pavletic, Z. S., & Armitage, J. O. (1996). Bone marrow transplantation for cancer - An update. The oncologist, 1(3), 159–168.

434. Payton, M., & Coats, S. (2002). Cyclin E2, the cycle continues. The international journal of biochemistry & cell biology, 34(4), 315–320. https://doi.org/10.1016/s1357-2725(01)00137-6

435. Pejin, B., Kojic, V., & Bogdanovic, G. (2014). An insight into the cytotoxic activity of phytol *at in vitro* conditions. Natural product research, 28(22), 2053–2056. https://doi.org/10.1080/14786419.2014.921686

436. Pérez, R., Rodríguez, H., Pérez, E., Suárez, M., Reyes, O., González, L. J., López de Cerain, A., Ezpelata, O., Pérez, C., & Ochoa, C. (2000). Study on the decomposition products of thiadiazinthione and their anticancer properties. Arzneimittel-Forschung, 50(9), 854–857. https://doi.org/10.1055/s-0031-1300301

437. Pettit, G. R., Numata, A., Iwamoto, C., Morito, H., Yamada, T., Goswami, A., Clewlow, P. J., Cragg, G. M., & Schmidt, J. M. (2002). Antineoplastic agents. 489. Isolation and structures of meliastatins 1-5 and related euphane triterpenes from the tree *Melia dubia*. Journal of natural products, 65(12), 1886–1891. https://doi.org/10.1021/np020216+

438. Pfeffer, C. M., & Singh, A. T. K. (2018). Apoptosis: A Target for Anticancer Therapy. International journal of molecular sciences, 19(2), 448. https://doi.org/10.3390/ijms19020448

439. Philchenkov, A., Zavelevich, M., Kroczak, T. J., & Los, M. (2004). Caspases and cancer: mechanisms of inactivation and new treatment modalities. Experimental oncology, 26(2), 82–97.

440. Pieters, L., Nahrstedt, A., Hamburger, M., Barz, W., Bauer, R., Butterweck, V., Calixto, J. B., Efferth, T., Jaroszewski, J. W., Khan, I., Kreis, W., Merfort, I., Schmidt, K., Simmet, T., Stuppner, H., Wu, Y.-C., & Ye, Y. (Eds.). (2011). 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research. Planta Medica, 1229–1472. https://ga-online.org/wp-content/uploads/2021/01/c6241c_90020aa3781b4262ae0de84291747969.pdf

441. Pistritto, G., Trisciuoglio, D., Ceci, C., Garufi, A., & D'Orazi, G. (2016). Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging, 8(4), 603–619. https://doi.org/10.18632/aging.100934

442. Pluskal, T., , & Weng, J. K., (2018). Natural product modulators of human sensations and mood: molecular mechanisms and therapeutic potential. Chemical Society reviews, 47(5), 1592–1637. https://doi.org/10.1039/c7cs00411g

443. Polat, D. Ç., İlgün, S., Karatoprak, G. Ş., Akkol, E. K., & Capasso, R. (2022). Phytochemical Profiles, Antioxidant, Cytotoxic, and Anti-Inflammatory Activities of Traditional Medicinal Plants: Centaurea pichleri subsp. pichleri, Conyza canadensis, and Jasminum fruticans. Molecules (Basel, Switzerland), 27(23), 8249. https://doi.org/10.3390/molecules27238249

444. Pommier Y. (2006). Topoisomerase I inhibitors: camptothecins and beyond. Nature reviews. Cancer, 6(10), 789–802. https://doi.org/10.1038/nrc1977

445. Prasad, D. X. (2024). Exploring the Phytochemical Profile of Melia dubia Seeds and Evaluating their Biological Properties.

446. Prinja, S., Dixit, J., Gupta, N., Dhankhar, A., Kataki, A. C., Roy, P. S., Mehra, N., Kumar, L., Singh, A., Malhotra, P., Goyal, A., Rajsekar, K., Krishnamurthy, M. N., & Gupta, S. (2023). Financial toxicity of cancer treatment in India: towards closing the cancer care gap. Frontiers in public health, 11, 1065737. https://doi.org/10.3389/fpubh.2023.1065737

447. Prinja, S., Singh, M. P., Aggarwal, V., Rajsekar, K., Gedam, P., Goyal, A., & Bahuguna, P. (2023). Impact of India's publicly financed health insurance scheme on public sector district hospitals: a health financing perspective. The Lancet Regional Health-Southeast Asia, 9.

448. Purushothaman, A., Nandhakumar, E., Shanthi, P., & Sachidanandam, T. P. (2016). Shemamruthaa, a herbal formulation induces apoptosis in breast cancer cells and inhibits tumor progression in rats. Journal of evidence-based complementary & alternative medicine, 21(4), NP1–NP10. https://doi.org/10.1177/2156587215607779

449. Qian, S., Wei, Z., Yang, W., Huang, J., Yang, Y., & Wang, J. (2022). The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Frontiers in oncology, 12, 985363. https://doi.org/10.3389/fonc.2022.985363

450. Qui P, Guan H, Dong P, et al (2011). The p53-, Bax- and p21-dependent inhibition of colon cancer cell growth by 5-hydroxy polymethoxyflavones. Mol Nutr Food Res, 55, 613-22.

451. Raffo, A. J., Perlman, H., Chen, M. W., Day, M. L., Streitman, J. S., & Buttyan, R. (1995). Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. Cancer research, 55(19), 4438–4445.

452. Rai, K. R., Moore, J., Wu, J., Novick, S. C., & O'Brien, S. M. (2008). Effect of the addition of oblimersen (Bcl-2 antisense) to fludarabine/cyclophosphamide for relapsed/refractory chronic lymphocytic leukemia (CLL) on survival in patients who achieve CR/nPR: Five-year follow-up from a randomized phase III study. Journal of Clinical Oncology, 26(15_suppl), 7008-7008.

453. Rajagopal, S., Kumar, R. A., Deevi, D. S., Satyanarayana, C., & Rajagopalan, R. (2003). Andrographolide, a potential cancer therapeutic agent isolated from

Andrographis paniculata. Journal of experimental therapeutics & oncology, 3(3), 147–158. https://doi.org/10.1046/j.1359-4117.2003.01090.x

454. Rajendran, V., & Jain, M. V. (2018). *In vitro* tumourigenic assay: Colony forming assay for cancer stem cells. Methods in molecular biology (Clifton, N.J.), 1692, 89–95. https://doi.org/10.1007/978-1-4939-7401-6_8

455. Raju, J., Patlolla, J. M., Swamy, M. V., & Rao, C. V. (2004). Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 13(8), 1392–1398.

456. Raman, B. V., Samuel, L. A., Saradhi, M. P., Rao, B. N., Krishna, N. V., Sudhakar, M., & Radhakrishnan, T. M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian J Pharm Clin Res, 5(2), 99-106.

457. Ramawat, K. G., Dass, S., & Mathur, M. (2009). The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. Herbal drugs: ethnomedicine to modern medicine, 7-32.

458. Ramos-Silva, A., Tavares-Carreón, F., Figueroa, M., De la Torre-Zavala, S., Gastelum-Arellanez, A., Rodríguez-García, A., Galán-Wong, L. J., & Avilés-Arnaut, H. (2017). Anticancer potential of *Thevetia peruviana* fruit methanolic extract. BMC complementary and alternative medicine, 17(1), 241. https://doi.org/10.1186/s12906-017-1727-y

459. Rana, A., Samtiya, M., Dhewa, T., Mishra, V., & Aluko, R. E. (2022). Health benefits of polyphenols: A concise review. Journal of Food Biochemistry, 46(10). https://doi.org/10.1111/jfbc.1426

460. Ranganathan, P., Sengar, M., Chinnaswamy, G., Agrawal, G., Arumugham, R., Bhatt, R., Bilimagga, R., Chakrabarti, J., Chandrasekharan, A., Chaturvedi, H. K., Choudhrie, R., Dandekar, M., Das, A., Goel, V., Harris, C., Hegde, S. K., Hulikal, N., Joseph, D., Kantharia, R., Khan, A., … National Cancer Grid of India (2021). Impact of COVID-19 on cancer care in India: a cohort study. The Lancet. Oncology, 22(7), 970–976. https://doi.org/10.1016/S1470-2045(21)00240-0

461. Ranganathan, S., Halagowder, D., & Sivasithambaram, N. D. (2015). Quercetin Suppresses Twist to Induce Apoptosis in MCF-7 Breast Cancer Cells. PloS one, 10(10), e0141370. https://doi.org/10.1371/journal.pone.0141370

462. Rauf, A., Imran, M., Butt, M. S., Nadeem, M., Peters, D. G., & Mubarak, M. S. (2018). Resveratrol as an anti-cancer agent: A review. Critical reviews in food science and nutrition, 58(9), 1428–1447. https://doi.org/10.1080/10408398.2016.1263597

463. Ravichandiran, V., Shanmugam, K., & Solomon, A. P. (2013). Screening of SdiA inhibitors from *Melia dubia* seeds extracts towards the hold back of uropathogenic E.coli quorum sensing-regulated factors. Medicinal chemistry (Shariqah (United Arab Emirates)), 9(6), 819–827. https://doi.org/10.2174/1573406411309060006

464. Ravindran, J., Prasad, S., & Aggarwal, B. B. (2009). Curcumin and cancer cells: how many ways can curry kill tumor cells selectively?. The AAPS journal, 11(3), 495–510. https://doi.org/10.1208/s12248-009-9128-x

465. Razak, N. B. A., Jones, G., Bhandari, M., Berndt, M. C., & Metharom, P. (2018). Cancer-Associated Thrombosis: An overview of mechanisms, risk factors, and treatment. Cancers, 10(10), 380. https://doi.org/10.3390/cancers10100380

466. Rehman Khan, R. A., Afzal, S., Aati, H. Y., Aati, S., Rao, H., Ahmad, S., Hussain, M., & Khan, K. U. R. (2024). Phytochemical characterization of *Thevetia peruviana* (lucky nut) bark extracts by GC-MS analysis, along with evaluation of its biological activities, and molecular docking study. Heliyon, 10(13), e33151. https://doi.org/10.1016/j.heliyon.2024.e33151

467. Ren, X., Duan, L., He, Q., Zhang, Z., Zhou, Y., Wu, D., Pan, J., Pei, D., & Ding, K. (2010). Identification of niclosamide as a new small-molecule inhibitor of the STAT3 signaling pathway. ACS medicinal chemistry letters, 1(9), 454–459. https://doi.org/10.1021/ml100146z.

468. Ren, Y., de Blanco, E. J. C., Fuchs, J. R., Soejarto, D. D., Burdette, J. E., Swanson, S. M., & Kinghorn, A. D. (2019). Potential anticancer agents characterized from selected tropical plants. Journal of natural products, 82(3), 657–679. https://doi.org/10.1021/acs.jnatprod.9b00018

469. Rezadoost, M. H., Kumleh, H. H., & Ghasempour, A. (2019). Cytotoxicity and apoptosis induction in breast cancer, skin cancer and glioblastoma cells by plant extracts. Molecular biology reports, 46(5), 5131–5142. https://doi.org/10.1007/s11033-019-04970-w

470. Ribatti, D., Tamma, R., & Annese, T. (2020). Epithelial-mesenchymal transition in cancer: A historical overview. Translational oncology, 13(6), 100773. https://doi.org/10.1016/j.tranon.2020.100773

471. Ricciuti, B., Genova, C., Crinò, L., Libra, M., & Leonardi, G. C. (2019). Antitumor activity of larotrectinib in tumors harboring NTRK gene fusions: a short review on the current evidence. OncoTargets and therapy, 12, 3171–3179. https://doi.org/10.2147/OTT.S177051

472. Rieger, A. M., Nelson, K. L., Konowalchuk, J. D., & Barreda, D. R. (2011). Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. Journal of visualized experiments: JoVE, (50), 2597. https://doi.org/10.3791/2597

473. Rippin, T. M., Bykov, V. J., Freund, S. M., Selivanova, G., Wiman, K. G., & Fersht, A. R. (2002). Characterization of the p53-rescue drug CP-31398 in vitro and in living cells. Oncogene, 21(14), 2119–2129. https://doi.org/10.1038/sj.onc.1205362

474. Rodríguez-García, C., Sánchez-Quesada, C., & J Gaforio, J. (2019). Dietary flavonoids as cancer chemopreventive agents: An updated review of human Studies. Antioxidants (Basel, Switzerland), 8(5), 137. https://doi.org/10.3390/antiox8050137

475. Rohn, J. L., & Noteborn, M. H. (2004). The viral death effector Apoptin reveals tumor-specific processes. Apoptosis : an international journal on programmed cell death, 9(3), 315–322. https://doi.org/10.1023/b:appt.0000025808.48885.9c

476. Roth, J. A., Nguyen, D., Lawrence, D. D., Kemp, B. L., Carrasco, C. H., Ferson, D. Z., Hong, W. K., Komaki, R., Lee, J. J., Nesbitt, J. C., Pisters, K. M., Putnam, J. B., Schea, R., Shin, D. M., Walsh, G. L., Dolormente, M. M., Han, C. I., Martin, F. D., Yen, N., Xu, K., … Cai, D. (1996). Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nature medicine, 2(9), 985–991. https://doi.org/10.1038/nm0996-985

477. Rudzińska, A., Juchaniuk, P., Oberda, J., Wiśniewska, J., Wojdan, W., Szklener, K., & Mańdziuk, S. (2023). Phytochemicals in cancer treatment and cancer prevention-Review on epidemiological data and clinical trials. Nutrients, 15(8), 1896. https://doi.org/10.3390/nu15081896

478. Saber, M. M., Mirtajani, S. B., & Karimzadeh, K. (2018). Green synthesis of silver nanoparticles using *Trapa natans* extract and their anticancer activity against A431 human skin cancer cells. Journal of Drug Delivery Science and Technology, 47, 375–379. https://doi.org/10.1016/j.jddst.2018.08.004

479. Sadeghi-Aliabadi, H., Ghasemi, N., & Kohi, M. (2009). Cytotoxic effect of Convolvulus arvensis extracts on human cancerous cell line. Research in Pharmaceutical Sciences, 3(1), 31-34.

480. Sadeghnia, H. R., Ghorbani Hesari, T., Mortazavian, S. M., Mousavi, S. H., Tayarani-Najaran, Z., & Ghorbani, A. (2014). *Viola tricolor* induces apoptosis in cancer cells and exhibits antiangiogenic activity on chicken chorioallantoic membrane. BioMed research international, 2014, 625792. https://doi.org/10.1155/2014/62579

481. Salas, J. A., & Méndez, C. (1998). Genetic manipulation of antitumor-agent biosynthesis to produce novel drugs. Trends in biotechnology, 16(11), 475–482. https://doi.org/10.1016/s0167-7799(98)01198-6

482. Salehi, B., Krochmal□Marczak, B., Skiba, D., Patra, J. K., Das, S. K., Das, G., ... & Martorell, M. (2020). Convolvulus plant—A comprehensive review from phytochemical composition to pharmacy. Phytotherapy Research, 34(2), 315-328.

483. Salim, M., Kabeer, T. K., Nair, S. A., Dan, M., Sabu, M., & Baby, S. (2016). Chemical profile, antiproliferative and antioxidant activities of rhizome oil of Zingiber anamalayanum from Western Ghats in India. Natural product research, 30(17), 1965–1968. https://doi.org/10.1080/14786419.2015.1094802

484. Salmikangas, P., Kinsella, N., & Chamberlain, P. (2018). Chimeric Antigen Receptor T-Cells (CAR T-Cells) for Cancer Immunotherapy - Moving Target for Industry?. Pharmaceutical research, 35(8), 152. https://doi.org/10.1007/s11095-018-2436-z

485. Saman, H., Raza, S. S., Uddin, S., & Rasul, K. (2020). Inducing Angiogenesis, a Key step in cancer vascularization, and treatment approaches. Cancers, 12(5), 1172. https://doi.org/10.3390/cancers12051172

486. Sánchez, M., Romero, M., Gómez-Guzmán, M., Tamargo, J., Pérez-Vizcaino, F., & Duarte, J. (2019). Cardiovascular effects of flavonoids. Current medicinal chemistry, 26(39), 6991–7034. https://doi.org/10.2174/0929867326666181220094721

487. Santos, L. K. B., Veras, M. D. A., Marques, K. K. G., DE Moraes Alves, M. M., Mendes, A. N., DE Amorim Carvalho, F. A., Sobral, M. V., Chaves, M. H., & GonÇalves, J. C. R. (2020). Assessment of *in vitro* anti-melanoma potential of *Ephedranthus pisocarpus* R.E.Fr. Anticancer research, 40(9), 5015–5024. https://doi.org/10.21873/anticanres.14504

488. Sarkar, F. H., Li, Y., Wang, Z., & Kong, D. (2009). Cellular signaling perturbation by natural products. Cellular signalling, 21(11), 1541–1547. https://doi.org/10.1016/j.cellsig.2009.03.009

489. Saslis-Lagoudakis, C. H., Savolainen, V., Williamson, E. M., Forest, F., Wagstaff, S. J., Baral, S. R., Watson, M. F., Pendry, C. A., & Hawkins, J. A. (2012). Phylogenies reveal predictive power of traditional medicine in bioprospecting. Proceedings of the National Academy of Sciences of the United States of America, 109(39), 15835–15840. https://doi.org/10.1073/pnas.1202242109

490. Saxena, M., Stephens, M. A., Pathak, H., & Rangarajan, A. (2011). Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell death & disease, 2(7), e179. https://doi.org/10.1038/cddis.2011.61

491. Schiff, P. B., & Horwitz, S. B. (1981). Taxol assembles tubulin in the absence of exogenous guanosine 5'-triphosphate or microtubule-associated proteins. Biochemistry, 20(11), 3247–3252. https://doi.org/10.1021/bi00514a041

492. Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. Cold Spring Harbor perspectives in medicine, 5(4), a006098. https://doi.org/10.1101/cshperspect.a006098

493. Shah, S. N., Wani, T. A., Ram, B., Koul, M., Awasthi, P., Rajput, D. S., & Reddy, G. R. S. (2016). An efficient protocol for *in vitro* organogenesis and antioxidant studies in *Melia dubia* Cav. African Journal of Biotechnology, 15(19), 768-775.DOI:10.5897/AJB2015.15153

494. Shahzad, N., Khan, W., Md, S., Ali, A., Saluja, S. S., Sharma, S., Al-Allaf, F. A., Abduljaleel, Z., Ibrahim, I. A. A., Abdel-Wahab, A. F., Afify, M. A., & Al-Ghamdi, S. S. (2017). Phytosterols as a natural anticancer agent: Current status and future perspective. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 88, 786–794. https://doi.org/10.1016/j.biopha.2017.01.068

495. Shanmugam, M. K., Rane, G., Kanchi, M. M., Arfuso, F., Chinnathambi, A., Zayed, M. E., Alharbi, S. A., Tan, B. K., Kumar, A. P., & Sethi, G. (2015). The multifaceted role of curcumin in cancer prevention and treatment. Molecules (Basel, Switzerland), 20(2), 2728–2769. https://doi.org/10.3390/molecules20022728

496. Shapiro, G. I., & Harper, J. W. (1999). Anticancer drug targets: cell cycle and checkpoint control. The Journal of clinical investigation, 104(12), 1645–1653. https://doi.org/10.1172/JCI9054

497. Sharma, A., Tiwari, P., Arora, R., & Sankaranarayanan, A. (2022). Madagascar periwinkle alkaloids: biosynthesis, ethnobotanical attributes, and pharmacological functions. South African Journal of Botany, 151, 108-115.

498. Sharma, H., Sen, S., Lo Muzio, L., Mariggiò, A., & Singh, N. (2005). Antisense-mediated downregulation of anti-apoptotic proteins induces apoptosis and sensitizes head and neck squamous cell carcinoma cells to chemotherapy. Cancer biology & therapy, 4(7), 720–727. https://doi.org/10.4161/cbt.4.7.1783

499. Sharma, S., Kelly, T. K., & Jones, P. A. (2010). Epigenetics in cancer. Carcinogenesis, 31(1), 27–36. https://doi.org/10.1093/carcin/bgp220

500. Shati, A. A., Alkahtani, M. A., Alfaifi, M. Y., Elbehairi, S. E. I., Elsaid, F. G., Prasanna, R., & Mir, M. A. (2020). Secondary metabolites of *Saussurea costus* Leaf extract induce apoptosis in breast, liver, and colon cancer cells by caspase-3-dependent intrinsic pathway. BioMed research international, 2020, 1608942. https://doi.org/10.1155/2020/1608942

501. Shen, Q., Liu, X., Li, W., Zhao, X., Li, T., Zhou, K., & Zhou, J. (2022). Emerging role and mechanism of circRNAs in pediatric malignant solid tumors. Frontiers in genetics, 12, 820936. https://doi.org/10.3389/fgene.2021.820936

502. Sheng-Ji P. (2001). Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharmaceutical biology, 39 Suppl 1, 74–79. https://doi.org/10.1076/phbi.39.s1.74.0005

503. Shikov, A. N., Pozharitskaya, O. N., Makarov, V. G., Wagner, H., Verpoorte, R., & Heinrich, M. (2014). Medicinal plants of the Russian Pharmacopoeia; their history and applications. Journal of ethnopharmacology, 154(3), 481–536. https://doi.org/10.1016/j.jep.2014.04.007

504. Shin, J. Y., Kim, H. S., Lee, K. S., Kim, J., Park, J. B., Won, M. H., Chae, S. W., Choi, Y. H., Choi, K. C., Park, Y. E., & Lee, J. Y. (2000). Mutation and expression of the p27KIP1 and p57KIP2 genes in human gastric cancer. Experimental & molecular medicine, 32(2), 79–83. https://doi.org/10.1038/emm.2000.14

505. Shoaib, M., Shah, I., Ali, N., Adhikari, A., Tahir, M. N., Shah, S. W. A., Ishtiaq, S., Khan, J., Khan, S., & Umer, M. N. (2017). Sesquiterpene lactone! a promising antioxidant, anticancer and moderate antinociceptive agent from *Artemisia macrocephala* jacquem. BMC Complementary and Alternative Medicine, 17(1). https://doi.org/10.1186/s12906-016-1517-y

506. Shomar, A., Barak, O., & Brenner, N. (2022). Cancer progression as a learning process. iScience, 25(3), 103924. https://doi.org/10.1016/j.isci.2022.103924

507. Siebzehnrubl, F. A., Silver, D. J., Tugertimur, B., Deleyrolle, L. P., Siebzehnrubl, D., Sarkisian, M. R., Devers, K. G., Yachnis, A. T., Kupper, M. D., Neal, D., Nabilsi, N. H., Kladde, M. P., Suslov, O., Brabletz, S., Brabletz, T., Reynolds, B. A., & Steindler, D. A. (2013). The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. EMBO molecular medicine, 5(8), 1196–1212. https://doi.org/10.1002/emmm.201302827

508. Singh, A. T., Ghosh, M., Forte, T. M., Ryan, R. O., & Gordon, L. I. (2011). Curcumin nanodisk-induced apoptosis in mantle cell lymphoma. Leukemia & lymphoma, 52(8), 1537–1543. https://doi.org/10.3109/10428194.2011.584253

509. Singh, R., Letai, A., & Sarosiek, K. (2019). Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nature reviews. Molecular cell biology, 20(3), 175–193. https://doi.org/10.1038/s41580-018-0089-8

510. Singh, R., Rai, C., & Ishan, R. (2021). Impact of COVID-19 lockdown on patients with cancer in North Bihar, India: A phone-based survey. Cancer Research, Statistics, and Treatment, *4*(1), 37-43.

511. Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D., & Dirks, P. B. (2004). Identification of human brain tumour initiating cells. Nature, 432(7015), 396–401. https://doi.org/10.1038/nature03128

512. Sinkule J. A. (1984). Etoposide: a semisynthetic epipodophyllotoxin. Chemistry, pharmacology, pharmacokinetics, adverse effects and use as an antineoplastic agent. Pharmacotherapy, 4(2), 61–73. https://doi.org/10.1002/j.1875-9114.1984.tb03318.x

513. Sirisoma, N., Pervin, A., Zhang, H., Jiang, S., Willardsen, J. A., Anderson, M. B., Mather, G., Pleiman, C. M., Kasibhatla, S., Tseng, B., Drewe, J., & Cai, S. X. (2009). Discovery of N-(4-methoxyphenyl)-N,2-dimethylquinazolin-4-amine, a potent apoptosis inducer and efficacious anticancer agent with high blood brain barrier penetration. Journal of medicinal chemistry, 52(8), 2341–2351. https://doi.org/10.1021/jm801315b

514. Sitarek, P., Merecz-Sadowska, A., Śliwiński, T., Zajdel, R., & Kowalczyk, T. (2020). An *in vitro* evaluation of the molecular mechanisms of action of medical plants from the Lamiaceae family as effective sources of active compounds against human cancer cell lines. Cancers, 12(10), 2957. https://doi.org/10.3390/cancers12102957

515. Siva, B., Venkanna, A., Poornima, B., Divya Reddy, S., Boustie, J., Bastien, S., Jain, N., Usha Rani, P., & Suresh Babu, K. (2017). New seco-limonoids from Cipadessa baccifera: Isolation, structure determination, synthesis and their antiproliferative activities. Fitoterapia, 117, 34–40. https://doi.org/10.1016/j.fitote.2017.01.003

516. Slatter, T. L., Hung, N., Campbell, H., Rubio, C., Mehta, R., Renshaw, P., Williams, G., Wilson, M., Engelmann, A., Jeffs, A., Royds, J. A., Baird, M. A., & Braithwaite, A. W. (2011). Hyperproliferation, cancer, and inflammation in mice expressing a Δ133p53-like isoform. Blood, 117(19), 5166–5177. https://doi.org/10.1182/blood-2010-11-321851

517. Slika, H., Mansour, H., Wehbe, N., Nasser, S. A., Iratni, R., Nasrallah, G., Shaito, A., Ghaddar, T., Kobeissy, F., & Eid, A. H. (2022). Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 146, 112442. https://doi.org/10.1016/j.biopha.2021.112442

518. Sommers, C. L., Heckford, S. E., Skerker, J. M., Worland, P., Torri, J. A., Thompson, E. W., Byers, S. W., & Gelmann, E. P. (1992). Loss of epithelial markers and acquisition of vimentin expression in adriamycin- and vinblastine-resistant human breast cancer cell lines. Cancer research, 52(19), 5190–5197.

519. Soucheray, M., Capelletti, M., Pulido, I., Kuang, Y., Paweletz, C. P., Becker, J. H., Kikuchi, E., Xu, C., Patel, T. B., Al-Shahrour, F., Carretero, J., Wong, K. K., Jänne, P. A., Shapiro, G. I., & Shimamura, T. (2015). Intratumoral heterogeneity in EGFR-mutant NSCLC results in divergent resistance mechanisms in response to EGFR tyrosine kinase Inhibition. Cancer research, 75(20), 4372–4383. https://doi.org/10.1158/0008-5472.CAN-15-0377

520. Soumya, T., Lakshmipriya, T., Klika, K. D., Jayasree, P. R., & Manish Kumar, P. R. (2021). Anticancer potential of rhizome extract and a labdane diterpenoid from *Curcuma mutabilis* plant endemic to Western Ghats of India. Scientific reports, 11(1), 552. https://doi.org/10.1038/s41598-020-79414-8

521. Spatafora, C., & Tringali, C. (2012). Natural-derived polyphenols as potential anticancer agents. Anti-cancer agents in medicinal chemistry, 12(8), 902–918. https://doi.org/10.2174/187152012802649996

522. Steele, R. J., Thompson, A. M., Hall, P. A., & Lane, D. P. (1998). The p53 tumour suppressor gene. The British journal of surgery, 85(11), 1460–1467. https://doi.org/10.1046/j.1365-2168.1998.00910.x

523. Steinbuch, S. C., Lüß, A. M., Eltrop, S., Götte, M., & Kiesel, L. (2024). Endometriosis-Associated Ovarian Cancer: From Molecular Pathologies to Clinical Relevance. International journal of molecular sciences, 25(8), 4306. https://doi.org/10.3390/ijms25084306

524. Stewart, Z. A., Westfall, M. D., & Pietenpol, J. A. (2003). Cell-cycle dysregulation and anticancer therapy. Trends in pharmacological sciences, 24(3), 139–145. https://doi.org/10.1016/S0165-6147(03)00026-9

525. Stojanović-Radić, Z., Pejčić, M., Dimitrijević, M., Aleksić, A., V Anil Kumar, N., Salehi, B.,& Sharifi-Rad, J. (2019). Piperine-a major principle of black pepper: a review of its bioactivity and studies. Applied Sciences, 9(20), 4270

526. Strober, W. (1997). Monitoring cell growth. Current protocols in immunology, 21(1), A-3A.

527. Suffness, M. (1993). Chapter 32. TaxOL: From Discovery to Therapeutic use. In Annual reports in medicinal chemistry (pp. 305–314). https://doi.org/10.1016/s0065-7743(08)60902-1

528. Sun, H., Liu, L., Lu, J., Qiu, S., Yang, C. Y., Yi, H., & Wang, S. (2010). Cyclopeptide Smac mimetics as antagonists of IAP proteins. Bioorganic & medicinal chemistry letters, 20(10), 3043–3046. https://doi.org/10.1016/j.bmcl.2010.03.114

529. Sun, Y., Sun, F., Jin, J., Xu, W., & Qian, H. (2023). Exosomal LncRNAs in Gastrointestinal Cancer: Biological Functions and Emerging Clinical Applications. Cancers, 15(3), 959. https://doi.org/10.3390/cancers15030959

530. Süntar, I. (2019). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews, 19(5), 1199–1209. https://doi.org/10.1007/s11101-019-09629-9

531. Susheela, T., Balaravi, P., Theophilus, J., Reddy, T. N., & Reddy, P. U. M. (2008). Evaluation of hypoglycaemic and antidiabetic effect of *Melia dubia* CAV fruits in mice. Current Science, 94(9), 1191–1195. http://www.jstor.org/stable/24100701

532. Suttaratrungse, K., Chanhan, P., Kanokmedhakul, K., Moosophon, P., & Kanokmedhakul, S. (2015). Two new flavonols from flowers of *Getonia floribunda* Roxb. Phytochemistry Letters, 11, 316-319.

533. Suzuki, K., & Matsubara, H. (2011). Recent advances in p53 research and cancer treatment. Journal of biomedicine & biotechnology, 2011, 978312. https://doi.org/10.1155/2011/978312

534. Suzuki, K., Shono, F., Kai, H., Uno, T., & Uyeda, M. (2000). Inhibition of topoisomerases by fatty acids. Journal of enzyme inhibition, 15(4), 357–366. https://doi.org/10.1080/14756360009040693

535. Svane, I. M., Pedersen, A. E., Johnsen, H. E., Nielsen, D., Kamby, C., Gaarsdal, E., Nikolajsen, K., Buus, S., & Claesson, M. H. (2004). Vaccination with p53-peptide-pulsed dendritic cells, of patients with advanced breast cancer: report from a phase I study. Cancer immunology, immunotherapy : CII, 53(7), 633–641. https://doi.org/10.1007/s00262-003-0493-5

536. Swanton, C., Bernard, E., Abbosh, C., André, F., Auwerx, J., Balmain, A., Bar-Sagi, D., Bernards, R., Bullman, S., DeGregori, J., Elliott, C., Erez, A., Evan, G., Febbraio, M. A., Hidalgo, A., Jamal-Hanjani, M., Joyce, J. A., Kaiser, M., Lamia, K., Locasale, J. W., … Hanahan, D. (2024). Embracing cancer complexity: Hallmarks of systemic disease. Cell, 187(7), 1589–1616. https://doi.org/10.1016/j.cell.2024.02.009

537. Syed Najmuddin, S. U., Romli, M. F., Hamid, M., Alitheen, N. B., & Nik Abd Rahman, N. M. (2016). Anti-cancer effect of *Annona Muricata* Linn Leaves Crude Extract (AMCE) on breast cancer cell line. BMC complementary and alternative medicine, 16(1), 311. https://doi.org/10.1186/s12906-016-1290-y

538. Takahashi, M., Inafuku, K. I., Miyagi, T., Oku, H., Wada, K., Imura, T., & Kitamoto, D. (2007). Efficient preparation of liposomes encapsulating food materials using lecithins by a mechanochemical method. Journal of Oleo Science, 56(1), 35-42.

539. Tandrasasmita, O. M., Lee, J. S., Baek, S. H., & Tjandrawinata, R. R. (2010). Induction of cellular apoptosis in human breast cancer by DLBS1425, aPhaleria macrocarpacompound extract, via down-regulation of PI3-kinase/AKT pathway. Cancer Biology & Therapy, 10(8), 814–823. https://doi.org/10.4161/cbt.10.8.13085

540. Tang, X. L., Yang, X. Y., Kim, Y. C., Kim, S. Y., Kang, B. D., Choi, D. Y., Kim, O. J., Park, W. C., & Park, H. (2012). Protective effects of the ethanolic extract of *Melia toosendan* fruit against colon cancer. Indian journal of biochemistry & biophysics, 49(3), 173–181.

541. Tanwer, B. S., & Vijayvergia, R. (2014). Biological evaluation of *Alangium salviifolium* (LF) Wangerin. Journal of Chemical and Pharmaceutical Research, 6(12), 611-618.

542. Tayoub, G., Al-Odat, M., Amer, A., Aljapawe, A., & Ekhtiar, A. (2018). Antiproliferative Effects of Pancratium Maritimum Extracts on Normal and Cancerous Cells. Iranian journal of medical sciences, 43(1), 52–64.

543. Teicher, B. A., Holden, S. A., Ara, G., & Chen, G. (1996). Transforming growth factor-beta in in vivo resistance. Cancer chemotherapy and pharmacology, 37(6), 601–609. https://doi.org/10.1007/s002800050435

544. Teo, M. Y. M., Fong, J. Y., Lim, W. M., & In, L. L. A. (2022). Current Advances and Trends in KRAS Targeted Therapies for Colorectal Cancer. Molecular cancer research : MCR, 20(1), 30–44. https://doi.org/10.1158/1541-7786.MCR-21-0248

545. The International Agency for Research on Cancer (IARC). (n.d.). Global Cancer Observatory. https://gco.iarc.who.int/en

546. Thépot, A., Desanlis, A., Venet, E., Thivillier, L., Justin, V., Morel, A. P., Defraipont, F., Till, M., Krutovskikh, V., Tommasino, M., Damour, O., & Hainaut, P. (2011). Assessment of transformed properties in vitro and of tumorigenicity in vivo in primary keratinocytes cultured for epidermal sheet transplantation. Journal of skin cancer, 2011, 936546. https://doi.org/10.1155/2011/936546

547. Thompson, C. A., Zhang, Z. F., & Arah, O. A. (2013). Competing risk bias to explain the inverse relationship between smoking and malignant melanoma. European journal of epidemiology, 28(7), 557–567. https://doi.org/10.1007/s10654-013-9812-0

548. Thozhukkad Moosaripparambil, S., & Vadakkadath Meethal, K. (2024). Unveiling the anticancer potential of *Anamirta cocculus* (L.) Wight& Arn.: Evidences from cytotoxicity studies, apoptosis analysis, and molecular docking. 3 Biotech, 14(10), 245. https://doi.org/10.1007/s13205-024-04096-2

549. Timko Olson, E. R., Olson, A. A., Driscoll, M., & Vermeesch, A. L. (2023). Nature-Based Interventions and Exposure among Cancer Survivors: A Scoping Review. International journal of environmental research and public health, 20(3), 2376. https://doi.org/10.3390/ijerph20032376

550. Timko Olson, E. R., Olson, A. A., Driscoll, M., & Vermeesch, A. L. (2023). Nature-based interventions and exposure among cancer survivors: A scoping review. International journal of environmental research and public health, 20(3), 2376. https://doi.org/10.3390/ijerph20032376

551. Timmerman, L. A., Grego-Bessa, J., Raya, A., Bertrán, E., Pérez-Pomares, J. M., Díez, J., Aranda, S., Palomo, S., McCormick, F., Izpisúa-Belmonte, J. C., & de la Pompa, J. L. (2004). Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes & development, 18(1), 99–115. https://doi.org/10.1101/gad.276304

552. To, K. K. W., & Cho, W. C. S. (2021). Flavonoids Overcome Drug Resistance to Cancer Chemotherapy by Epigenetically Modulating Multiple Mechanisms. Current

cancer drug targets, 21(4), 289–305. https://doi.org/10.2174/1568009621666210203111220

553. Toden, S., Okugawa, Y., Jascur, T., Wodarz, D., Komarova, N. L., Buhrmann, C., Shakibaei, M., Boland, C. R., & Goel, A. (2015). Curcumin mediates chemosensitization to 5-fluorouracil through miRNA-induced suppression of epithelial-to-mesenchymal transition in chemoresistant colorectal cancer. Carcinogenesis, 36(3), 355–367. https://doi.org/10.1093/carcin/bgv006

554. Toh, J. W. T., & Wilson, R. B. (2020). Pathways of Gastric Carcinogenesis, Helicobacter pylori Virulence and Interactions with Antioxidant Systems, Vitamin C and Phytochemicals. International journal of molecular sciences, 21(17), 6451. https://doi.org/10.3390/ijms21176451

555. Tringale, K. R., Pang, J., & Nguyen, Q. T. (2018). Image-guided surgery in cancer: A strategy to reduce incidence of positive surgical margins. Wiley interdisciplinary reviews. Systems biology and medicine, 10(3), e1412. https://doi.org/10.1002/wsbm.1412

556. Trott, O., Olson, A. J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 31(2), 455–461. https://doi.org/10.1002/jcc.21334

557. Trujillo-Alonso, V., Pratt, E. C., Zong, H., Lara-Martinez, A., Kaittanis, C., Rabie, M. O., Longo, V., Becker, M. W., Roboz, G. J., Grimm, J., & Guzman, M. L. (2019). FDA-approved ferumoxytol displays anti-leukaemia efficacy against cells with low ferroportin levels. Nature nanotechnology, 14(6), 616–622. https://doi.org/10.1038/s41565-019-0406-1

558. Trung, H. T., Purnomo, K. A., Yu, S. Y., Yang, Z. J., Hu, H. C., Hwang, T. L., Tuan, N. N., Tu, L. N., Duc, D. X., Quang, L. D., Backlund, A., Thang, T. D., & Chang, F. R. (2023). Anti-inflammatory and Antiphytopathogenic Fungal Activity of 2,3-seco-Tirucallane Triterpenoids Meliadubins A and B from Melia dubia Cav. Barks with ChemGPS-NP and In Silico Prediction. ACS omega, 8(40), 37116–37127. https://doi.org/10.1021/acsomega.3c04657

559. Tsimberidou, A. M. (2015). Targeted therapy in cancer. Cancer chemotherapy and pharmacology, 76, 1113-1132.

560. Tufail, M., Huang, Y. Q., Hu, J. J., Liang, J., He, C. Y., Wan, W. D., Jiang, C. H., Wu, H., & Li, N. (2024). Cellular Aging and Senescence in Cancer: A Holistic Review of Cellular Fate Determinants. Aging and disease, 10.14336/AD.2024.0421. Advance online publication. https://doi.org/10.14336/AD.2024.0421

561. Turgeon, M., Perry, N. J. S., & Poulogiannis, G. (2018). DNA damage, repair, and cancer metabolism. Frontiers in Oncology, 8. https://doi.org/10.3389/fonc.2018.00015

562. Tyagi, A. K., Prasad, S., Yuan, W., Li, S., & Aggarwal, B. B. (2015). Identification of a novel compound (β-sesquiphellandrene) from turmeric (Curcuma longa) with anticancer potential: comparison with curcumin. Investigational new drugs, 33(6), 1175–1186. https://doi.org/10.1007/s10637-015-0296-5

563. Ulrich-Merzenich, G., Panek, D., Zeitler, H., Vetter, H., & Wagner, H. (2010). Drug development from natural products: exploiting synergistic effects. Indian journal of experimental biology, 48(3), 208–219.

564. Umezawa H. (1982). Low-molecular-weight enzyme inhibitors of microbial origin. Annual review of microbiology, 36, 75–99. https://doi.org/10.1146/annurev.mi.36.100182.000451

565. Umthong, S., Phuwapraisirisan, P., Puthong, S., & Chanchao, C. (2011). In vitro antiproliferative activity of partially purified Trigona laeviceps propolis from Thailand on human cancer cell lines. BMC complementary and alternative medicine, 11, 37. https://doi.org/10.1186/1472-6882-11-37

566. Uramoto, H., Iwata, T., Onitsuka, T., Shimokawa, H., Hanagiri, T., & Oyama, T. (2010). Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma. Anticancer research, 30(7), 2513–2517.

567. Valastyan, S., & Weinberg, R. A. (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell, 147(2), 275–292. https://doi.org/10.1016/j.cell.2011.09.024

568. Valentina, P., Ilango, K., Kiruthiga, B., & Parimala, M. J. (2013). Preliminary phytochemical analysis and biological screening of extracts of leaves of Mella dubia Cav.International Journal of Research in Ayurveda and Pharmacy, 4(3), 417–419. https://doi.org/10.7897/2277-4343.04322

569. Valter, K., Chen, L., Kruspig, B., Maximchik, P., Cui, H., Zhivotovsky, B., & Gogvadze, V. (2017). Contrasting effects of glutamine deprivation on apoptosis induced by conventionally used anticancer drugs. Biochimica et biophysica acta. Molecular cell research, 1864(3), 498–506. https://doi.org/10.1016/j.bbamcr.2016.12.016

570. van den Born, B. J., van der Hoeven, N. V., Groot, E., Lenting, P. J., Meijers, J. C., Levi, M., & van Montfrans, G. A. (2008). Association between thrombotic microangiopathy and reduced ADAMTS13 activity in malignant hypertension. Hypertension (Dallas, Tex. : 1979), 51(4), 862–866. https://doi.org/10.1161/HYPERTENSIONAHA.107.103127

571. Vazquez-Martin, A., Oliveras-Ferraros, C., Cufí, S., Del Barco, S., Martin-Castillo, B., & Menendez, J. A. (2010). Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial-mesenchymal transition (EMT) status. Cell cycle (Georgetown, Tex.), 9(18), 3807–3814.

572. Venditto, V. J., Haydar, D., Abdel-Latif, A., Gensel, J. C., Anstead, M. I., Pitts, M. G., Creameans, J., Kopper, T. J., Peng, C., & Feola, D. J. (2021). Immunomodulatory Effects of Azithromycin Revisited: Potential Applications to COVID-19. Frontiers in immunology, 12, 574425. https://doi.org/10.3389/fimmu.2021.574425

573. Venkateshwarlu, R., Gopal, Y. V., Raju, A. B., & Prasad, K. B. (2012). Antitumor activity of Alangium salvifolium against Dalton's ascitic Lymphoma. *Medicinal Chemistry & Drug Discovery*, *3*(2), 122-33.\

574. Vermeij, R., Leffers, N., van der Burg, S. H., Melief, C. J., Daemen, T., & Nijman, H. W. (2011). Immunological and clinical effects of vaccines targeting p53-overexpressing malignancies. Journal of biomedicine & biotechnology, 2011, 702146. https://doi.org/10.1155/2011/702146

575. Verpoorte, R. (2005). Alkaloids. In Elsevier eBooks (pp. 56–61). https://doi.org/10.1016/b0-12-369397-7/00010-8

576. Vignais, M., & Fafet, P. (2013). TGFB-dependent Epithelial-Mesenchymal Transition. Madame Curie Bioscience Database - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK6525/

577. Vijayan, N., Swapna, T. S., Seghal Kiran, G., & Abdulhameed, S. (2017). Medicinal plants of India: Implications in modern medicine. Bioresources and Bioprocess in Biotechnology: Volume 1: Status and Strategies for Exploration, 421-443.

578. Vijayarathna, S., Oon, C. E., Chen, Y., Kanwar, J. R., & Sasidharan, S. (2017). *Polyalthia longifolia* Methanolic Leaf Extracts (PLME) induce apoptosis, cell cycle arrest and mitochondrial potential depolarization by possibly modulating the redox status in hela cells. Biomedicine & Pharmacotherapy, 89, 499–514. https://doi.org/10.1016/j.biopha.2017.02.075

579. Vikhanskaya, F., Lee, M. K., Mazzoletti, M., Broggini, M., & Sabapathy, K. (2007). Cancer-derived p53 mutants suppress p53-target gene expression--potential mechanism for gain of function of mutant p53. Nucleic acids research, 35(6), 2093–2104. https://doi.org/10.1093/nar/gkm099

580. Visvader, J. E. (2011). Cells of origin in cancer. Nature, 469(7330), 314–322. https://doi.org/10.1038/nature09781

581. Vllasaliu, D., Fowler, R., & Stolnik, S. (2014). PEGylated nanomedicines: recent progress and remaining concerns. *Expert opinion on drug delivery*, *11*(1), 139-154.

582. Vucic, D., & Fairbrother, W. J. (2007). The inhibitor of apoptosis proteins as therapeutic targets in cancer. Clinical cancer research : an official journal of the American Association for Cancer Research, 13(20), 5995–6000. https://doi.org/10.1158/1078-0432.CCR-07-0729

583. Wagner, K. D., & Wagner, N. (2022). The Senescence markers p16INK4A, p14ARF/p19ARF, and p21 in organ development and homeostasis. Cells, 11(12), 1966. https://doi.org/10.3390/cells11121966

584. Wajdy, J., Tayseer, A., & Dheyaa, K. (2021). Constituents, pharmacological and toxicological effects of *Melia azadirachta*-A review. Sapporo Med. J, 55.

585. Wall, M. E., & Wani, M. C. (1996). Camptothecin. Discovery to clinic. Annals of the New York Academy of Sciences, 803, 1–12. https://doi.org/10.1111/j.1749-6632.1996.tb26371.x

586. Wang X, Zhuang Y, Wang Y, Jiang M, Yao L. The recent developments of camptothecin and its derivatives as potential anti-tumor agents. Eur J Med Chem. 2023 Nov 15;260:115710. doi: 10.1016/j.ejmech.2023.115710. Epub 2023 Aug 4. PMID: 37595544

587. Wang, C., & Youle, R. J. (2009). The role of mitochondria in apoptosis*. Annual review of genetics, 43, 95–118. https://doi.org/10.1146/annurev-genet-102108-134850

588. Wang, D., Tang, L., Wu, Y., Fan, C., Zhang, S., Xiang, B., Zhou, M., Li, X., Li, Y., Li, G., Xiong, W., Zeng, Z., & Guo, C. (2020). Abnormal X chromosome inactivation and tumor development. Cellular and molecular life sciences : CMLS, 77(15), 2949–2958. https://doi.org/10.1007/s00018-020-03469-z

589. Wang, G., Feng, C. C., Chu, S. J., Zhang, R., Lu, Y. M., Zhu, J. S., & Zhang, J. (2015). Toosendanin inhibits growth and induces apoptosis in colorectal cancer cells through suppression of AKT/GSK-3β/β-catenin pathway. International journal of oncology, 47(5), 1767–1774. https://doi.org/10.3892/ijo.2015.3157

590. Wang, H., Zhou, Y., Acosta, J. A. T., & Fowke, L. C. (2007). CDK inhibitors. Annual Plant Reviews Volume 32: Cell Cycle Control and Plant Development, 62-86.

591. Wang, L. S., Huang, Y. W., Liu, S., Chang, H. L., Ye, W., Shu, S., Sugimoto, Y., Funk, J. A., Smeaks, D. D., Hill, L. N., & Lin, Y. C. (2006). Conjugated linoleic acid (CLA) modulates prostaglandin E2 (PGE2) signaling in canine mammary cells. Anticancer research, 26(2A), 889–898.

592. Wang, S. S., Jiang, J., Liang, X. H., & Tang, Y. L. (2015). Links between cancer stem cells and epithelial-mesenchymal transition. OncoTargets and therapy, 8, 2973–2980. https://doi.org/10.2147/OTT.S91863

593. Wang, Y. C., & Bachrach, U. (2002). The specific anti-cancer activity of green tea (-)-epigallocatechin-3-gallate (EGCG). Amino acids, 22(2), 131–143. https://doi.org/10.1007/s007260200002

594. Wang, Y., Cui, R., Xiao, Y., Fang, J., & Xu, Q. (2015). Effect of carotene and lycopene on the risk of prostate cancer: a systematic review and dose-response meta-analysis of observational studies. PloS one, 10(9), e0137427. https://doi.org/10.1371/journal.pone.0137427

595. Wani, M.Y., Malik, M.A. (2021). Cancer. In: Gold and its complexes in anticancer chemotherapy. Springer, Singapore. https://doi.org/10.1007/978-981-33-6314-4_1

596. Weaver B. A. (2014). How Taxol/paclitaxel kills cancer cells. Molecular biology of the cell, 25(18), 2677–2681. https://doi.org/10.1091/mbc.E14-04-0916

597. Wee, P., & Wang, Z. (2017). Epidermal growth factor receptor cell proliferation signaling pathways. Cancers, 9(5), 52. https://doi.org/10.3390/cancers9050052

598. Wei, Y. M., Zhang, X., Cen, F. L., Du, Y. Y., Liu, Y. L., Han, Q. Y., Yu, Z. X., & Chen, G. Y. (2024). Structurally diverse isoquinoline alkaloids from the barks of *Alangium salviifolium* (L. f.) Wangerin and their cytotoxicity. Phytochemistry, 223, 114139. https://doi.org/10.1016/j.phytochem.2024.114139

599. Weigelt, B., Glas, A. M., Wessels, L. F., Witteveen, A. T., Peterse, J. L., & van't Veer, L. J. (2003). Gene expression profiles of primary breast tumors maintained in distant metastases. Proceedings of the National Academy of Sciences of the United

States of America, 100(26), 15901–15905. https://doi.org/10.1073/pnas.2634067100

600. Weinberg, E., Maymon, T., & Weinreb, M. (2014). AGEs induce caspase-mediated apoptosis of rat BMSCs via TNFα production and oxidative stress. Journal of molecular endocrinology, 52(1), 67–76. https://doi.org/10.1530/JME-13-0229

601. Welch, D. R., & Hurst, D. R. (2019). Defining the hallmarks of metastasis. Cancer research, 79(12), 3011-3027.

602. Wong R. S. (2011). Apoptosis in cancer: from pathogenesis to treatment. Journal of experimental & clinical cancer research: CR, 30(1), 87. https://doi.org/10.1186/1756-9966-30-87

603. World Health Organization: WHO. (2023, January 30). Integrating traditional medicine in health care. https://www.who.int/southeastasia/news/feature-stories/detail/integrating-traditional-medicine#:~:text=More%20than%2080%25%20of%20the,and%20acupressure%2C%20and%20indigenous%20therapies.

604. World Health Organization: WHO. (2023b, August 10). Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise. https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise

605. Wu, J., Zhang, H., Xu, Y., Zhang, J., Zhu, W., Zhang, Y., Chen, L., Hua, W., & Mao, Y. (2017). Juglone induces apoptosis of tumor stem-like cells through ROS-p38 pathway in glioblastoma. BMC neurology, 17(1), 70. https://doi.org/10.1186/s12883-017-0843-0

606. Wu, S. B., Su, J. J., Sun, L. H., Wang, W. X., Zhao, Y., Li, H., Zhang, S. P., Dai, G. H., Wang, C. G., & Hu, J. F. (2010). Triterpenoids and steroids from the fruits of *Melia toosendan* and their cytotoxic effects on two human cancer cell lines. Journal of natural products, 73(11), 1898–1906. https://doi.org/10.1021/np100566v

607. Wu, W. K., Wang, X. J., Cheng, A. S., Luo, M. X., Ng, S. S., To, K. F., Chan, F. K., Cho, C. H., Sung, J. J., & Yu, J. (2013). Dysregulation and crosstalk of cellular signaling pathways in colon carcinogenesis. Critical reviews in oncology/hematology, 86(3), 251–277. https://doi.org/10.1016/j.critrevonc.2012.11.009

608. Wu, X., Liu, X., Sengupta, J., Bu, Y., Yi, F., Wang, C., Shi, Y., Zhu, Y., Jiao, Q., & Song, F. (2011). Silencing of Bmi-1 gene by RNA interference enhances sensitivity to doxorubicin in breast cancer cells. Indian journal of experimental biology, 49(2), 105–112.

609. Wu, Y., Ginther, C., Kim, J., Mosher, N., Chung, S., Slamon, D., & Vadgama, J. V. (2012). Expression of Wnt3 activates Wnt/β-catenin pathway and promotes EMT-like phenotype in trastuzumab-resistant HER2-overexpressing breast cancer cells. Molecular cancer research: MCR, 10(12), 1597–1606. https://doi.org/10.1158/1541-7786.MCR-12-0155-T

610. Wu, Z., Li, S., & Zhu, X. (2021). The mechanism of stimulating and mobilizing the immune system enhancing the anti-tumor immunity. Frontiers in immunology, 12, 682435.

611. Xie, M., He, C. S., Wei, S. H., & Zhang, L. (2013). Notch-1 contributes to epidermal growth factor receptor tyrosine kinase inhibitor acquired resistance in non-small cell lung cancer *in vitro* and *in vivo*. European journal of cancer (Oxford, England : 1990), 49(16), 3559–3572. https://doi.org/10.1016/j.ejca.2013.07.007

612. Xiu, Z., Zhu, Y., Han, J., Li, Y., Yang, X., Yang, G., Song, G., Li, S., Li, Y., Cheng, C., Li, Y., Fang, J., Li, X., & Jin, N. (2022). Caryophyllene oxide induces ferritinophagy by regulating the NCOA4/FTH1/LC3 pathway in hepatocellular carcinoma. Frontiers in pharmacology, 13, 930958. https://doi.org/10.3389/fphar.2022.930958

613. Yahfoufi, N., Alsadi, N., Jambi, M., & Matar, C. (2018). The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients, 10(11), 1618. https://doi.org/10.3390/nu10111618

614. Yallapu, M. M., Nagesh, P. K., Jaggi, M., & Chauhan, S. C. (2015). Therapeutic Applications of Curcumin Nanoformulations. The AAPS journal, 17(6), 1341–1356. https://doi.org/10.1208/s12248-015-9811-z

615. Yamabe, K., Shimizu, S., Ito, T., Yoshioka, Y., Nomura, M., Narita, M., Saito, I., Kanegae, Y., & Matsuda, H. (1999). Cancer gene therapy using a pro-apoptotic gene, *caspase-3*. Gene therapy, 6(12), 1952–1959. https://doi.org/10.1038/sj.gt.3301041

616. Yamasaki, M., Chujo, H., Koga, Y., Oishi, A., Rikimaru, T., Shimada, M., Sugimachi, K., Tachibana, H., & Yamada, K. (2002). Potent cytotoxic effect of the trans10, cis12 isomer of conjugated linoleic acid on rat hepatoma dRLh-84 cells. Cancer letters, 188(1-2), 171–180. https://doi.org/10.1016/s0304-3835(02)00459-7

617. Yang, B., Chen, R., Liang, X., Shi, J., Wu, X., Zhang, Z., & Chen, X. (2019). Estrogen enhances endometrial cancer cells proliferation by upregulation of prohibitin. Journal of Cancer, 10(7), 1616–1621. https://doi.org/10.7150/jca.28218

618. Yang, C. T., Li, J. M., Weng, H. H., Li, Y. C., Chen, H. C., & Chen, M. F. (2010). Adenovirus-mediated transfer of siRNA against survivin enhances the radiosensitivity of human non-small cell lung cancer cells. Cancer gene therapy, 17(2), 120–130. https://doi.org/10.1038/cgt.2009.55

619. Yap, T. A., & Workman, P. (2012). Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. Annual review of pharmacology and toxicology, 52, 549–573. https://doi.org/10.1146/annurev-pharmtox-010611-134532

620. Yates, L. R., Knappskog, S., Wedge, D., Farmery, J. H. R., Gonzalez, S., Martincorena, I., Alexandrov, L. B., Van Loo, P., Haugland, H. K., Lilleng, P. K., Gundem, G., Gerstung, M., Pappaemmanuil, E., Gazinska, P., Bhosle, S. G., Jones, D., Raine, K., Mudie, L., Latimer, C., Sawyer, E., … Campbell, P. J. (2017). Genomic Evolution of Breast Cancer Metastasis and Relapse. Cancer cell, 32(2), 169–184.e7. https://doi.org/10.1016/j.ccell.2017.07.005

621. Ye, Y. X., Zhou, J., Zhou, Y. H., Zhou, Y., Song, X. B., Wang, J., Lin, L., Ying, B. W., & Lu, X. J. (2014). Clinical significance of BCR-ABL fusion gene subtypes in chronic myelogenous and acute lymphoblastic leukemias. Asian Pacific journal of cancer prevention APJCP, 15(22), 9961–9966. https://doi.org/10.7314/apjcp.2014.15.22.9961

622. Yip, H. Y. K., & Papa, A. (2021). Signaling Pathways in Cancer: Therapeutic Targets, Combinatorial Treatments, and New Developments. *Cells*, *10*(3), 659. https://doi.org/10.3390/cells10030659

623. Yip, K. W., & Reed, J. C. (2008). Bcl-2 family proteins and cancer. Oncogene, 27(50), 6398–6406. https://doi.org/10.1038/onc.2008.307

624. Yoneda, T., Imaizumi, K., Oono, K., Yui, D., Gomi, F., Katayama, T., & Tohyama, M. (2001). Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. The Journal of biological chemistry, 276(17), 13935–13940. https://doi.org/10.1074/jbc.M010677200

625. Yoo, Y. A., Kang, M. H., Lee, H. J., Kim, B. H., Park, J. K., Kim, H. K., Kim, J. S., & Oh, S. C. (2011). Sonic hedgehog pathway promotes metastasis and lymphangiogenesis via activation of Akt, EMT, and MMP-9 pathway in gastric cancer. Cancer research, 71(22), 7061–7070. https://doi.org/10.1158/0008-5472.CAN-11-1338

626. Yook, J. I., Li, X. Y., Ota, I., Fearon, E. R., & Weiss, S. J. (2005). Wnt-dependent regulation of the E-cadherin repressor snail. The Journal of biological chemistry, 280(12), 11740–11748. https://doi.org/10.1074/jbc.M413878200

627. Yoshida, T., Song, L., Bai, Y., Kinose, F., Li, J., Ohaegbulam, K. C., Muñoz-Antonia, T., Qu, X., Eschrich, S., Uramoto, H., Tanaka, F., Nasarre, P., Gemmill, R. M., Roche, J., Drabkin, H. A., & Haura, E. B. (2016). ZEB1 Mediates Acquired Resistance to the Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer. PloS one, 11(1), e0147344. https://doi.org/10.1371/journal.pone.0147344

628. Youssef, A. M. M., Maaty, D. A. M., & Al-Saraireh, Y. M. (2023). Phytochemical Analysis and Profiling of Antioxidants and Anticancer Compounds from *Tephrosia purpurea* (L.) subsp. *apollinea* Family Fabaceae. Molecules (Basel, Switzerland), 28(9), 3939. https://doi.org/10.3390/molecules28093939

629. Youssef, A. M. M., Maaty, D. A. M., & Al-Saraireh, Y. M. (2023). Phytochemical Analysis and Profiling of Antitumor Compounds of Leaves and Stems of *Calystegia silvatica* (Kit.) Griseb. Molecules (Basel, Switzerland), 28(2), 630. https://doi.org/10.3390/molecules28020630

630. Yu, F., Lu, S., Yu, F., Shi, J., McGuire, P. M., & Wang, R. (2008). Cytotoxic activity of an octadecenoic acid extract from *Euphorbia kansui* (Euphorbiaceae) on human tumour cell strains. The Journal of pharmacy and pharmacology, 60(2), 253–259. https://doi.org/10.1211/jpp.60.2.0015

631. Yu, Q., Zhang, K., Wang, X., Liu, X., & Zhang, Z. (2010). Expression of transcription factors snail, slug, and twist in human bladder carcinoma. Journal of

experimental & clinical cancer research: CR, 29(1), 119. https://doi.org/10.1186/1756-9966-29-119

632. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. Molecules (Basel, Switzerland), 21(5), 559. https://doi.org/10.3390/molecules21050559

633. Yukimune, Y., Hara, Y., Nomura, E., Seto, H., & Yoshida, S. (2000). The configuration of methyl jasmonate affects paclitaxel and baccatin III production in Taxus cells. Phytochemistry, 54(1), 13–17. https://doi.org/10.1016/s0031-9422(00)00006-6

634. Yusuf, H., Husna, F., Gani, B. A., & Garrido, G. (2021). The chemical composition of the ethanolic extract from Chromolaena odorata leaves correlates with the cytotoxicity exhibited against colorectal and breast cancer cell lines. Journal of Pharmacy & Pharmacognosy Research, 9(3), 344-356.

635. Zeimet, A. G., Reimer, D., Radl, A. C., Reinthaller, A., Schauer, C., Petru, E., Concin, N., Braun, S., & Marth, C. (2009). Pros and cons of intraperitoneal chemotherapy in the treatment of epithelial ovarian cancer. Anticancer research, 29(7), 2803–2808.

636. Zhang JY, Huang WJ, Sun HM, Liu Y, Zhao XQ, Tang SL, Sun MN, Wang S, Li JJ, Zhang LL, Zhou JH, Pan QR, Chen HB (2017) Structure Identification, and In Vitro Anticancer Activity of Lathyrol-3-phenylacetate-5,15-diacetate. Molecules (Basel, Switzerland) 22(9):1412. https://doi.org/10.3390/molecules22091412

637. Zhang, J. H., & Xu, M. (2000). DNA fragmentation in apoptosis. Cell research, 10(3), 205–211. https://doi.org/10.1038/sj.cr.7290049

638. Zhang, X., Li, N., Wang, Y. H., Huang, Y., Xu, N. Z., & Wu, L. Y. (2009). Zhonghua zhong liu za zhi [Chinese journal of oncology], 31(3), 174–177.

639. Zhang, Y., Tang, C., Ke, C., Li, X., Xie, H., & Ye, Y. (2011). Limonoids from the fruits of *Melia toosendan*. Phytochemistry, 73, 106–113. https://doi.org/10.1016/j.phytochem.2011.10.001

640. Zhang, Z., Wang, C. Z., Du, G. J., Qi, L. W., Calway, T., He, T. C., Du, W., & Yuan, C. S. (2013). Genistein induces G2/M cell cycle arrest and apoptosis via ATM/p53-dependent pathway in human colon cancer cells. International journal of oncology, 43(1), 289–296. https://doi.org/10.3892/ijo.2013.1946

641. Zhao, X., Zhu, Y., Hu, J., Jiang, L., Li, L., Jia, S., & Zen, K. (2018). Shikonin Inhibits Tumor Growth in Mice by Suppressing Pyruvate Kinase M2-mediated Aerobic Glycolysis. Scientific reports, 8(1), 14517. https://doi.org/10.1038/s41598-018-31615-y

642. Zhao, Z., Cheng, X., Wang, Y., Han, R., Li, L., Xiang, T., He, L., Long, H., Zhu, B., & He, Y. (2014). Metformin inhibits the IL-6-induced epithelial-mesenchymal transition and lung adenocarcinoma growth and metastasis. PloS one, 9(4), e95884. https://doi.org/10.1371/journal.pone.0095884

643. Zheng, X., Carstens, J. L., Kim, J., Scheible, M., Kaye, J., Sugimoto, H., Wu, C. C., LeBleu, V. S., & Kalluri, R. (2015). Epithelial-to-mesenchymal transition is

dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature, 527(7579), 525–530. https://doi.org/10.1038/nature16064

644. Zhou, Y., Liang, C., Xue, F., Chen, W., Zhi, X., Feng, X., Bai, X., & Liang, T. (2015). Salinomycin decreases doxorubicin resistance in hepatocellular carcinoma cells by inhibiting the β-catenin/TCF complex association via FOXO3a activation. Oncotarget, 6(12), 10350–10365. https://doi.org/10.18632/oncotarget.3585

645. Zhou, Z., Wang, C., Liu, H., Huang, Q., Wang, M., & Lei, Y. (2014). Retraction: Cadmium induced cell apoptosis, DNA damage, decreased DNA repair capacity, and genomic instability during malignant transformation of human bronchial epithelial cells. International journal of medical sciences, 11(3), 246. https://doi.org/10.7150/ijms.8533

646. Zhu L. (2005). Tumour suppressor retinoblastoma protein Rb: a transcriptional regulator. European journal of cancer (Oxford, England : 1990), 41(16), 2415–2427. https://doi.org/10.1016/j.ejca.2005.08.009

647. Zia, A., Farkhondeh, T., Pourbagher-Shahri, A. M., & Samarghandian, S. (2021). The role of curcumin in aging and senescence: Molecular mechanisms. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 134, 111119. https://doi.org/10.1016/j.biopha.2020.111119

Presentations and Publications

PRESENTATIONS

- Presented a paper in the International Conference on the role of AI and Multidisciplinary Research in Shaping a Sustainable Future on the title "A preliminary investigation of the synergistic effect of methotrexate and glucosamine on human colon adenocarcinoma cell line, HT29" in 2024.
- Presented a paper in the 42nd Annual conference of the Society of Toxicology, India (STOX 2023) on “Differential cytotoxicity of *Anamirta cocculus* (L.) Wight & Arn. leaf extracts on cancer cell lines” in 2023.
- Presented a paper in the international conference on Recent Innovations in Biological Research (ICRIBR) entitled “In-vitro cytotoxic, antioxidant and GC-MS studies on *Hewittia malabarica* L. Suresh” in 2022.
- Presented a paper entitled “Cytotoxicity of selected plant extracts on HT29, colorectal carcinoma cell line” in the International virtual conference on biological innovations and computational exploration for Pandemic challenges (BICPAC’22) in 2022.