HOLISTIC APPROACH ON BIOACTIVITIES, PHYTOCHEMICALS, NANOPARTICLE BIOSYNTHESIS AND ENDOPHYTE ISOLATION FROM TWO *FICUS* SPECIES

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DOCTOR OF PHILOSOPHY IN BOTANY

by

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CERTIFICATE

This is to certify that the thesis entitled **"Holistic approach on bioactivities, phytochemicals, nanoparticle biosynthesis and endophyte isolation from two *Ficus* species"** submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is a record of original research work done by **Nushiba Naser P. T.,** during the period of study (2019-2024) at the Cell and Molecular Biology Division, Department of Botany, University of Calicut under my supervision and guidance and it has not formed the basis for the award of any degree or diploma. I also certify that the contents in the thesis are subjected to plagiarism check using the software iThenticate and no text or data is reproduced from other works.

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Dr. John E. Thoppil
Supervising Teacher

DECLARATION

I, Nushiba Naser P. T., hereby declare that the thesis entitled **"Holistic approach on bioactivities, phytochemicals, nanoparticle biosynthesis and endophyte isolation from two *Ficus* species"** submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is a record of original research work done by me under the supervision and guidance of Dr. John E. Thoppil, Senior Professor, Department of Botany, University of Calicut and that it has not formed the basis for the award of any degree/diploma to any candidate of any University. I also declare that this thesis is free from AI generated contents.

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ABSTRACT

Holistic approach on bioactivities, phytochemicals, nanoparticle biosynthesis and endophyte isolation from two *Ficus* species

Ficus plants are known for their therapeutic benefits and symbolic significance in religion and culture. They have played a significant role in ancient indigenous medical methods, such as Ayurveda, Siddha, Unani, and Homoeopathy. The fruit of various *Ficus* plants, particularly the fig *Ficus carica*, is rich in antioxidants and health-benefitting polyphenols. Members of this genus contribute to various medicines in ayurveda, such as "Pancha Valkala Kashaya". However, most research focuses on a small number of plants, so there are a number of *Ficus* species that are overlooked. *Ficus drupacea* Thunb. and *Ficus exasperata* Vahl, comes under this type of plants. The present study encompasses two different phases and each phase contain various parts. The first phase contains major three parts such as phytochemical profiling, bioactivity studies and silver nano particle's formulation. The second phase contain three parts in which isolation and identification of the fruit fungal endophytes from the two species is the first part, biochemical analysis of the fruit fungal endophytes comes as the second part and nanoparticle biosynthesis and associated studies is the third part.

Phytochemical assays were conducted as two part using preliminary studies with three solvents based on their polarity *viz* hexane, methanol and water. Polar solvents were good at eluting various phytochemical groups and so among the polar solvents water was selected due to its nontoxic nature towards biological system. Phytochemical delineation of the four aqueous extracts such as leaf and fruit of *F. drupacea* and *F. exasperata* revealed ninety-two non-volatile and ten volatile compounds, which falls under the major phytochemical groups, alkaloids, phenols, terpenoids, glycosides etc. Major two activities such as antioxidant and anticancer activities were studied using the extracts. In the antioxidant study four important in vitro antioxidant assays such as DPPH radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay and superoxide radical scavenging assays were conducted. Even though concentration dependent variation, ie., (an increasing percentage inhibition with increasing extract concentration) was reported, over all ability to scavenge free radicals seems to be moderate with the four-extracts used in the study. Anticancer activity was also tested using the four aqueous extracts on MCF-7 breast cancer cell lines. Toxicity studies were also conducted on the normal

cell line L929 to assess the ability of the extract to distinguish and save the normal cells while harming only the cancer cells. Anticancer assay revealed potential anticancer activity with the four extracts. Among the four extracts, *F. drupacea* fruit extract was found to be very effective with its anticancer ability specifically to target the cancer cells and giving least cytotoxic effects on the normal cell lines. This extract was chosen for further studies. Apoptosis detection was done using double stain method as well as comet assay. The study revealed that the cause of antiproliferative action was due to apoptosis but not necrosis. Studies continued to analyze the ability of the fruit extract in causing cell cycle arrest and gene modulation. Cell cycle arrest was reported with the G1 phase which was supported by the cell cycle regulation assay by flow cytometry. The gene expression studies on both p53 and STAT 3 further confirmed the anticancer activity of the cells with increased expression of P53 and down regulation of STAT 3 which were the basis of apoptosis and cell cycle arrest.

The third part of the first phase was bio fabrication of nanoparticle from the four aqueous extracts (leaf and fruit extracts of *F. exasperata* and *F. drupacea*). Silver nanoparticles were readily synthesized from the four-extract considered in the current study. Visual indication of colour change was further confirmed by the UV-vis spectroscopy as well as SEM imaging. The nanoparticles biosynthesized were able to produce a constitutive peak for silver in the UV- vis range and nearly spherical shaped nanoparticles that falls under the size range of 20-50 nm were produced. When these nanoparticles were tested for their antimicrobial efficacy, they seem to be better antimicrobial agents in the higher concentration and they could produce zone of inhibition nearly comparable to the positive control used in the study.

In phase two the study revealed the presence of fungal endophytes from the two plants both from fruits and leaves. 11 fungal endophytes were isolated from the leaves and fruits. Fruits were reported to have a single endophyte which is similar for both *F. exasperata* as well as *F. drupacea*. All the 10 fungal endophytes from the leaves of both plants were identified to the species level using the BLAST analysis based on ITS and LSU region. All the fungal isolates were from the division Basidiomycota and Ascomycota. Fruit fungal endophyte of *F. drupacea* was subjected to phytochemical profiling and 34 compounds were reported. This fungal extract was used in the synthesis of gold and silver nanoparticles. These nanoparticles were found to have anti-microbial action against two gram negative bacteria *E. coli* and *Salmonella paratyphi.*

സംഗ്രഹം

രണ്ട് ഫൈക്കസ് സ്പീഷീസുകളിൽ നിന്നുമുള്ള ബയോആക്ടിവിറ്റികൾ ഫൈറ്റോകെമിക്കൽസ്, നാനോപാർട്ടിക്കിൾ ബയോസിന്തസിസ് എൻഡോഫൈറ്റ് ഐസോലേഷൻ എന്നിവയെക്കുറിച്ചുള്ള സമഗ്ര പഠനം.

ഫൈക്കസ് സസ്യങ്ങൾ അവയുടെ ചികിത്സാഗുണങ്ങൾക്കും മതത്തിലും സംസ്കാരത്തിലുമുള്ള പ്രാധാന്യത്തിനും പേരുകേട്ടവയാണ്. ആയുർവേദം, യുനാനി, സിദ്ധ തുടങ്ങിയ പ്രാചീന തദ്ദേശീയ ചികിത്സാരീതികളിൽ അവയ്ക്ക് ഒരു പ്രധാന പങ്കുണ്ട്. ഫൈക്കസ് ഗണത്തിൽപ്പെടുന്ന അംഗങ്ങൾ ആയുർവേദത്തിലെ 'പഞ്ചവൽക്കല കഷായം' പോലുള്ള വിവിധ മരുന്നുകൾക്ക് ഒഴിച്ചു കൂടാനാവാത്തതാണ്. (വിവിധ ആൽ മരങ്ങളുടെ പഴങ്ങൾ, പ്രത്യേകിച്ച് ഫൈക്കസ് കാരിക്കയുടെ പഴമായ അത്തിപ്പഴം, ആന്റി ഓകാസിഡന്റുകളാലും ആരോഗ്യദായകമായ പോളി ഫിനോളുകളാലും സമ്പുഷ്ടമാണ്). എന്നിരുന്നാലും ഈ വിഭാഗത്തിലെ ചെടികളെക്കുറിച്ചുള്ള പഠനം ചുരുക്കം ചില സസ്യങ്ങളിൽ മാത്രം ശ്രദ്ധ കേന്ദ്രീകരിക്കുന്നു. അതിനാൽ തന്നെ ശ്രദ്ധിക്കപ്പെടാതെ പോകുന്ന നിരവധി ഫൈക്കസ് ചെടികൾ ഉണ്ട്. ഫൈക്കസ് ട്രൂപേസിയ, ഫൈക്കസ് എക്സാസ്പെരേറ്റ തുടങ്ങിയവ ഇതിൽപ്പെടുന്നു. ഈ രണ്ട് സസ്യങ്ങളാണ് നിലവിലെ പഠനത്തിനായി ഉപയോഗിച്ചിരിക്കുന്നത്.

ഈ പഠനം രണ്ട് വ്യത്യസ്ത ഘട്ടങ്ങളിലായാണ് പൂർത്തീകരിച്ചിരിക്കുന്നത്. ഓരോ ഘട്ടത്തിനെയും വ്യത്യസ്ത ഭാഗങ്ങൾ ആക്കി തിരിച്ചിരിക്കുന്നു. ആദ്യ ഘട്ടത്തിൽ പ്രധാന മൂന്നു ഭാഗങ്ങളാണുള്ളത്, ഫൈറ്റോകെമിക്കലുകളെക്കുറിച്ചുള്ളപഠനവും ജൈവ പ്രവർത്തനങ്ങളെ ക്കുറിച്ചുള്ള പഠനവും, വെള്ളിക്കണങ്ങളെക്കുറിച്ചുള്ള പഠനവുമാണ് അവ. രണ്ടാംഭാഗം പ്രധാനമായും ഈ പഠനത്തിനുപയോഗിച്ച രണ്ട് സസ്യങ്ങളിലെ എൻഡോഫൈറ്റുകളെ ക്കുറിച്ചുള്ള പഠനമാണ്. ഈ ഘട്ടവും മൂന്നു ഭാഗങ്ങളായി തിരിച്ചിരിക്കുന്നു. എൻഡോഫൈറ്റുകളെ കണ്ടെത്തലും തിരിച്ചറിയലുമാണ് ആദ്യഘട്ടമെങ്കിൽ പഴങ്ങളിൽ നിന്നു വേർതിരിച്ച

എൻഡോഫൈറ്റുകളുടെ ബയോകെമിക്കലുകളെക്കുറിച്ചുള്ള പഠനമാണ് രണ്ടാംഭാഗം. ഈ പഴത്തിൽ നിന്നു വേർതിരിച്ച ഫ്ലെബിയ ഇനത്തിൽപ്പെടുന്ന എൻഡോഫൈറ്റിനെ ഉപയോഗിച്ച് സ്വർണ്ണവും വെള്ളിയും കണികകൾ നിർമ്മിക്കുന്നതാണ് മൂന്നാം ഭാഗമായി പഠനത്തിൽ ഉൾപ്പെടുത്തിയിരിക്കുന്നത്.

ആദ്യ ഘട്ടത്തിലെ ഫൈറ്റോകെമിക്കലുകളുടെ പഠനം വിശകലനം ചെയ്യുമ്പോൾ മൂന്നു വ്യത്യസ്ത ലായകങ്ങൾ ഉപയോഗിച്ച് നടത്തിയ പ്രാഥമിക പഠനത്തിന്റെ അടിസ്ഥാനത്തിൽ (വെള്ളം, മെത്തനോൾ, ഹെക്സേൻ എന്നിവ ആയിരുന്നു ഈ മൂന്നു ദ്രാവകങ്ങൾ). കൂടുതൽ ഫൈറ്റോ കെമിക്കലുകളെ ചേർത്ത് നിർത്താൻ കഴിവുള്ളതിനാലും മറ്റു രണ്ട് ദ്രാവകങ്ങളെ അപേക്ഷിച്ച് ഒട്ടും വിഷമില്ലാത്തതിനാലും തുടർപഠനത്തിനായി വെള്ളം തിരഞ്ഞെടുത്തു.

വെള്ളമുപയോഗിച്ച് നടത്തിയ ഫൈറ്റോ കെമിക്കൽ പഠനത്തിൽ രണ്ട് സസ്യങ്ങളിൽ നിന്നുമായി ലഭിച്ച നാലു എക്സ്ട്രാക്റ്റുകളിൽ നിന്നുമായി തൊണ്ണൂറ്റിരണ്ട് സ്ഥിര സംയുക്തങ്ങളും പത്ത് അസ്ഥിര സംയുക്തങ്ങളെയും കണ്ടെത്തി. ഇവ പ്രധാന ഫൈറ്റോകെമിക്കൽ ഗ്രൂപ്പുകളായ ആൽക്കലോയിഡുകൾ, ഫിനോൾസ്, ടെർപിനോയിഡുകൾ, ഗ്ലൈക്കോസൈഡുകൾ മുതലായവയിൽ ഉൾപ്പെടുന്നവയാണ്. ബയോആക്ടിവിറ്റി പഠനത്തിന്റെ ഭാഗമായി ഈ രണ്ട് ചെടികളിൽ നിന്നുമുള്ള എക്സ്ട്രാക്റ്റുകളുടെ ആന്റി ഓക്സിഡന്റുകളായി പ്രവർത്തിക്കാനുള്ള കഴിവും ക്യാൻസർ കോശങ്ങളെ നശിപ്പിക്കാനുള്ള കഴിവുമാണ് പഠന വിധേയമാക്കിയത്. ഫൈക്കസ് ട്രൂപേസിയായുടെ ഇലച്ചാറിനാണ് ആന്റിഓക്സിഡന്റായി പ്രവർത്തിക്കാനുള്ള കഴിവ് കൂടുതലായി കാണപ്പെട്ടത്.

ആന്റി ഓക്സിഡന്റുകളായി പ്രവർത്തനക്ഷമത പരിശോദിക്കുന്നതിനായി നാലുതരം പരീക്ഷണങ്ങളാണ് ഉപയോഗിച്ചത്. ടി പി പി എച്ച് അസ്സേ, സൂപ്പർഓക്സൈഡ് അസ്സേ, നൈട്രിക്ക് ഓക്സൈഡ് അസേ, ഹൈഡ്രോക്സിൽ റാഡിക്കൽ അസേ, മുതലായവയായിരുന്നു അവ.

എന്നാൽ ക്യാൻസർ കോശങ്ങളുമായി ബന്ധപ്പെട്ട പഠനത്തിൽ ഫൈകസ് ട്രൂപേസിയായുടെ പഴച്ചാറാണ് മുന്നിൽ വന്നത്. ഇവയ്ക്ക് സാധാരണ കോശങ്ങളെ ദോഷമായി

ബാധിക്കാതെ ക്യാൻസർ കോശങ്ങളെ മാത്രമായി നശിപ്പിക്കാനുള്ള കഴിവ് മറ്റുള്ളവയെ അപേക്ഷിച്ച് കൂടുതലാണെന്നു കണ്ടെത്തി.

ക്യാൻസർ കോശങ്ങളെ നശിപ്പിക്കാൻ കഴിവുള്ള ഈ പഴച്ചാറാണ് പിന്നീട് തുടർപഠനത്തിനുപയോഗിച്ചത്. കോമറ്റ് അസ്സേയും, ഡബിൾ സ്റ്റെയിനിംഗ് അസേയുമാണ് ആദ്യം നടത്തിയത്. ക്യാൻസർ വിരുദ്ധ പ്രവർത്തനം ഈ പഴച്ചാർ നടത്തുന്നത് അപോപ്പടോസിസ് എന്ന പ്രവർത്തനം വഴി ആണെന്ന് ഉറപ്പിക്കുകയായിരുന്നു ലക്ഷ്യം. (കോശ ആത്മഹത്യ). ഫൈക്കസ് ട്രൂപേസിയയുടെ പഴച്ചാറുകൾക്ക് ക്യാൻസർ കോശങ്ങളിൽ ആപ്പോപ് ടോസിസ് നടത്താനുള്ള കഴിവുണ്ടെന്ന് ഈ പഠനം സമർത്ഥിക്കുന്നു.

ഈ അപോപ് ടോസിസിന്റെ കാരണം വിശകലനം ചെയ്യുന്നതിലേക്കായി ജനിതക പഠനമാണ് പിന്നീട് നടത്തിയത്. P53, STAT 3 എന്ന രണ്ട് ജീനുകളുടെ പ്രവർത്തനങ്ങളെ ഫൈക്കസ് ട്രൂപേസിയയുടെ പഴച്ചാർ എത്തരത്തിൽ ബാധിക്കുന്നു എന്ന ചോദ്യത്തിനു ഉത്തരമെന്നോണം ഈ രണ്ടു ജീനുകളുടെ പ്രവർത്തനങ്ങളിലും മാറ്റം സംഭവിച്ചതായി കണ്ടെത്തി. ഫൈക്കസ് ട്രൂപേസിയയുടെ പഴച്ചാറുകൾ p53 ജീനിന്റെ പ്രവർത്തനത്തെ നിയന്ത്രിച്ച് കുറക്കുന്നതായും കണ്ടെത്തി. ഈ രണ്ട് ജീനുകളുടെ പ്രവർത്തനത്തിൽ വന്ന മാറ്റംമൂലം സെൽ സൈക്കിൾ റെഗുലേഷൻ സംബന്ധിച്ച പഠനത്തിൽ G1 സ്റ്റേജിൽ സെൽ സൈക്കിൾ നിലച്ചുപോവുന്നതായും കണ്ടെത്തി. ഫൈക്കസ് ട്രൂപേസിയയുടെ പഴച്ചാറിനു ബ്രെസ്റ്റ് ക്യാൻസർ കോശങ്ങളെ നശിപ്പിക്കാനുള്ള കഴിവ് ഉണ്ടെന്ന് ഈ പഠനം വിലയിരുത്തുന്നു. ബാക്ടീരയകളെയും ഫംഗസുകളെയും നശിപ്പിക്കാൻ കഴിവുള്ള വെള്ളി നാനോ കണങ്ങൾ ഉൽപ്പാദിപ്പിക്കാനും ഈ രണ്ട് സസ്യങ്ങളുടെ ഇലച്ചാറും പഴച്ചാറും ഉപയോഗിക്കാമെന്നും നിലവിലെ പഠനത്തിൽനിന്നും മനസ്സിലാക്കാം.

രണ്ടാം ഘട്ടമായ എൻഡോഫൈറ്റുകളെക്കുറിച്ചുള്ള പഠനത്തിൽ എൻഡോഫൈറ്റുകളുടെ സ്വാധീനം ഈ രണ്ട് ചെടികളുടെ ഇലകളിലും പഴങ്ങളിലും കണ്ടെത്തി. ഫംഗസ് വിഭാഗത്തിൽ വരുന്ന എൻഡോഫൈറ്റുകളിൽ മാത്രമാണ് നിലവിലെ പഠനം ശ്രദ്ധ കേന്ദ്രീകരിച്ചത്. DNA യിലെ ITS

ഭാഗത്തിനെ കുറിച്ചുള്ള പഠനം വഴിയാണ് എൻഡോഫൈറ്റുകളെ തിരിച്ചറിയാൻ സാധിച്ചത്. പഴത്തിൽ നിന്നും വേർതിരിച്ച എൻഡോ ഫൈറ്റുകളെ ബയോകെമിക്കൽ പഠനത്തിനു വിധേയമാക്കുകയും അവയിൽ നിന്നും 34 ബയോകെമിക്കലുകളെ കണ്ടെത്തുകയും ചെയ്തു. ഈ ഫ്ലെബിയ എന്ന എൻഡോഫൈറ്റിനും ആന്റി ബാക്ടീരിയൽ ആക്ടിവിറ്റിയുള്ള ജൈവ കണങ്ങൾ (സ്വർണം, വെള്ളി) ഉൽപ്പാദിപ്പിക്കാനുള്ള കഴിവുണ്ടെന്നും ഈ പഠനത്തിൽ നിന്നും മനസിലാക്കാം.

സൂചക പദങ്ങൾ : ഫൈക്കസ്, സ്തനാർബുദം, എം. സി. എഫ്-7, നാനോപാർട്ടിക്കിൾ, എൻഡോഫൈറ്റുകൾ.

CONTENTS

ABBREVIATIONS

Ag	:	Silver
AgNP	:	Silver nanoparticle
$AlCl_3$	:	Aluminium Chloride
amu	:	Atomic Mass Unit
ANOVA	:	Analysis of Variance
AO	:	Acridine Orange
Au Cl_4	:	Tetrachloroaurate
Bcl-2	:	B Cell Lymphoma 2
BLAST	:	Basic local alignment search tool
$CaCl_2$	:	Calcium Chloride
cDNA	:	Complementary DNA
cm	:	Centimetre
CO_2	:	Carbon Dioxide
COX	:	Cyclooxygenase
CUPRAC	:	Cupric ion Reducing Antioxidant Capacity
DLS	:	Dynamic Light Scattering
DMEM	:	Dulbecco's Modified Eagle's medium
DMRT	:	Duncan's Multiple Range Test
DMSO	:	Dimethyl Sulphoxide
dNTP	:	Deoxy Nucleotide Triphosphate
DPPH	:	2, 2-Diphenyl-1- Picrylhydrazyl
EDTA	:	Ethylene diamine Tetra Acetic Acid
ELISA	:	Enzyme Linked Immunosorbent Assay
ER	:	Estrogen Receptor
EtBr	:	Ethidium Bromide
eV	:	Electron Volt
FEFE	:	*Ficus exasperata* fruit extract
FELE	:	*Ficus exasperata* leaf extract
FE-SEM	:	Field Emission Scanning Electron Microscope
$FeCl_3$	:	Ferric Chloride
FDFE	:	*Ficus drupacea* fruit extract
FDLE	:	*Ficus drupacea* leaf extract

Fig.	:	Figure
FRAP	:	Ferric Reducing Ability of Power
FTIR	:	Fourier Transform Infrared Spectroscopy
FWHM	:	Full Width at Half Maximum Intensity
g	:	Gram
GC-MS	:	Gas Chromatography - Mass Spectrometry
H	:	Hour
H_2O_2	:	Hydrogen Peroxide
H_2SO_4	:	Sulphuric Acid
HCl	:	Hydrochloric Acid
HE Agar	:	Hektoen Enteric Agar
HPLC	:	High Performance Liquid Chromatography
HRLC-MS	:	High Resolution Liquid Chromatography - Mass Spectrometry
IC_{50}	:	Inhibition Concentration 50%
ITS	:	Internal transcribed spacer
KCl	:	Potassium Chloride
KOH	:	Potassium Hydroxide
L	:	Litre
L929	:	Normal Fibroblast Cell Line
LC_{50}	:	Least Concentration 50%
LDH	:	Lactate Dehydrogenase
LDLs	:	Low Density Lipoproteins
m/z	:	Mass-to-Charge
M	:	Molar
MCF-7	:	Michigan Cancer Foundation-7 (Human breast cancer cell line)
MDA-MB 231	:	M. D. Anderson-Metastatic Breast 231 (Human breast cancer cell line)
mg	:	Milligram
MHI agar	:	Muller Hinton Agar Medium
Min	:	Minute
mL	:	Millilitre
mm	:	Millimetre
mM	:	Millimolar
MTT	:	3-(4,5-Dimethylthiazol-2-yl),2-5-

		Diphenyltetrazolium Bromide
N	:	Normal
NaCl	:	Sodium Chloride
NADH	:	transcription 3 signal transducer and activator
Na_2CO_3	:	Sodium Carbonate
$NaNO_2$	:	Sodium Nitrate
NaOH	:	Sodium Hydroxide
NBT	:	Nitroblue Tetrazolium
NCCS	:	National Centre for Cell Sciences
NIST	:	National Institute of Standards and Technology
nm	:	Nanometre
NO	:	Nitric Oxide
ORAC	:	Oxygen Radical Absorbance Capacity
PBS	:	Phosphate Buffered Saline
PR	:	Progesterone Receptor
RNS	:	Reactive Nitrogen Species
ROS	:	Reactive Oxygen Species
rpm	:	Rotations Per Minute
RT	:	Retention Time
RT-qPCR	:	Quantitative Reverse Transcription Polymerase Chain Reaction
s	:	Seconds
SE	:	Standard Error
SEM	:	Scanning Electron Microscopy
STAT 3	:	Signal transducer and activator of transcription 3
SPR	:	Surface Plasmon Resonance
SPSS	:	Statistical Package for Social Sciences
TBA	:	Thio Barbituric Acid
TBARS	:	Thiobarbituric Acid Reactive Substances
TCA	:	Trichloro Acetic Acid
TE	:	Tris EDTA
TEAC	:	Trolox Equivalent Antioxidant Capacity
TEM	:	Transmission Electron Microscopy
TGA	:	Thermogravimetric Analysis
TGF-β	:	Transforming Growth Factor-β
TNF	:	Tumour Necrosis Factor

TRAIL	:	TNF Related Apoptosis Inducing Ligand
TRAP	:	Total Radical Trapping Antioxidant Parameter
UV	:	Ultraviolet
UV-Vis	:	Ultraviolet-visible
v/v	:	Volume Per Volume
V	:	Volt
w/v	:	Weight Per Volume
WHO	:	World Health Organization
XRD	:	X-ray Diffraction
µg/mL	:	Microgram per Millilitre
µg	:	Microgram
µl	:	Microlitre
µm	:	Micrometre
µM	:	Micromolar
^{o}C	:	Degree Celsius

INTRODUCTION

Life on Earth began approximately 3.7 billion years ago. From that time onwards, all life forms are intertwined by the strands of evolution. The 600-million-year-old transition of an algae form freshwater to land, laid the groundwork for the diversification and evolution of all ecosystems (terrestrial and aquatic ecosystems) on Earth (Morris et al., 2018). The evolution of flowering plants, which began some 90 million years ago, resulted in angiosperms dominating all other plant forms such as gymnosperms, ferns, and bryophytes (Morris et al., 2018). Plants always had a significant impact on biogeochemical processes. A separate terrestrial and aquatic environment was created as a result of the climate changes that occurred during the Paleozoic era, which is thought to be due to releasing oxygen into the atmosphere and taking in carbon dioxide by the plants as part of photosynthesis (Dahl & Arens, 2020). Land plant's evolution was the key to all terrestrial life forms by providing niche on earth from time to time.

The existence of humans has been dependent on the life of plants since antiquity, using them for clothing, shelter, food, medicine, and other purposes, which is why human life has become increasingly intertwined with plants throughout history. The significance of plants has remained constant since then, even if their scope has changed over time. Plants have a rich history in the evolution of life on Earth. They play a significant part in the ecosystem by delivering essential ecological services. The best of which is the provision of oxygen, food, and medicine, which makes them unavoidable contributors of the earth.

When ailments were becoming an issue, people started to look for the cure around them and reached towards the traditional medical systems. Locally available plants became the pillars for the system. During earlier times, experience and observation was the major ways to understand the properties and usage of anything as there were no literature available. The oldest known record of written drug formulation was discovered on a Sumerian clay slab from Nagpur that dates back to

5000 years. China's ruler Shen Nung wrote a treatise titled Pen T'Sao about 2500 BC that discussed 365 prescriptions made from medicinal plants. Around 1550 BC, the Ebers Papyrus was composed of 800 proscriptions that listed 700 different plant species and medications. The book De Materia Medica, written by Discorides, the military physician of Neros army in the Roman Empire, and regarded as the father of pharmacognosy, described 944 drugs, 657 of which are solely plant-based and include information on the appearance, location, method of collection, preparation, and medicinal properties of the herbs. The Indian classic Susruta-samhita, written around 600 BC, provided valuable information about 700 medicinal plants, much to the benefit of naturalists of the day. Understanding and application of therapeutic plants appear to have advanced by the early 1800s. The first isolation of active principles from the plants were reported between 1815 to 1820 (Sinha et al., 2023).

Alkaloids were initially detected in plants such as poppies and pomegranates. Even though the use of *Opium* was reported from the earliest civilisations the reports on crystalline isolation of active ingredient from *Opium* was done in 1803 in Paris by Jean-Francois Derosne. Strychnine, veratrum alkaloids, colchicine, caffein and quinine was isolated within the era of 1800. However, the isolation of various other phytochemicals in the early 19^{th} century paved the way for the development of scientific pharmacy. More phytochemicals such as atropine, codine, papaverine, curcumin etc. were discovered after understanding the uses of these drugs progressed (Petrovska, 2012). Developments in other fields, such as instrumentation, aided in the industrialization of drug production and contributed significantly to modern medicines. The aforementioned historical accounts make it clear that the usage of medicinal plants dates back to the beginning of human history and has occasionally been documented in scientific publications. Most plants appear to be used in their unrefined state to gain the aforementioned benefits and extract the active component. These notions of understanding, employing, and producing herbal formulations from various plants throughout the world eventually formed the genuine birth of the present branch of phytochemistry.

Primary metabolites are substances that participate in the biosynthesis or breakdown of numerous biomolecules and are directly related to energy production, use, and cell viability. Secondary metabolites, on the other hand, are classes of molecules that play no direct role in cell survival but are synthesized within the organism as part of its defence or adaptation process to its surroundings. Secondary metabolites and its pathways are often individualistic. Biosynthesis of these secondary metabolites or natural products is inextricably linked to primary metabolism; intermediates in the primary metabolic pathway led to the formation of numerous secondary metabolites in distinct ways via particular secondary metabolic pathways.

Such examples are acetyl coenzyme A (acetyl-CoA), shikimic acid, and mevalonic acid, which are the main sources of secondary metabolites produced by certain routes such as the mevalonic acid and shikimic acid pathways. Despite the fact that precursors are same in all plants, the ultimate product they can create is quite varied and can even be used to identify a single plant or family. This can be attributed to chemotaxonomy, which identifies taxonomically related plants based on the specific and distinct bioactive compounds they generate. These particular secondary metabolites appear to be the active components that led to the categorization of medicinal plants and the use of various plant parts and decoctions for various diseases from ancient times. The significance of plant biomolecules in taxonomy, medicine, and other fields necessitated the creation of a branch that could effectively oversee all plant metabolite-related research, which ultimately explains the importance of phytochemistry. Phytochemistry is now concerned with a thorough understanding of each biomolecule, which includes structural confirmation, biosynthetic route elucidation, role, and even mode of action in living systems. Thanks to advances in equipment and chemistry, which now contribute significantly to the aforementioned functions, as well as the particular identification, isolation, and manufacture of these natural substances as needed. Technologies, such as HR-LC/MS, GC/MS, and HPLC methods, have made it easier to understand the bioactive phytochemicals found in each plant. Studying phytochemistry has always been a vital issue, dating back to the beginning of human civilization. More understanding is always necessary for the

discovery of new phytochemicals as well as the impact of any specific plant biochemical on biological systems. In the current study, the phytochemical composition of *Ficus exasperata* and *Ficus drupacea* fruit and leaves were detailed using different phytochemical analyses, including preliminary analysis with various biochemical assays as well as HR-LC/MS and GC-HRMS analysis to properly elucidate the major and minor fractions of the extract considered for the study.

Once identified, phytocomponents from diverse fractions must be tested for a variety of bioactivities. Antioxidant activities were studied utilizing a variety of biochemical tests. Even though oxygen is necessary for aerobic organisms, it causes problems by causing oxidative stress inside the system. Antioxidants are substances that, at low concentrations, have the ability to delay, diminish, or completely scavenge and eliminate molecules or atoms that can cause oxidation in a system, preferably belonging to the free radical group. These free radicals are routinely produced in all plant systems under physiological and pathological situations, including aerobic respiration and antimicrobial mechanisms. So, effective free radical eradication should be used to remove any oxidation-related damage to cellular macromolecules such as DNA, RNA, lipids, sugars, and so on. Every plant has one or more mechanisms for accomplishing this, and antioxidant generation plays a significant part. In the human body, several forms of free radicals are created during respiration and metabolism. In modest amounts, some of these free radicals operate as signalling molecules in the system. However, excess production or accumulation contributes to oxidative stress. This is counterbalanced by the body's powerful antioxidant systems.

Enzymatic and nonenzymatic antioxidant regulation, such as glutathione and uric acid, is frequently utilized to aid in cellular redox balance. However, there may be occasions when the equilibrium is disrupted and reactive species accumulate owing to a variety of factors including aging, environmental pollution, or disease states, which may necessitate the intake of exogenous antioxidants via diet. Dietary antioxidants that appear to have been thoroughly explored include vitamin E, vitamin C, beta carotene, oxycarotenoids, and numerous other carotenoids such as lycopene.

Vitamin E is an excellent chain-breaking antioxidant that can protect cell membranes by efficiently stopping lipid peroxidation in the membranes (Yadav et al., 2016).

Oxidative damage is defined as the presence of an excess of free radicals or oxidants that surpass the cellular limitations of the free radicals and cannot be neutralized by the system's natural antioxidant molecules. Even though there are numerous ways for these oxidants to be produced in the living system, their accumulation can be caused by external factors such as environmental pollution or exposure to harmful radiations, as well as diseases or cellular impairment. Even while reactive oxygen species are the primary source of oxidants, reactive nitrogen molecules also contribute to oxidative damage within the cell. The most oxidation-prone biomolecules are always fatty acids, however practically all biomolecules within cells are sensitive to free radical oxidation.

Numerous medical problems, including neurological diseases like Parkinson's and Alzheimer's, are linked to damage caused by free radicals. Redox imbalance in the body appears to have a part in inflammatory disorders such as diabetes, cancer, immunodeficiency syndrome, and age-related eye problems. Biological aging is typically caused by free radicals mediated by the mitochondrial electron transport chain (Pisoschi & Pop, 2015).

Plant-based goods, such as fruits, vegetables, seeds, and even the leaves of certain plants, are rich in phytochemicals such as phenols, flavonoids, carotenoids, microelements, and vitamins. Human tradition has included the incorporation of plant-based antioxidant-rich foods in diet. Human evolution can be traced back two million years, when macro and micronutrient requirements were mostly met by herbivorous diets. Antioxidants have been incorporated into diets since the Paleolithic era, and their content was far higher than that of modern human diets. Organised agriculture, which began 12000 years ago, has contributed to the development of modern human diets and is regarded to be the cause of civilizational sickness, such as some deeply rooted prevalent ailments found in the population.

The mismatch between the old diet and the modified current diet may have contributed significantly to various types of illnesses. Even though there is evidence

that consuming an antioxidant-rich diet or taking antioxidant supplements can improve longevity, reduce aging, and increase life expectancy by lowering illness risk, there is no acknowledged label for any single antioxidant.

An antioxidant is said to be effective when it has several radical capture sites that can detect and eliminate diverse free radicals from multiple locations. Understanding antioxidants in nature and seeking antioxidant enrichment through diet modification using underexplored natural delicacies that are widely available is becoming increasingly important. This work contributes to a better understanding of the antioxidant activity of two common fig species in both their fruits and leaves. The study included four *in vitro* assays, including the DPPH assay, which can help researchers comprehend the very low levels of antioxidants in the system. Hydroxyl radicals generated in the system are extremely hazardous because they are highly reactive and cause a variety of cell damage when present. Another antioxidant assay used in the study is the hydroxyl radical assay, which involves the formation of hydroxyl radicals via the Fenton reaction. The Fenton reaction is typically observed in the liver as part of the breakdown of drugs or poisons. In this procedure, hydrogen peroxide combines with ferrous ions to produce hydroxyl radicals, and the ability of the selected extracts to scavenge these free radicals was tested *in vitro*. Another investigation on superoxide radical scavenging *in vitro* assay was conducted with the ability to reduce NBT. Superoxide radicals are produced inside the mitochondrion of the cells during the electron transport chain and might be harmful to the cellular system. Super oxide radicals are not dangerous themselves, but they can quickly transform into more hazardous hydroxyl, singlet, or even peroxy nitrite radicals. For this reason, it's critical to get rid of any superoxide radicals that have developed inside the system.

In the normal development of organisms there is a wellset boundaries for each and every functions. Every function in the cells is genetically monitored very well. Birth and death of every cell is counterbalanced well according to the needs, usually most of the cells in adult bodies are not in a position to divide unless they are healing cells like hepatocytes or heart muscle cells. Still there are cells like blood cells,

intestinal epithelial cells etc. that are kept dividing as part of the need for tissue renewal. This is all monitored perfectly by the growth signals, which may be growth promoting or inhibiting and by death signals. When this regulatory mechanism starts malfunctioning, the cells start to divide abnormally surpassing all the control. Certain cells, such as intestinal epithelial cells and blood cells, continue to divide in order to maintain tissue renewal. All of this is flawlessly observed by the growth signals, which can either promote or impede growth, as well as the death signals. Cells begin to divide erratically, outpacing any control, when this regulatory mechanism malfunctions.

Cancer develops when any cell in the body multiplies abnormally, failing to respond appropriately to growth signals and eventually invading nearby tissues and organs before potentially spreading across the entire body. This is the end result of accumulating anomalies in the regulatory systems. Tumours can be benign or malignant; benign tumours are restricted to a specific place and never spread to adjacent tissues. However, when a tumour becomes malignant, it might evade any region of the body by losing cell-to-cell adhesion and spread to various organs via metastasis. According to the GLOBCAN data, India has the third-highest number of cancer patients worldwide, after the United States and China. The country will experience a growth in cancer patients to 2.08 million, or roughly 57.5 percent more cases of the disease (Sathishkumar et al., 2022). Males are mostly affected by stomach, prostate, lung, colorectal, and liver cancers; in contrast, females are more likely to get breast, cervix, colorectal, and thyroid cancers (Bray et al., 2018). Appropriate researches are constantly required to treat and eradicate the disease from the public, as different forms and subtypes of cancer contribute to one of the leading causes of mortality in the human population. Cancer is classified into numerous categories and subtypes according to the organs and tissues that are afflicted. These subtypes include sarcoma, lymphoma, leukaemia, adenoma and carcinoma. Environmental variables have a significant role in the onset of cancer, even if the accumulation of different mutations in the cell is the primary cause of various malignancies. Chemical exposure that causes cancer can contribute directly or indirectly to the development of malignant characteristics in cells. In rare

circumstances, environmental radiation, bacteria or viruses can potentially aid in the development of cancer.

Breast cancer is the most common cancer among women, and it has become a serious public health concern, particularly in developing nations such as India. Breast cancer cells grow within the milk ducts or milk producing lobules and eventually elude the breast tissues, causing lumps or thickness in the breast. This is treatable in about 80 percent of early-stage instances, but in invasive metastatic late-stage cases, it appears to be incurable and generally fatal. Breast cancer can be caused by a variety of factors, including a family history of the disease, early puberty, hormonal therapy, dense breast tissue, infertility, not having children before the age of thirty, not breastfeeding. Cigarette smoking, alcoholism, lack of exercise, and other lifestyle choices all dramatically raise the risk of developing breast cancer. Despite the fact that all of these factors contribute to breast cancer, the genetic basis of the disease is mainly due to the mutation in tumour suppressor gene BRCA or the activation of human epidermal growth factor receptor 2. The current study focuses on the antineoplastic activity of four chosen extracts on *in vitro* breast cancer MCF 7 cell lines. This is a model cell line that expresses estrogen and progesterone receptors while remaining HER negative. They are classified as luminal type A malignancies and have been identified from the pleural effusion of a metastatic breast cancer. They are said to have made significant contributions to our understanding of the numerous factors involved in the development and treatment of breast cancer.

Treatments with extracts were found to help reduce the uncontrolled proliferation of MCF 7 cells in culture. The reason for anticancer activity is investigated in depth here by studying some of the molecular pathways. The effect of several therapies on the expression fold change of the two genes, P53 and STAT 3, was investigated using GAPDH as an internal control. P53, sometimes known as the "guardian of the genome," plays a key role in tomour suppression and cell cycle regulation. When there is any damage or errors with the cellular DNA, the gene P53 helps to pause and attempt to repair the damage using repair mechanisms. If the damage is irreversible, P53 triggers an apoptotic programme that allows the cell to be

appropriately disposed off by permanently stopping the cell cycle. To comprehend the molecular level interaction of the chosen extract on the antiproliferative action, another molecular target, STAT 3 (Signal transducer and activator of transcription 3), activity was also investigated. This gene, like P53, has a significant impact on cellular function. STAT 3 plays a direct function in tomourigenesis when it is elevated. STAT 3 has a function in cell proliferation, metastasis, apoptosis, epigenetic control, cancer stem cell renewal, tumour inflammation, and other processes (Hu et al., 2024). The growth and invasion of cancer can be reduced if the activity of STAT 3 in a cancer cell is altered by any chemicals or medications. This will prevent the cells from dividing uncontrollably. Anti-cancer medications employ chemicals that specifically target STAT 3. In recent years, herbal medicine has gained significant traction in the treatment of a variety of malignancies. A major contribution of the drugs that we use nowadays for survival is mainly contributed by the herbal medicine used in the ancient time, but finding a cancer therapy medication that works well and doesn't have any major side effects appears to be one of the goals of scientific research. hence it is somewhat comparable to grasp the biological pathways underlying cancer cells. Numerous natural compounds and phytochemicals with antineoplastic activity have already been discovered in earlier cancer research investigations; some of these are quite successful in treating the malignant growth in a variety of cancer types. Among them are vinca alkaloids, taxol, epipodophyllotoxins, camptothecin, and other substances that are widely used. Phytochemicals have demonstrated a range of therapeutic potential and are already established a place in the scientific literature for the treatment of a variety of health concerns, whether in the traditional or allopathic medical systems. The goal of this work is to better understand how phytochemicals from the fruits and leaves of *Ficus exasperata* and *Ficus drupacea* act against MCF 7 breast cancer cell lines using both morphological and molecular techniques.

Richard Feynman's 1959 address at the American Physical Society's annual conference introduced the concept of nanotechnology. However, the word nanotechnology was coined in 1974 by a Japanese scientist Norio Taniguchi (Hulkoti & Taranath, 2014). Nanos is a Greek term that means dwarf and was subsequently adopted as a prefix for the term nanotechnology. The two main strategies for

synthesizing nanoparticles are top down and bottom up. The bottom-up method produces nanosized particles by assembling atoms collectively to form the unique size materials, but in top-down methods, the nano size of particles is obtained by the breakdown of materials, eventually giving them the desired size of nanoparticles. The production of nanoparticles using green method along with some other physicochemical methods such as precipitation, laser, aerosol, pyrolysis, chemical, and electrochemical methods, and vapor deposition are coming under the process of bottom-up synthesis. Sputtering, ball milling, thermal/laser ablation etc. are coming under the process of top to bottom synthesis of nanoparticles.

Nanotechnology is concerned with the extraordinary properties of materials having dimensions less than 100 nm. The material's unique qualities might be as a result of its small size and increased surface area to volume ratio. This nanoparticle nature also adds to remarkable magnetic, optical, and electrical properties. Characterization synthesis and application of these nanomaterials in diverse disciplines has made significant contributions to science over the last several decades. The usage of nanotechnology is widespread, which include drug or gene delivery, optics, medical field, electronics and communication technology, antimicrobial products, food, cosmetics, and textiles. Despite the fact that nanotechnology has been around for many years and has many advantages, it was primarily created by synthetic means, which may be costly and often not environmentally friendly. This is where green nanotechnology emerges. The science of producing nanoparticles utilizing biological resources in an economical and environmentally beneficial manner is known as "green nanotechnology." Plant components, algae, fungus, bacterial, and other extracts were frequently employed in green nanotechnology to produce nanoparticles. This area has received a lot of research since it can be applied to a variety of fields, particularly medicine, to prevent any potential harm from the artificial synthesis of nanoparticles. Among the numerous types of nano particles generated, metallic nano particles were determined to be effective due to their various features. Particularly in the context of biomedical applications, non bio toxic and relatively less reactive metals are important to consider. In order to create biologically useful nanoparticles that can be effectively

incorporated into the biological system, silver and gold appear to be the best sources of metals. Humans have used silver for a variety of purposes ever since the beginning of humanity. Some people used it as jewellery, some as exquisite cutlery, and some in the treatment of different ailments, most notably as an antiseptic for wounds. During World War I, soldiers' wounds were dressed with silver. This silver nitrate solution was administered as an eye drop to the infants to prevent *Neisseria gonorrhoea* infections. Silver was shown to be the most effective metal with antibacterial properties among all other metals due to its biosafety. With its remarkable antibacterial characteristics, strong conductivity, catalytic capabilities, and chemical durability, silver is an excellent choice for nanotechnology applications. Silver is utilized in infant pacifiers, computer keyboards, food storage, and textile sectors, as well as in antiseptic sprays and treatments to prevent infection. The toxicity of silver has not been established, however numerous organizations such as the US FDA, the US EPA, the SIAA of Japan, and others have allowed the use of silver nanoparticle-containing goods (Ahmed et al., 2016). The characterisation of nanoparticles after they have been generated is critical because it can provide structural and functional features to the nanoparticles synthesized. The main approach utilized in this is UV-vis spectroscopic analysis, which can produce a distinct peak for each single nanoparticle formed. The second step is to conduct a SEM or TEM investigation to determine the crystal structure and shape of the nanoparticles generated. Another method for characterizing nanoparticles is FTIR, which provides information on any unique functional groups of chemical or organic molecules that surround the nanoparticles created from a given extract. There are several characterisation methods available today, including AFM, XRD, and DLS, which are also effective ways for understanding the various properties of nanoparticles created, particularly through green synthesis. Silver nanoparticles are the most effective catalysts for dye reduction and removal. They were reported to be efficient biosensors. In addition, silver nanoparticles have been shown to have anti-inflammatory, anti-cancer, and antioxidant effects—even against germs that are resistant to many drugs.

Additionally, they are quite good in preventing the spread of some infectious viruses, such as the HIV virus. Silver nanoparticles have exceptional molecular

diagnostic capabilities, including the ability to characterize biomarkers, and analyse gene expression. Silver was previously given as silver nitrate to obtain its antibacterial characteristics; but, when silver was used in nanoparticle form, it became a more effective antimicrobial agent. The reduction in particle size allowed for the use of a larger surface area in bacterial fight. When these nanoparticles were biosynthesised, their attributes became depending on the temperature, size, shape, pH, and the capping agents derived from the bio extract employed in manufacture. The silver nanoparticles' antimicrobial and other significant bioactivities are still being investigated, but one possibility is that the positive charge on the silver ion played a role in disrupting the membrane. Additionally, the silver ions have the ability to interact with the nucleosides of the cell. The production of reactive oxygen species by nanoparticles damages bacterial cell biomolecules. Furthermore, the antimicrobial efficacy of silver nanoparticles is enhanced by ATP depletion, membrane perforations caused by high membrane permeability, and damage to DNA and RNA. Gram negative and gram positive bacteria were both effectively inhibited by silver nanoparticles' antibacterial properties. In today's environment, when the number of multidrug-resistant bacteria is increasing, the necessity for an effective antimicrobial agent is critical. In many circumstances, nanoparticles can attack MDRs on their own or in combination with some standard antibiotics, which will contribute significantly to the fight against known and new multidrug-resistant bacteria. Given the expanding importance of nanotechnology in modern life, it is the scientific community's responsibility to ensure that the most environmentally friendly model is used while developing nanomaterials. This has resulted in a greater emphasis on the development of green nanoparticles from easily accessible, locally available plants or critters. This study is aimed to create silver nanoparticles from *F. exasperata and F. drupacea* fruit and leaf extracts, and the results showed that the silver nanoparticles resulted from the current study were excellent antibacterial agents.

Endophytes, or microscopic organisms that live inside plants, are found in nearly every plant on the planet. They dwell inside the host for the entirety, or part, of their existence. The majority of them are either bacteria or fungi, while actinomycetes, algae, cyanobacteria, and other organisms are also considered as endophytes. These

organisms are kept within the plants in a certain equilibrium that prevents them from becoming pathogenic. The majority of the time, they never upset this balance or pose a harm to the host. Nearly all plant elements, including leaves, roots, fruits, flowers, stems, bark, etc., contain endophytes. Endophyte colonization depends on a variety of parameters, including biotic and abiotic environmental conditions, plant genotype and tissue type, and microbial community genotype.

These associations frequently benefited the host through a variety of mechanisms. They assist the host in overcoming typically adverse conditions like drought stress, salinity stress, and so on. They also aid in the mobilization and provision of nutrients in certain cases, while in others they operate as hormone sources and growth boosters, frequently supporting the plant in enhancing disease resistance. Endophytes are constantly in contact with the host's internal environment, which can be challenging because they frequently encounter different biomolecules, internal pH, or enzymes that the host creates for survival. The endophyte can interact with all of them. As a result, the biochemical output of an endophyte is considerably affected by the chemical composition of its host. This is why endophytes are extremely important. Any natural resource that is beneficial to human life has already been the subject of research by the scientific community into potential common uses. There are fewer reports from the plant world regarding new natural compounds that are beneficial to human health. However, when studying organisms that live in harsh or unnatural environments, their biochemical composition may differ significantly from that of other organisms, which is where endophytes come into play. There have been a number of novel chemicals described from endophytes to date. However, the number of plants studied for endophytes is insignificant when compared to the total number of plants on the globe. Furthermore, endophytes can often mimic the biochemicals produced by their hosts. This is particularly significant since the destruction of a whole canopy can provide a few milligrams or grams of a certain biochemical of interest, but synthesising the same biochemical using an endophyte in the laboratory will be a huge accomplishment. Penicillin, an antibiotic, griseofulvin, an antifungal, lovastatin, an immunosuppressant, and other medications were major discoveries that led scientists to search for novel pharmaceuticals in fungi or microorganisms rather

than plants. Endophytes can give many novel biochemicals to the scientific community, hence studying biochemicals from this category of microorganisms is quite important. Biochemical study frequently demonstrated the presence of biochemicals classified as major phytochemical classes, such as alkaloids, terpenoids, and phenols, in many endophytes. The present study is approaching in the understanding of the fungal endophytic community present in the two selected fig plants and addressing the biochemical components from the isolated fungal endophyte. This study is also focusing on the ability of these biochemicals in formulating gold and silver nanoparticles along with the antimicrobial ability of these particular green nano particles. Gold was one of the first metals discovered, and it appears that Indian, Chinese, and Arabian scientists employed it in colloidal form for medical applications as early as 5-14 BC (Dykman & Khlebtsov, 2011). Colloidal gold was utilized in Europe during the Middle Ages; Paracelsus used the word "potable gold," which he recommended for the treatment of a variety of mental illnesses, and also in the treatment of syphilis, leprosy, plague, epilepsy, and diarrhoea. Despite the fact that there are numerous accounts of the use of gold in traditional medicine dating back to the ancient times, Faulk and Taylor's 1971 study on the antibody conjugation of colloidal gold for electron microscopy visualisation of *Salmonella* bacterial surface antigens brought gold nanoparticle usage into the broad public domain. This opened the door to numerous studies on the use of metal nanoparticles, including gold, for a variety of applications (Dykman & Khlebtsov, 2011). Gold nanoparticles are very useful in various therapeutics and in biomedical field. They are promising nanoparticles due to the increased surface to volume ratio, less toxicity, and better biocompatibility. The redox ability of these particles is used in the field of electronics, surface plasmon resonance is utilised in the field of optics, and fluorescence quenching is used in the field of material science. Gold nanoparticles can be used for air cleaning, odor elimination, emissions control, water purification, power cells, and vital medicinal applications. These particles' small size allows them to enter tissues and assault immune cells, making them valuable in immunotherapy, medication delivery, and cancer therapy. They are also used as effective antidiabetic, antimicrobial, anticoagulant, thrombolytic agents (Firdhouse & Lalitha, 2022). There

are numerous reports on the biosynthesis of gold nanoparticles from fungus, bacteria, plants and other sources. Here in this work endophytic fungus of *F. drupacea* fruits were used in the biosynthesis of gold nanoparticles. The biochemicals produced by the endophytic fungus might have contributed to the reduction and caping of the gold nanoparticles and silver nanoparticles produced and they were characterised using UV-vis spectroscopy and SEM analysis to confirm the presence of nanoparticles. Activity of these nanoparticles were tested by using the antimicrobial assays conducted.

The Moraceae family, usually known as the fig or mulberry family, consists of approximately 37-43 genera and 1100-1400 species (Somashekhar et al., 2013). This family belongs to the Rosales order. Despite their cosmopolitan occurrence, they are most frequent in the tropics and subtropics and rarely seen in temperate regions. This family includes trees, shrubs, hemi epiphytes, wines, and, in rare cases, herbs. They have colourless or milky latex. Compound inconspicuous blooms and compound fruit are often distinguishing features. They are either monoecious (male and female flowers on the same plant) or dioecious. Plants from the family have been shown to offer a variety of medicinal properties, including anti-diabetic, antibacterial, anti-cancer, anti-inflammatory, and immunological modulatory actions. This family includes several plants which are a common and nutritious parts of people's diet, such as jackfruit, breadfruit, fig, mulberry, and so on. In this family the most important genus is *Ficus* as it is the biggest genus with the number of species it has and the benefits obtained from various plants present in the genus. These are the key stone species with fundamental duties in the ecosystem. Maximum diversity of the genus is found in the Asiatic main land, New Guinea and Borneo. In terms of its life forms and habitat, this genus is regarded as one of the most diverse genera. The genus contains 800 species that have been documented worldwide, with 100 additional species found in India (Mukhtar et al., 2018). With 43 distinct *Ficus* species, Meghalaya is regarded as a *Ficus* hot spot in India (Chaudhary et al., 2012). Their ability to spread across the globe, their territorial behaviour, and most amazingly their capacity to strangle members of the plant kingdom which originally began as an aerial epiphyte on well-established host trees all suggest that they belong to an aggressive genus (Ahmed et

al., 2012). This genus includes shrubs, herbs, climbers, creepers, deciduous and evergreen trees. The habits range from stranglers to upright trees, rheophytes, lithophytes, and epiphytes. This genus is categorized on the basis of its morphological traits and distributional patterns into 19 sections and 27 subsections. The national tree of India, the banyan tree (*Ficus benghalensis*) and the most worshiped tree in India *Ficus religiosa* comes under this genus. *Ficus* plants are commonly grown for both their therapeutic benefits and their symbolic significance in religion and culture. *Ficus* plants have an important role in ancient indigenous medical methods, such as Ayurveda, Siddha, Unani, and homoeopathy. The plants in this genus are very well documented with their therapeutic potential in various ailments. The fruit of various *Ficus* is edible especially the fig *Ficus carica* is a delicacy. Most of them are rich in antioxidants with health benefitting polyphenols. Members of this genus are also contributing to various medicines in ayurveda for example "Pancha Valkala Kashaya" (From *F. religiosa, F. benghalensis, F. glomerata, F. infectoria* and *Azadiracta indica*) (Singh et al., 2011). Despite the fact that the genus *Ficus* includes hundreds of plants in India, most research focuses on a small number of plants that are thought to be especially important in traditional systems. However, many common *Ficus* plants are ignored, since scarce literature is available on them. *Ficus drupacea* Thunb and *Ficus exasperata* Vahl are two plants that fall under this category. The current research is phytochemically delineating the leaves and fruits of these two *Ficus* plants and determining their fundamental bioactivities, as well as isolating and identifying fungal endophytes from them.

Major objectives of the study

- To analyse the phytochemicals from the fruits and leaves of the selected plants, viz *F. exasperata* and *F. drupacea.*
- To reveal the antioxidant capacities of the fruits and leaves.
- To test the antineoplastic ability of the selected extract on MCF 7 breast cancer cell lines.

- To delineate the cell cycle arrest and apoptosis induction ability of the selected extract on MCF 7 breast cancer cell lines.
- To conduct investigations on gene expression, and to comprehend the molecular underpinnings of the anticancer activity.
- Green synthesis of silver nanoparticles from the leaves and fruits of the two plants and antimicrobial efficacy determination of these phyto fabricated nanoparticles.
- Isolation and identification of the fungal endophytes present in the leaves and fruits of the two selected plants.
- Mycochemical analysis, silver and gold nanoparticle biosynthesis from the fruit endophyte *Phlebia* sp.

PHASE I

PART I

Traditional Aspects

Ficus is a genus having immense traditional importance. There are number of reports on various *Ficus* species having their usage from time immemorial. They have historically served as supplies of food and medicine, as well as decorative trees, sacred plants, lac hosts, fodder, fuel, for fences and also support local livelihoods and provide a significant supply of various foods. In South Asia, *Ficus* plants are widely grown both for their therapeutic properties and for their symbolic value in religion and culture. In traditional indigenous medical practices including Ayurveda, Siddha, Unani, and homoeopathy, *Ficus* plants play a significant role. *Ficus carica* is one of the earliest tree crops and medicinal plants utilized by humans. According to archaeological data, it has been farmed for more than 11,000 years and may have even predated cereal grains (Kislev et al., 2006). Some of the common plants from the genus with its traditional values are discussed here. Ayurveda, the traditional Indian medical system, has used the popular medicinal plant *Ficus racemosa* L. for many years to treat a variety of illnesses and conditions, including diabetes, liver disorders, diarrhoea, inflammatory conditions, haemorrhoids, respiratory, and urinary diseases (Ahmed & Urooj, 2010)

F. religiosa has become a reliable source of conventional medicine for the treatment of inflammatory disorders, infectious disorders, sexual disorders, epilepsy, diabetes, diarrhoea, and other conditions. Although majority of the experimental trials supported its historical applications as a medicine, they used unidentified crude extract. The bark, fruits, leaves, adventitious roots, latex, and seeds of this plant are frequently employed with other herbs for therapeutic purposes. The bark is a key component of many Ayurvedic formulations, including "Pancha Valkaladi Tailum" (oil containing *F. religiosa, F. benghalensis, F. glomerata, F. infectoria, Azadirachta*

indica, Curcuma longa, and *Hemidesmus indicus* R. Br.), and "Pancha Valkala Kashaya" (*F. religiosa, F. benghalensis, F.glomerata., F. infectoria* and *A. indica*) (Singh et al., 2011).

Ficus exasperata Vahl major parts are used in traditional medicine as analgesics, antiarthritics, diuretics, wound healers, antiparasitic, vermifuges, abortifacients, ecbolics, and to cure haemorrhoids and venereal illnesses. Additionally, the plant's leaves are used as animal feed. The leaves of *F. exasperata* are particularly significant in terms of traditional medicine. All over Africa, there have been significant reports of the use of ethnomedicine; the main centers are Nigeria, Cameroon, Ivory Coast, and Sierra Leone (Ahmed et al., 2012). The most well-known *Ficus* species, *F. carica,* is often referred by more than 135 different names. *F. carica* has been used traditionally for more than 40 different illnesses. It has been used to treat diseases of the immune system (skin disease, scabies, and gonorrhoea), reproductive system (menstrual pain), gastrointestinal tract (ulcer and vomiting), respiratory system (liver diseases, asthma, and cough), endocrine system (diabetes), and respiratory system. Additionally, it is employed in the treatment of urinary and digestive system infections (Badgujar et al., 2014).

Ficus benghalensis stem bark was used against diabetes, diarrhoea, and dysentery. The latex is used to treat rheumatism, burns, and wounds. Fruits can be eaten and used as tonics. The bark and leaf buds have properties that help stop bleeding. The leaf buds and latex are effective in treating dysentery. The latex, which has a milky consistency, is used to treat bruises, rheumatism, and is applied to wounds to remove worms. It's also used for gum bleeding and swelling. This milky latex is applied externally to alleviate pains, bruises, and is used as a soothing treatment for rheumatism and lumbago. Additionally, the bark and roots are used in antidiabetic preparations according to Sri Lankan Siddha Medicine (Logeshet al., 2023).

Phytochemical Profiling

Phytochemicals are components that naturally occur inside plants, within almost all plant parts such as leaf, fruit, bark, root, stem and even in flowers. They can be broadly divided into two; I) primary metabolites and II) secondary metabolites.

Primary metabolites are components such as carbohydrates, proteins, fats, sugars, lipids, esters, etc. with direct involvement in the cellular processes and thereby contributing to the growth and development. Secondary metabolites are phytochemicals produced from primary metabolites, and doesn't have particular nutritional benefits or direct influence on the growth or metabolism. Plant metabolites have been employed since 2600 BC, and throughout the next 4,000 years, secondary metabolites derived from plants were mostly used in medicine, food and poisoning (Twaij & Hasan, 2022). They are an essential part of the plant system as they have major role in fruit set, abscission, flowering stimulation, as well as to maintain perennial growth and signal transduction. They have functions in defence and protection, and can act as attractants or repellents. Secondary metabolites are intricate structures that are produced by complex biochemical processes. They accumulate in different concentrations within specialized tissues depending on their needs. **Fig. 1** show the schematic representation of synthesis of secondary metabolites from different metabolic pathways.

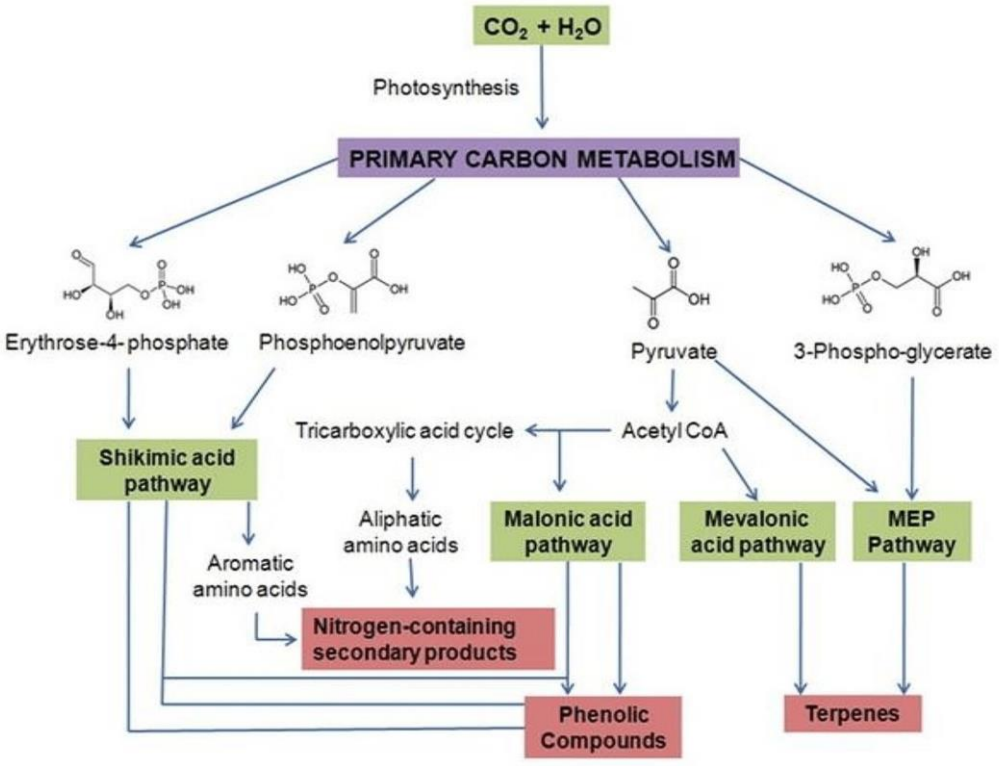


Fig. 1: Schematic representation of metabolic pathways in secondary metabolite synthesis (Twaij & Hasan, 2022).

The plant kingdom has yielded the discovery of approximately 50,000 secondary metabolites (Teoh & Teoh, 2016). These compounds are synthesised in reaction to external environmental conditions, such as pests, UV radiation, or illnesses. Majority of these secondary metabolites are beneficial against a range of maladies due to their medicinal properties. Secondary metabolites have been valued since time immemorial due to these activities. There are thousands of secondary metabolites, each with its own special qualities that affect the colour, flavour, and aroma of plants. Despite not being classified as essential nutrients, secondary metabolites have been proven to have important health-promoting qualities and have been practiced in various medicinal systems including traditional or folklore medicine. Examples include the anti-inflammatory, antioxidant, antibacterial, and anticancer properties found with numerous phytochemicals. Additionally, research into these natural substances has aided in the creation of pharmaceuticals that can both treat and prevent chronic disorders.

Phytochemicals also contribute to the nutritional value of plant-based foods. Plant-based diets high in phytochemicals have been linked to lower risk of serious conditions such as cardiovascular problems, cancer, neurological disorders, and diabetes. Identifying and understanding the phytochemicals responsible for these protective effects can help to develop dietary guidelines and functional foods to prevent and manage these diseases. They often have specific health benefits beyond basic nutritional requirements. Flavonoids found in fruits and vegetables can help strengthen the immune system and protect against oxidative stress. Lycopene carotenoid hydrocarbon responsible for the red colour in tomatoes are known for its antioxidant properties (Leh & Lee, 2022). Due to their multiple advantages for crop production and preservation, plant phytochemicals play a key role in agriculture. In order to reduce the use of synthetic pesticides and promote sustainable agricultural practices, researchers pinpoint potential phytochemicals that underlie insect resistance or plant defence mechanisms. They can also design environmentally friendly pest management and crop protection strategies. Neem oil, for example, which is extracted from the neem tree (*Azadirachta indica*), is employed as a natural insecticide. The therapeutic uses of certain plant chemicals, such as alkaloids, phenols, terpenoids,

phytosterols, and glycosides, have made them well-known and the subject of substantial investigation.

Alkaloids:

Alkaloids are a type of nitrogen-containing organic compounds that have a ubiquitous presence in plants. They are supposed to aid in germination as well as to protect plants from predators such as herbivores and microorganisms. They are found in around 20% of higher plants (Teoh & Teoh, 2016). They have a wide range of biological functions, and traditional medicine has used them for many years. Some alkaloids have anticancer, antimalarial, and antibacterial properties; however, morphine, codeine, and caffeine are frequently used for their euphoric effects. Vincristine, a significant example of an alkaloid and a chemotherapy medication for many cancers, is found in the Madagascar periwinkle (*Catharanthus roseus*) (Škubník et al., 2021).

Phenols:

Phenols are chemical compounds that have an aromatic ring and one or more hydroxyl groups attached. They are the largest group of secondary metabolites. They include coumarins, quinones, napthoquinones, anthraquinones and flavonoids that give plants their odour, and colour. These substances are prevalent in plants with a variety of roles, including antioxidant potential, growth and development regulation, pathogen and predator defence etc. Some phenolic substances, including resveratrol and curcumin, have been linked to important health advantages, including anti-inflammatory and anticancer characteristics. Eugenol, a component of clove oil that has antifungal, antibacterial, and antioxidant properties, is a convincing example of a phenolic compound with potential therapeutic activities (Ali et al., 2017).

Terpenoids:

An enormous and varied class of chemical molecules known as terpenoids is formed from isoprene units with five carbons. They are found in many plants and often contribute to floral fragrance, defence against diseases and herbivores, pollinator attraction and regulation of growth and development. Terpenoids with widespread

uses in the food, fragrance, and medicinal industries include menthol, camphor, limonene etc. Artemisinin, a key example of a terpene and a component of sweet wormwood *Artemisia annua* is used as an antimalarial medication (Weathers et al., 2010).

Flavonoids:

Plant-based foods include a large number of flavonoids, a diverse category of polyphenolic chemicals. Their effectiveness as antioxidants helps to protect cells from oxidative damage brought on by free radicals. By modifying cardiovascular functioning, demonstrating anticancer effects, and enhancing cognitive behaviour, flavonoids have significant influence on human health. Catechins, kaempferol, quercetin etc. are considered as some good examples demonstrating these qualities (Pandey & Rizvi, 2009).

Carotenoids:

The red, orange, and yellow hues of fruits and vegetables are imparted by the pigments known as carotenoids. They serve as antioxidants and are crucial for photosynthesis. Carotenoids, which serve as the body's precursors to vitamin A, have been associated with a number of health advantages, including the lower risk of age-related macular degeneration, cardiovascular disease, and several malignancies. Beta-carotene, lycopene, and lutein are examples of carotenoids (Krinsky et al., 2005).

Glycosides:

The group of chemicals which connects a sugar moiety to a non-sugar part is known as glycosides. They are famous for unique biological activities. Digoxin, a glycoside derived from *Digitalis purpurea* (foxglove), which is widely used to treat cardiac insufficiency, is one of the most notable instances (Mladěnka et al., 2018).

Phytosterols:

Phytosterols, which are sterols found in plants that resemble cholesterol in appearance, can lower blood cholesterol levels, when ingested as part of a healthy

diet. Phytosterols, such as sitosterol, are found in many plant-based sources, including nuts and seeds (Awad et al., 2007).

A great deal of interest has been aroused in the identification and separation of phytochemicals especially secondary metabolites from plants. This is because of the significant role that phytochemicals play in supporting human health. The growth of technology has made it easier to identify and separate these molecules in large quantities, making them easily available for research and in the understanding of novel therapeutic chemicals. The development of functional foods and nutraceuticals that include certain positive health benefits, have been made possible by the incorporation of certain phytochemicals into foods or dietary supplements. These specialised goods, are able to provide health benefits above and beyond basic nutrition. Examples include green tea, soy, tomatoes etc. Schematic representation of phytochemical analysis and its uses are given in the diagram **Fig. 2**.

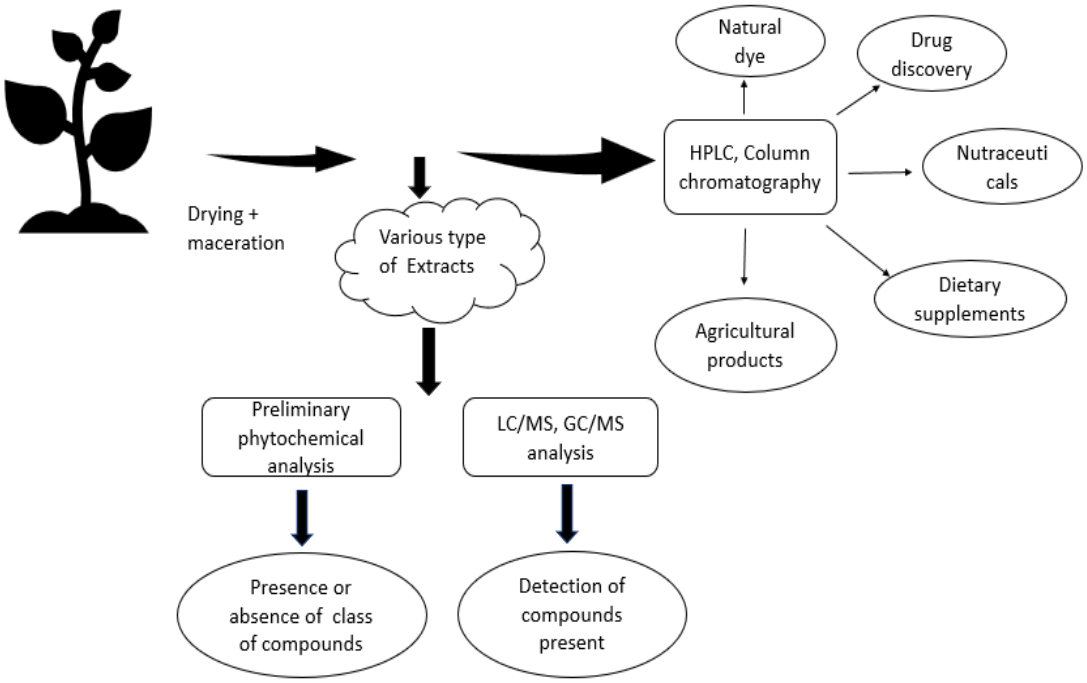


Fig. 2: Schematic representation of phytochemical analysis from plants.

Research on the leaves of *Ficus exasperata*, using both methanolic and aqueous extracts were utilised to figure out their anti-inflammatory properties. It was discovered that the aqueous extract contained more phytochemical component groups.

The methanolic extract fraction, were also revealed to have large number of phytocompounds, which were similar to the phytochemical groups obtained in the aqueous extract (Nworu et al., 2013). Six fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey were chosen to ascertain their phytochemical and antioxidant capabilities. Several assays were used to measure the antioxidant activity, total phenolic content, and total flavonoid content of the fig samples. The results demonstrated that the levels of antioxidants and phytochemicals varied throughout the fig accessions. "Krmz Siyah" was the accession with the highest phenolic content, and "Sarlop" had the highest flavonoid content. The antioxidant activity evaluation of the fig accessions was done using DPPH and ABTS assays. The results showed that the "Sarılop" accession exhibited the highest antioxidant activity in both assays (Çalışkan & Polat, 2011).

The leaf and bark powder of *Ficus infectoria* were extracted using a mixture of solvents including ethanol: water (50:50), petroleum ether, chloroform, and methanol. The examination revealed the presence of a variety of phytochemicals, including glycosides, alkaloids, proteins, carbohydrates, tannins, flavonoids, and phytosteroids in the bark extracts (Kumar et al., 2012). A novel sphingolipid called ficusamide was discovered in the methanolic extract of the stem bark of *Ficus exasperata*, along with nine known compounds, including three furanocoumarins: oxypeucedanin hydrate, bergapten, and six other known compounds. The structures of these compounds were determined. In the antimicrobial tests carried out by the researchers, ficusamide showed less antibacterial activity than the isolated furanocoumarins (Dongfack et al., 2012).

The antioxidant, α-amylase, and α-glucosidase activities of the crude acetone extract from the leaves of 10 different *Ficus* species were examined. *Ficus lutea* stood out among them with a remarkably high polyphenol content. The same plant also showed excellent antioxidant and α-glucosidase activity (Olaokun et al., 2013). The bioactive phytochemicals generated from *Ficus sycomorus* leaves were analysed using Gas chromatography-Mass spectrometry (GC-MS). The existence of 22 distinct phytochemicals was discovered, some of which demonstrated a wide range of

insecticidal and acaricidal effects. These natural substances have the potential to become an environmentally friendly alternative to synthetic pesticides in the control of some insects and mites. Notably, the leaf phytochemicals were observed to have a repellent effect on adult females of *Tetranychus utricae*, *Aphis craccivora*, and *Sitophilus oryzae*, even at very low concentrations (Romeh, 2013).

Bello et al. (2013) conducted a qualitative phytochemical analysis on extracts of *Ficus sycomorus* stem bark and *Piliostigma thonningii* roots. They employed the cold percolation method to get ethyl acetate, aqueous, chloroform, methanolic, and N-hexane extracts from both plant parts. Notably, the methanolic extract of the stem bark of *Ficus sycomorus* was found to contain flavonoids, alkaloids, reducing sugars, glycosides, resins, saponins, and tannins.

The leaves of *F. exasperata* were analysed to determine their nutritional and antinutrient phytochemical components. This research characterised the phytochemicals found in the leaves. A close examination of the leaves revealed several helpful phytonutrients such as dietary fibre carbohydrates, ascorbic acid, proteins etc. Antinutrients such as alkaloids, saponins, phytates, and tannins were also discovered in extremely low concentrations, similar to those seen in edible leafy greens (Bello et al., 2014). Phytochemical components of methanolic extracts of *Ficus hispida* stem bark were examined. In addition to several known chemicals including amyrin acetate, lupeol acetate, lupeol, and α-amyrin, two new compounds, α sitosteryl capriate and lanost-5-enyl caprylate, were discovered after the methanolic crude extracts were exposed to silica column. All compounds were structurally elucidated, and it was noted that these compounds were reported from plant bark for the first time (Chaudhary et al., 2014).

In another study six species of *Ficus* were examined to determine the various phytochemicals present in their leaves, using two solvent extracts: ethanol and chloroform. Among the six species studied, the ethanolic extract of *F. sycomorus* and *F. exasperata,* as well as the chloroform extract of *F. mucuso, F. glumosa,* and *F. vogelli*, were found to contain tannins. Meanwhile, flavonoids were present in the chloroform extract of all six species. Saponins and alkaloids were detected in *F.*

cordata, F. sycomorus, F. glumosa, and *F. vogelli,* while steroids and triterpenes were found in *F. mucuso, F. glumosa, F. vogelli,* and *F. exasperata*. Notably, none of the species studied were found to contain anthraquinones (Oladiipo et al., 2014). The methanol extracts of *Ficus benjamina* stem, root, and leaves were subsequently extracted using different solvents based on their polarity. GC-MS and HPLC techniques were used to examine the phytochemical content of each extract. The essential oil extracted from the stem and roots were analysed via GC-MS and revealed the presence of four and eight components respectively. The stem extract included four phenolic chemicals, but the leaf extract contained only one, which was caffeic acid. The root fractions had the most antioxidant activity, while the stem and leaves had strongest antibacterial activity (Imran et al., 2014).

Using petroleum ether, chloroform, and ethyl acetate as the three distinct solvents, the phytochemical makeup of *Ficus binnendijkii* leaves was examined. Numerous phytochemical groups, including steroids, carbohydrates, flavonoids, proteins, tannins, and alkaloids, were detected in the extracts after analysis. GC-MS examination of the petroleum ether extract indicated the presence of 46 phytochemicals, with the major ones being α-amyrin, phytol, moretenol, methyl hexadecanoate, methyl-9,12-octadecadienoate, and methyl tetra decanoate (El-Rafie & Sleem, 2016). Another study investigated the phytochemical composition and antioxidant capacity of various fig (*Ficus carica* L.) plant sections from three Tunisian fig types. The study focused on the peel, pulp, and seeds of the fruits, along with their respective juices. The results showed that all three fig types were high in tannins, flavonoids, and polyphenols with the "Bouhouli" variety's peel and pulp having the greatest total phenolic and flavonoid levels. The variety named "Bornio" had the most tannin content. Furthermore, the antioxidant activity was high in all parts and juices of the fig varieties implying their potential as natural source of antioxidants (Harzallah et al., 2016).

In Nigeria and other African nations, *Ficus capensis* is frequently used as a medicinal plant to cure a variety of illnesses and diseases, such as respiratory problems, stomach-aches, convulsions, and impending abortion. The ethanol extract

of this plant's leaves was subjected to a routine GC-MS analysis by researchers to provide an insight on its ethno-medical applications. The results of the analysis showed the presence of ten phytochemical components, including the major components 2, 2-dimethylbutane, 5-cyclo-methylidadiene hepta-3,5-diene-1-ol, heptanoic acid, 5-dien-1-ol, and hepta-1,5-1-ol (Aja et al., 2017). Through phytochemical analysis, it was discovered that the aqueous leaf extract of *Ficus capensis* contains significant amounts of flavonoids, terpenoids, tannins, and alkaloids. These substances are well known for their healing abilities and are frequently used as a home treatment for a variety of medical issues. Only traces of other phytochemicals, such as glycosides, saponins, and steroids, were found in the extract. This leaf's ability to serve as both a food source and a leafy vegetable is supported by the presence of additional vital nutrients like minerals and vitamins (Odoemenam & Ujam, 2017). GC-MS analysis of bioactive components of *Ficus mollis* leaves were done with gas chromatography-mass spectrometry (GC-MS) to investigate the phytochemical composition of its leaves. The study's goal was to identify and analyse principal phytochemical components found in *Ficus mollis* leaves, with emphasis on tannins, alkaloids, steroids, triterpenes, and saponins. The researchers were interested in these compounds because of their bioactive features and potential medicinal effects. This work adds to our understanding of the phytochemical composition of *Ficus mollis* leaves and provides useful information about the existence of bioactive chemicals. This study addresses a significant gap in the present literature by giving GC-MS data and conducting phytochemical analyses on *Ficus mollis*, which previously lacked scientific information (Priya & Abinaya, 2018).

Ten varieties of Algerian fig, *Ficus carica* belonging to uniferous, biferous, and caprifig trees were used to identify the phenolic and flavonoid contents as well as antioxidant and antimicrobial activities of its methanolic leaf extracts. Total phenolic contents were found to be the highest in the biferous leaf extract which was followed by the uniferous varieties. They also revealed the correlation between polyphenols present in the extract to that of the antioxidant activity. Higher the polyphenol content, higher the antioxidant activity expressed by the extracts (Mahmoudi et al., 2016).

LC-MS/MS and chemometric analysis were used to examine the phytochemical diversity of 13 different *Ficus* species. The findings revealed that a wide variety of phytochemicals, including flavonoids, phenolic acids, and alkaloids, were present in these *Ficus* species. Certain phytochemicals were found to be present in high levels in some species, for instance, *F. carica* demonstrated high levels of quercetin and kaempferol derivatives. The diverse plant species were also discovered to have varied antibacterial and quorum sensing inhibitory actions. According to the study, *Ficus* plants may be useful as sources of inherently antibacterial and quorum sensing inhibitory substances (Elhawary et al., 2018).

Palmeira et al. (2019) investigated the phytochemical content and bioactive qualities of various portions of a common fig variety from Portugal (*Ficus carica*). The scientists concentrated on the nutritional value, chemical makeup, and bioactive substances found in fruits, leaves, and latex. To detect its nutritional value and phytochemicals as well, proximate analysis and High-Performance Liquid Chromatography (HPLC) were utilised. According to the findings, figs are a good source of dietary fibre, carbohydrates, and minerals like, calcium, potassium and magnesium. The fruit was abundant in flavonoids, phenolic compounds, and antioxidants. Fig leaves were discovered to be high in phenolic compounds, notably flavonoids, which possess powerful antioxidant and antibacterial activities. Although present in lesser amounts, latex displayed significant antioxidant and antibacterial capabilities.

A more recent study optimised the microwave-assisted extraction of bioactive components from *Ficus racemosa* fruits. Several phytochemicals were discovered, including polyphenols (ascorbic acid, gallic acid, catechin, tannic acid, chlorogenic acid, ferulic acid, and quercetin). They could improve the circumstances to maximise the yield of each phytochemical. HPLC-DAD was employed to quantify the phytochemicals detected (Basista, et al., 2020).

The phenolic profile and related bioactivities of the *Ficus sycomorus* L., or sycomore fig, leaf and stem bark have been investigated in recent research. The goal of the study was to discover and measure the phenolic compounds in *Ficus sycomorus*

leaf and stem bark extracts in order to assess their potential for antioxidant and enzyme inhibition. In order to learn more about the biological functions and potential health advantages of the discovered phenolic compounds, they also conducted bioinformatics research. The phenolic components were identified and quantified using high-performance liquid chromatography (HPLC). The extracts of *Ficus sycomorus*'s leaf and stem bark were found to have a rich phenolic profile. Flavonoids, phenolic acids, and tannins etc. were some among the phenolic substances that were discovered (Suliman et al., 2021). Phytochemicals reported from the genus *Ficus* throughout the years is given in the **Table 1**.

Table 1: Phytochemicals reported from the genus *Ficus*

Sl No	Plant	Plant part	Phytochemical compounds	Reference
1	*F. carica*	Latex	1-butanol-3-methyl, 1-butanol-2-methyl,1-pentanol S,1-hexanol 1-heptanol, phenylethyl alcohol, phenylpropyl alcohol, α-thujene, α-pinene, β-pinene, limonene, eucalyptol, terpinolene, cis-linalool oxide, linalool, epoxylinalol α-guaiene, α-bourbonene, β-caryophyllene, trans-α-bergamotene, α-caryophyllene, τ-muurolene, germacrene, cadinene, α-calacorene, methyl salicylate, quinoline, psoralene	Oliveira et al., 2010
2	*F. amplissima*	Bark	(-)-(1r,3r) menthol, diisononyl phthalate, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, ethyl α-D glucopyranoside, Myristic acid, methyl hexadecanoate, N-hexadecanoic acid, ethyl hexadecanoate, isopropyl palmitate, phytol, 9,12-octadecadienoic acid, heptadecene-(8)-carbonic acid, Stearic acid, 2-butenedioic acid (e)-, bis(2-ethylhexyl) ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, stearaldehyde, 1,2-benzenedicarboxylic acid, 9-octadecenoyl chloride, (z), (-)-isolongifolol, acetate, γ-sitosterol, olean-12-en-3-one, lup-20(30)-en-3-one, 9, 19-cyclolanost-24-en-3-ol, (3.beta.), lupeol	Murugan et al., 2012

			lanosterol acetate, olean-12-en-3-yl acetate, lup-20(29)-en-3-yl acetate	
3	*F. retusa*	Leaf	1,2-benzenedicarboxylic acid dibutyl ester, phenol, 4-(2-aminopropyl butyrolactone,1,2-benzenedicarboxylic acid dibutyl ester	Aly et al., 2013
4	*F. deltoidea*	Leaf, Fruit	epicatechin, quercetin-3-rutinoside, quercetin 5,4'-di-O-beta-D-glucopyranoside, myricetin, naringenin	Dzolin et al., 2014
5	*F. carica*	Leaf	rutin, isoschaftoside, isoquercetin, chlorogenic acid, caffeoyl malic acid psoralen and bergapten, psoralic acid glucoside	Takahashi et al., 2014
6	*F. sycomorus*	Root	pentacyclic triterpenoid isomers	Abubakar et al., 2016
7	*F. arnottiana*	Leaf	hexadecen-1-ol eicosanoic acid, tetratriacontane, sulfurous acid, octadecyl 2-propyl ester, tritriacontane, octadecane, 3-ethyl-5-(2-ethylbutyl)-oxirane, heptadecyl tritriacontane, cyclotrisiloxane, hexamethyl hop-22(29)-en-3 beta-ol, pentadecanal lup-20(29)-en-3-ol acetate, (3 beta)-ethanol, 2-hydroxyethylhydrazine, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 2,6-lutidine 3,5-dichloro-4-dodecylthio cyclotrisiloxane, hexamethyl tetratriacontane	Babu et al., 2017
8	*F. carica*	Leaf	myristic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, cerotic acid, linoleic acid, α-linolenic acid, stearyl alcohol, behenyl alcohol, pentacosan-1-ol, ceryl alcohol, montanyl alcohol, melissyl alcohol, phytol, stigmasterol, β-Sitosterol, lanosterol, cycloartenol, squalene, α-amyrin, β-amyrin, germanicol, lupeol	Ivanov et al., 2018
9	*F. religiosa, F. benghalensis* *F. palmata* subsp.*virgata* *F. virens* *F. lyrata* *F. benjamina* *F. hispida,* *F. racemosa*	Root Stem Root	quinic acid, protocatechuic acid, chlorogenic acid, mangiferin, catechin, epicatechin, rutin, hispidine, 7-hydroxycoumaric acid, ferulic acid, vanillic acid, luteolin, quercetin, apigenin, kaempferol, chrysin, betulinic acid, ursolic acid, oleanolic acid	Kumar et al., 2018

10	*F. curtipes*	Stem, Bark, Leaf	flavan-3-ols, apigenin derivatives, apigenin-di-glycosides	Andrade et al., 2019
11	*F. sycomorus*	Leaf, Fruit	benzoic acid, vanillic acid, caffeine, ellagic acid, rutin, chlorgenic acid, pyrogallol, p-hydroxybenzoic acid quinol, o- coumaric acid, myricetin, cinnamic acid, quercitin, rosmarinic acid, neringenin, kaempferol, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, campesterol, stigmasterol, lanosterol	El-Beltagi et al., 2019
12	*F. carica*	Fruit	protocatechuic acid, 4-O-caffeoylquinic acid 3, p-coumaric acid, trans cinnamic trans ferulic acid, salviolinic acid, rutin, luteolin-7-o-glucoside, apigenin-7-o-glucoside, quercetin, kaempferol, naringenin, apigenin, luteolin, cirsiliol, gallic acid, protocatechuic acid, 4-O-caffeoylquinic acid, p-coumaric acid, luteolin-7-o-glucoside, apigenin-7-o-glucoside, naringin quercetin, kaempferol, naringenin, apigenin, luteolin	Khadhraoui et al., 2019
13	*F. glomerata*	Leaf	3 methoxycatechol, 7-methoxychromone, p-cymene, anthole, 13-hydroxy-trideccanoic acid, 10-hydroxy-undecanoic acid, 4-(2hydroxypropoxy)-3,5-dimethyl-phenol,4,9-octadecadiynoic acid, phthalic acid, ethyl ehexyl ester, khayanthone	Shaikh et al., 2020
14	*F. sycomorus*	Stem Bark	heterocyclic members of pyrazole, imidazole, triazole, thiazole, 1, 3-benzenedicarboxylic acid, bis (2-hethylhexyl) ester	Saheed et al., 2020
15	*F. hirta*	Root	epicatechin, chlorogenic acid, epiafzelechin, psoralenoside, methoxypsoralenoside, dihydrogenpsoralenoside, pelargonidin 7-glucoside, aloin A, isoliquiritigenin vitexin, picraquassioside A, isoeugenitol, kaempferol, naringenin, apigenin, bergapten, resveratrol, pinolenic acid	Tang et al., 2020
16	*F. hirta*	Fruit	methyl-1,2,3,4-tetrahydro--carboline-3-carboxylic acid, dihydrophaseic acid, vomifoliol, dehydrovomifoliol, pubinernoid A, 2-phenylethyl-O- d-	Chen et al., 2020

			glucoside, 1-O-trans-cinnamoyl--D-glucopyranosyl (1-6) glucopyranoside, 4-O-benzoyl-quinic acid 3-O-benzoyl-quinic acid, benzyl-d-glucopyranoside, 2-O-benzoyl-butanedioic acid-4-methyl ester pinocembrin-7-O--d-glucoside, naringenin-7-O--d-glucoside, eriodictyol-7-O--d-glucoside, luteolin apigenin, umbelliferone	
17	*F. auriculata*	Fruit	5-hydroxy-2-(4-hydroxy-2-methyl-2H-pyran-6-yl)-9-methyl-2H,3H,4H-naphtho[1,2-b] pyran-4-one,	Chandran et al., 2022
18	*F. exasperata*	Leaf	nonadecane, pentadecane, 1-dodecanol, 2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-d), Isopropyl myristate, n-hexadecanoic acid, behenic alcohol, oleic acid, propanoic acid, decyl ester, octacosanol, bis(2-ethylhexyl) phthalate	Mohammed et al., 2022
19	*F. thonningii*	Leaf	phenolic glycosides, quassin	Muhammad et al., 2022
20	*F. platyphylla*	Bark	2,4-dimethoxyamphetamine, 4-phenoxyphenethylamine, 2-octylphenylketone, ethanol, 2,2-(dodecylamino) bisphenyl acetic acid, ethylmalonic acid, 2-uorophenyloctylester, isonipecotic acid, N-(4-uoro-2-tri uoromethylbenzoyl), pentyl ester, sarcosine, N-(4-ethyl benzoyl) - dodecylester, astilbin, succinic acid, 3-ethyl phenyl heptadecyl ester, triacontylbenzene, octaethylene glycol	Hassan et al., 2022

PART II
Antioxidant Activities

Compounds having the ability to inhibit oxidation is termed as antioxidants. They are of two types, endogenous and exogenous. Exogenous antioxidants are that enter the system by food or supplements, vitamin E, vitamin C, carotenoids, polyphenols, etc. Endogenous antioxidants are produced in the body by metabolism, which may be enzymatic or non-enzymatic. One of the major endogenous enzymatic antioxidants include super oxide dismutase (SOD), catalase (CAT), glutathione

peroxidase (GPx) glutathione reductase (GR) and peroxiredoxins (Flieger et al., 2021).

Synergetic action of all these antioxidants helps in free radical quenching while they exceed the limit. Antioxidants protect cells by preventing free radical reactions, repairing the oxidative damage and by inactivating the product of free radical reactions. Free radicals such as ROS (reactive oxygen species), and RNS (reactive nitrogen species) will be produced in all aerobic organisms during mitochondrial respiration during ATP production (Pham Huy et al., 2008). Unhealthy diet, exposure to pollutants, radiation etc. are also leading to the formation of large amount of ROS and RNS in the body. When they exceed the biological limit of the cells, this will lead to oxidative stress which in turn results in the oxidation of cellular components, and lead to a number of non-communicable diseases. ROS and RNS have different beneficiary roles in the cells when they are perfectly balanced with their production and quenching. They have role in cell metabolism, growth, differentiation and migration. ROS can activate genes, and contribute to apoptosis by regulating EDRF (endothelium derived relaxing factor) in normal cells. They are also an essential part of the innate immune system in the fight against pathogens (Satish et al., 2013).

The polyphenols and flavonoids that are said to be abundant in the genus *Ficus* are in charge of its antioxidant properties. Various solvent extracts of nearly all plant parts, including leaves, bark, fruits and roots have been proven for their antioxidant properties. **Fig. 3** represents the cases of ROS production and the damage induced by this ROS in the biological system. This review includes research on the antioxidant capacity of the genus *Ficus*, based on numerous *in vitro* assays and diverse extracts from different plant parts.

Antioxidant potential of six commercial fig varieties with different colours were analysed by Solomon et al. (2006). They discovered a significant correlation between colour, the overall quantity of polyphenols, flavonoids, anthocyanins, and antioxidant capacity. In darker varieties, these phytochemicals were more prevalent. Fruit skins contained the greatest quantity of phytochemicals and antioxidant activity.

The quantity of anthocyanins and polyphenols had a direct impact on the antioxidant capacity. The anthocyanin component considerably increased the Mission and Brown-Turkey cultivars' overall antioxidant capacity. In comparison to other fig varieties, the Mission type has the highest levels of polyphenols, flavonoids, anthocyanins, and overall antioxidant capacity.

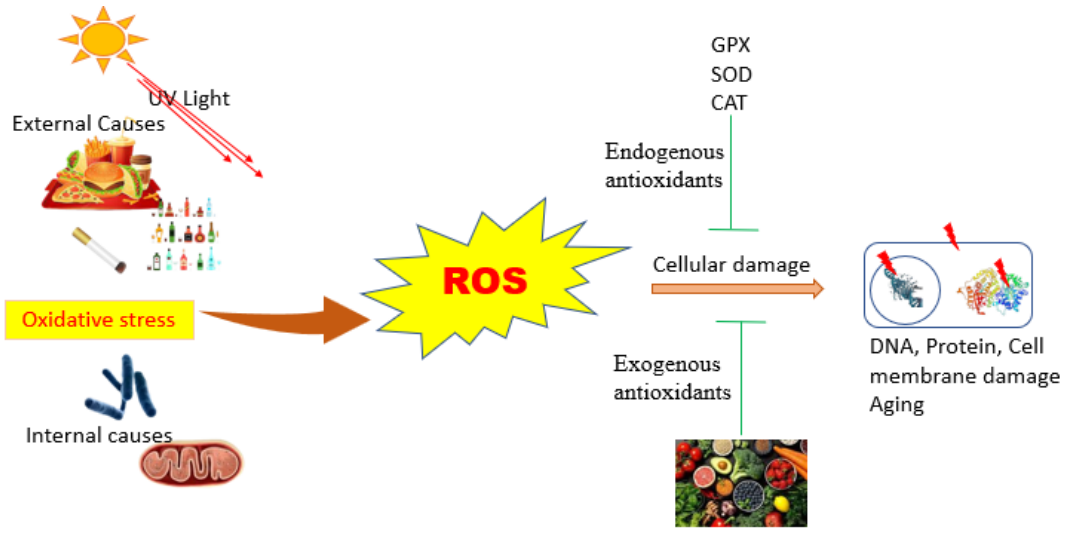


Fig. 3: Causes and effects of reactive oxygen species in biological system

Methanolic and acetone extracts of *F. benghalensis* aerial root and *F. racemosa* stem bark was tested for reducing power, ABTS, and DPPH radical scavenging assays. Methanolic extracts of *F. benghalensis* and acetone extracts of *F. racemosa* were found to have higher quantities of polyphenols. Despite the fact that methanolic plant extracts have a strong hydrogen donating ability, both extracts showed a dose dependent increase in the reducing power for all antioxidant assays (Manian et al., 2008). Methanolic extracts of bark, fruits and leaves of *F. microcarpa* was found to have excellent antioxidant power whereas the ethyl acetate fraction showed higher number of polyphenols and strong ability in the antioxidant assays tested such as ABTS, DPPH etc. (Ao et al., 2009).

ABTS, DPPH, hydroxide radical, and super oxide radical scavenging assays were used to evaluate the potential of *F. racemosa* ethanolic and aqueous extracts to scavenge free radicals. Ethanolic extracts were found to be superior antioxidants, with a concentration-dependent improvement in free radical scavenging capacity

(Veerapur et al., 2009). Antioxidant studies were conducted on the effects of *F. racemosa* fruit ethanolic extract and its fractions. The ethanol extract significantly reduced the number of free radicals in the DPPH experiment. For the first time, 3-O-(E)-caffeoyl quinate was isolated from this plant, which also exhibited strong antioxidant properties. The antioxidant and immune-boosting properties of water extract (WE) and crude hot-water soluble polysaccharide (PS) from *F. carica* were studied by researchers. The antioxidant ability of the extracts was assessed using the DPPH, superoxide, hydroxyl radicals, and reducing power tests. Significant scavenging behaviours on DPPH were shown by both WE and PS. PS, on the other hand, exhibited greater scavenging efficacy against superoxide and hydroxyl radicals (Yang et al., 2009).

In vitro experimental models were used to examine the antioxidant capacity of ethanolic leaf extract of *Ficus exasperata*. Using the DPPH and lipid peroxidation assays, reducing activity was evaluated. In rat brain homogenates, the extract scavenged 2,2-diphenyl-1-picryl hydrazyl (DPPH) (IC 50.0499 ± 0.302) and inhibited lipid peroxidation (IC50 1.283 ± 0.923). The extract had a moderate level of antioxidant activity when compared to the standard (n-propyl gallate) used in the study, which were comparably high (Abotsi et al., 2010). Antioxidant assays were performed on several *F. carica* L. accessions. The ferric-reducing antioxidant power assay indicated that among the numerous accessions studied, black-skinned fig fruits have potential antioxidant activity. According to the study antioxidant activity of fruits are also connected to their polyphenol and anthocyanin concentration (Çalişkan et al., 2011). *F. banghalensis* latex methanolic extract was examined for free radical scavenging activities using DPPH, ferric chloride, and phosphor molybdenum scavenging tests.

The studies revealed that the latex was of high potential ability in free radical scavenging (Yadav et al., 2011). Utilizing a variety of *in vitro* assays, Ali et al. (2012) investigated the antioxidant activity of *Ficus carica* hydro-alcoholic leaf extracts and discovered that the plant extract's flavonoids make the hydro alcoholic extract an effective antioxidant. Trolox equivalent antioxidant capacity and ferric reducing

antioxidant power assays were used by the researchers to investigate the antioxidant capacity of several fig (fruit) genotypes and cultivars growing in north eastern Turkey. The test results revealed that local fig genotypes have better overall antioxidant capacities than cultivars. These findings imply that the genotype plays a major role in determining the bioactive chemical content of figs. The study offers insightful data on the genotypic variety of locally grown figs and its health advantages (Ercisli et al., 2012).

Comparing the anti-oxidant properties of *Ficus benghalensis* (FBH) and *Moringa oleifera* (MRH) root extracts, the antioxidant properties of the FBH extract were more potent and showed concentration-dependent reduction of microsomal lipid peroxidation (Satish et al., 2013). Jain et al. (2013) examined DPPH radical scavenging, reducing power assay, and iron chelating activities of *Ficus benjamina*. The DPPH radical scavenging experiment was used to examine the antioxidant capacity of the *Ficus exasperata* stem bark after it had been extracted using three different solvents. When contrasted with the employed petroleum ether extract, it was discovered that the chloroform and ethanol extracts had better antioxidant activity (Amponsah et al., 2013).

Using the ABTS, ferric reducing antioxidant power assay, dried fruits of *Ficus carica* were evaluated for their antioxidant capacity. These fruits were having enormous antioxidant potential (Akanni et al., 2014). The *in vitro* antioxidant assays DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and reducing power assay were used to assess the extracts of *Artocarpus altilis* (stem bark), *Ficus exasperata* (leaves), and *Khaya africana* (fruits). Results showed that all three plant extracts had good antioxidant activity in all the assays that were studied. N-butanol extracts of *Ficus exasperata* leaves were found to exhibit free radical scavenging activity, despite the separated components such as apigenine, glucoside, isoquercetin, hydroxy benzoate etc. were not having antioxidant potential (Taiwo & Igbeneghu, 2014).

Several bioassays were performed on the aqueous leaf extract of *Ficus asperifolia* to test its antioxidant capabilities. The ferric reducing power assay (FRAP)

indicates its ability to convert ferric ions to ferrous ions and act as an antioxidant. The extract's DPPH radical scavenging potential was examined, revealing its ability to neutralise free radicals. The extract also revealed the potential to scavenge nitric oxide and hydroxyl radicals (Ojo & Akintayo, 2014). Tests on 10 different *Ficus carica* cultivar's antioxidant capacity using DPPH free radicle assay revealed that they have high phenolic content and high antioxidant capacity. Biferous and uniferous cultivars among them were noted to have higher phenolic contents. Free radical scavenging ability was correlated with the number of phenols found in each plant variety (Mahmoudi et al., 2016).

Antioxidant activity of petroleum ether and chloroform extracts of *F. krishnae* stem bark was analysed using various *in vitro* assays such as DPPH radical-scavenging activity, ABTS radical scavenging assay, phosphomolybdenum assay and ferric reducing power assay. The results they obtained suggests that *F. krishnae* stem bark extracts possess considerable antioxidant activity. The chloroform extract exhibited higher antioxidant potential compared to the petroleum ether extract (Kanjikar et al., 2017). *F. benghalensis* bark extracts demonstrated DPPH radical scavenging capacity in a concentration dependent manner, with values ranging from 21.42 ± 0.457 % to 82.6 ± 2.395%. The study also discovered that, among the extracts tested methanolic extract had the highest capacity for free radical scavenging (Raheel et al., 2017). DPPH free radical scavenging assay and NO scavenging assay were used to test the antioxidant activities of *F. infectoria*, *F. krishnae*, and *Ficus benjamina* leaf methanolic extracts. *Ficus benjamina* and *F. infectoria*, displayed highest levels of activity in DPPH assays and nitric oxide scavenging assays respectively (Jassal & Sharma, 2019). The antioxidant capacity of aqueous leaf extracts from *Ficus exasperata*, *Moringa oleifera*, and *Jatropha tanjorensis* was investigated. The research found that all three plants exhibit antioxidant activity, but that the antioxidant activity of *Moringa oleifera* and *Jatropha tanjorensis* was higher than that of *Ficus exasperata*. Significant radical scavenging activity was demonstrated by *Ficus exasperata* (FE) against DPPH and NO radicals. DPPH and NO scavenging activities of FE had IC50 values of 12.5 and 15.0 mg mL^{-1}, respectively. The most effective plant for scavenging free radicals was *Moringa olifera* (Anigboro et al., 2019). Hydro-

alcoholic extracts of *F. bangalensis* were evaluated by Khanal & Patil (2020) with seven distinct antioxidant assays, including the metal chelating assay. They discovered that the extract had the highest level of free radical scavenging in the ABTS assay and had strong chelating properties. When the antioxidant activity of *F. benghalensis* leaf ethanol extract was tested against DPPH assay, it was discovered to be an efficient antioxidant with a 75.5% ability to scavenge DPPH free radicals (Abdel Rahman et al., 2021).

Anticancer Activities

Cancer is a condition in which a cell loses its ability to respond to signals that normally lead to cell growth and division. This will cause the tumour to develop out of control, invade neighboring healthy tissues, and eventually spread across the entire body. This loss of control is frequently brought on by potentially dynamic genetic alterations. There may be series of mutations which lead to change in cellular functions. The main mutation that causes cancer to develop is a gain of function mutation for oncogenes and a loss of function mutation for tumour suppressor genes. Cancer is not only seen in humans, but also in every other species with the exception of a few primitive mammals and hemichordates (Aktipis et al., 2015). In 2020, there were 19.3 million new instances of cancer (18.1 million excluding non-melanoma skin cancer) and about 10.0 million deaths from cancer (9.9 million excluding non-melanoma skin cancer) globally (Ferlay et al., 2021). Cancer can develop from a variety of tissues or cells, resulting in the emergence of more than 100 unique types. Tumour can be benign or malignant, benign tomours are the uncontrolled growth which will remain confined to the place of formation and will not spread or evade to other locations. Malignancy differs from benign tumour in their ability to invade nearby tissues and migrate to any other part of the body. Benign tomours can be easily removed by a surgery or other methods like radiation.

Tomours can be classified according to the cells of origins such as carcinomas, sarcomas, leukemias and lymphomas. 90 % of human cancers are carcinomas which are the malignancies found on the epithelial cells, whereas sarcomas are rare type of

tomour in humans which affect the connective tissues such as bone, muscle, cartilage and fibrous tissue. Leukemias and lymphomas which contribute to 8% of human cancers arise from the blood forming tissues and immune cells respectively. These four types of cancers are again classified according to their occurrence in different organs for example breast or lung carcinoma (Cooper, 2000). The prostate, lung, bronchus, colon, rectum, and urinary bladder have the largest percentages of cancer types in men. Breast, lung, bronchus, colon, rectum, uterine corpus, and thyroid cancer are the most common in women (Hassanpour & Dehghani, 2017). Mutations, chemical substances, smoking, some viral and bacterial infections, epigenetics etc. are the common cause behind most of the tomour development. When cells become malignant, their properties change correspondingly. Hanahan & Weinberg (2000) outline the major characteristics of cancer cells that allow them to multiply indefinitely, which are briefly discussed here in **Fig. 4**.

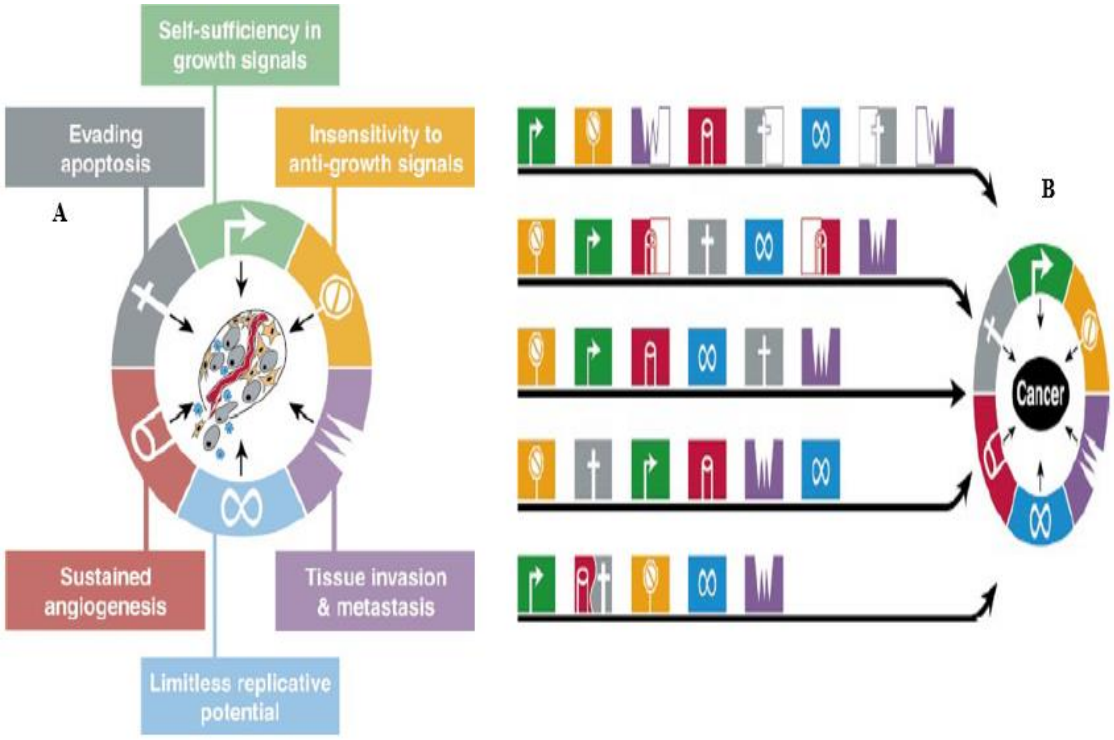


Fig. 4: A: Representation of the acquired capabilities of cancer cells and B: Represent the parallel pathways of cancer (Hanahan &Weinberg, 2000).

Cancer cells become independent and they no longer require an external signal for growth or division, and they become resistant to growth halt signals. These cells also evade the apoptotic systems. The replicative potential of the cells increases to the point where they will never stop dividing and collect a large number of mutations. They may produce new blood vessels for the site of growth by angiogenesis, which will nourish these unconditionally dividing cells (**Fig. 4**). They can also become metastatic and invasive. The cells exhibit the Warburg effect, in which they rely on glycolysis, which does not use oxygen in energy production. They can also infiltrate the immune system and obtain cancer cells that are not killed by the patient's defence system. They also exhibit dysregulated metabolism. Even though the capabilities of cancer cells are almost similar in most cases, the order of accumulation of these capacities are varying in various types and subtypes of tumours (**Fig. 4**). Occasionally, a single mutation might result in the aggregation of several capacities in the cancer cell. A prime example is the loss of function mutation in the P53 gene, which causes angiogenesis and apoptosis resistance.

Apoptosis is the process of self-control of a cell in regulating its division. Apoptosis was derived from a Greek word with meaning dropping of and was first described by Kerr and his colleagues in the 1970 (Wong, 2011). Understanding the mechanisms behind apoptosis is crucial as it will help in treating cancer by targeting genes or proteins regulating apoptosis. There are two main initiation pathways and another less described pathway for apoptosis. The intrinsic and extrinsic path ways of apoptosis is represented in **Fig. 5**.

Extrinsic pathway starts when death receptor (TNFR1) binds to the death ligand (TNF & FasL) (Hengartner, 2001). Intra cellular death domain of death receptor recruit adapter proteins TRADD, FADD and Caspase 8 (Schneider & Tschopp, 2000). This whole ligand receptor adapter protein complex is now called death inducing signalling complex (DISC), which will activate pro caspase 8. Caspase 8 initiates apoptosis by cleaving the executioner caspases (Karp, 2009). Intrinsic pathway starts with any internal stimuli such as irreplaceable DNA damage, oxygen limited conditions, extreme oxidative stress etc. (Karp, 2009).

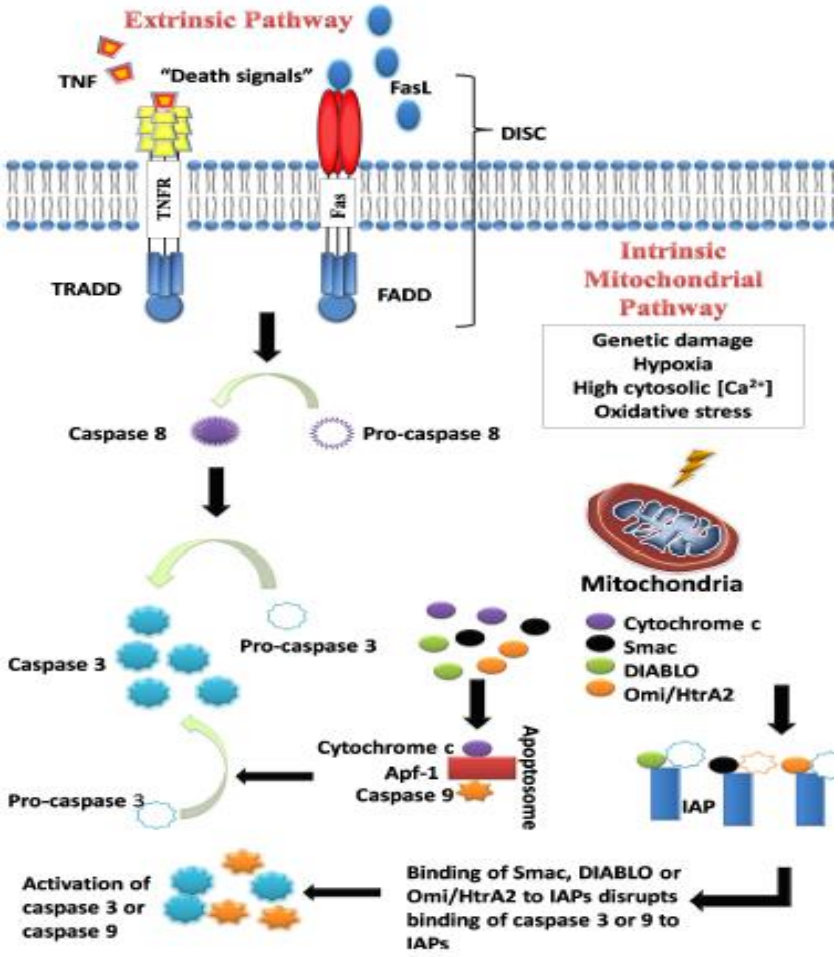


Fig. 5: The intrinsic and extrinsic path ways of apoptosis (Hengartner, 2001)

The Bcl-2 family of proteins, which are divided into two categories, monitors the intrinsic pathway. They are specifically, proapoptotic (such as Bax, Bid, Bik, Bim, and Hrk) and antiapoptotic (such as Bcl-2, Bcl-X_L, Bcl-W, Bfl-1, and Mcl-1) proteins (Reed, 1997). In this case, mitochondria are implicated, their permeability rises, and pro-apoptotic chemicals like cytochrome C, and apoptotic factors such as high temperature requirement protein A (HtrA2), mitochondria derived activator caspase (Smac), direct inhibitor of apoptosis protein binding protein, and apoptosis inducing factor (AIF) are released into the cytoplasm (Kroemer et al., 2007). Antiapoptotic genes control apoptosis by preventing the release of cytochrome C into the cytosol, a process that is induced by proapoptotic proteins. When cytochrome c is released, this will form an apoptosome complex with Apaf-1, and caspase 9, and this complex will activate caspase 3 (Kroemer et al., 2007). By attaching to IAPs, Smac and DIABLO

encourage caspase activation (Kroemer et al., 2007). Third and less discussed pathway of apoptosis is intrinsic endoplasmic reticulum pathway, in which mitochondria is not involved, but when endoplasmic reticulum is disrupted due to any extreme condition it leads to this path way by involvement of caspase cascade (Wong, 2011). Apoptosis being the natural death process of damaged or defective cells in a system, it is the best tool in the treatment of cancer. Identifying the molecular targets in the apoptotic pathway is a best method in developing drugs for cancer treatment.

Cancer patients those who are already struggling with the disease and are being burdened by drug-induced side effects are seeking aid from complementary and alternative medicine that has no, or fewer adverse consequences. Many studies are already reported on the importance of plant-based medicines in cancer treatment. WHO reported many countries which still use the traditional herbal medicines effectively and developing countries are studying about that system. In the last 20 years, over 25% of medicine has been sourced from plants, with the remaining 25% coming from chemically altered natural substances. Nine plant-derived chemicals have been approved for use as anticancer treatments, including vinblastine, vincristine, etoposide, teniposide, taxol, navelbine, taxotere, topotecan, and irinotecan (Cravotto, 2010). Campothecine and taxols should be mentioned here because they were extremely strong phytochemicals with effective anticancer properties (Greenwell & Rahman, 2015). There is an urgent need for an effective natural substance to halt the unchecked spread of cancer cells. Studies on plant biochemicals in the regulation of cancer are of utmost importance since they are less harmful to the environment and have less adverse effects than conventional goods.

Breast Cancer Research

The tissues that comprise the human breast range from thick to fatty. When healthy breast cells proliferate uncontrollably, they form a tumour, which may or may not become cancerous. Non-cancerous breast tumours are anomalous growths that stay inside the breast. While benign breast lumps are not fatal, they do increase a woman's chances of acquiring breast cancer. Numerous areas of the breast can develop cancer, including the lobules (lobular cancer), ducts (ductal cancer), nipples (paget

disease), which is relatively rare, the fat connective tissue (stoma), which can also develop the less common phyllodes tumour, and the lining of blood or lymph vessels in the breast (angiosarcoma). Men can develop breast cancer, though they are far less likely than women. Older age, BRCA2/BRCA1 mutations, elevated oestrogen levels, Klinefelter syndrome, a family history of breast carcinoma, and radiation exposure are significant risk factors for breast cancer in men (Giordano, 2018). According to GLOBOCAN 2020 estimates, there are presently an estimated 2.3 million new cases of breast cancer worldwide, making it one of the most frequently diagnosed malignancies and the fifth leading cause of cancer-related deaths (Sung, 2021). There are a number of modifiable and nonmodifiable risk factors present in the case of breast cancer, which are given in the **Table 2**.

Table 2: Modifiable and nonmodifiable risk factors in the onset of breast cancer (Łukasiewicz et al., 2021).

Non-Modifiable Factors	Modifiable Factors
Female sex	Hormonal replacement therapy
Older age	Diethylstilbestrol
Family history (of breast or ovarian cancer)	Physical activity
Genetic mutations	Overweight/obesity
Race/ethnicity	Alcohol intake
Pregnancy and breastfeeding	Smoking
Menstrual period and menopause	Insufficient vitamin supplementation
Density of breast tissue	Excessive exposure to artificial light
Previous history of breast cancer	Intake of processed food
Non-cancerous breast diseases	Exposure to chemicals
Previous radiation therapy	Other drugs

Genetic mutations, which fall under the category of non-modifiable risk factors, are a major contributor to the development of breast cancer. BRCA1 and BRCA2, which are found on chromosomes 17 and 13, respectively, are two high penetrance genes inherited in an autosomal dominant manner (Shiovitz & Korde,

2015). Breast cancer may result from mutations in these two genes, which are actively involved in DNA repair and cell cycle regulation (Hoskins et al., 2008). Other genes such as TP53, CDH1, PTEN, and STK1 are also very important breast cancer genes. TP53 is involved in DNA repair, cell cycle regulation, inception of apoptosis, onset of senescence and maintenance of cellular metabolism. CDH1 is involved in control of cellular adhesions, proliferation and motility of epithelial cells. Whereas PTEN is involved in the cell cycle regulations (Shahbandi et al., 2020; Corso et al., 2016; Kechagioglou et al., 2014). Breast carcinogenesis has been linked to a sizable number of DNA repair genes, such as ATM, PALB2, BRIP1, or CHEK2, which can interact with the BRCA genes (Shiovitz & Korde, 2015). Certain events, such as pregnancy, breast feeding, the first menstrual cycle, and menopause, as well as their duration and any accompanying hormonal imbalance, may be used to potentially induce carcinogenic processes in the breast microenvironment (Łukasiewicz et al., 2021). Over 300 primary tumours were comprehensively profiled (at DNA, RNA, and protein levels) and grouped into biologically similar tumour types for "The Cancer Genome Atlas Project (TCGA)". Based only on mRNA gene expression levels, the identification of the four primary intrinsic breast cancer subtypes (Luminal A, Luminal B, HER2-enriched, and basal-like) was reported by "The Cancer Genome Atlas Network". A combined investigation of human and mouse mammary tumours led to the discovery of the fifth intrinsic subtype—claudin-low breast cancer (Herschkowitz et al., 2007). Breast cancer subtypes and their major characteristics are given in the **Table 3** given below.

Even if the number of breast cancer cases among women in high-income nations is higher, research has shown that these women have good survival rates and a low death rate (Ginsburg, 2017). However, the majority of deaths occur among the breast cancer patients in low-income countries. This alone demonstrates the critical need for breast cancer research, particularly from a nation like India. Medical research nowadays focuses on finding a low side effect cost effective natural product (from biochemicals) that can effectively combat various types of cancer. This is the ground of the current research which mainly focuses on phytochemicals of *F. exasperata* and *F. drupacea* in combat of MCF breast cancer cell lines. This review is mainly focusing

on the anticancer activities of the genus *Ficus* in various cancer models both *in vivo* and *in vitro*.

Table 3: Breast cancer subtypes and its characteristics (Łukasiewicz et al., 2021).

SL No	Brest cancer subtypes	Characteristics
1	Luminal A & B	Comprise 70% of all reports
	A	ER &/PR positive, HER negative. Low grade and slow growing, ER genes expressed
	B	ER genes expressed, Low expression of OXA1, PR genes and high expression of MKI67 and AURKA. ER positive, PR/&HER negative
2	HER2-Enriched Breast Cancer	Comprise 10-15 % of all reports. Fast growing, ERBB2/HER2 and GRB7 genes are highly expressed
3	Basal-Like/Triple-Negative Breast Cancer	Comprise 20 % of all reports. ER, PR & HER negative. Found in women younger than 40. Contribute to approximately 80% of cancers from BRCA1 mutations. High grade and aggressive. Sub divided into 6 subtypes
4	Claudin-Low Breast Cancer	Comprise 7-14 % of all reports. EMT genes are highly expressed. Claudins 3, 4, and 7, occludin, and E-cadherin genes are less expressed.

The antibacterial and cytotoxic effects of ethanol extracts of *F. septica* and *Sterculia foetida* leaves were examined. The plant extracts were found to be effective with potential antimicrobial and cytotoxic capacity selectively against the studied cancer cell lines. Several bacteria and fungi, including *Staphylococcus aureus, Escherichia coli, Candida albicans,* and *Trichomonas vaginalis,* were inhibited in their growth by this extract (Vital et al., 2010).

70% ethanol was utilised as the extraction solvent as researchers investigated the anticancer efficacy of the *Ficus religiosa* (FR) and *Tephrosia purpurea* (TP)

fractions on MCF-7. With an IC50 value of 152.4 μM, TP fractions were the most active and had the highest anticancer activity, while FR had an IC 50 of 160.3 μM. The apoptosis induction caused by the extracts were the basis of the anticancer action. Therefore, both the plants ethanol extract fractions worked well as anticancer agents (Gulecha & Sivakumar, 2011).

The cytotoxicity of *F. carica* latex, fruit and the leaf extracts against HeLa cell lines was examined. The methanolic extract was the last of the three to exhibit anticancer activity, with latex exhibiting the highest level of activity. It was discovered that the anticancer activity was dosage dependent and increased with concentration. The specific cytotoxicity against the cancer cells was caused by the latex and its extracts inducing apoptosis (Khodarahmi et al., 2011).

Ficus religiosa aqueous and alcoholic extracts have been investigated for their antioxidant and anticancer properties. The ORAC test was used by the authors to determine the antioxidant activity of the *F. religiosa* aqueous and alcoholic extracts. Both extracts of *F. religiosa* displayed cytotoxic effect against HeLa cervical cancer cells (Choudhariet al., 2011).

The effects of *F. carica* tree latex on stomach cancer cell lines were studied by Hashemi et al. (2011). The latex was observed to have a concentration-dependent impact, with larger concentrations producing better outcomes. The maximum activity was recorded at a concentration of 5 mg/ml. They discovered that latex selectively targets malignant cells and is not harmful to normal cell types. The primary factors controlling the proliferation of cancer cell lines were the stimulation of apoptosis and the suppression of angiogenesis. It was discovered that the latex of the *F. carica* was highly effective against stomach cancer cell lines.

In another study, researchers tested the anticancer potential of *Ficus religiosa* bark aqueous extract against two human cervical cancer cell lines, SiHa (HPV-16 positive) and HeLa (HPV-18 positive). A dose-dependent increase in activity was reported. The extract triggered G1/S phase cell cycle arrest in SiHa cells by upregulating p53 and p21 proteins and downregulating phospho-Rb protein. The extract triggered apoptosis in HeLa cells by activating caspase-3 and releasing

cytochrome C from mitochondria. In both SiHa and HeLa cells, the extract reduced the production of the viral oncoproteins E6 and E7 (transformation of cervical cancer cells). The extract also had shown antioxidant, anti-inflammatory, and analgesic action, making it a useful medicinal agent (Choudhari et al., 2013).

F. racemosa leaves, bark, and fruit ethanol extracts were examined for their phytochemicals, antibacterial, antioxidant, and cytotoxic properties. Alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids were only a few of the phytochemical components reported. The extract found to exhibit cytotoxicity against a variety of cancer cells, such as HeLa cervical cancer cells, MCF-7 breast cancer cells, and HT-29 colon cancer cells. This extract showed antimicrobial ability against a number of organisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella typhi* (Kambli et al., 2014).

To identify the active components of chloroform extract of *F. religiosa* leaves, dried pulverised leaves were analysed using HPLC-DAD. The extract was also tested for cytotoxicity against human cancer cell lines such as prostate cancer cells (PC3 and PC3-TxR), leukaemia cancer cells (K562 and K562Dox), and breast cancer cells (MCF7). The chloroform extract of *F. religiosa* leaves was found to have cytotoxic effects on all three types of cancer cells. Serotonin and tannic acid, or their isomers, were identified as the active molecules responsible for the cytotoxicity (Abbooda, 2014).

After extracting the *Ficus religiosa* (FR) leaves with ethanol, the leaves were divided into three fractions: chloroform, ethyl acetate, and water. Several assays were used to determine the cytotoxicity of FR extracts on human foreskin surface epithelial (hFSE) cells and prostate cancer cells (PRST2). Ethyl acetate, chloroform, and crude ethanol extracts had shown considerable cytotoxicity towards PRST2 cells that was dosage dependant. According to the trypan blue exclusion assay and the Live/Dead® viability assay, the viability of PRST2 cells treated with FR extracts was noticeably reduced in a concentration-dependent manner. Additionally, the APO-BrdU TUNEL assay revealed that the apoptotic activities of the FR extract increased in a

concentration-dependent manner. On hFSE cells treated with the same extracts, no similar activities were reported (De Las Llagas, 2014).

Using hydrodistillation, the essential oils from *Ficus mucoso* and *Casuarina equisetifolia* leaves were extracted. Analysis revealed that 13.0 % phellandrene, 11.3 % p-cymene, 10.5 % germacrene D, 9.7 % caryophyllene, 9.5 % 1,8-cineole, and 8.7 % copaene were present in *F. mucoso* essential oil. 40.6 % -phellandrene, 15.7 % p-cymene, 14.1 % 1,8-cineole, and 8.4 % terpinolene made up the *C. equisetifolia* essential oil. With an IC50 value of 98.18 µg/mL, *F. mucoso* demonstrated good anticancer activity against Hs578T human breast cancer cells. However, *C. equisetifolia* had no discernible action. *F. mucoso* essential oil was proved to be effective against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus fumigatus* when tested for its antibacterial properties (Essien et al., 2016).

The research team examined the impact of *F. exasperata* (also known as sandpaper fig) leaf extract on the A2780 ovarian cancer cell line. The methanol extract was the most effective, inhibiting A2780 cell growth by 80% at a dosage of 1 mg/mL. A2780 cell growth was suppressed by the extracts in a dose-dependent manner. The extracts had the ability to trigger the cancer cells apoptosis, selectively eliminating the malignancy without causing any harm to healthy tissues (Bafor et al., 2017). Another study investigated the cytotoxic potential of an 85% ethanol extract of *F. religiosa* leaves on the Jurkat T-cell leukaemia cell line. The extract inhibits cell proliferation and promotes apoptosis in a dose-dependent manner. The highest effective dosage was determined to be 100 g/mL, and the extract did not cause any cytotoxicity in the normal cell lines (Maity et al., 2017).

Kumaresan et al. (2018) conducted research on the antioxidant and cytotoxic activity of extracts prepared using *F. religiosa and F. benghalensis* leaves. They combined both the leaf extract to form a mixed extract. The mixed extract was found to have potent antioxidant properties, which also suppress HeLa cell proliferation in a dose-dependent manner. The combined extracts of *F. religiosa* and *F. benghalensis* leaves were found to have the potential to be used as a natural anticancer drug for the

treatment of cervical cancer after further research. Using several cell lines, the cytotoxic effects of *Ficus benghalensis* latex were examined. Three different solvents, such as ethanol, methanol and chloroform, were used to create latex extracts. Cell lines of choice were HeLa, MCF-7, Jurkat, and HL-60. The efficiency of the extracts were in the order ethanol, methanol, and chloroform. HeLa was discovered to be the most vulnerable cell type, followed by MCF-7, Jurkat, and lastly HL-60. According to the report, more research is required to determine whether *Ficus benghalensis* latex has any potential as a chemotherapeutic agent (Tulasi et al., 2018).

Tulasi et al. (2018) investigated the anti-cancer activity of *Ficus religiosa* and *Ficus benghalensis* latex extracts on the human breast cancer cell line MCF-7. The scientists discovered that both latex extracts had high cytotoxic activity against MCF-7 cells, with the IC50 for *F. religiosa* ethanolic extract being 101.55 g/mL and the IC50 for *F. benghalensis* methanolic extract being 75.66 µg/mL. *Ficus benghalensis* was shown to have more activity since its methanolic latex extract had a lower IC 50 than the other plant. MCF-7 cells were morphologically altered by latex extracts, by showing cell shrinkage, nuclear fragmentation, and apoptotic bodies.

Part III

Phytofabrication of Nanoparticles

Research breakthroughs during the past many years have made us aware of the versatility of metals, particularly metal nanoparticles. There have been accounts of different metal elements being used to treat various ailments (Prasad & Elumalai, 2011). Five thousand years ago when the term "nano" wasn't even coined, but Ayurveda described the use of tiny metal particles (ayaskriti) in its therapeutic recommendations (Ruddaraju et al., 2020). Plant extracts have been known to convert metals into nanoforms since the early 1900s (Mittal et al., 2013), but widespread use and a plethora of data were not made public until recently. The number of scientific studies on nanoparticle synthesis increased from 3 to 4 times in 2018 compared to reports from 2009, while at the same time, biosynthesis reports increased to 6-7 times (Bao & Lan, 2019). Due to its capacity for producing and utilizing materials in the nanoscale as well as its prospective applications in the disciplines of electronics,

chemistry, energy, and medicine, nanotechnology is currently attracting a lot of scientific attention (Saxena et al., 2012). NPs are particles having a size between 1 and 100 nm. Their small size significantly increases the surface area to volume ratio, which in turn improves their characteristics such as thermal conductivity, catalytic activity, non-linear optical performance, chemical stability, etc. (Marslin et al., 2018). Even at tiny target locations, nanoparticles can assure site-specific and safer medicine release. If the particles have magnetic capabilities, they can also be externally directed to the target site (Ahmad et al., 2017).

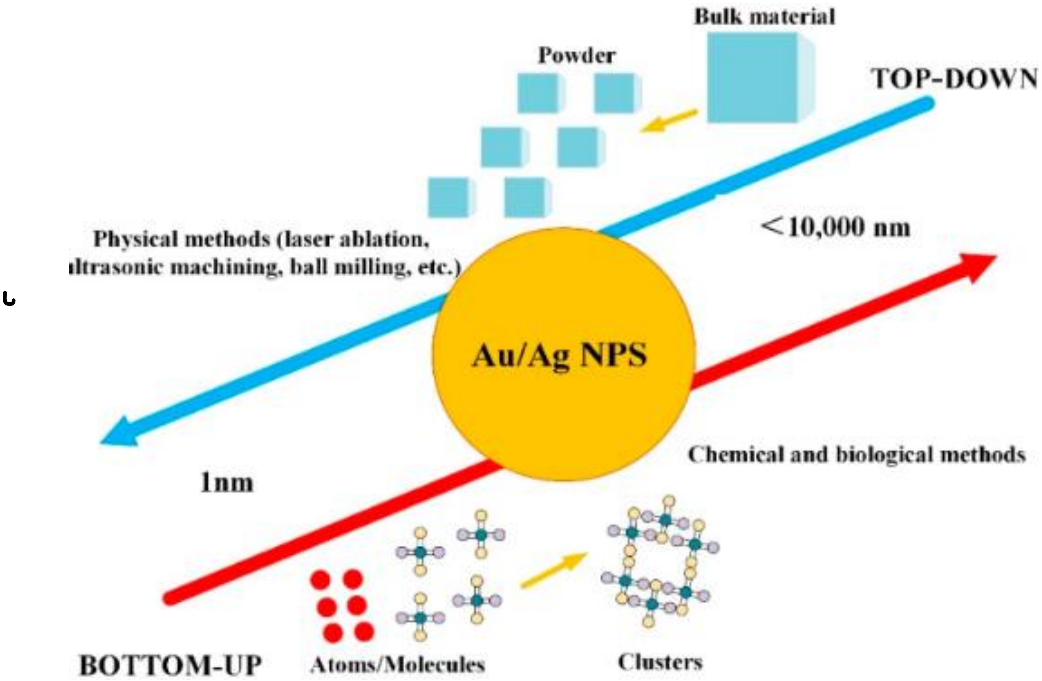


Fig. 6: Two different methods to fabricate Au, Ag NPs top-down and bottom-up. (Jiang et al., 2022).

There are numerous ways to create metal nanoparticles, including spray pyrolysis, vapour deposition, mechanical grinding, and chemical precipitation (Kurihara et al., 1995), electromagnetic levitation technique (Halali & Malekzadeh, 2013), ball milling process (Purushotham & Krishna, 2014), and reduction in presence of various chemicals (Banne et al., 2017; Sun et al., 2003). Production of nanoparticles biologically from plants, bacteria, fungi, algae, etc. is regarded as a green synthesis technique. It appears to be an environmentally benign alternative to other physicochemical processes because it uses fewer potentially

harmful chemicals and requires less energy to operate. Paul Anastas proposed 12 principles of green chemistry (Beach et al., 2009), and chemical technology now places a great deal of emphasis on these for a clean environment. These factors undoubtedly make green synthesis important for its wide variety of applications.

In general, there are two ways to synthesise nanoparticles: top down and bottom up (**Fig. 6**). The top-down approach produces nanoparticles by degrading their larger counterparts, which may involve conventional physical processes like milling, pyrolysis, etching, etc. Common bottom-up methods include spray pyrolysis, polyol reduction, microwave assisted synthesis, and biological or chemical synthesis by agglomeration of tiny particles as a result of specific reactions (Ruddaraju et al., 2020). Researchers are concentrating on developing the field of nanobiotechnology so that it might be a blessing to the entire globe because biosynthesis appears to be the more affordable and environmentally pleasant way. There is still room for new plants to be discovered for the synthesis of nanoparticles, especially in India, one of the biodiversity paradises. Reviewing literature from the genus *Ficus* reveals their potential in formulating bio nanoparticles for the application in several fields. There are few reports from the genus which, mentions about the production of various metal nanoparticles. Due to the expanded use in the medical field, particularly as an antibacterial agent even in solid state, silver nanoparticles appear to be the best investigated among papers on the green synthesis of metal nanoparticles (Siddiqi & Rao, 2018).

For the green manufacturing of different metal nanoparticles, researchers used fruit, leaf, bark, and latex extracts. The process of biosynthesis appears to be straightforward, requiring neither harsh experimental conditions nor causing any harm to the environment. From species to species, as well as depending on the plant parts employed, there were variations in temperature, pH, and incubation time. Alkaline pH frequently encouraged nanoparticle biosynthesis in *Ficus*. Metal oxide solution was the frequently used chemical solution for nanoparticle biosynthesis. This genus has also been linked to reports of metal oxide nanoparticle formation, in which the reaction took place in a nitrogen atmosphere with a somewhat high temperature**.**

Numerous plant extracts produce nanoparticles, although the precise mechanisms involved have not been fully understood. According to the findings, the oxidation of metal ions to metal nanoparticles is mediated by reactive hydrogen species generated during the transformation of flavonoids from enol to keto tautomeric shift and ketones to carboxylic acid (Makarove et al., 2014). According to Si & Mandal (2007), proteins containing at least one tryptophan at their c-terminus have the ability to convert metal ions into metal nanoparticles. Schematic representation of the phytofabrication of nanoparticles are given in the **Fig. 7**

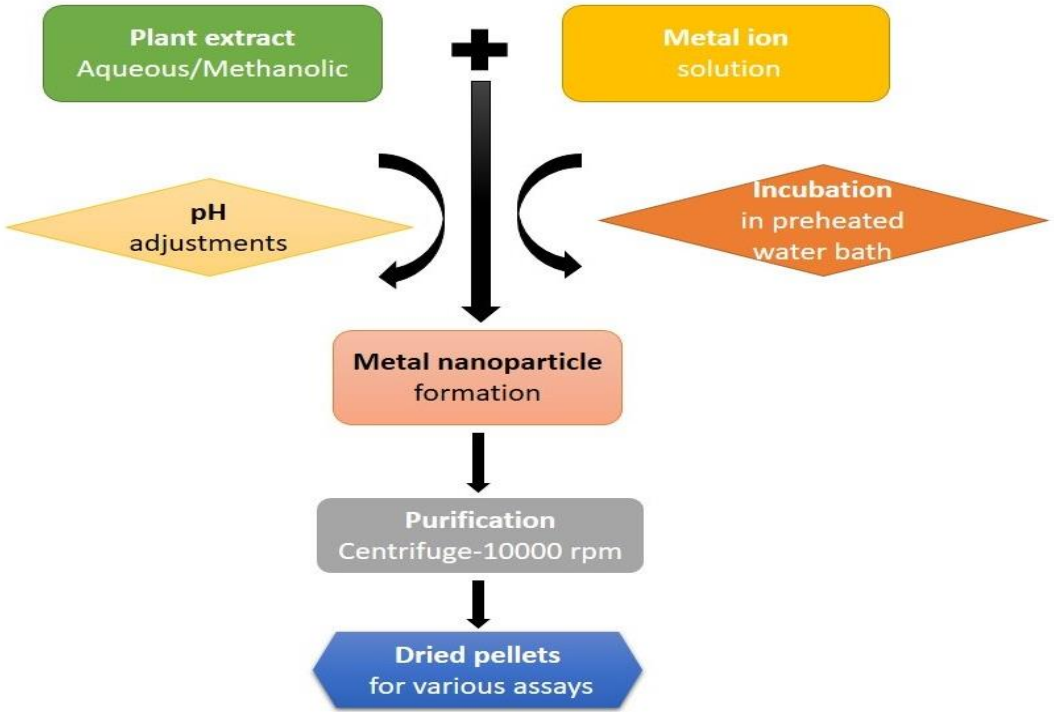


Fig. 7: Schematic representation of green synthesis of metal nanoparticles

In just five minutes, Saxena et al. (2012) were able to create nanoparticles measuring about 16 nm in size using a simple approach for the green production of silver nanoparticles from *Ficus benghalensis* leaf extracts. This investigation came to the conclusion that the synthesized AgNPs had effective antibacterial action against the tested bacterial strains. According to Singh & Bhakat (2012), studies on the biosynthesis of gold and silver nanoparticles from the bark and leaf extracts of *Ficus carica* were a simple and effective way of nano metal biosynthesis from aqueous solutions of gold and silver. Silver nanoparticles with effective antibacterial action

were produced with the fruit extract from *F. carica* (Logaranjan et al., 2012). The examined gram positive and gram negative organisms were both effectively killed by the biosynthesized nanoparticles, which were 20–25 nm in size.

Ficus benjamina leaf extracts were successfully used by researchers to create silver nanoparticles. The particles were found to be 20–30 nm in size. Within the HEK293 normal cell line and the HeLa cancer cell line, the cytotoxic effects of these nanoparticles were examined. The anticancer potential of silver nanoparticles from *F. benjamina* spp. was established by this research (Bhakat et al., 2012). The fourth instar larvae of *Culex gelidus* and *Culex quinquefasciatus*, the vectors of filariasis and Japanese encephalitis, were used as a test subject for the larvicidal activity of green synthesized silver nanoparticles from *Ficus racemosa* bark extracts. The AGNPs created from different concentrations of bark extracts as well as the crude bark aqueous extract were shown to have the highest efficiency after being treated for 24 hours (Velayutham et al., 2013). Green silver nanoparticles with sizes ranging from 5 to 35 nm were produced from *Ficus religiosa* leaf extracts and used to treat mice with induced Dalton's ascites lymphoma. The produced AGNPs were found to greatly resuscitate the model organism (Antony et al., 2013).

From the latex of the *F. carica* plant, Borase et al. (2013) was able to create silver nanoparticles with a diameter of 167 nm that can efficiently decrease ferric ions and ascorbic acid. Silver nanoparticles with a size range of 5–50 nm was synthesized from *F. reliogiosa* leaf extracts. The chemical substances that reduced silver ions to nano silver form were proteins, quinones, and flavonoids (Saware & Venkataraman, 2014). In an earlier reports, the researchers employed *Ficus benghalensis* leaf extract in the green synthesis of silver nanoparticles, which were successfully characterized and were determined to be in the size range of 10-15 nm (Saware et al., 2014). In another study scientists created silver nanoparticles from *Ficus carica* leaf extracts using irradiation synthesis, under a range of irradiances (between 6.5 mW/cm^2 and 13.3 mW/cm^2) within the wavelength of 330-550 nm. The irradiation synthesis was found to be more successful when compared with synthesis using sunlight and synthesis in the dark. They were able to create nanoparticles as small as 13 nm (Ulug et al., 2015). Silver nanoparticles with a size range of 100 and 20 nm were produced using latex and leaf extracts of *Ficus sycomorus*. They were discovered to be effective antimicrobials against nine gram-negative human pathogens and one-gram positive

bacteria (Salem et al., 2014). *Ficus religiosa* leaf extracts were used by Sankar et al. (2014) to successfully synthesize copper nanoparticles with in a wavelength of 577 nm. They were shown to be very potent anti-cancerous agents that can cause apoptosis by generating ROS to disturb the potential of the mitochondrial membrane. Silver nanoparticles were successfully produced by Mondal et al. (2011) using the latex of the *Ficus religiosa* plant. *Ficus ampilissima* leaf extract was used to successfully convert silver nitrate solution into AgNPs with a diameter of 13–51 nm (Johnson & Prabu, 2015).

The influence of *Ficus racemosa* latex on the production of silver and gold nanoparticles was examined (Tetgure et al., 2015). Latex was found to be an effective reducing agent, which generated AUNPs with a size range of 20–50 nm and AGNPs of 50–120 nm. This study discovered that the pH dependent amino acid binding effectiveness of these nanoparticles was appealing. *F. microcarpa* leaf extracts were able to produce green silver nanoparticles that were efficient against bacteria such as *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Praba et al., 2015). Streptomycin's antibacterial activity was significantly enhanced by biosynthesized silver nanoparticles with a size range of 40.9 to 29 nm from *Ficus virens* leaf extracts. Nanoparticles are formed by molecules having C=O, -OH, and N-H groups, which are stabilized by proteins.

When the fruit extract from *F. carica* was utilized to make silver nanoparticles, it was discovered that particles of diverse sizes and shapes were formed by heat and ultrasonication procedures (Salar et al., 2015). While nanoparticle synthesis was done by using both ultrasonication and thermal techniques, ultrasonication produced smaller (10-13 nm) spherical and non-aggregated nanoparticles and thermal technique produced larger (20-80 nm) spherical and aggregated nanoparticles (Kumar et al., 2016). *F. reliogiosa* leaf extracts were utilized to create silver nanoparticles that were 21 nm in size and were discovered to be antibacterial as well as cytotoxic to several cancer cell types. Histochemical examination after *in vivo* oral administration of silver nano particles in the model test mice showed that 89 days of washout time was sufficient to completely eliminate silver from the cells (Nakkala et al., 2017).

The dried fruit extracts of *F. carica* were used by scientists to create silver nanoparticles. UV absorption spectroscopy and SEM examination were used to

describe the characteristics of synthesized particles. These AgNPs ranged in size from 54 to 89 nm and had a spherical form. Oral dosing was found to be safe by toxicology studies, which also demonstrated considerable toxicity in MCF7 cell lines (Jacob et al., 2017). Solid lipid nanoparticles made from *Ficus benjamina* were tested against cardio renal harm brought on by the administration of alcohol and disulfiram. These solid lipid nanoparticles were discovered by researchers to have hepatoprotective properties. The ability of these plants to protect against alcohol abuse-related damage was demonstrated by a decrease in the buildup of aldehydes in the liver tissues and the restoration of abnormal cardio-renal biomarkers and cellular structures (Sharma et al., 2017). The leaf extracts of the *F. hispda* were successfully used to create magnetite (Fe_3O4) nanoparticles. These 10.96 nm-sized, spherical nanoparticles have the capacity to adsorb methylene blue (Ramesh et al., 2018). In another study for the synthesis of silver nanoparticles, *F. hispida* leaf extracts were employed, and it was discovered that they were highly effective against tested gram positive and gram-negative bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Bacillus subtilis*) (Pranitha et al., 2017). *Ficus retusa* leaf extracts were used in the green production of silver nanoparticles. Synthesized nanoparticles with a size range of 5-35 nm were found to be successful antibacterial agents against tested microbial strains. In addition, they effectively decolored eriochrome black T when sodium borohydride was present (Singhal et al., 2017).

Scientists discovered the capacity of green synthesized silver nanoparticles from *Ficus benjamina* (within a size range of 60-105 nm), in the heavy metal cadmium elimination. According to Al-Qahtani (2017), these nanoparticles were quite effective at treating cadmium-containing wastewater. Kanjikar et al. (2018) focused on the environmentally friendly synthesis of silver nanoparticles from *Ficus krishnae* bark extracts. They discovered that the synthesized nanoparticles had a face-centered cubic shape and ranged in size from 160 to 260 nm. The MTT assay in ovarian cancer cell lines confirmed its excellent cytotoxicity. Ramesh et al. (2018) was successful in creating silver nanoparticles from *F. hispida* leaf extracts that were spherical and 20 nm in size. Effectively converting 4-nitrophenol into 4-aminophenol, these AgNPs additionally, had strong antibacterial impact on both the gram-positive and gram-negative microorganisms. Studies on *F. benjamina* leaf extracts could effectively

synthesize AgNPs with 13.2 nm size. With an inhibitory zone ranging from 7 mm to 13 mm, these nanoparticles were effective antimicrobial agents against a variety of pathogenic bacteria, including *Bacillus subtilis, Pasteurella multocida, Staphylococcus aureus,* and *Enterobacter aerogenes* (Ashraf, 2018).

Usman et al. (2019) employed green synthesis of copper nanoparticles by *F. carica* fruit extract to successfully eliminate harmful organic dye Alizarin Yellow R. The photocatalytic activity of synthesized nanoparticles with a size range of roughly 60 to 80 nm and a spherical shape was responsible for the degradation of these dyes. They succeeded in achieving a performance maximum degradation of 89.71%. The green manufacture of silver nanoparticles with antibacterial activity against both gram positive and gram negative bacteria and protozoa was successfully accomplished using extracts from *Ficus copiosa* leaves. They revealed the antimicrobial efficacy of these green nanoparticles against the test organisms such as *Trichomonas vaginalis,* a protozoan, as well as *Bacillus subtilis, Staphylococcus aureus, E. coli,* and *Streptococcus pneumoniae* (Timi et al., 2019). Bimetallic zero valent Fe/Cu nanoparticles with a size range of 19–63 nm was made by Abdel Aziz et al. (2019). They were able to successfully remove Carbamazepine from aqueous media using these green synthesized nanoparticles. By changing the trial parameters, including the dosage, removal time, and pH, they were able to increase the removal effectiveness to 95%. Bhat et al. (2019) synthesized *F. racemosa* leaf extract-mediated silver nanoparticles. According to the findings, the AgNPs were cubic in shape and had a diameter ranging from 2.04 to 3.64 nm. They also had a high absorption peak at 400 nm. *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis* were the five bacterial species against which the AgNPs had shown good anti-bacterial efficacy. The leaf methanol extract was discovered to be the most effective against all of the species, followed by the aqueous and $AgNO_3$ extracts. Microwave irradiation was used as a novel technique for producing silver nanoparticles (AgNPs). The technique was discovered to be more effective than conventional heating techniques, producing smaller AgNPs with better characteristics. Several bacterial strains, including *P. aeruginosa, B. cereus, C. jejuni,*

B. subtilis, L. monocytogenes, and C. perfringenes, were found to be susceptible to the antibacterial effects of the AgNPs (Khan et al., 2011).

Silver nanoparticles were synthesized from *Ficus palmata* leaf extract by green chemical methods. Using UV-Vis spectroscopy, XRD, SEM, and TEM, these AgNPs were characterized. The outcomes demonstrated that the AgNPs were crystalline with a face-centered cubic shape and had a maximum absorption peak at 452 nm. AgNPs had a spherical form and were typically 10–20 nm in size. They have antibacterial efficacy against *Klebsiella pneumoniae* and *Bacillus cereus*. The minimum inhibitory concentration (MIC) of the AgNPs against both bacteria was 100 µg/mL, indicating substantial antibacterial action (Kumar et al., 2021).

Fig (*Ficus carica*) and rosemary (*Salvia rosmarinus*) leaf aqueous extracts were used to study the green production of silver nanoparticles. The reduction of aqueous silver nitrate by the leaf extracts led to the production of AgNPs with an average size of 22 nm. Transmission electron microscopy, energy-dispersive X-ray spectroscopy, UV-Vis spectrophotometry, Fourier transform infrared spectroscopy, and dynamic light scattering experiments were used to characterize these AgNPs. The outcomes demonstrated that the AgNPs were stable for several weeks and had good dispersibility. Additionally, it was discovered that the AgNPs have antibacterial action against a variety of Gram-positive and Gram-negative bacterial strains, including *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* (Almasoud et al., 2021).

Silver nanoparticles were synthesised using a leaf extract from the *Ficus variegata* plant. Transmission electron microscopy (TEM) analysis of the silver nanoparticles showed that they were primarily spherical, with an average diameter of 26.507 nm. Both Gram-positive *Staphylococcus aureus* and *Gram-negative Escherichia coli* bacterial growth was subsided by these biosynthesised silver nanoparticles. Notably, the disc diffusion approach revealed that the nanoparticles were more successful at preventing the growth of *E. coli* than *S. aureus* (Wattimena et al., 2022). *Ficus deltoidea* Jack var. *kunstleri* have therapeutic effects across its leaf and root components. Research into the synthesis of AgNPs from this plant revealed the effective biosynthesis of silver nanoparticles. Researchers tried to produce

nanoparticles from various parts (AgNP-Leaf, AgNP-Stem, AgNP-Fig, and AgNP-Root). According to measurements made with a transmission electron microscope (TEM), the diameters of the particles were as follows: AgNP-Root (15.4 ± 3.4 nm), AgNP-Stem (20.5 ± 2.4 nm), AgNP-Fig (21.3 ± 4.2 nm), and AgNP-Leaf (22.9 ± 4.3 nm). AgNP-Root showed more pronounced antibacterial effectiveness against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion technique (Din et al., 2022). Major properties and application of nanoparticles in various field is depicted here in the image **Fig. 8**. The present study is an attempt in the fabrication of silver nanoparticles from the leaves and fruits of two therapeutically important and locally available *Ficus* species (*F. exasperata* and *Ficus drupacea).* Characterisation was done by using UV-vis spectroscopy and Scanning electron microscopy. The ability of phytofabricated silver nanoparticles from the four extracts in microbial growth inhibition was also studied using some gram-negative and gram-positive bacteria along with two fungi.

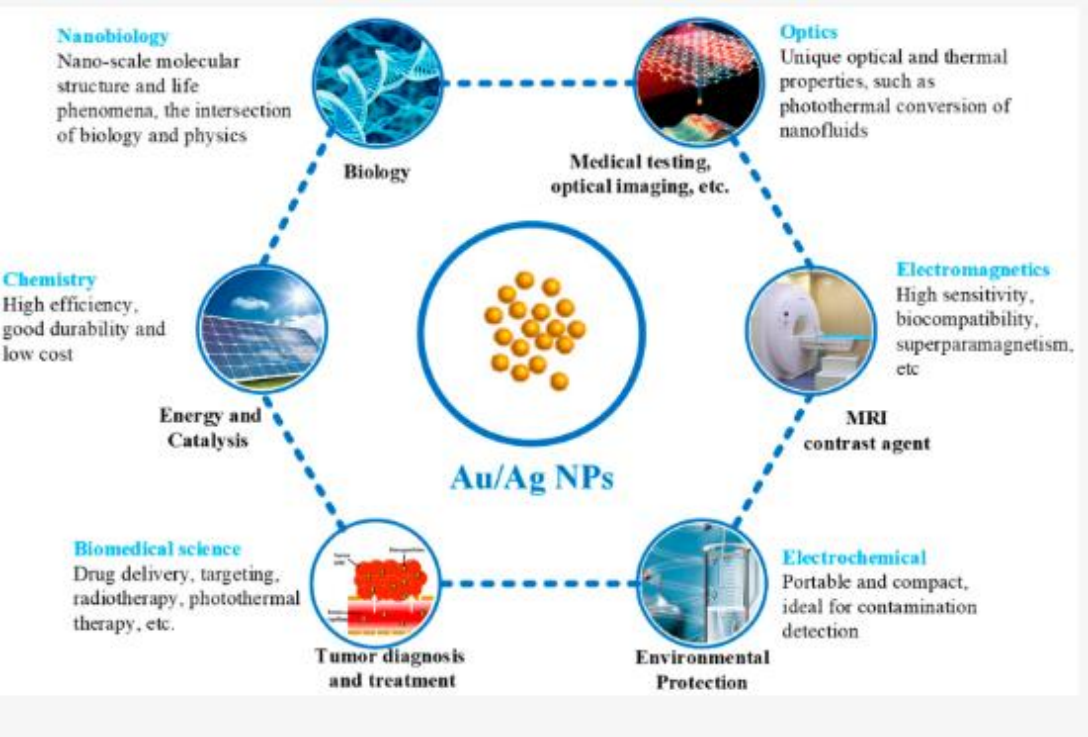


Fig. 8: Properties and application of Au/Ag NPs in different fields (Jiang et al., 2022).

PHASE II

Fungal endophyte isolation

Endophytes are endosymbiotic organisms residing in intra or inter cellular regions of the host species without making apparent symptoms of disease. They include viruses, bacteria, archaea, fungus, oomycetes, and other microbial taxa which can colonise plant tissues. This review discusses about the endophytic fungi and bacteria. De Barry coined the word endophyte in 1866 to describe organisms that proliferate inside host cells intra or intercellularly without ever producing disease signs (Anand et al., 2023). The term endophyte is derived from the Greek prefixes endo (within) and phyton (plant). A plant's caulosphere (stem), phyllosphere (leaf), anosphere (flowers), spermosphere (seeds), and carposphere (fruit) are the few sections from which endophytes can be isolated. These microorganisms influence plants by colonising and residing inside the plant's internal environment, forming a plant microbial endosphere. Endophytic bacteria were divided into four categories. In contrast to facultative endophytes, which reside in the host on a voluntary basis, obligatory endophytes are organisms that are inextricably linked to a plant throughout their life cycle. Opportunistic endophytes are organisms that join the host on occasion and benefit from its internal environment, whereas passenger endophytes are endophytes that enter the plant by accident (Hardoim et al., 2008). The capacity of an endophyte to colonise a host species is affected by a variety of factors, including plant tissue type, genotype, microbial strain type, biotic and abiotic environmental factors (Hardoim et al., 2015). Important techniques in identifying endophytes include SEM (scanning electron microscopy), CLSM (Confocal laser scanning microscopy) and the molecular identifications based on the analysis of 16S ribosomal RNA genes in bacteria and 18S rRNA or internal transcribed spacer (ITS) in fungal endophytes.

Endophytes are gaining much attention nowadays because of their ability to mimic the host biochemicals and their ability to form entirely new natural products which may be worthy in therapeutics (Zhou et al., 2010). Interaction between hosts and endophytes is incredibly versatile and codependent. A slight alteration in the host gene expression will influence the endophyte's gene expression, and vice versa (Moricca & Ragazzi, 2008).

This is the reason why endophytes can be used to produce important host biochemicals. Using endophytes to produce any plant phytochemical is the best

method which is cheap but environmental friendly. Taking the natural production of taxol (Paclitaxel) as an example, to produce 1kg of taxol drug, it costs 10 tons of plant bark or 300 yew trees which are slow growing and endangered (Zhou et al., 2010). Almost all the species of plants screened for endophytes were found to have one or more of them during any stages of their life cycle (Govindappa et al., 2014). Utilizing endophytes in the production of valuable phytochemicals is the best method to be explored. Though the bioactivity studies including phytopathogenic, antimicrobial, anticancerous, stress related activities of the endophytes are explored, molecular mechanisms contributing towards these complex interactions are poorly understood. Physiological aspects of endophyte-host interactions, genes implicated in the advantageous interaction must be identified, isolated, and characterised in detail. Different forms of omic investigations including genomics, proteomics, transcriptomics, metabolomics, comparative genomics, genome sequencing, microarray, and next-generation sequencing research are contributing to our understanding of the mechanisms behind these interactions (Kaul et al., 2016).

Endophytes have more genes involved in biosynthetic processes and functions, while phytopathogens have more genes involved in degradation and host invasion. The ability of bacteria and fungi to colonise the endosphere of plants is mostly due to lateral gene transfer, which is facilitated by mobile components like plasmids and genomic islands. Endophytes must overcome the host's first line of defence by recognising MAMPs (microbe associated molecular patterns) in order to colonise effectively (Salvi et al., 2022). Comparative genomics analysis conducted by Hardoim et al. (2015) revealed that genes involved in motility and chemotaxis (eg: Tar,Tap), genes responsible for signal trasnduction (eg: evgS - evgA, regB - regA, ntrY- ntrX), genes involved in transcriptional regulation (eg: nifA, norR, sdiA), genes which mediate detoxification and stress-related enzyme production (eg: btuE, gst, katE, norR), genes involved in protein production for transport system (eg: kpsT,thiY, pot D, dppF, metN) and genes involved in plant growth promoting properties (eg: nifH, acds, budc, alsD, butB) etc. are discovered to be present in endophytes in substantially higher levels. Genes involved in secretion systems (eg: yscJ) was found to be lesser in endophytic community than those of pathogens and other members of the microbial community which is in close connection with the host plants. Plant endophytes are benefitting the host in various ways including reduction in herbivory,

priming of host plants defensive responses, abiotic and biotic stress remediation, protection from ROS etc. are represented on the **Fig. 9**.

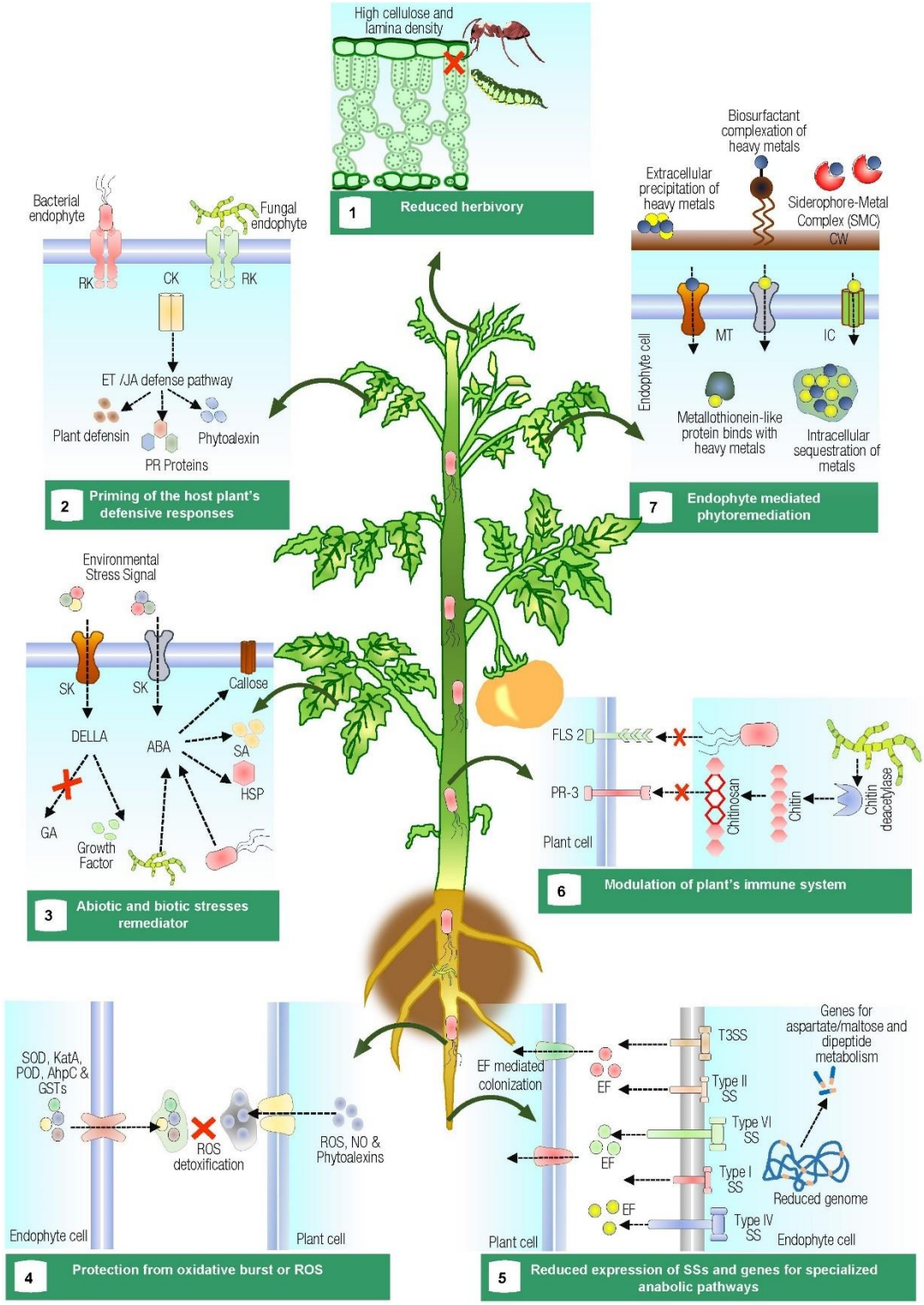


Fig. 9: Various benefits of endophytes in the host plant (Khare et al., 2018).

Even though endophytes are not extensively studied from the genus *Ficus* there are a number of reports on both bacterial and fungal endophytes from the genus which is having various biological activities including antimicrobial, antifungal and anticancerous activities. Available literature on endophytic studies within the genus is briefly discussed here. *Bacillus subtilis* K1 was a highly antifungal endophytic bacteria that was isolated from *Ficus bhangalensis,* and the bioactive chemicals were classified as surfactins, iturins, and fengycins. In addition, they discovered that these isolated chemicals had strong emulsification and stabilising properties. The biosurfactant activity of the separated chemical groups, which remained stable even at 100^0C under laboratory conditions, demonstrated the potential of this bacteria for oil recovery (Pathak et al., 2009).

From the leaves of the *Ficus carica,* scientists were able to isolate and distinguish endophytic *Aspergillus* sp. From the fungal extract, novel indole alkaloids were extracted and identified via spectroscopic analysis. These biochemicals were quite effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* (Prabhavathy & Nachiyar, 2011). Endophytic fungus *Aspergillus tamarii* was isolated from the leaves of *Ficus carica* L. The fungus was shown to be a good source of indolyl diketopiperazines (IDPs), and six IDPs were identified: verruculogen, fumitremorgin C, cyclotryprostatins B, tryprostatin A, tryprostatin B, and fumitremorgin B. These IDPs were employed once more to investigate their antibacterial properties. The biochemicals from *A. tamari* were found to be a potent phytopathogen inhibitor, particularly against *Fusarium oxysporum, Botrytis cinerea,* and *Phytophthora infestans* (Zhang et al., 2012).

From the leaves of *Ficus carica* researchers isolated an endophytic strain *of Fusarium solani*. This fungal extract was screened for antimicrobial activity. Promising antimicrobial activity was shown against number of organisms, such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. Two antimicrobial compounds fuscidin A and B were identified from the extract which might be responsible for the antimicrobial activity of this fungal endophyte (Zhang et al., 2012). Rakshith et al. (2013) reported an enophytic fungus

from the stem bark of *Ficus pumila*. Morphological and molecular identification of the fungus revealed that the organisms belonged to the genus *Phomopsis* showed antibacterial ability against number of gram positive and gram-negative bacteria. In a study by Solis et al. (2014), foliar endophytic yeasts were reported from three tropical *Ficus* species. Among these,15 percent of the colonies were belonging to the class *Cryptococcus* and *Rhodotorula*. Site specific and host specific variations were found in the species richness, and abundance. *Ficus carica* leaves were used to isolate an endophytic fungus called *Fusarium* sp, which could produce a variety of biochemicals, including three helvolic acid compounds, one of which was discovered to be novel to science and was called helvolic acid methyl ester. All the three compounds isolated were effective with their antifungal and antibacterial properties (Liang et al., 2016).

Researchers isolated 29 endophytic bacteria from the leaves, stem, aerial root, and fruit of *F. variegate*. Morphological, biochemical, and molecular (16S rRNA) studies were used to characterise every endophyte. Antimicrobial studies on these isolated endophytic bacteria were undertaken against four bacteria types: *Staphylococcus aureus, Bacillus subtillis, Escherichia coli,* and *Pseudomonas aeruginosa*. Of all the endophytes tested, only one had antibacterial activity against the tested strains. This bacterium shared 99% of its traits with strain SV1 of *Pseudomonas aeruginosa* (Leonita et al., 2016).

Ten different *Ficus* species in Costa Rica were used to isolate 120 endophytic bacterial species. The identification process included 16S rRNA sequencing, which revealed that all of these species fall within 10 genera, with three of them being the most prevalent. They were *Pantoea, Klebsiella oxytoca, and Pseudomonas* sp. The ability of these endophytes to promote plant growth and health, including the production of growth hormone IAA, was examined. The findings suggest that these endophytic bacteria are helping the plant's health and development (Alvarado Marchena et al., 2016).

Endomelanconiopsis endophytica, an endophytic fungus isolated from *Ficus hirta*, produced two novel xyloketals. K and L xyloketals were isolated using a mix

of chromatography and solvent extraction procedures. Extensive spectroscopic investigation was used to determine the structures of the xyloketals. These compounds were tested for cytotoxicity against four human tumour cell lines (SF-268, MCF-7, NCI-H460, and HepG-2) and showed no significant action (Sun et al., 2016).

The roots of *Ficus carica* were used to isolate the endophytic fungus *Aspergillus tamarii*. Malformin E, a brand-new cyclic pentapeptide from the species, was extracted, and its structure was revealed using NMR spectroscopy, HRMS, UV, and Marfey's analysis. These biochemicals demonstrated remarkable antimicrobial properties against a wide range of bacteria and fungi, including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Penicillium chrysogenum, Candida albicans, and Fusarium solani*. They were also cytotoxic against human cancer cell lines MCF-7 and A549 (Ma et al., 2016).

On the basis of phylogenetic, chemotaxonomic, and phenotypic data, an endophytic bacterial strain CBS5Q-3T from *Ficus microcarpa* was reported. They suggested naming the species *Jiella endophytica* because the bacterium was found to be a new species and showed 98.1% genetic similarity with *Jiella aquimaris* (Tuo et al., 2019). *Ficus elastica* leaves revealed 42 filamentous endophytic fungi, which were included in seven taxa after ITS-rDNA sequencing. The fungi with superior bioactivities were *Penicillium funiculosum* and *Trichoderma harzianum*. Isolated fungal strains were also created a large number of biochemicals, including two novel isocoumarins that were identified from *T. harzianum*. This novel chemical had anti *E. coli* action (Ding et al., 2019). Endophytes can aid the plant in overcoming biotic and abiotic stress, hence boosting the host's vitality. In the root, leaf, and stem of the *Ficus carica*, 11 fungal endophytes were discovered. Of these endophytes, two exhibited antagonistic behaviour against the *phytopathogens Magnaporthe oryzae, Ganoderma boninense,* and *Fusarium verticillioides*. Eight of the isolated endophytes had a maximum PSI value of 3.02 ± 0.05 for their ability to solubilize phosphate (Rosli et al., 2020).

Endophytic bacteria were identified from *Ficus septica* leaves growing in tannery waste-contaminated soil. Tolerance to Cr (VI) in bacterial isolates was tested,

and three isolates were shown to be tolerant up to 900 mg/L. In addition, the bacteria were discovered to be capable of producing IAA, EPS, and phosphate solubilizing enzymes (Rohmah et al., 2020). Scientists have discovered endophytic bacteria on *Ficus minahassae's* leaves. According to identification results based on the 16S rRNA gene marker, the yellow pigmented bacteria obtained were 100% equivalent to *Brachybacterium muris* (YL1), and the beige pigmented bacteria (YL2) were 99.80% similar to *Pseudacidovorax intermedius*. The antibacterial testing was conducted using *S. aureus* and *E. coli* which revealed that YL2 was effective against *S. aureus*, while YL1 was ineffectual against the two bacterial strains tested. The formation of secondary metabolites by the endophytes, such as indole-3-acetic acid (IAA) and siderophores, was thought to be the cause of their antibacterial activity (Tallei et al., 2020).

Aspergillus neonigrans were isolated from *Ficus carica*, which was found to be antimicrobial and anticancerous from the preliminary studies. Four new metabolites, named aurasperone D, asperpyrone D, dianhydroaurasperone C, and aurasperone A, from *A. neoniger*. Aurasperone D and asperpyrone D were found to be cytotoxic to human erythroleukaemia cells 562 and human umbilical vein endothelial cells. While dianhydroaurasperone C and aurasperone were having weak cytotoxic activity. All four compounds were also found to have antimicrobial activity against the plant pathogen *Fusarium oxysporum*. In addition to the isolated new metabolites, several known bioactive compounds were also reported in the extract of *A. neoniger*, such as aurafuranone, and auranopin. These compounds were previously reported to have a variety of pharmacological activities. The presence of these known bioactive compounds in the extract of *A. neoniger* suggests that the fungus may have a number of potential therapeutic applications (Abdou et al., 2021). Plants such as *Acalypha ornata, Albizia zygia, Alchornea cordifolia, Chrysophyllum albidum, Ficus exasperata, Gomphrena celosioides, Millettia thonningii, and Newbouldia laevis* were chosen for the isolation of endophytes. There were 18 different types of fungi that were isolated, and the extract from each one had a strong ability to chelate iron, as well as some that could scavenge free radicals. Phytol, dl-alpha-tocopherol, oleamide, methyl stearate, oleic acid, palmitic acid, campesterol, stigmasterol, -

sitosterol, lupeol, and other compounds were found in the chosen active extracts (Ibrahim et al., 2021). There are ten endophytic fungi that have been isolated from the *Ficus religiosa* tree. These fungi can produce a wide range of biochemicals, including phenols, flavonoids, alkaloids, steroids, terpenes, and terpenoids. Extracts from isolated fungal strains *Curvularia lunata* were very effective in inhibiting alpha amylase activity and was very good at free radical scavenging ability. The remaining nine isolates, on the other hand, were discovered to be displaying only moderate activity in the inhibition of alpha amylase and in the free radical scavenging activity (Jayant & Vijayakumar, 2021).

From the aerial sections of the *Ficus carica*, the following endophytes viz., *Chaetomium globosum, Penicillium oxalicum, Alternaria alternata, Fusarium proliferatum,* and *Aspergillus neoniger* were identified. These fungi were highly efficient in controlling the cell division of HUVEC, K-562, and HeLa cell lines. It was revealed that endophytes of *Ficus carica* play a substantial role in the ability of the plants therapeutic potential in various fields and functions (Abdou et al., 2021).

An endophytic strain of *Alternaria alternata* was isolated from the leaves *of Ficus carica*. This fungal extract was tested for cytotoxicity and antifungal activity against a panel of test organisms, and it demonstrated promising antifungal and cytotoxic activity. This fungus yielded four noval alternariol derivatives: alternariol, alternariol-5-O-sulfate, alternariol-5-O-methyl ether, and alternariol-5-O-methyl ether-4'-O-sulfate (Abdou et al., 2022). Even though there are number of reports available on the endophytes from the genus *Ficus*, most of them are focused only on few species of the genus. This makes the research on endophytes from locally available yet therapeutically significant *Ficus* species from India, a *Ficus* hotspot an important work.

MATERIALS AND METHODS

This research is completed in two phases. Phase one was divided into three sections, the first of which focused on the phytochemicals present in aqueous extracts of the leaves and fruits of two selected *Ficus* species, *F. exasperata* and *F. drupacea*. Part two helped to comprehend bioactivities such as antioxidant and anticancer capabilities, as well as the molecular expression of the genes responsible for this anticancer activity. Part three focuses on the ability of plant extracts to create biological silver nanoparticles. The second phase of the research focuses exclusively on the fungal endophytes isolated from the two plants mentioned above. This step is broken down into three parts: endophyte isolation and identification, phytochemical characterization, and nanoparticle biosynthesis.

Phase I

- Part I: Phytochemical analysis was carried out as preliminary biochemical assay using conventional procedures, followed by GC-HRMS and HR-LCMS.
- Part II: Bioactivity studies were conducted using antioxidant and antiproliferative tests on the MCF-7 breast cancer cell line to the gene expression level.
- Part III: Silver nanoparticle biosynthesis and its antimicrobial efficacy determination.

Phase II

- Part I: Endophytic fungal isolation and identification was done from the two *Ficus* plants used in the present study.
- Part II: Fruit endophyte *Phlebia* sp. was subjected to phytochemical characterization using preliminary assays followed by HR-LCMS analysis.
- Part III: Biosynthesis of silver nanoparticles and their effectiveness assessed through antibacterial activity tests.

PHASE 1

Plant Materials

Ficus drupacea and *Ficus exasperata* leaves and fruits were the raw materials selected for the study (**plate 1-2)**. Both plants have similar cycle for fruit setting and were collected in March-April and leaves were collected during November – December from various parts of southern Kerala. Collected leaves were shade dried and fruits were oven dried in controlled temperature. Materials were authenticated by Dr. A. K. Pradeep, Associate Professor in the Department of Botany, University of Calicut. Voucher specimens were deposited in the herbarium of the Department of Botany, University of Calicut (CALI). The plants were assigned voucher numbers as follows: *F. exasperata* CALI cumber 7217 and *F. drupacea* CALI number 7218.

A. Systematic Name: *Ficus drupacea* Thunb.

Class : Equisetopsida

Sub class : Magnoliidae

Super order : Rosanae

Order : Rosales

Family : Moraceae

Genus : *Ficus* L.

Species : *Ficus drupacea* Thunb.

Plant description

Synonyms: *Urostigma pilosum*, *Ficus citrifolia*, *Ficus mysorensis*, *Ficus drupacea* var. *mysorensis*

Common Name: Kallaal, Kaattaal, Brown woolly fig, Hairy fig

Habitat: Semi-evergreen and moist deciduous forests, also in the plains

Distribution: India, China, Sri Lanka, Bangladesh, Laos, queensland

Flowering & Fruiting: January - April

Plant Description: Trees grow up to 25 m high; aerial roots numerous, arising in tufts from the stout branches; bark surface greyish-brown, smooth; exudation milky; young shoots brown pubescent. Leaves simple, alternate, spiral, sub-distichous; stipule 10-25 mm long, lateral, broadly lanceolate, tomentose; petiole 1.2-3.5 cm long, stout, grooved above, glandular at apex below, tomentose; lamina 10-22 x 6-15 cm, ovate or elliptic-ovate, base round or subcordate, apex abruptly acuminate, margin entire, tender leaves tomentose below, glabrous above and scurfy tomentose beneath when mature, coriaceous; 3-5-ribbed from base, lateral nerves 9-13 pairs, parallel, prominent beneath, intercostae reticulate, prominent. Flowers unisexual; inflorescence a syconia, sessile, in axillary pairs, ellipsoid-globose, thick walled, tomentose without, at first covered by stout conical tomentose stipule; basal bracts 3, 2-6 mm, orbicular, concave, brown-pilose without, obtuse, orifice umbonate, closed by 3-4 apical bracts, not forming a flat disc; internal bristles a few; flowers of 4 kinds; male flowers disperse, numerous; pedicel to 4 mm long; tepals 2-3, free, brown, acute; stamen 1, exserted; filament sessile; tepals 3-4, free, brown, acute, ovary superior, obovoid, 0.7 mm, brown; style filiform 2 mm; gall flowers pedicellate; pedicel 0.2-3.5 mm; tepals 3, free; ovary obovoid; style short, subterminal. Syconium 1.5-2 cm across, orange red when ripe; achene smooth.

Systematic name: *Ficus exasperata* Vahl

Class	:	Equisetopsida
Sub class	:	Magnoliidae
Superorder	:	Rosanae
Order	:	Rosales
Family	:	Moraceae
Genus	:	*Ficus* L.
Species	:	*Ficus exasperata* Vahl

Synonyms: *Ficus asperrima, Ficus hispidissima, Ficus silicea, Ficus scabra, Ficus punctifera*

Common Name: Parakam, Therakam, Sand paper tree

Habitat: Moist deciduous forests, also in the plains

Distribution: East Africa, Arabia, India and Sri Lanka

Flowering & Fruiting: February - April

Plant description: Deciduous trees grow up to 18 m high; aerial roots none; bark 5-6 mm thick, greenish-white, smooth, punctiform lenticellate, fibrous; blaze creamy white, exudation watery; all parts coarsely and harshly scabrid with stout white hairs. Leaves simple, laxly alternate spiral to opposite or sub distichous; stipules short, paired, lateral, cauducous; petiole 1-6.5 cm long, slender, not articulated, lamina 5.5-19 x 3-9 cm, elliptic, ovate, oblong-lanceolate, or obovate, basal acute, round or cuneate, apex acute to shortly acuminate, margin denticulate or sinuate-crenate to serrate, scabrid on both surfaces, without, coriaceous, 3-ribbed from base, glands at nerve axils; lateral nerves 3-6 pairs, pinnate, prominent, inter costae scalariform; leaves of saplings and coppice shoots often lobed. Flowers unisexual; inflorescence a syconia, axillary, solitary, harshly scabrid; peduncle to 1.5 cm with 2-3 small scattered, lateral bracts, sometimes more or less aggregated into a collar, body sub globose or ellipsoid with scattered small lateral bracts, apical bracts projecting 1-2 mm; internal bristles copious, white, shorter than flowers; flowers of 4 kinds; male flowers sessile, ostiolar, in 1-2 rings; tepals 3-6, oblong-spathulate, white hairy; stamen 1; filament 0.5 mm; anther oblong, parallel; female flowers sessile; tepals 4-7, linear-spathulate, white hairy; ovary superior, obovoid; style filiform, lateral, puberulous, stigma clavate; gall flowers sessile to pedicellate, tepals 4-6, lanceolate, white hairy; ovary white, sessile, style terminal, puberulous, stigma dilated. Syconium 0.7-1.5 x 1-1.5 cm, yellow or purple when ripe; achene oblong, slightly keeled, reticulate.

PART 1

Methodology

Extract Preparation

Leaf extract

Healthy leaves of *F. drupacea* and *F. exasperata* were collected from the tree, washed in running tap water to remove debris and dirt. The leaves were then dried using a blotting paper and dried in an oven with controlled temperature of 38^0 C. Completely dried leaves were then pulverized using a clean kitchen blender to get fine powder which is then stored in an air tight container. This powder is used in the synthesis of various extracts.

Hexane Extract

Dry leaf powder (mentioned above) from *F. exasperata* and *F. drupacea* were weighed to about 5 mg and soaked in 100 ml of hexane overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used for the preliminary analysis for phytochemicals.

Methanol Extract

Dry leaf powder (mentioned above) from *F. exasperata* and *F. drupacea* were weighed (5 mg each) and soaked in 100 ml of methanol overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used in the preliminary analysis for phytochemicals.

Aqueous Leaf Extract

Dry leaf powder (mentioned above) from *F. exasperata* and *F. drupacea* were weighed (5 mg) and soaked in 100 ml of sterilized double distilled water overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used for the preliminary analysis for phytochemicals.

Fruit Extract Preparation

Fully ripened healthy fruits of *F. drupacea* and *F. exasperata* were collected from the tree and washed in running tap water to remove debris and dirt. The fruits were then dried using a blotting paper. Fruits were then cut open before drying to avoid rotting as they are flushy and dried in an oven with controlled temperature of 41^0 C. Completely dried fruits were then pulverized using a kitchen blender to get fine powder which is then stored in an air tight container. This powder is used in the synthesis of various extracts.

Hexane Extract

Dry fruit powder (mentioned above) from *F. exasperata* and *F. drupacea* were weighed. 5 mg of each were soaked in 100 ml of hexane overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used for the preliminary analysis for phytochemicals.

Methanol Extract

Dry fruit powder (mentioned above) from *F. exasperata* and *F. drupacea* are weighed to about (5 mg) and soaked in 100 ml of methanol overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used in the preliminary analysis for phytochemicals.

Aqueous Leaf Extract

Dry fruit powder (mentioned above) from *F. exasperata* and *F. drupacea* are weighed (5 mg) and soaked in 100 ml of sterilized double distilled water overnight, heated to boil and filtered through Whatman no.1 filter paper. This extract is used for the preliminary analysis for phytochemicals.

PART 1

Phytochemical Profiling

Qualitative phytochemical screening

i. Test For Alkaloids

The dried extract of selected plant parts (50 mg) was diluted in weak hydrochloric acid and filtered. This filtrate was utilized in subsequent chemical tests to detect the presence of alkaloids.

Wagner's test (Shanmugam et al., 2019)

An aliquot of the extract is mixed with 3-5 drops of Wagner's reagent (Appendix 1). The production of a reddish-brown precipitate suggested the presence of alkaloids.

Hagger's test

A portion of the methanolic extract was treated with saturated picric acid solution. An orange-yellow precipitate confirms the presence of alkaloids.

ii. Test For Flavonoids (Harbourne, 1998)

Alkaline Reagent Test

A tiny amount of extract was added to sodium hydroxide solution. A yellowish solution will be generated. Then a few drops of weak hydrochloric acid were added, and the colour disappeared, indicating the presence of flavonoids.

Lead Acetate Test

A few drops of 10% lead acetate solution were mixed with the extract. A yellow precipitate demonstrates the presence of flavonoids in the extract.

iii. Test for Phenols (Harborne, 1998; Kumar et al., 2007)

$FeCl_3$ Test

A few drops of 0.1% ferric chloride solution were mixed with the extract. A deep blue colour suggested the presence of phenolic compounds.

Phenol Test

The solvent-free extract is spread on filter paper, and a drop of phosphomolybdic acid reagent was added. Exposing the spot to ammonia vapours causes blue coloration, indicating the presence of phenols.

iv. Test for Tannins (Evans & Evans, 2002)

The solvent-free extract (0.5 g) was combined with 10 millilitres of distilled water and filtered. The extracted filtrate was utilized to identify the presence of tannins.

Ferric Chloride Test

A few drops of 1% ferric chloride solution were added to 2 milliliters of filtrate. The presence of a blue-black, green, or blue-green precipitate indicates tannins.

v. Test for Terpenoids

The Salkowski Test (Shanmugam et al., 2019)

Approximately 2 ml of chloroform was applied to 1 ml of the sample along the test tube's sidewalls. After that, 2 ml of concentrated sulphuric acid was added and carefully allowed to stand. A yellow color with green fluorescence indicates the presence of terpenoids.

vi. Test for Phytosterols

Liebmann-Burchard Reaction (Siddiqui & Ali, 1997)

The methanolic extract was chloroform-treated and then filtered. It was filled with around 2 ml of acetic anhydride, followed by 0.5 g of the extract and 2 ml of sulphuric acid. Changes in color from violet to blue indicate the presence of phytosterols.

vi. Test for Saponins

Foam test (Adegoke et al., 2010)

In a test tube, 5 ml of distilled water was combined with approximately 0.5 g of methanolic extract. Thus, the produced suspension was vigorously shaken before being examined for a stable, continuous foam. A layer of foam was maintained, indicating the presence of saponins.

vii. Test for Cardiac Glycosides (Harborne, 1998)

Keller Killiani's Test

A test tube containing 5 mL of extract was treated with 2 mL of glacial acetic acid before adding a drop of ferric chloride solution. It was cautiously treated with 1 mL of pure sulphuric acid. The appearance of a brown ring at the interface indicated the existence of cardiac glycosides, which are distinguished by their deoxy sugar.

viii.Phlobatannin Test (Edeoga et al., 2005)

HCl test

Approximately 2 ml of extract was heated with 1 ml of 1% aqueous hydrochloric acid. A crimson precipitate shows the presence of phlobatannins.

ix. Test for Anthraquinones (Kumar et al., 2007)

Borntrager's Test

50 mg of the solvent-free extract was treated with 1 mL of 10% ferric chloride solution and 1 mL of concentrated HCl. Finally, the solution was boiled, cooled, and filtered. Then, add an equal amount of diethyl ether to the filtrate and shake thoroughly. The ether extract was then treated with powerful ammonia. A pink or deep red colouration in the aqueous layer indicated the presence of anthraquinones.

x. Test for Proteins and Amino Acid

To 2 ml of extract, 2 drops of ninhydrin solution were added. The creation of a purple colour signalled the presence of proteins and amino acids.

xi. Test for Coumarins

Sodium Hydroxide Test

Approximately 1 ml of the extract was treated with a 10% sodium hydroxide solution. The formation of yellow colour suggested the presence of coumarins.

xii. Test for Resins

Approximately 0.5 g of the extract was combined with 10 mL of distilled water. A turbidity was generated, indicating the presence of resin.

HR-LCMS (High-Resolution-Liquid Chromatography Mass Spectrometry)

Biopharma Q-Exactive Plus - High Resolution LCMS was used for the separation of non-volatile components which are identified and separated using Orbitrap. There was a direct infusion mass with both positive and negative mode ionization (APCI) and ESI. It was capable of up to 280,000 resolutions. The highest scan rate was 12 Hz. To boost sensitivity, an ion source called an RF-lens was employed. For high flux ion sources, advanced active beam guide intelligent ion beam management was employed. Its resolution was 1,40,000 @ m/z 200 and its mass range (m/z) was up to 50-8000 amu.

GC-HRMS (Gas Chromatography High-Resolution Mass Spectrometry)

Examination of gas chromatography mass spectrometry (GC-MS) The volatile components in the extract were identified and separated with an Agilent Model 8890 GC system equipped with a single quadrupole mass spectrometer (5977B MSD) analyzer. The GC oven was operating at about 450^0C. Area reproducibility was less than half of the chromatographic performance, and retention time repeatability was less than 0.008%. 7500:1 was the inlet split ratio. Accurate mass measurement: 1 µL injection of 100 pg/µL OFN; standard scanning starting at 50.350 u will yield its monoisotope at m/z 271<0.005. 150–350°C was its ion source temperature, while 106–200°C was its quadrupole temperature. Database and spectrum library were created using a licensed copy of the NIST 2017 library.

PART II

Free Radical Scavenging Activity of Selected *Ficus* Species

Antioxidants are substances with the ability to scavenge free radicals produced by diverse metabolic processes. Plants and animals produce these chemicals to help balance oxidative stress. In the current study, four *in vitro* antioxidant assays are utilized to determine the effect of plant extract on scavenging free radicals produced by substrates such as DPPH, Fe3+ - ascorbate - EDTA - H_2O_2, sodium nitroprusside, and potassium ferricyanide.

I. DPPH Radical Scavenging Assay

The DPPH assay was used to measure the plant extracts' capacity to scavenge radicals (Chang et al, 2001). The absorption of the DPPH solution was seen to significantly decrease at 517 nm following the addition of an antioxidant. As a standard, ascorbic acid (10 mg/mL DMSO) was employed. When scavenged, the pink-colored stable free radical 1, 1-diphenyl-2-picryl hydrazyl turns yellow. The fundamental properties of DPPH include exhibiting free radical scavenging activity. The following can be used to express the scavenging reaction between an antioxidant (H-A) and DPPH:

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

When antioxidants react with DPPH, they might be converted to DPPH-H, which lowers absorbance. Following that, the discoloration indicates the scavenging power of the extracts or antioxidants via hydrogen donating ability.

The preferred concentrations (12.5 µg/mL - 200 µg/mL) were obtained from the stock concentration (10 mg/mL) and brought up to the final volume of 20 µl with DMSO. 1.48 ml of 0.1 mM DPPH solution was added. The control was an equivalent volume of distilled water without test solution, and the standard was ascorbic acid. The reaction mixture was incubated at room temperature for 20 minutes in the dark. After that, the absorbance of the reaction mixture was measured at 517 nm using 3 ml of DPPH as a blank.

The percentage of inhibition was estimated using the calculation shown below:

$$\text{Percentage inhibition} = \frac{AC-AS}{AC} \times 100$$

Where AC represents the control absorbance and AS represents the sample absorbance.

Each extract was tested in triplicates, and the standard errors were calculated.

ii. Hydrogen Radical Scavenging Assay

The basic concept of this assay is based on the breakdown product of 2 deoxyribose via TBA condensation (Elizabeth and Rao,1990). The Fe3+ - ascorbate - EDT - H2O2 system (the Fenton reaction) produces the hydroxyl radical. Gallic acid (10 mg/mL DMSO) was used as the reference. Samples of varying concentrations (12.5-200 µL) were obtained from a stock concentration (10 mg/mL). Mix 500 µL of a reaction mixture containing 2 deoxy 2 ribose (2.8 mM), $FeCl_3$ (100 µM), EDTA (100 µM), H_2O_2 (1.0 mM), and ascorbic acid (100 µM) in KH_2PO_4 - KOH buffer (20 mM pH 7.4) to a final volume of 1 mL.

As a control, the same volume of distilled water was used but without the test solution. Subsequently, the reaction mixture was incubated at 37°C for one hour. One millilitre of 2.8% TCA and one millilitre of 1% aqueous TBA were then added. To develop the colour, the mixture was again incubated for 15 minutes at 90°C. Following cooling, the mixture's absorbance at 532 nm was measured and found to be different from a suitable blank solution (Kumaran and Karunakaran, 2006).

This is how the percentage of inhibition was calculated:

$$\text{Percentage inhibition} = \frac{AC-AS}{AC} \times 100$$

Where AC represents the control absorbance and AS represents the sample absorbance.

Each extract was tested in triplicates, and the standard errors were calculated.

iii. Nitric Oxide Scavenging Assay

The nitric oxide scavenging assay's fundamental idea is that sodium nitroprusside in an aqueous solution at physiological pH spontaneously produces nitric oxide, which reacts with oxygen to form nitrite ions, which can then be measured using Griess reagent (Appendix 2). Effective nitric oxide scavengers diminish nitrite ions by competing with oxygen. Sodium nitroprusside (5 mM) was combined with varying concentrations of the sample, ranging from 125 µg/ml to 2000 µg/ml, from a stock concentration of 10 mg/ml (Appendix 3) (pH = 7.4), and the mixture was incubated at 25°C for 150 minutes (Kumaran & Karunakaran, 2006). The control was the reagent that did not contain the test solution.

After 30 minutes, 1.5 mL of the incubated solution was taken out and mixed with 1.5 mL of Griess reagent, which is composed of 0.1% N-1-naphthyl ethylene diamine dihydrochloride, 2% phosphoric acid, and 1% sulphanilamide. The absorbance was measured at 546 nm with gallic acid serving as the reference (Valentao et al., 2002).

This is how the proportion of scavenging activity was determined:

$$\text{Percentage inhibition} = \frac{\text{AC}-\text{AS}}{\text{AC}} \times 100$$

Where AC represents the control absorbance and AS represents the sample absorbance.

Each extract was tested in triplicates, and the standard errors were calculated.

IV: Superoxide Free Radical Scavenging Assay

Superoxide is important in biology as it can produce singlet oxygen and hydroxyl radicals. Redox imbalance may result from excessive superoxide anion generation, which might have negative physiological repercussions. In the riboflavin-NADH system, super oxide anion is created by NADH oxidation and measured by NBT reduction, which results in the creation of a blue formazan product. Ascorbic acid (10 mg/mL) served as the reference. In a test tube, 125-2000 µg/ml samples from

a stock solution of 10 mg/ml were combined with 0.05 ml of riboflavin (0.12 mM), 0.2 ml of EDTA [0.1 M], and 0.1 ml of NBT [1.5 mM]. The reaction mixture was then diluted to 2.64 ml with phosphate buffer [0.067 M]. Distilled water was used as the control. After 5 minutes of incubation in fluorescent light, absorbance was measured at 560 nm on a UV visible spectrophotometer, as well as after 30 minutes of illumination. Finally, OD was computed by Valentão et al. (2002).

Percentage inhibition was computed as follows:

$$\text{Percentage inhibition} = \frac{\text{AC}-\text{AS}}{\text{AC}} \times 100$$

Where AC represents the control absorbance and AS represents the sample absorbance.

Each extract was tested in triplicates, and the standard errors were calculated.

***In Vitro* Cytotoxicity Evaluation by MTT Assay (**L929)

The L929 (Fibroblast) cell line was obtained from the National Centre for Cell Sciences (NCCS) Pune, India, and maintained in Dulbecco's modified Eagles medium (DMEM) (Sigma-Aldrich, USA). The cell line was grown in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing Penicillin (100 U/ml), Streptomycin (100 µg/ml), and Amphotericin B (2.5 µg/ml). Cell lines were cultured at 37ºC in a humidified incubator with 5% CO_2 (NBSE Eppendorf, Germany). The vitality of cells was determined by direct observation using an inverted phase contrast microscope, followed by the MTT assay method.

Cells Seeding in 96 Well Plate:

Trypsinized confluent monolayer cells were suspended in 10% growth medium after two days. A 100µl cell suspension (5 x 103 cells/well) was then seeded in a 96-well tissue culture plate and cultured at 37ºC in a humidified 5% CO_2 incubator.

Compound Stock Preparation involved weighing and dissolving 1 mg of sample in 1 mL of 0.1% DMSO with a cyclomixer. To guarantee sterility, the sample solution was filtered using a 0.22 µm Millipore syringe filter.

***In Vitro* Cytotoxicity Evaluation:**

The growth medium was removed after 24 hours, and each freshly prepared compound in DMEM was serially diluted five times by a two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µl of DMEM). Three duplicates of each concentration of 100µl were added to each well, and the mixture was then incubated at 37ºC in an incubator with 5% CO_2 that was humidified. Control cells that had not been treated were also kept.

Direct Microscopic Observation: After treatment for 24 hours, the entire plate was examined under an Olympus CKX41 inverted phase contrast tissue culture microscope equipped with an Optika Pro5 CCD camera. Images of the microscopic observation were captured. Any noticeable alterations in the morphology of the cells, such as granulation and vacuolization in the cytoplasm of the cells, as well as rounding or shrinkage of the cells, were regarded as markers of cytotoxicity.

***In vitro* Cytotoxicity determination by MTT Method:** Filter sterilization was used to completely dissolve 15 mg of MTT (Sigma, M-5655) in 3 ml of PBS.

The sample material in the wells was removed after the 24-hour incubation period, and 30 µl of reconstituted MTT solution was added to each test and cell control well. The plate was then gently shaken well and incubated for four hours at 37ºC in a humidified 5% CO_2 incubator. In order to solubilize the formazan crystals, the supernatant was withdrawn after the incubation period and 100 µl of MTT solubilization solution (Dimethyl sulphoxide: DMSO, Sigma Aldrich, USA) was added. The wells were then gently agitated by pipetting up and down. At a wavelength of 540 nm, the absorbance values were determined using a microplate reader (Riss et al., 2013).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = Mean\ OD\ sample \div Mean\ OD\ control \times 100$$

***In Vitro* Anticancer Effect Determination by MTT Assay (**MCF-7**)**

The National Centre for Cell Sciences (NCCS), Pune, India, originally provided the MCF-7 (human breast cancer) cell line, which was cultured in Dulbecco's modified Eagles medium (DMEM) of Sigma Aldrich, USA. A 25 cm^2 tissue culture flask containing DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany), and an antibiotic solution containing 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 2.5 µg/ml of amphoteracin B was used to cultivate the cell line. Cell lines that were cultured were maintained at 37ºC in an NBS Eppendorf, Germany, humidified 5% CO_2 incubator. The MTT assay method was used to assess the vitality of the cells after they were directly observed using an inverted phase contrast microscope (Riss et al., 2013).

Cells Seeding in 96 Well Plate: The process of seeding cells in a 96-well plate involved trypsinizing a confluent monolayer of cells that was two days old. The cells were then suspended in 10% growth media. A 100 µl cell suspension (5x103 cells/well) was then planted in the tissue culture plate, which was then placed in an incubator with 5% CO_2 humidity at 37ºC.

Compound stock preparation involved weighing and dissolving 1 mg of sample in 1 ml of 0.1% DMSO with a cyclomixer. To guarantee sterility, the sample solution was filtered using a 0.22 µm Millipore syringe filter.

Anticancer Evaluation: The growth medium was removed after 24 hours, and newly prepared compounds in DMEM were serially diluted five times by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µl of DMEM). Three duplicates of each concentration of 100µl were then added to the corresponding wells, and the mixture was incubated at 37ºC in an incubator with 5% CO_2 humidified. Additionally, untreated control cells were kept.

Anticancer Assay by Direct Microscopic Observation: After a 24-hour treatment period, the entire plate was examined using an Olympus CKX41 inverted phase contrast tissue culture microscope equipped with an Optika Pro5 CCD camera. Images of the microscopic observation were captured. Any observable alterations in the morphology of the cells, such as granulation and vacuolization in the cytoplasm of the cells, as well as rounding or shrinkage of the cells, were regarded as markers of cytotoxicity.

Anticancer Assay by MTT Method: Using the MTT method, an anticancer assay was conducted by 15 mg of MTT (Sigma, M-5655) in 3 ml PBS until it was completely dissolved, and then it was sterilized using filter sterilization. The sample material in the wells was removed after the 24-hour incubation period, and 30 µl of reconstituted MTT solution was added to each test and cell control well. The plate was then gently shaken well and incubated for four hours at 37ºC in a humidified 5% CO_2 incubator. Following the incubation period, the supernatant was discarded, and 100 µl of MTT solubilization solution (DMSO, dimethyl sulphoxide, Sigma Aldrich, USA) was added. The formazan crystals were then gently mixed by pipetting up and down in the wells. At a wavelength of 540 nm, the absorbance values were determined using a microplate reader.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability } = Mean\ OD\ sample \div Mean\ OD\ control \times 100$$

Determination of Apoptosis by Acridine orange (AO) and Ethidium Bromide (EtBr) Double Staining

DNA-binding dyes, such as ethidium bromide (EtBr) (Appendix 7) (Sigma, USA), and acridine orange (AO), were used to accomplish the morphological identification of necrotic and apoptotic cells (Gherghi et al, 2003). Both living and non-living cells can detect the green fluorescence that AO produces when it intercalates into double-stranded DNA. On the other hand, when EtBr intercalates into DNA, it is absorbed by non-viable cells only and produces red fluorescence. Following a 24-hour exposure to the material at LC50 concentration, the cells

underwent a gentle washing with cold PBS and were subsequently stained for 10 minutes at room temperature using a mixture of AO (100 µg/mL) and EtBr (100 µg/mL). Following a 1 x PBS wash, the stained cells were examined using an Olympus CKX41 fluorescent microscope equipped with a blue filter and an Optika Pro5 camera. Living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation), and necrotic cells (uniformly orange-stained cell nuclei) can all be independently scored into four groups.

Comet Assay

The MCF-7 cell line was cultivated in accordance with the previously mentioned standard protocols, treated with a drug at their LC50 concentration (DRPFT-144.59219 µg/mL), and then incubated for a whole day. The cells were employed for the comet assay after being trypsinized and cleaned in fresh medium. One millilitre of 1% normal melting point agarose (NMA Invitrogen, USA) was used to pre-coat fully frozen microscope slides, which were then kept at 4ºC. 1×104/5–30µl of cell suspensions were combined with 10 µl of low melting point agarose (Novex, Invitrogen), pipetted over the agarose layer, covered with a cover slip, and kept at 4ºC. NMA (1 ml) served as the last line of defence. The slides were incubated at 4ºC for 20 minutes following each stage to allow the agarose to set.

Before being used, slides were immersed in a cold lysing solution that contained 12.5 µM NaCl, 100 mM Na_2EDTA, 10 mM Tris Base pH 10, and 1% SDS. Immediately before usage, 10% DMSO and 1% Triton X 100 were added. After two hours, slides were left in the 300 mM NaOH and Na_2EDTA pH13 electrophoresis buffer for 20 minutes to allow the DNA to unwind. Using an electrical source (Power case, Life Technologies), an electric current of 25V (300 mA) was applied for 20 minutes to perform electrophoresis in the same buffer.

Ultimately, slides were cleaned three times for five minutes each in neutralization buffer (0.4 M Tris, pH 7.5), dried, and stained with fifty microliters of ethidium bromide (20 µg/ml). Using an Opitka Pro5 CCD camera coupled to an Olympus CKX41 inverted epifluorescent microscope, the slides were captured on

camera. Tritek comet scoring software was used to score the comets, and statistical correlation was found.

Analysis of DNA Content and Cell Cycle Distribution Using Cell Cycle Kit by Flow Cytometry

During toxicity studies, it is essential to track a cell's capacity for proliferation in order to evaluate its overall health. Direct measurement of DNA synthesis is the most accurate way to achieve this. Standard ethanol fixation and detergent permiabilization are the fundamental components of the MUSE cell cycle kit, and they are adequate to access DNA during an active cell cycle. Propidium iodide (PI), a nuclear DNA intercalating stain that differentiates cells at different stages of the cell cycle based on the differential DNA content in the presence of RNAase to increase the specificity of DNA staining in each phase (G0/G1, S, and G2/M), is one of the premixed reagents used in the kit.

The MCF-7 cell line was cultivated using the regular protocols previously mentioned, and it was treated with a chemical at its LC50 concentration (DRPFT-144.59219 µg/mL) for a full day of incubation. The sample of cells was moved to a 50 ml conical flask or a 12 x 75 mm polystyrene tube. For fixation in a tube, 1×106 cells is the bare minimum advised. After that, the samples were centrifuged for five minutes at 3000 rpm. The particle was not disturbed during the removal of the supernatant. The cell pellet either produces a visible pellet or a white film on the tube bottom following centrifugation.

Each tube was filled with the appropriate amount of PBS (1 millilitre for every 106 cells), and the mixture was gently vortexed or pipetted multiple times. For five minutes, the cells were centrifuged at 3000 rpm. With the cell pellet intact, the supernatant was removed, leaving around 50 µl of PBS per 1×106 cells. Pipette several times to resuspend the pellet in the leftover PBS, or gently vortex to do so. Drop by drop, the reconstituted cells were added to the tube holding 1 millilitre of ice-cold 70% ethanol while being vortexed at a medium pace. Close the tube and freeze it at -20ºC.

Staining of cell cycle

The samples were centrifuged at 3000 rpm for 5 minutes at room temperature following the overnight incubation. After removing the supernatant, 250 µl of PBS was added to the pellet. Next, centrifugation was carried out once more at the same speed and duration. Once the supernatant was discarded, 250 µl of cell cycle reagent was applied to the pellet. For thirty minutes, this was incubated at dark (which is light sensitive). A flow cytometer was then used to evaluate it. The samples were examined and the gating process was carried out in relation to untreated control cells.

Gene Expression Studies

Using the total RNA isolation kit and the manufacturer's instructions, total RNA was isolated (Invitrogen - Product code 10296010). When TRIzol solution is added, cells are disrupted and RNA is released. Proteins are found in the interphase, whereas chloroform is extracted only in the aqueous phase after centrifugation. RNA precipitates as a white pellet on the tube's side and bottom when it is mixed with isopropanol.

The MCF-7 cell line was cultivated in accordance with the previously mentioned standard protocols, treated with a drug at their LC50 concentration (DRPFT-144.59219 µg/mL), and then incubated for a whole day. Additionally, untreated wells were kept up. Following five minutes of incubation, 1 millilitre of TRIzol reagent was applied to the culture well plate and the DMEM medium was aseptically removed. Next, the contents were moved into a brand-new, sterile Eppendorf tube. After adding 200µL of chloroform and vigorously shaking for 15 seconds, the mixture was incubated for two to three minutes at room temperature. It was then centrifuged for 15 minutes at 40^0C at 14000 rpm. After gathering the aqueous layer, 500 µL of 100% isopropanol was added. After 10 minutes of room temperature incubation, it was centrifuged at 14,000 rpm.

After 10 minutes of room temperature incubation, it was centrifuged for 15 minutes at 40^0C at 14000 rpm. After discarding the supernatant, 200 µL of 75% ethanol (Merck) was used to wash the particle that had been produced. After that, it was centrifuged in a cooling centrifuge (Remi CM12) for five minutes at 40^0C at 14000 rpm. After drying, the RNA pellet was suspended in TE buffer.

cDNA Synthesis

Trizol was used to extract total RNA (Invitrogen, USA). Purity and concentration of isolated total RNA was estimated. Using the cDNA preparation kit (G BIOSCIENCES, Product code: 786-5019s, 786-5020, master premix for first-strand cDNA synthesis), template complementary DNA was produced. Approximately 5 µl of RT An RNAse-free tube was filled with Easy Mix, 0.5 µl of oligo dT, and 2 µl of RNA template (0.5µg of total RNA). Next, sterile distilled water was added to get the reaction volume up to 10 µl. Pipetting the fluid gently up and down allowed it to be combined. The Eppendorf Master Cycler, a thermal cycler, was configured to synthesise cDNA. We cycled for 20 minutes at 42^0 C and 5 minutes at 85^0 C.

Step	**Temperature ºC**	**Time (minutes)**	**Number of cycles**
cDNA synthesis	42	20	1
Inactivation	85	5	1

Gene Expression Analysis By RT-qPCR

SYBR Green Master Mix (G BIOSCIENCES, Product code-786-5062) was used in a real-time qRT-PCR assay using a Light cycler 96 (Roche). Every reaction was carried out in triplicate, and the ΔΔCt method was used to analyse the data (using Light Cycler 96SW1.1Software). The table provides information on the different steps involved, the time required, and the temperature at each phase.

Steps	**Time required**	**Temperature**
Initial activation step	2 minutes	95ºC
3 step cycling		
Denaturation	10 seconds	95ºC
Annealing	1 minute	58ºC
Extension	1 min/kb	72ºC
Number of cycles	40 cycles	68ºC
End of PCR cycling	Indefinite	4ºC

The table gives the details of forward and reverse primers.

OLIGO NAME	FORWARD		REVERSE	
	SEQUENCE (5' ->3')	Tm	SEQUENCE (5' ->3')	Tm
H-GAPDH	ACTCAGAAGACTGTGGATGG	57.3	GTCATCATACTTGGCAGGTT	55.3
p53	CCACCATGAGCGCTGCTCA	71.3	GCAGGGGAGGGAGAGATG	65
STAT	GGAGGAGTTGCAGCAAAAAG (20)	57.3	TGTGTTTGTGCCCAGAATGT (20)	55.3

AGAROSE GEL ELECTROPHORESIS

DNA fragments can be separated and seen using agarose gel electrophoresis. When exposed to an electric field, the fragments are sorted by size, and charge and flow through the agarose gel matrix. Applying potential across an electrolyte solution (buffer) creates the electric field. Boiling in an aqueous buffer causes agar to dissolve, which then forms into a gel when cooled. A 1.5% agarose gel was made with 1x TE buffer and melted at 90°C in a hot water bath. The melted agarose was then cooled to 45^0 C. After adding 6 µl of 10 mg/mL ethidium bromide, the gel comb-containing gel casting device was filled. The comb was taken out of the gel after it had set. The platform containing the gel was submerged in the electrophoresis buffer by pouring it into the gel tank. Samples were put onto the gel, which was then operated for 30 minutes at 50 V. Using a gel documentation system (E gel imager, Invitrogen), the stained gel was visualized.

PART III

Phytofabrication of Silver Nanoparticles

Eight millilitres of 10 mM $AgNO_3$ were mixed with two millilitres of plant extracts each (FDFE, FDLE, FEFE, FEFL) to produce green silver nanoparticles. A 2:8 ratio was kept for the large-scale production of silver nanoparticles from the extract. After vortexing, the mixture's pH was brought to 8, and it was then incubated for 15 minutes at 60^0C. The reaction mixture's colour changed to reddish brown, making the creation of silver nanoparticles evident. For 15 minutes at 40^0C, this

solution was centrifuged at 13,000 rpm in order to extract the nanoparticles from the reaction mixture. These pellets were gathered and resuspended in sterile distilled water for purification. Pellets that had been purified and centrifuged were dried in a hot air oven at a controlled temperature. Dried nanoparticle powder was stored for characterization and stock preparation (1 mg/ml).

Characterization of Green Nanoparticles

UV-Vis Spectroscopy

A UV-Vis spectroscopic analysis of the green nanoparticles was carried out using the reaction mixture following the development of brick red colour, which acts as a visual cue for nanoparticle creation. The absorbance between 300 and 700 nm was measured using a spectrophotometer (Cary 5000).

FE-SEM

Finely powdered and oven-dried nanoparticles were employed for FE-SEM inspection (Geminisem 300, with magnification of 12 X - 2,000,000 X, acceleration voltage was 0.02 – 30 kv, and the Inlens BSE resolution was 1.2 nmat 1 kv. To determine the average size measurement and to further demonstrate nanoparticle formation, 100 µg of these samples were evaluated using this equipment.

ANTIMICROBIAL ACTIVITY

Antibacterial activity

33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) was dissolved in 1000 ml of distilled water to create the medium. The dissolved medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. While still molten, the autoclaved media was well combined and then poured onto 100 mm petri plates (25–30 ml/plate).

Bacterial cultures of *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), and *Streptococcus mutans* (ATCC 25175) were seeded into petri plates containing 20 millilitres of Muller Hinton Agar

Medium (growth of culture regulated according to Mc Farlands Standard, 0.5%). Sterile discs with the appropriate test samples were positioned on plates. After that, the plates were incubated for 24 hours at 37°C. The diameter of the inhibitory zone that developed around the discs was used to measure the antibacterial activity (Hudzicki, 2009).

Antifungal activity

39 g of the commercially available Potato Dextrose Agar Medium (Hi Media) were dissolved in 1000 ml of distilled water to create the medium. The dissolved medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. While still molten, the autoclaved media was well combined and then poured onto 100 mm Petri plates (25–30 ml/plate). *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231) two overnight-grown fungal species, were swabbed from potato dextrose agar plates. Sterile discs with the appropriate test samples were positioned on plates. After being incubated at room temperature for the entire night, the zone of inhibition was determined and compared to that of the common antifungal drug, Clotrimazole (NCCLS, 1993).

PHASE II

PART I

Endophyte Isolation

Materials and methods

PDA, purified agar, KH_2PO_4, $MgSO_4$ and peptone, were obtained from Hi Media Lab chemicals. Leaves and fruits were collected from the Calicut University Botanical Garden. Plant parts collected were washed, surface sterilised and processed within 24 hours from collection.

Media Preparation

Specially prepared media were used for the isolation of both fruit and leaves. For isolation, dried fruit powder (0.02 mg/ml), dried leaf powder (0.02 mg/ml), KH_2PO4 (0.0018 mg/ml), $MgSO_4$ (0.0001 mg/ml), Peptone (0.005 mg/ml), and purified agar (0.02 mg/ml) were used. All of these ingredients were thoroughly combined with distilled water and autoclaved for 15 minutes at 121^0Celsius and 15 pounds of pressure.

Leaves

Collected leaves of *F. exasperata* and *F. drupacea* were washed thoroughly in running tap water and surface sterilized using 3% sodium hypochlorite (3 minutes) and washed in sterile distilled water followed by 75 % Ethyl alcohol for 5 minutes. Again, washed in sterile distilled water and taken into the sterilised LAF. Sterilized leaves were cut into 3 cm square pieces and inoculated to potato dextrose agar plates.

Fruits

Hand-picked fully ripened fruits of *F. drupacea* and *F. exasperata* were placed in sterile plastic bags after being properly cleaned under running tap water and immersed in 3% sodium hypochlorite for five minutes. After that, the fruits were sliced open and immersed in 3% sodium hypochlorite for an additional three minutes. They were soaked in 75% ethyl alcohol for four minutes, cleaned in sterile distilled

water, and then rinsed again. In sterile LAF, sterilised fruits are introduced. To extract the water droplets, use sterilized blotting paper. The skin and exposed sections were removed with a sterile scalpel. The fragments are then cut into 3 mm pieces and injected into potato dextrose agar media under sterile circumstances. Strict adherence to sterilization was maintained to prevent contamination at any point in the process.

Identification

DNA Isolation Using NucleoSpin® Plant II Kit (Macherey-Nagel)

About 100 mg of the endogenous tissue/mycelium is homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 is added and vortexed for 1 minute. Ten microlitres of RNase A solution was added and inverted to mix. The homogenate was incubated at 65ºC for 10 minutes. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow through liquid was collected and the filtrate was discarded. Four hundred and fifty microlitres of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 minute and the flow through liquid was discarded. Four hundred microlitre buffer PW1 was added to the column, centrifuged at 11000 x g for 1 minute and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid was discarded. Finally, 200 µl of PW2 was added and centrifuged at 11000 x g for 2 minutes to dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 µl of buffer PE was added and incubated at 65ºC for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4ºC.

Target	**Primer Name**	**Direction**	**Sequence (5' → 3')**
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

Sequencing Using Big Dye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufacturer's protocol.

Sequencing PCR amplification profile

96^{o}C	-	2 min	
96^{o}C	-	30 sec	30 cycles
50^{o}C	-	40 sec	
60 ^{o}C	-	4 min	

Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1

PART II

Biochemical Analysis of the Fruit Endophyte

The biochemical examination of the extract is important because it can reveal the many biochemicals that a particular organism creates. In order to do this, 3 ml of the sieved extract had to be collected and put in an Eppendorf tube for an HR-LCMS analysis.

Biochemical Profiling

Qualitative Biochemical Screening

i. Test for Alkaloids

The dried extract of selected plant parts (50 mg) was diluted in weak hydrochloric acid and filtered. This filtrate was utilized in subsequent chemical tests to detect the presence of alkaloids.

Wagner's test (Shanmugam et al., 2019)

An aliquot of the extract is mixed with 3-5 drops of Wagner's reagent (Appendix 1). The production of a reddish-brown precipitate suggested the presence of alkaloids.

Hagger's test

A portion of the methanolic extract was treated with saturated picric acid solution. An orange-yellow precipitate confirms the presence of alkaloids.

ii. **Test For Flavonoids** (Harbourne, 1998)

Alkaline Reagent Test

A tiny amount of extract was added to sodium hydroxide solution. A yellowish solution will be generated. Then a few drops of weak hydrochloric acid were added, and the colour disappeared, indicating the presence of flavonoids.

Lead Acetate Test

A few drops of 10% lead acetate solution were mixed with the extract. A yellow precipitate demonstrates the presence of flavonoids in the extract.

iii. Test for Phenols (Harborne, 1998; Kumar et al., 2007)

$FeCl_3$ Test

A few drops of 0.1% ferric chloride solution were mixed with the extract. A deep blue colour suggested the presence of phenolic compounds.

Phenol Test

The solvent-free extract is spread on filter paper, and a drop of phosphomolybdic acid reagent was added. Exposing the spot to ammonia vapours causes blue coloration, indicating the presence of phenols.

iv. **Test for Tannins** (Evans & Evans, 2002)

The solvent-free extract (0.5 g) was combined with 10 millilitres of distilled water and filtered. The extracted filtrate was utilized to identify the presence of tannins.

Ferric Chloride Test

A few drops of 1% ferric chloride solution were added to 2 milliliters of filtrate. The presence of a blue-black, green, or blue-green precipitate indicates tannins.

v. **Test for Terpenoids**

The Salkowski Test (Shanmugam et al., 2019)

Approximately 2 ml of chloroform was applied to 1 ml of the sample along the test tube's sidewalls. After that, 2 ml of concentrated sulphuric acid was added and carefully allowed to stand. A yellow color with green fluorescence indicates the presence of terpenoids.

vi. **Test for Phytosterols**

Liebmann-Burchard Reaction (Siddiqui & Ali, 1997)

The methanolic extract was chloroform-treated and then filtered. It was filled with around 2 ml of acetic anhydride, followed by 0.5 g of the extract and 2 ml of sulphuric acid. Changes in color from violet to blue indicate the presence of phytosterols.

vi. Test for Saponins

Foam test (Adegoke et al., 2010)

In a test tube, 5 ml of distilled water was combined with approximately 0.5 g of methanolic extract. Thus, the produced suspension was vigorously shaken before being examined for a stable, continuous foam. A layer of foam was maintained, indicating the presence of saponins.

vii. **Test for Cardiac Glycosides** (Harborne, 1998)

Keller Killiani's Test

A test tube containing 5 mL of extract was treated with 2 mL of glacial acetic acid before adding a drop of ferric chloride solution. It was cautiously treated with 1

mL of pure sulphuric acid. The appearance of a brown ring at the interface indicated the existence of cardiac glycosides, which are distinguished by their deoxy sugar.

viii. **Phlobatannin Test** (Edeoga et al., 2005)

HCl test

Approximately 2 ml of extract was heated with 1 ml of 1% aqueous hydrochloric acid. A crimson precipitate shows the presence of phlobatannins.

ix. **Test for Anthraquinones** (Kumar et al., 2007)

Borntrager's Test

50 mg of the solvent-free extract was treated with 1 mL of 10% ferric chloride solution and 1 mL of concentrated HCl. Finally, the solution was boiled, cooled, and filtered. Then, add an equal amount of diethyl ether to the filtrate and shake thoroughly. The ether extract was then treated with powerful ammonia. A pink or deep red colouration in the aqueous layer indicated the presence of anthraquinones.

x. **Test for Proteins and Amino Acid**

To 2 ml of extract, 2 drops of ninhydrin solution were added. The creation of a purple colour signalled the presence of proteins and amino acids.

xi. Test for Coumarins

Sodium Hydroxide Test

Approximately 1 ml of the extract was treated with a 10% sodium hydroxide solution. The formation of yellow colour suggested the presence of coumarins.

xii. Test for Resins

Approximately 0.5 g of the extract was combined with 10 mL of distilled water. A turbidity was generated, indicating the presence of resin.

A. HR-LCMS (High-Resolution Liquid Chromatography Mass Spectrometry)

Biopharma Q-Exactive Plus - High Resolution LCMS was used to reveal non-volatile components were identified and separated using Orbitrap. There was a direct infusion mass with both positive and negative mode ionization (APCI) and ESI. It was capable of up to 280,000 resolutions. The highest scan rate was 12 Hz. To boost sensitivity, an ion source called as RF-Lens was employed. For high flux ion sources, advanced active beam guide intelligent ion beam management was employed. Its resolution was 1,40,000 @ m/z 200 and its mass range (m/z) was up to 50-8000 amu.

PART III

Nanoparticles Biosynthesis

Silver nanoparticles: After treating 50 millilitres of sieved fungal extract with 10 millilitres of a 1 mM silver nitrate solution, the mixture was heated for 15 minutes in a water bath.

Gold Nanoparticles: A 100 ml Erlenmeyer flask was filled with 60 ml of sieved fungal extract, and 5 ml of a 1 mM concentration of $AuCl_4$ solution was added. The solution was held for five minutes in a water bath that has been heated to 60^0C.]

Nanoparticle Characterization

UV-vis Spectrophotometric Analysis

Spectrophotometer analysis was used for primary analysis following the colour change indication for the creation of nanoparticles. Spectrophotometer (Cary 5000) was utilized to detect absorbance between 300 and 700 nm in wavelength.

SEM Analysis

Finely powdered and oven-dried nanoparticles were examined using a scanning electron microscope (FE-SEM; Siemens Geminisem 300, with magnification of 12X -2,000,000X, acceleration voltage 0.02 - 30kv, and the Inlens BSE resolution of 1.2 nmat 1 kv).

Antimicrobial Activity

Agar plates were prepared using LB agar medium for *E. coli* (MTCC 452) and HE agar medium for *S. paratyphi* (MTCC 3220). The particular agar plates were infected with bacteria that had developed overnight. The fungus extract was used to create nanoparticles, which were then ground up, dissolved in sterile distilled water, and impregnated in the sterile discs. To evaluate the antibacterial activity of the nanoparticles, these sterile discs were inserted into agar plates seeded with bacterial cultures.

PHASE I

PART I

Phytochemical Delineation

Phytochemical analysis of the selected four extracts from the two *Ficus* species were conducted by qualitative analysis using various assays including GC-HRMS and HR-LCMS. Volatile components were identified through GC-HRMS and non-volatile components were revealed by HR-LCMS.

Qualitative Phytochemical Analysis

The presence or absence of various important phytochemical groups were analyzed using a number of biochemical assays. The assays for alkaloids, flavonoids, phenols, glycosides, tannins, saponins etc. were conducted using three different solvent extracts. Methanol, water and hexane were the three solvents selected. The results indicated the presence of all major classes of secondary metabolites in the polar solvents such as water and methanol. Methanol and water seem to be similar in eluting various class of secondary metabolites. Hexane extracts could only report the presence of alkaloids, flavonoids and steroids. But aqueous as well as methanolic extracts reported secondary metabolites such as alkaloids, phenols, flavonoids, glycosides, proteins, coumarins, saponins and terpenoids. As methanolic extract is toxic to the biological system, water was selected as the solvent for further studies. The results of preliminary phytochemical analysis are detailed in the **Table 4.**

Table 4: Preliminary phytochemical analysis using three different solvent extracts of fruits and leaves of *F. exasperata* and *F. drupacea*

Sl No	Class of compounds	Chemical tests	FDFE			FDLE			FEFE			FELE		
			Aqs	Meth	Hex	Aqs	Meth	Hex	Aqs	meth	Hex	Aqs	Meth	Hex
1	Alkaloids	Wagners test	+	+	–	+	+	+	+	+	+	+	+	–
		Hagers test	+	+	+	+	+	+	+	+	–	+	+	+
2	Flavonoids	Alkaline reagent test	+	+	–	+	+	–	+	+	+	+	+	+
		Lead acetate test	+	+	+	+	+	+	+	+	–	+	+	–
3	Phenols	Ferric chloride test	+	+	–	+	+	–	+	+	+	+	+	–
P	Tannins	Braymers test	–	–	–	+	+	–	–	–	–	+	+	–
5	Terpenoides	Salkowski test	+	+	–	+	+	–	+	+	–	+	+	–
6	Steroids	Liebermann Burchard test	–	–	+	–	–	+	–	–	–	–	–	+
7	Saponins	Foam test	–	–	–	–	–	–	–	–	–	–	–	–
8	Glycosides	Keller Killiani test	+	+	–	+	+	–	+	+	–	+	+	–
9	Phlobatannins	Precipitation test	–	–	–	–	–	–	–	–	–	–	+	–
10	Anthraquinones	Borntragers test	–	–	–	+	+	–	–	+	–	–	+	–
11	Proteins and aminoacids	Ninhydrin test	+	+	–	+	+	–	+	+	–	+	+	–
12	Coumarins	Alcoholic NaOH test	+	+	–	+	+	–	+	+	–	–	+	–
13	Resins	Turbidity test	–	–	–	–	–	–	–	–	–	–	–	–

Aqs: Aqueous extract, Meth: Methanolic extract, Hex: Hexane extract, FDLE: *F. drupacea* leaf extract, FDFE: *F. drupacea* fruit extract, FELE: *F. exasperata* leaf extract, FEFE: *F. exasperata* fruit extract

Gas Chromatograph with High Resolution Mass Spectrometer (GC-HRMS) Analysis of Aqueous Plant Extracts

GC-HRMS analysis of phytochemicals from the four extracts of *Ficus drupacea and Ficus exasperata* revealed the presence of 10 phytochemicals. These comes under phenols, organic compounds, purines and fatty alcohols. The results are tabulated in **Table 5.** Compounds like 2 hydroxy-3-pentanone, catechol, 2,3-dihydrobenzo furan and 2-methoxy-4-vinylphenol were present in the *F. drupacea* leaf extract. Among these compounds catechol and 2-methoxy-4-vinylphenol are phenols, one is a benzofuran and others belong to organic compounds. Undecane, 1,2,3,5-Cyclohexanetetrol were the only compounds revealed by the GC-HRMS analysis of *F. drupacea* fruit, showing presence of least number of compounds among the four types of extracts used in the study. *Ficus exasperata* leaf extract revealed the presence of four phytochemicals such as decane, undecane, hydroquinone, 2-tridecanol. Among these compounds, 2-tridecanol is a fatty alcohol and hydroquinone is a phenol derivative, the rest of them are organic compounds. Catechol, 2,3-dihydrobenzo furan and hydroquinone were the compounds with maximum area percentage. Catechol had the highest area percentage with a value of 83.83% followed by 2,3- dihydrobenzo furan with an area percentage of 9.72 %. Structure of each compound with its MS spectrum are given from **Fig. 10 (i)-10 (iii).**

Table 5: GC-HRMS analysis result of leaves and fruits aqueous extract of *F. exasperata* and *F. drupacea*

Sl No.	RT	Compound	Area %			
			FELE	FEFE	FDLE	FDFT
1	4.075	2-Hydroxy-3-pentanone			2.82	
2	4.169	Decane	3.15	10		1.9
3	6.031	Undecane	2.83	8.6		1.84
4	8.219	hydroquinone	65.71	79.97		
5	8.226	Catechol			83.83	
6	8.682	2,3-dihydrobenzofuran			9.72	
7	9.138	2-Tridecanol	26.31			
8	11.051	2-methoxy-4-vinylphenol			3.29	
9	17.721	1,2,3,5-Cyclohexanetetrol				95.95
10	17.827	Guanosine			5.49	

HR-LCMS Analysis of Phytochemicals

HR-LCMS analysis revealed the presence of a total of 92 to phytochemicals from the four types of extract studied, which falls under the major secondary metabolite's groups such as alkaloids, phenols, polyphenols, flavonoids, terpenoids, glycosides, coumarins, Isoquinolines, proteins, ether, carbohydrates, fatty acids and carboxylic acids. When the number of phytochemical compounds from each plant is analyzed, higher number of compounds were revealed from the fruits and leaves of *Ficus exasperata*. The leaf extract of *F. exasperata* revealed the presence of 53 compounds which was found to be the highest number of phytochemicals from the four extracts studied. Least number of phytochemicals were present in the leaf extract of *F. drupacea* with 19 compounds. When compared to the GC-HR/MS of the four extracts, the results of HR-LCMS revealed higher number of compounds. Phenolic compounds such as caffeic acid, chlorogenic acid, gallic acid, vanillic acid; alkaloids such as isocarbostyril, isococculidine, anhalonidine, solanocapsine; flavonoids such as biorobin, hespiridine, sophorol; terpenoids such as inundatine, vulgarin, armillane, cynaroside A, ophiobolin, madlongiside C, alpha-campholene acetate; glycosides such as lusitanicoside; coumarins such as osthenol-7-O-beta-D-gentiobioside; and a number of carboxylic acids such as corchorifatty acid D, 7-iso-Jasmonic acid, 16-hydroxyhexadecanoic acid etc. were the major compounds revealed by the HR-LCMS analysis. The details of the HR-LCMS analysis with the retention time and class of compounds are given in the **Table 6**. Structure of each compound with its MS spectrum are given from **Fig.11 (i)-11 (xxiii).**

Table 6: HR-LCMS analysis result of leaves and fruits aqueous extract of *F. exasperata* and *F. drupacea*

Sl No.	Compound	RT	Class of compounds	FDFE	FDLE	FEFE	FELE
1	2,3-Dihydroxyacetophenone	0.778	Aromatic ketone	-	-	-	+
2	(+/-)3(2-Methyl-3 furyl)thio)2-butanone	0.802	Aryl sulphide	-	+	+	+
3	Norcotinine	0.805	Pyrrolidines	+	-	-	-
4	Hamamelose	0.99	Aldopentose	-	+	-	-
5	Quinic acid	1.046	Carboxylic acid	+	+	-	-
6	Retronecine	1.172	Retronecine	-	-	-	+
7	Isocitrate	1.243	Organic compound	-	-	-	+
8	Miserotoxin	1.257	Beta-D-glucoside	-	-	+	-
9	8-Hydroxyadenine	1.4	Oxopurine	-	-	-	+
10	Phenylacetaldehyde	1.45	Aldehyde	-	-	-	+
11	Anhalonidine	1.52	Alkaloid	+	-	-	+
12	2-Methylbenzaldehyde	1.744	Aldehyde	-	-	-	+
13	Gallic acid	1.829	Polyphenol	+	+	-	-
14	Leu-gly-pro	1.886	Tripeptide	-	-	+	-
15	N-(1-Deoxy-1-fructosyl) phenylalanine	1.964	Protein derivative	+	-	+	-
16	Isocarbostyril	2.22	Alkaloid	+	-	+	-
17	Isococculidine	2.276	Alkaloid	-	-	-	+
18	Actinidine	2.557	Cyclopenta pyridines	-	+	-	+
19	2,6-Dihydroxybenzoic acid	2.887	Carboxylic acid	-	-	-	+
20	Glaudine	2.998	Alkaloid	+	-	-	-
21	Ankorine	3.028	Pyrido isoquinoline			+	
22	N2-(2-Carboxymethyl-2-hydroxysuccinoyl) arginine	3.113	Protein Derivative	-	-	+	-
23	Phenylbutyrylglutamine	3.211	Protein Derivative	-	-	+	+

24	Chlorogenic acid	3.248	Polyphenol	+	-	-	+
25	Indoleacrylic acid	3.271	Indole	+	+	+	-
26	M Hydroxy benzoic acid	3.412	Benzene carboxylic acid	-	-	-	+
27	Syringic acid	3.469	Ether	-	-	-	+
28	Armillane	3.523	Diterpenoid	-	-	-	+
29	Salicylic acid	3.728	Carboxylic acid	+	-	-	-
30	Cynaroside A	3.747	Sesquiterpene	+	+	-	-
31	Dihydroferulic acid 4-Oglucuronide	4.019	Glycoside	+	+	-	+
32	Pantoyllactone glucoside	4.084	Carbohydrate	+	-	-	-
33	Ophiobolin	4.145	Sesterterpenoid	-	-	+	-
34	N-(3-Oxohexanoyl) homoserine lactone	4.221	Monocarboxylic Acid	-	-	-	+
35	Caffeic acid	4.525	Polyphenol	+	-	-	+
36	Biorobin	4.642	Flavonoids	-	+	-	+
37	5-(3,4-diacetoxybut-1-ynyl)-2,2'-bithiophene	4.692	Carboxylic acid ester	-	-	+	-
38	1,5, hydroxymarasmen-3one	4.807	Stilbene Glycosides	-	-	+	-
39	Osthenol-7-O-beta-D-gentiobioside	4.81	Coumarin	-	-	+	-
40	Histidinyl methionine	4.837	Pepetide	-	-	-	+
41	Ascorbyl stearate	4.964	Fatty acid Ester	-	-	-	+
42	Mono trans-p-coumaroylmesotartaric acid	5.015	Carboxylic Acid	-	-	-	+
43	Quercetin 3,7-dirhamnoside	5.071	Flavonoid Glycosides	-	+	-	-
44	Chromone	5.265	Chromones	-	-	-	+
45	Enol phenyl pyruvate	5.443	Organic compound	-	-	-	+
46	1-Sinapoyl-D-glucose	5.468	Carboxylic acid	+	-	-	-
47	1-0-E-cinnamoyl -6-(arabinosyl glucose)	5.64	Organic compound	-	-	-	+
48	Vanillic acid	5.648	Phenolic compound	-	-	+	-

49	Lusitanicoside	5.681	Glycoside	-	+	-	-
50	Genistein 8-c-glucoside	5.707	Isoflavones	-	-	-	+
51	Solanocapsine	5.718	Alkaloid	-	+	-	-
52	Chalcomoracin	5.725	Benzofurans	-	-	+	+
53	Tabernamine	5.907	Alkaloid	-	-	+	-
54	Lepidine E	5.943	Aromatic Ether	-	-	+	-
55	Ferulic acid	5.958	Phenol	-	-	-	+
56	Fabianine	5.997	Quinolines	-	-	-	+
57	Madlongiside C	6.203	Triterpenoid	-	-	+	-
58	Leuteolin-4-o-glucoside	6.349	Glycosyl oxyflavone	-	-	-	+
59	Inundatine	6.57	Sesquiterpenoid	-	-	-	+
60	Spirasine I	6.716	Diterpenoid	-	-	+	-
61	trans-Grandmarin isovalerate	6.802	Coumarins	+	-	+	-
62	Naltrindole	6.947	Isoquinolines	-	-	+	-
63	Hesperidin	7.203	Flavanone Glycoside	-	-	+	-
64	Gravolenic acid	7.237	Coumaric acid	+	-	-	-
65	(+) Sophorol	7.304	Isoflavanones	+	-	-	-
66	Alpha-Campholene acetate	7.439	Monoterpenoid	-	-	+	-
67	Vulgarin	7.451	Sesquiterpenoid	-	-	+	-
68	Jasmine ketolactone	8.062	Oxacycle	-	-	-	+
69	Icaceine	8.08	Diterpene alkaloid	-	-	-	+
70	Isoartocarpesin	8.224	Flavone	+	-	-	-
71	8'-Episesaminone	8.273	Lignans	+	-	-	-
72	16-oxopalmitate	8.637	Omega-oxo fatty acid	-	-	+	-
73	Corchorifatty acid D	8.677	Carboxylic acid	-	-	-	+
74	Borrelidin	8.765	Polyketides	-	-	-	+
75	Hulupone	8.994	Alkaloids	-	+	-	-
76	17-Hydroxylinolenic acid	9.183	Hydroxy fatty acid	-	-	-	+
77	16-Hydroxy-10-oxohexadecanoic acid	9.199	Fatty acid	-	-	-	+

78	9,10-Dihydroxy-12,13-epoxyoctadecanoate	9.793	Fatty acid	-	-	-	+
79	(+)-7-iso-Jasmonic acid	9.933	Carboxylic acid	-	+	-	-
80	Linalyl phenylacetate	10.216	Monoterpenoid	-	-	-	+
81	Nigakihemiacetal A	10.763	Quassinoid	+	-	-	-
82	16-hydroxy hexadecanoic acid	11.358	Carboxylic acid	-	-		+
83	Nigakilactone B	11.824	Triterpenoid	-	+	-	+
84	Austinol	12.754	Monoterpenoid	+	-	-	+
85	1,4-beta-D-Glucan	12.854	Oligosaccharide	+	-	-	-
86	Methyl 2-furoate	14.545	Ester	+	-	-	-
87	Ganoderic acid F	17.628	Triterpenoid	-	-	-	+
88	Ganosporelactone	18.186	Withanolide	-	-	-	+
89	N-Hexa decanoyl pyrrolidine	18.92	Alkaloid	-	+	-	-
90	Octadecyl fumarate	19.651	Ester	-	-	+	-

PART II

Antioxidant activities

Antioxidants are substances that aid in the scavenging of free radicals in a system. The antioxidant potential of *F. drupacea* extracts as well as *F. exasperata* extracts were investigated. The free radical scavenging ability of the fruit and leaf extracts are investigated here. The analysis employed four distinct assays: DPPH radical scavenging activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity, and super oxide radical scavenging activity.

DPPH Radical Scavenging Activity

When the four plant extracts FDFE, FDLE (fruit and leaf extracts of *F. drupacea)* and FEFE, FELE (fruit and leaf extracts of *F. exasperata*) were exposed to DPPH scavenging assays at concentrations ranging from 12.5 to 200 µg/Ml, the ability to scavenge free radicals increased with concentration. FDLE extract was discovered to have the highest inhibition percentage with a value of 57.84 ± 0.453% at the highest test concentration of 200 µg/mL. FELE had the lowest activity with a value of

53.76 ±1.132%. Ascorbic acid was utilized as the standard here, activity of the standard was results were high in comparison to the extract studied, with a value of 93.15% in the highest test concentration of 200 µg/mL. When the IC 50 values derived from the tests were anlysed, FDLE extract showed a comparatively better IC50 values (93.882 µg/mL.), which were three times greater than the control ascorbic acid IC50 values (32.17 µg/mL). When the IC50 values of the standard were compared to the test samples, it was shown that all four aqueous extracts had modest antioxidant activity (**Fig. 12)**. The standard, a pure antioxidant compound, is compared with the crude extracts, which may contain many components other than antioxidants that may interfere with the activities; however, the extracts were able to show comparable values to the standard, indicating the crude extract's potential antioxidant ability (**Fig. 13)**. FDFE's IC 50 value was found to be the highest, with 114.553 µg/mL. The IC 50 of FEFE was determined to be 99.042 µg/mL, whereas that of FELE was 104.468 µg/mL.

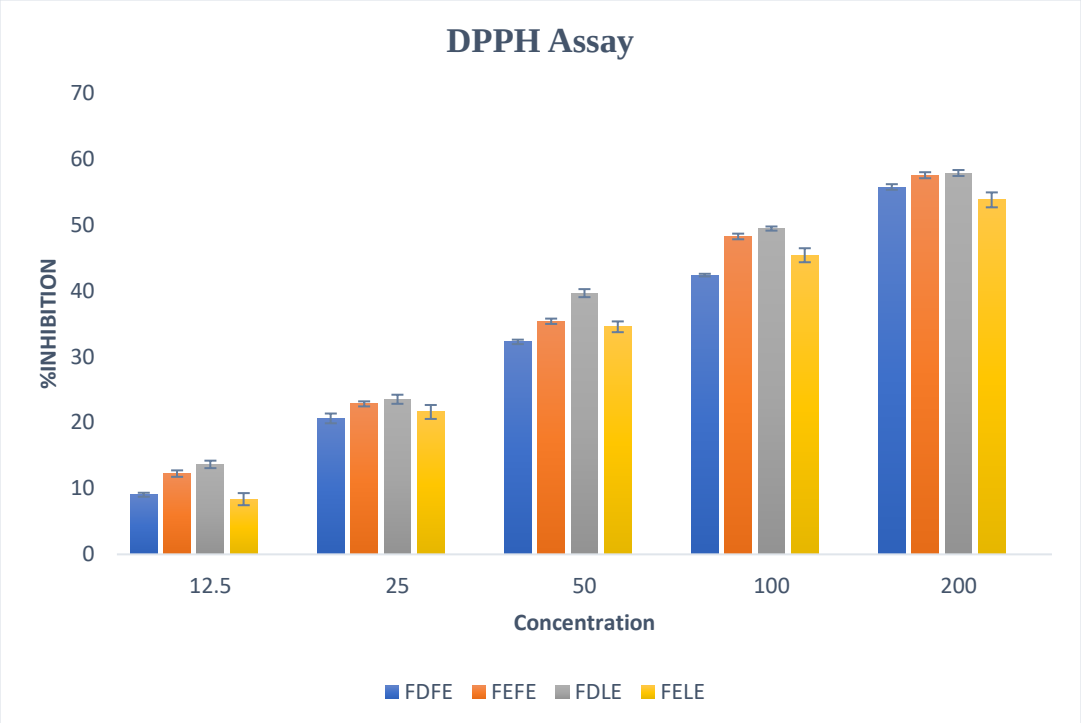


Fig. 12: DPPH radical scavenging activity of the leaf and fruit extracts of *F. exasperata*, and *F. drupacea*

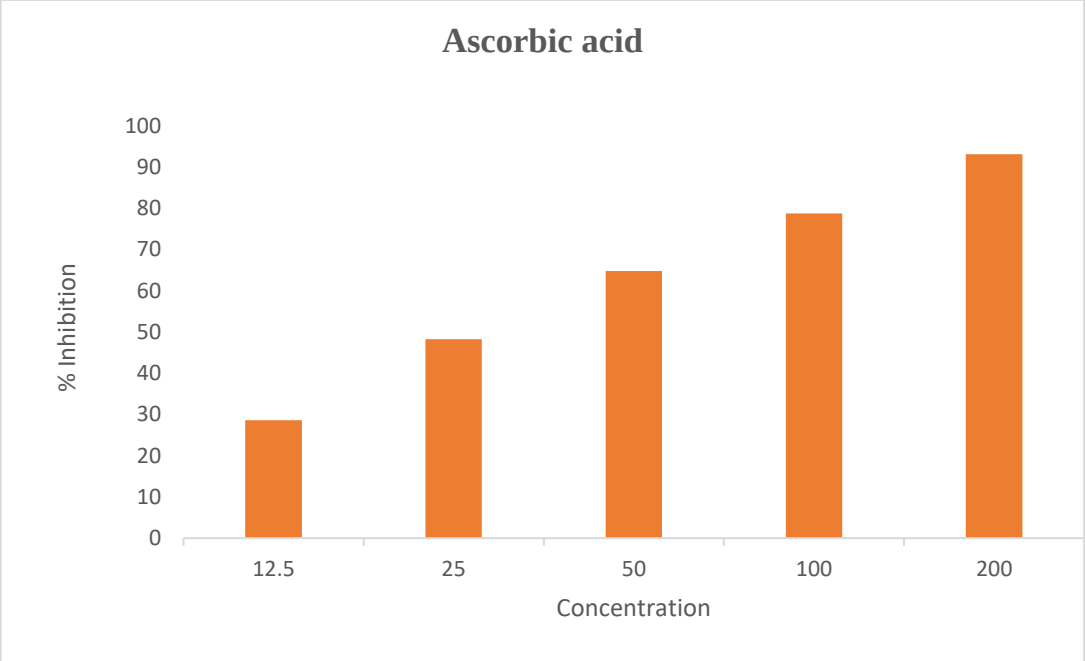


Fig. 13: DPPH radical scavenging activity of the standard ascorbic acid

Hydroxyl Radical Scavenging Activity

The ability of *F. drupacea* and *F. exasperata* leaf and fruit extracts to scavenge hydroxyl radicals was investigated. The concentrations used in the tests were 125, 250, 500, 1000, and 2000 µg/mL. FDFE extract demonstrated the highest percentage of inhibition in the hydroxyl radical scavenging assay, with an inhibition percentage of 64.4122 ± 0.714 µg/mL. The extract with the lowest IC50 value of 850 ± 070 µg/mL was FDLE with an inhibition % of 62.1176. FELE showed lowest inhibition percentage of 53.46 ± 0.986 µg/mL with an IC 50 value of 1603.062 µg/mL. The inhibition percentage for FEFE was 55.515 ± 0.663, with an IC 50 value of 1602.543 µg/mL **(Fig. 14)**. The standard gallic acid's IC 50 value was 305.93 µg/mL; based on this value, it is clear that the test samples were not as potent as the standard, and the test extracts were found to have moderate to low antioxidant activity (**Fig. 15)**.

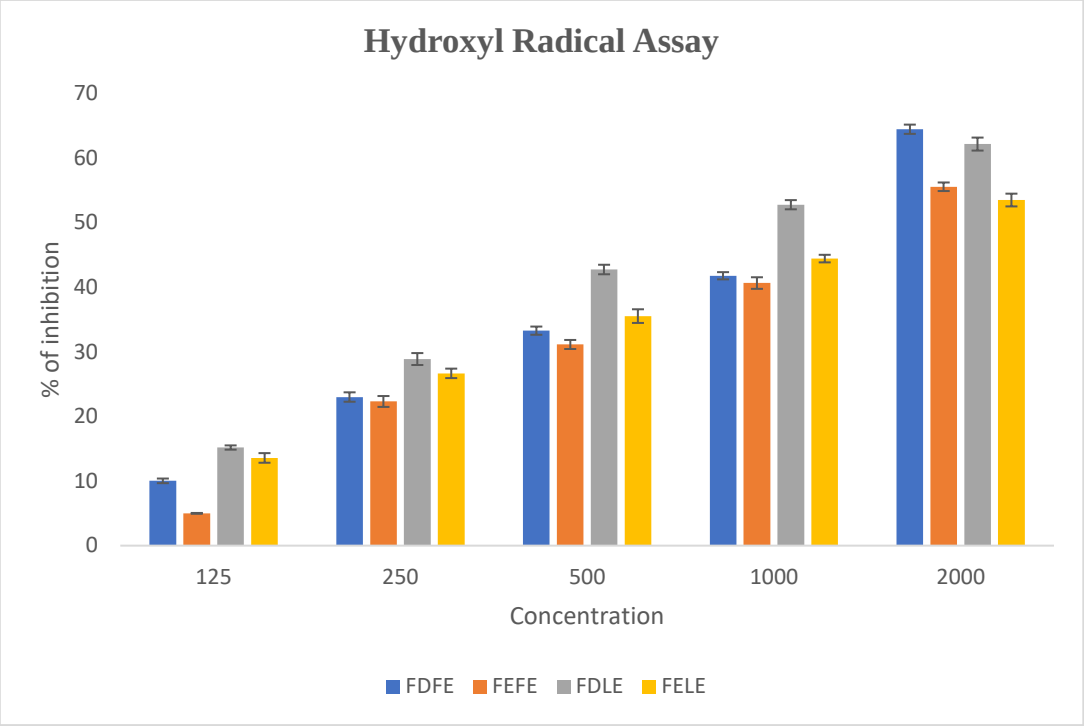


Fig. 14: Hydroxyl radical scavenging activity of the leaf and fruit extracts of *F. exasperata*, and *F. drupacea*

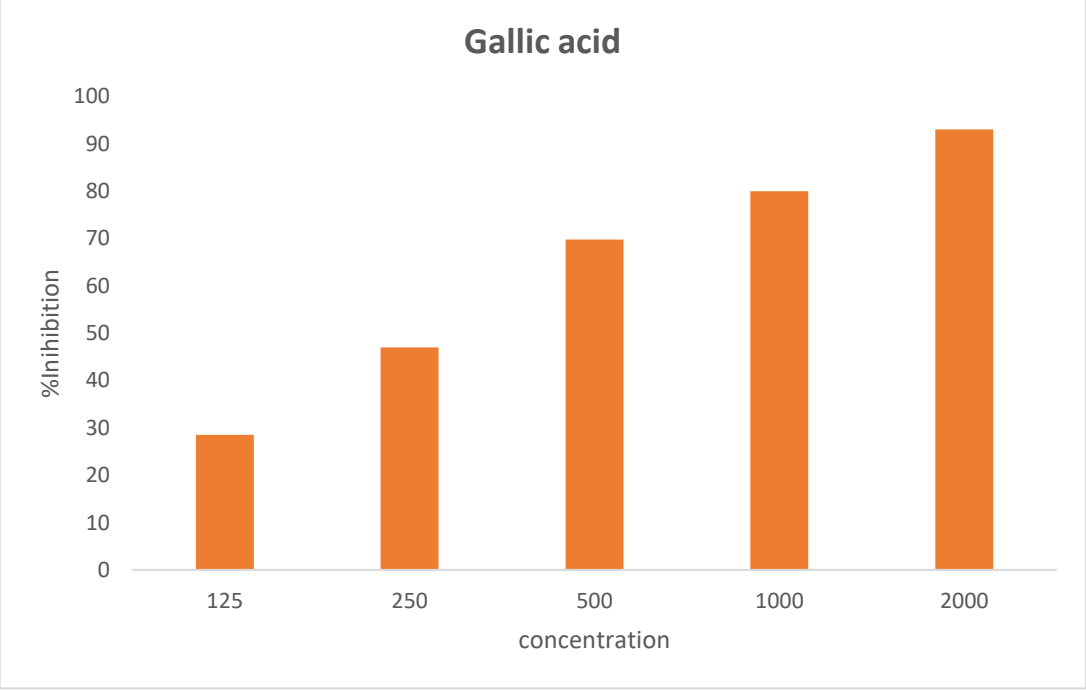


Fig. 15: Hydroxyl radical scavenging activity of the standard gallic acid.

Nitric oxide scavenging assay

Nitric oxide scavenging assays were performed with five test concentrations (125, 250, 500, and 1000 g/mL) for the four extracts FDFE, FDLE, FEFE, and FELE. FDLE had the highest inhibition percentage with a value of 58.806 ± 0.566% and FDFE had the lowest with 50.712 ± 0.7659% inhibition. FEFE and FELE were inhibited by 55.323 ± 0.629% and 53.806 ± 0.4722%, respectively. Gallic acid was used as the control, which was having an IC 50 value of 226.136 µg/mL. All four extracts had IC 50 values greater than the standard (Fig. 17). FDLE extract exhibited the lowest IC 50 value of 1451.633 µg/mL. FDFE, FEFE, and FELE have IC 50 values of 1818.576 µg/mL, 1633.370 µg/mL, and 1613.872 µg/mL, respectively **(Fig.16).**

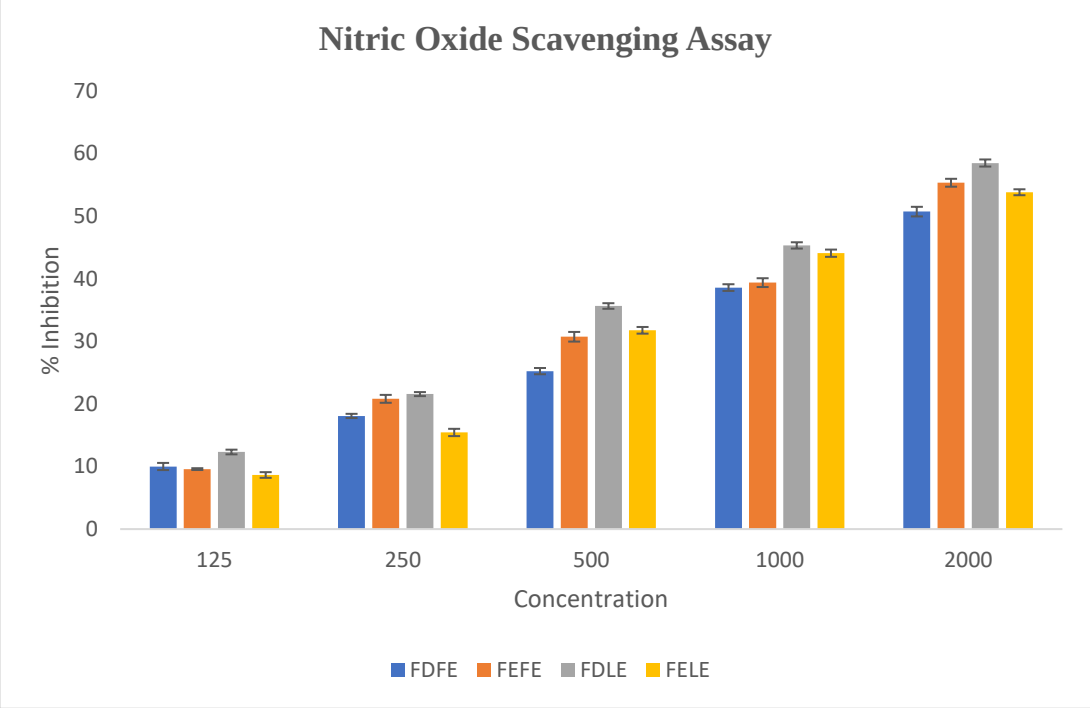


Fig. 16: Nitric oxide radical scavenging activity of the leaf and fruit extracts of *F. exasperata,* and *F. drupacea*

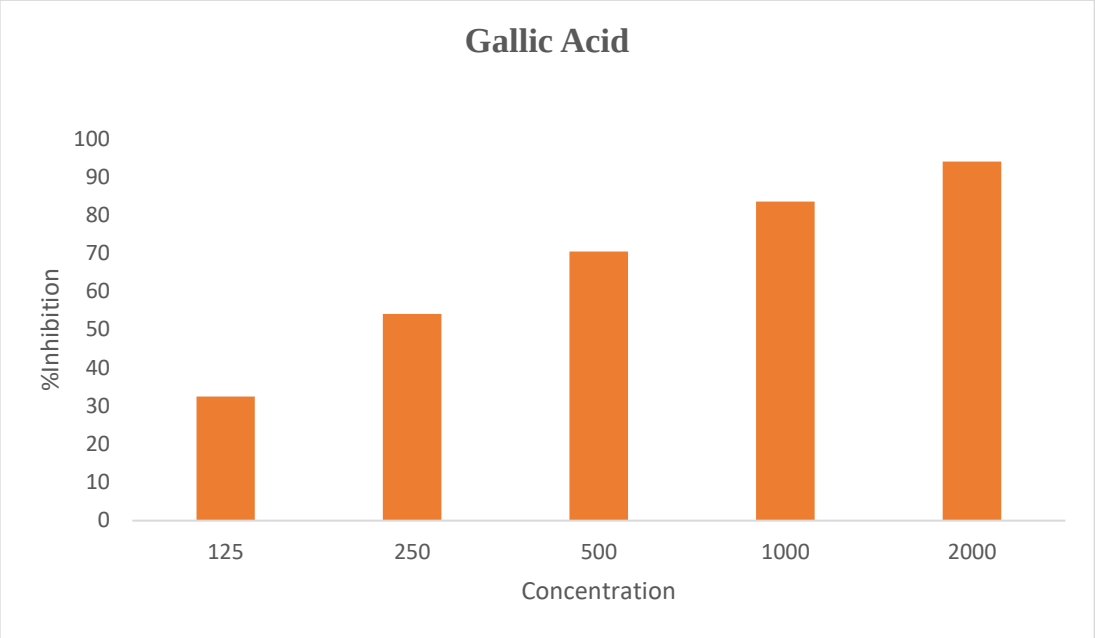


Fig. 17: Nitric oxide radical scavenging activity of the standard gallic acid

Super Oxide Radical Scavenging Activity

The super oxide radical scavenging ability was tested against four extracts: FDFE, FDLE, FEFE, and FELE with test concentrations of 125, 250, 500, 1000, and 2000 µg/mL. The superoxide radical scavenging activity increased with increasing concentration showing concentration dependent variations. The highest inhibition percentage was found in the FDLE extract, which had a value of 63.157 ± 0.5330, while the lowest inhibition percentage was found in the FEFE extract, which had a value of 56.280 ± 0.4066%. The remaining extracts, FDFE and FELE, showed inhibition percentages of 60.584 ± 0.3497 and 56.865 ± 0.5086%, respectively **(Fig. 18)**. The standard ascorbic acid's IC 50 value was 344.424 µg/mL (**Fig. 19)**. This is comparable to the IC50 of FDLE extract, which is 783.003 µg/mL. The antioxidant capacity for the four extracts to scavenge super oxide radical was moderate in action when compared to the control. IC50 value of the other three extracts FDFE, FELE, FEFE were 1339.96 µg/mL, 1544.5 µg/mL, 1552.16 µg/mL respectively.

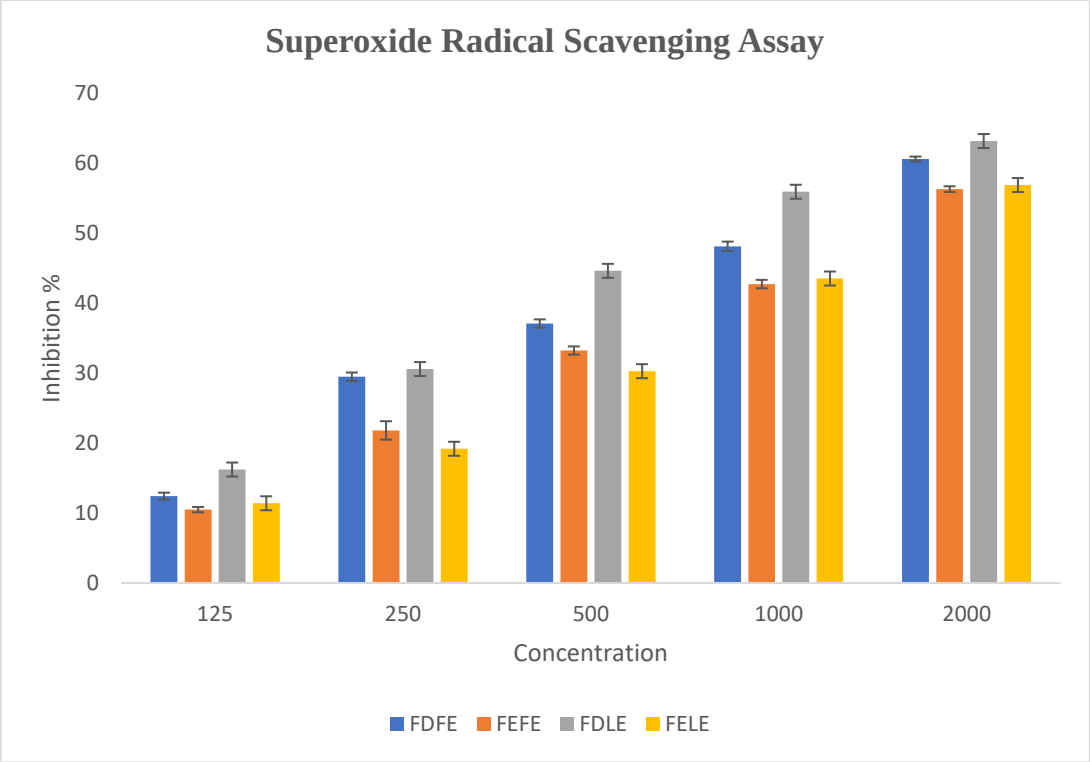


Fig. 18: Super oxide radical scavenging activity of the leaf and fruit extracts of *F. exasperata* and *F. drupacea*

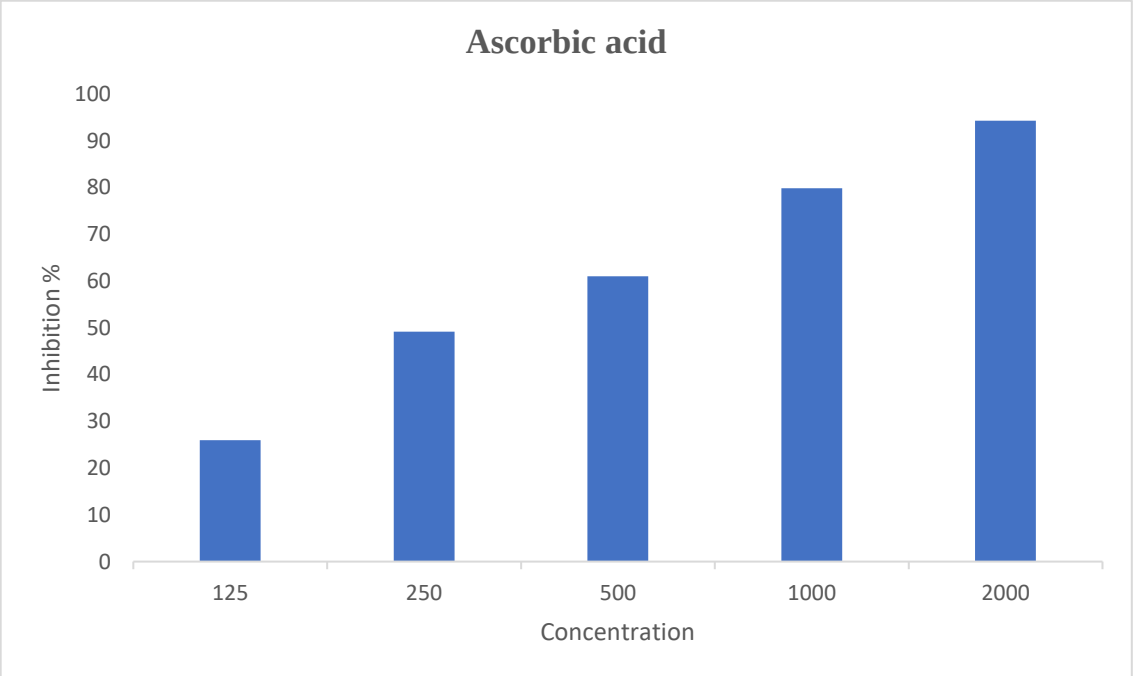


Fig. 19: Super oxide radical scavenging activity of the standard ascorbic acid

Analyzing the free radical scavenging activity of aqueous extracts of *F. exasperata* and *F. drupacea* using various assays revealed that the extracts have a modest antioxidant action. When compared to the values of its standards, the IC 50 and percentage inhibition values depicted in each experiment appear to be relevant. Among the four assays conducted, all the four extracts produced better results especially with two assays (DPPH radical scavenging assay and superoxide radical scavenging assay). All of the experiments revealed a concentration-dependent increase in anti-oxidant capacity. In the hydroxyl radical scavenging experiment, FDFE performed better, the leaf aqueous extract of *F. drupacea* showed a higher percentage inhibition in all three assays (DPPH, Superoxide, Nitric oxide scavenging assay).

Antiproliferative Efficacy Determination

***In vitro* Cytotoxicity Determination on L929 Cell Lines**

The four extracts (FDLE, FELE, FEFE, and FDFE) were tested for cytotoxicity on normal cell lines (L929) before their anticancer capabilities were investigated. 6.25, 12.5, 25, 50, and 100 µg/ml of concentration were chosen for the research. The results were inspected in an inverted phase contrast microscope after a 24-hour incubation period to test for any microscopic visual modifications, which revealed only minor visual alterations even at the highest concentrations. Lower concentrations revealed cells that were not damaged and were similar to untreated controls. MTT test is then used to calculate the cell viability percentage. When live cell mitochondrial enzymes react with the yellow tetrazolium salt (MTT), purple formazan crystals are created. The MTT assay uses a colorimeter to detect the absorbance of these crystals. In this assay, cells with more colour intensity will absorb more light, which represents a higher percentage of live cells. This experiment demonstrated that L929 cells had a high viability percentage following treatment.

The plant extracts were shown to be less cytotoxic on the cell lines tested in the study. However, larger concentrations had an impact on cytotoxicity. The viability percentage decreased as the concentrations increased from 6.25 to 100 µg/ml. Decreased percentage viability from 97.17 ± 0.497278 µg/ml to 61.45 ± 0.676704

µg/ml were reported with *F. exasperata* fruit extract which was having the lowest IC 50 value of 116 ± 5102 µg/ml among the four extracts studied. Highest IC 50 value of 207 ± 52842 was shown by the FDLE extract, with an inhibition percentage of 96.09 ± 0.547366 µg/ml for the lowest test concentration. 74.34 ± 0.251301 µg/ml was the inhibition percentage with the highest test concentration. Moderate cytotoxicity was observed with the rest of the extracts such as FDFE and FELE with an IC 50 value of 175 ± 0.95556 µg/ml, 194 ± 0.1828 µg/ml respectively. All the microscopic observations were represented in **Plates 3-6** and the IC50 value of both L929 as well as MCF-7 is represented in **Table 7**. FDFE and FELE extracts were having a viability percentage of 73.08 µg/ml, 74.23 µg/ml respectively with the highest test concentrations **(Fig. 20)**. One way ANOVA resulted with a p value of $p< 0.001$ when compared to the control. This indicates the potential of FDFE and FELE extracts in having a less cytotoxic effect and high percentage viability in normal cells so they can be chosen for antiproliferation studies on cancer cell lines.

ii. *In vitro* Cytotoxicity Determination on MCF Cell Lines

The cytotoxicity experiments on breast cancer cell line (MCF) were performed with four extracts (FDLE, FELE, FEFE, and FDFE) to ascertain their *in vitro* antiproliferative effects. Various concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml were used for the study. Duration of 24 hours was utilized to examine the effects of specified extracts on the selected cell line. The microscopic visual outcomes were examined using an inverted phase contrast microscope after 24-hour incubation; which revealed alterations within the cells, such as rounding of cells, granulation, echinoid spikes, and vacuolization in the cytoplasm, which were thought to be indicators of cytotoxicity.

MTT assay were conducted to determine the cytotoxic action of the selected extract on MCF-7 cell line. Dose dependent increase in the antiproliferative action against human breast cancer cell lines MCF-7 was revealed with the four extracts FELE, FDLE, FEFE and FDFE. The FDFE extract demonstrated the greatest *in vitro* cytotoxic action at the highest test concentration of 100 µg/ml with a viability percentage as low as 37.35929 ± 0.43124 and an IC 50 value of 144.5922 µg/ml. This

extract was found to be the most effective and better than FELE, as its IC 50 values on both MCF-7 and L929 indicates that it is less cytotoxic to L929 normal cell lines and most cytotoxic to MCF-7 cancer cell lines. This extract has been chosen for anticancer research spanning from detecting apoptosis to studying gene expression. The least effective extract was FELE with an inhibition percentage of 46.9019 ± 0.24809 µg/ml and an IC 50 value of 171.0563 µg/ml. The other two extracts had shown effective *in vitro* cytotoxicity, with 44.105 ± 0.4926 µg/ml and 43.91252 ± 0.25376 µg/ml for FDLE and FEFE extracts, respectively **(Fig. 21)**. One way ANOVA resulted with a p value of $p < 0.001$ when compared to the control for the highest test concentration. The IC50 value for both L929 and MCF-7 was recorded in **Table 7**, and all microscopic observations were displayed on **Plates 7-10.**

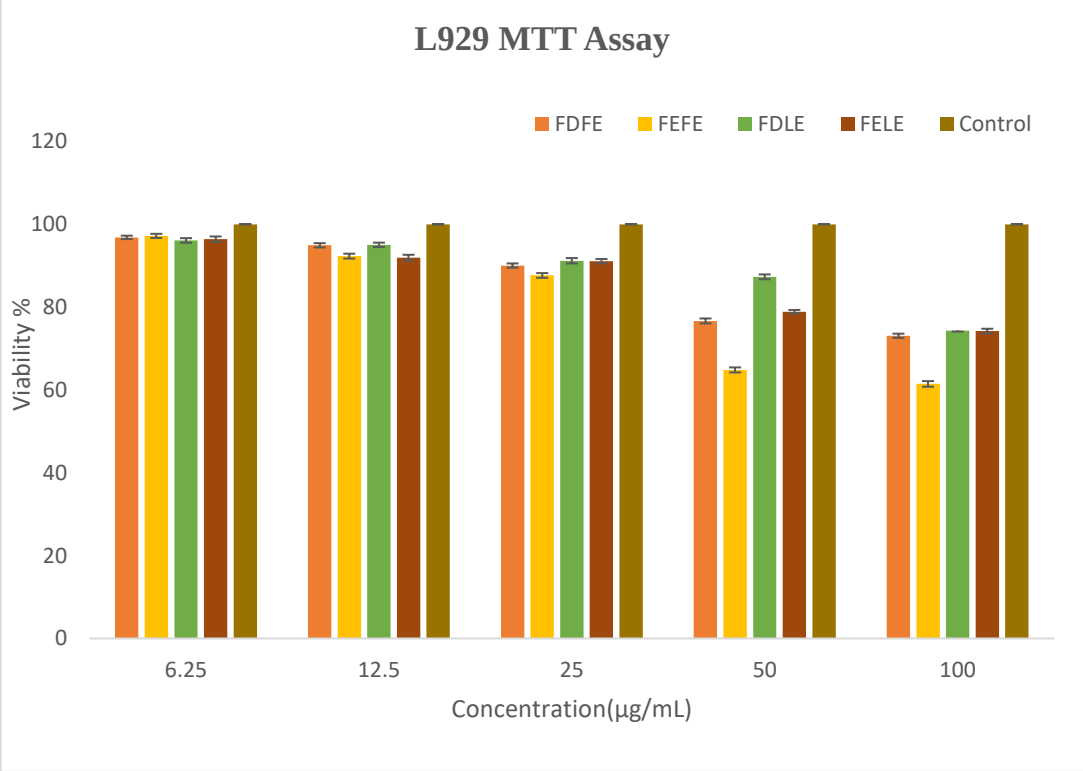


Fig. 20: Determination of cytotoxicity on L929 cell lines by the four selected *Ficus* extracts using MTT assay (FDFE: *F. drupacea* fruit extract, FEFE: *F. exasperata* fruit extract, FDLE: *F. drupacea* leaf extract, FELE: *F. exasperata* leaf extract)

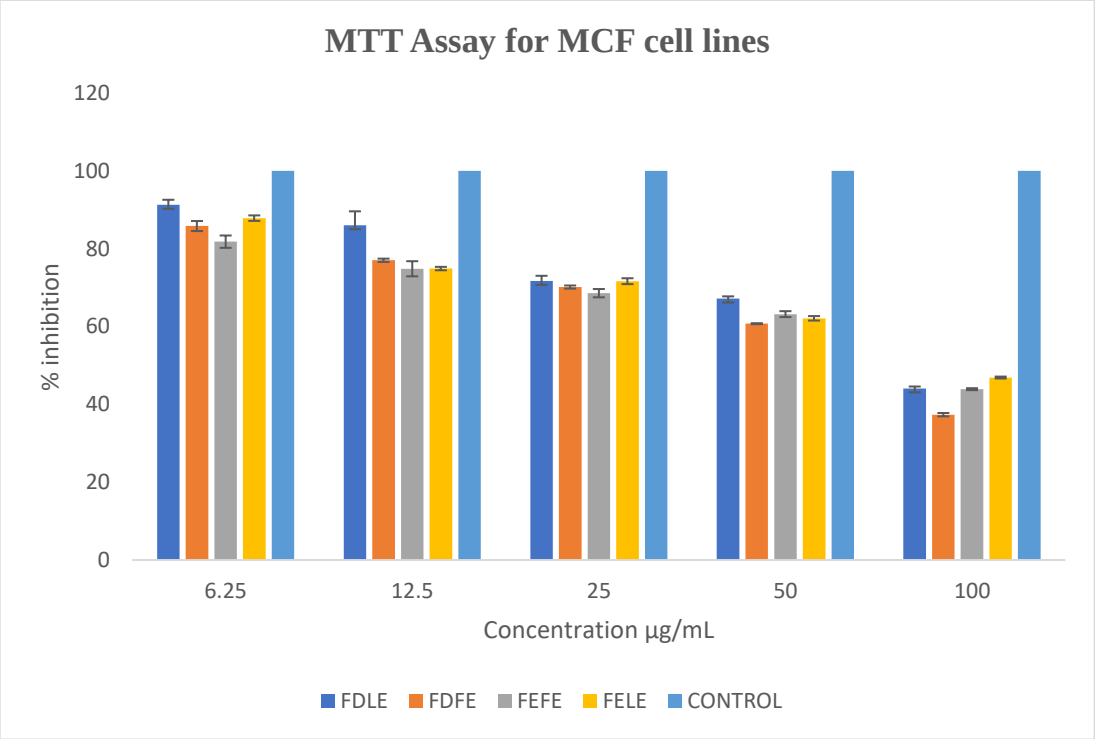


Fig. 21: Indication of cytotoxicity on MCF cell lines by the four selected plant extracts using MTT assay (FDFE: *F. drupacea* fruit extract, FEFE: *F. exasperata* fruit extract, FDLE: *F. drupacea* leaf extract, FELE: *F. exasperata* leaf extract)

Table 7: Lethal concentrations of selected species of *Ficus* in L929 and MCF 7 cell lines

Plant extracts	IC50 (µg/ml)	
	L929	MCF
FDFE	175.9556	144.5922
FEFE	116.5102	162.9488
FDLE	207.5284	168.5904
FELE	194.1828	171.0563

Apoptosis Detection

As genotoxicity is prominent with the extract, method of its antiproliferative action need to be further studied. Apoptosis is a well-managed process of cell death

that occurs as a normal aspect of development. Apoptosis that is improperly regulated is linked to diseases like Alzheimer's and cancer. Apoptosis differentiates itself from necrosis, or accidental cell death, by distinct morphological and biochemical changes, such as nuclear chromatin compaction and fragmentation, cytoplasm shrinkage, and loss of membrane asymmetry. To find weather this antiproliferative activity is attributable to apoptosis, double staining and comet assays were conducted.

Comet Assay

Comet assay is a simple reliable method used for the detection of DNA damages especially due to the genotoxicity. This is also called single cell electrophoresis as it is used in cellular level. Genotoxicity induction on MCF-7 cell lines by *F. drupacea* was analyzed using comet assay. DNA degradation was found to be prominent, which may be an indication of the cells directing towards apoptosis. There were a number of comets detected through fluorescence microscopy (**Plate 11 (ii))**. Comet length is considered as the measure of DNA damage present in the cells. The control cells only produced a comet length of 48.17 ± 0241, but the treated cells were having a comet length of 188.2 ± 2.192, tail length of the control was 1.38 microns and tail length of the treated cells were very high with a value of 38.8 microns (**Fig. 22-23)**. Percentage of DNA present in the comet was also very high in the treated cells when compared it to that of the control **(Fig. 24)**. DNA percentage was found to be 38.08541 ± 0.8954 % in the treatment whereas 1.50761 ± 0.0354 % was in the control. The results suggest that the extract of *F. drupacea* fruits imparted genotoxicity to the MCF-7 cell lines and it could produce DNA strand breaks due to the treatment. This might be an indication that cells are getting directed to apoptosis by the treatment with the fruit extract.

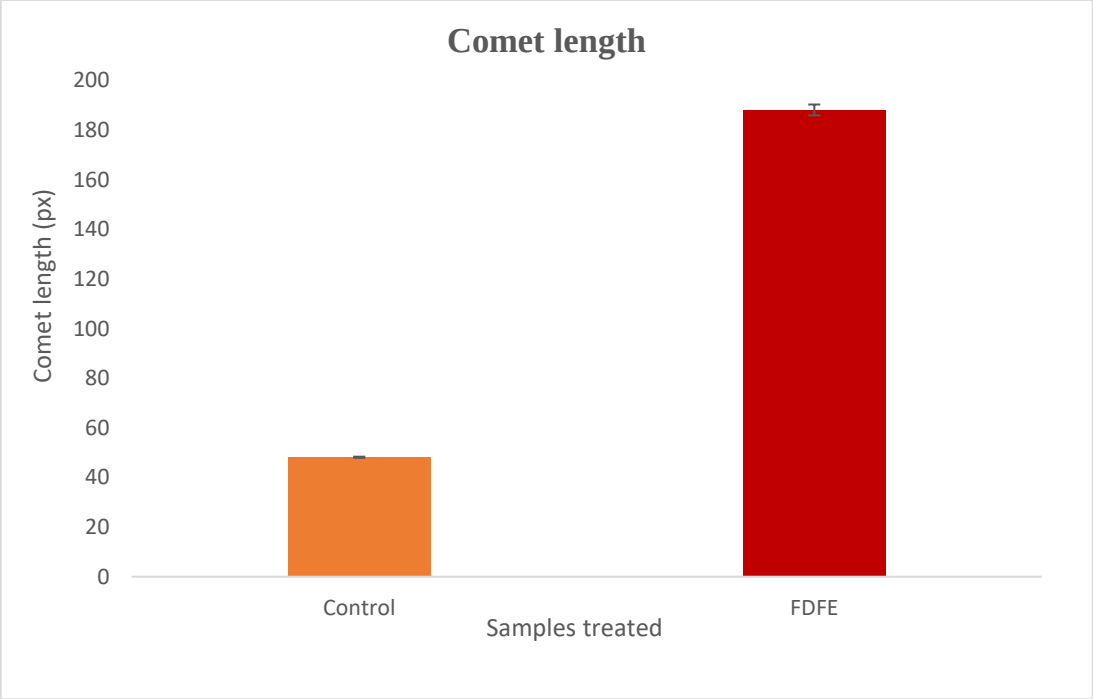


Fig. 22: Length of the comet depicting genotoxicity induced by *F. drupacea* fruit extract on MCF-7 cell lines

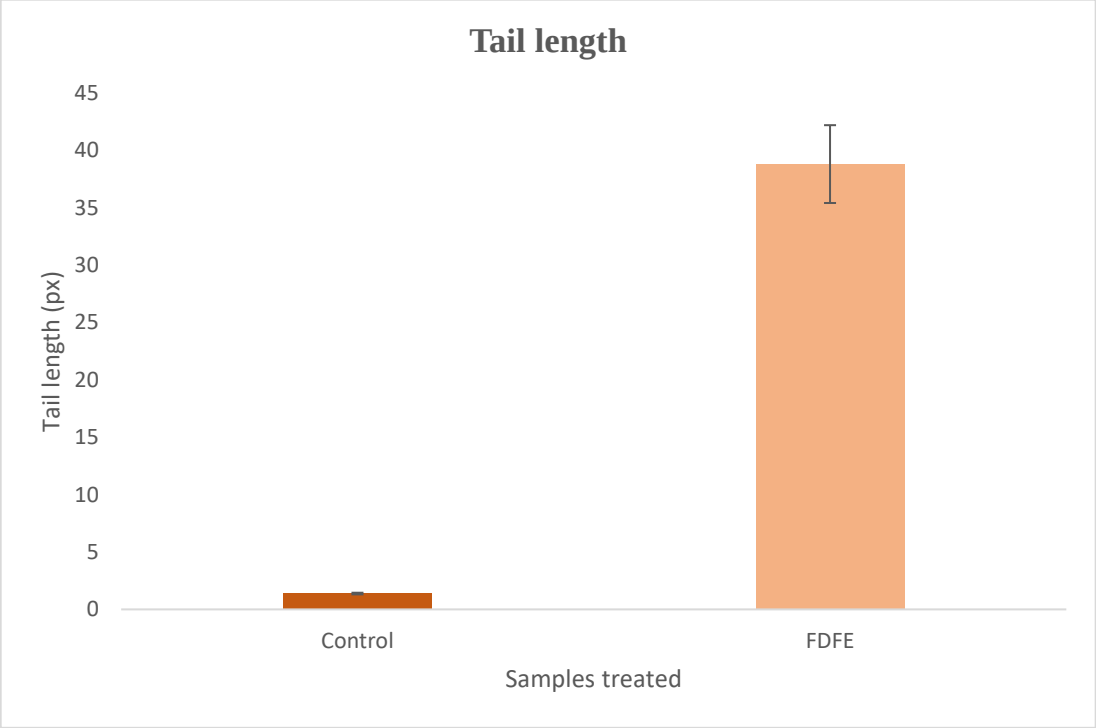


Fig. 23: Tail length of comet assay depicting genotoxicity induced by *F. drupacea* fruit extract on MCF-7 cell lines

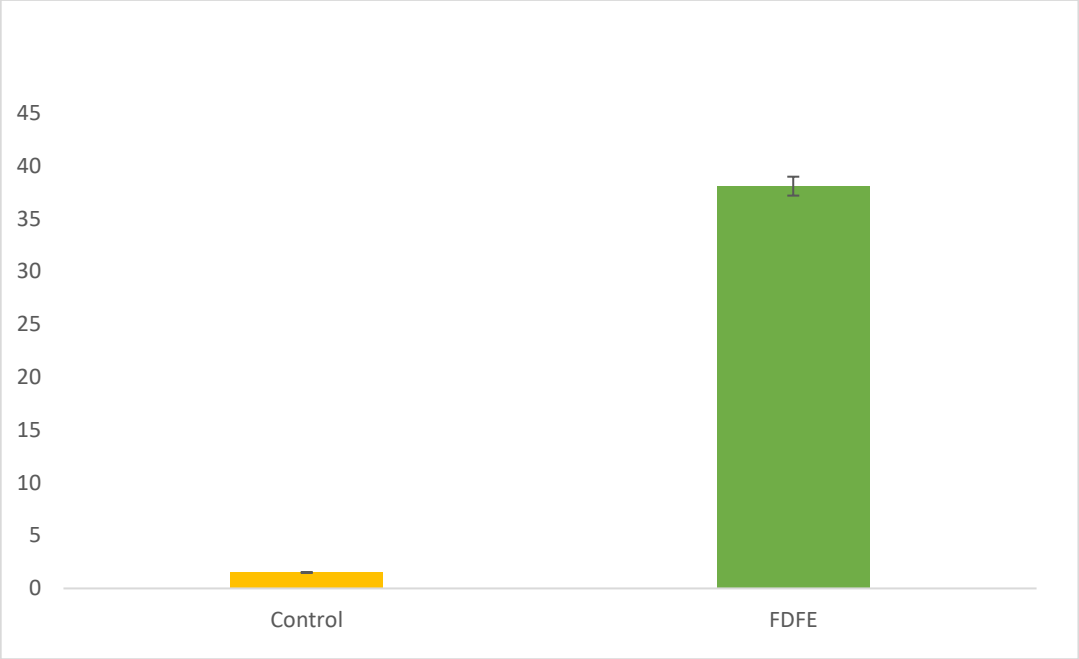


Fig. 24: DNA percentage of comet assay depicting genotoxicity induced by *F. Drupacea* fruit extract on MCF-7 cell lines

Double Staining

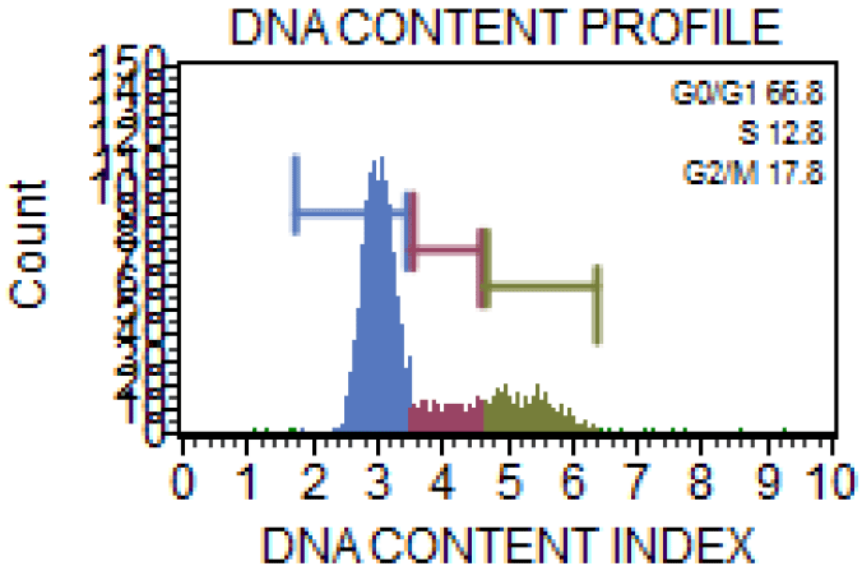
Acridine Orange (AO) and Ethidium Bromide (EtBr) are dyes commonly used to identify cells undergoing apoptosis or necrosis. When AO binds to double-stranded DNA, it emits green fluorescence and can enter both living and non-living cells. On the other hand, EtBr is only taken up by non-living cells and generates red fluorescence when it intercalates with DNA. After treatment, based on their characteristics, the cells were divided into four groups: Living cells are distinguished by their distinctive green nucleus. Apoptotic cells having vivid orange green nuclei with constricted or broken chromatin. Cells in late apoptosis with orange-stained nuclei and chromatin condensation or disintegration. Necrotic cells with uniformly red stained intact cell nuclei. The fluorescent images of MCF-7 cells after treatment with the IC 50 concentration of FDFE (144.5922 µg/mL) extract demonstrated an increase in the proportion of cells with yellow orange colour, indicating nearly 70 % of the cells are in late apoptotic or apoptotic stage. The image itself demonstrates the loss of cell viability and membrane integrity **(Plate 12)**. The cells in the control appear

to show a single green colour, indicating that the control cells are all alive and undamaged.

Cell cycle regulation

Effect of *F. drupacea* fruit extract on the cell cycle regulation of MCF-7 cell lines were determined by treating the cells with the IC 50 (144.5922 µg/mL) concentration of the extract for 24 hours. The DNA content profile and flow cytometry histogram are represented in the **Fig. 25**. In the tested samples DNA content was high in G1, but decreased in all other phases of the cell cycle in comparison with that of the control. The treated cell line was having 86.8% DNA content in the G1 phase, which was higher than the control value of 66.8%. During S phase, percentage of DNA in the treated cell line was reduced from 12.8 % to 5.8 %. When the DNA proportion of control and treated cells at the G2/M phase is compared, the DNA content was found to be decreased in the treatment with a value of 6.5% which was 17.8% in the control (**Fig. 25, Fig. 27)**. This demonstrates that the cell cycle was arrested in the treated cells at the G1 phase. Significant cell accumulation and higher DNA content was present in the G1 phase. Decreased percentage of DNA content was reported in the G2/M phase, indicating that the cell cycle was arrested at G1 in the case of the treated cells. When the cell population profile is taken into account, the control population profile shows a more dispersed pattern, whereas the treatment population profile initially shows clustered cells (**Fig. 26)**. This cell aggregation could indicate cell cycle arrest in the early phases of the cell cycle.

A)



B)

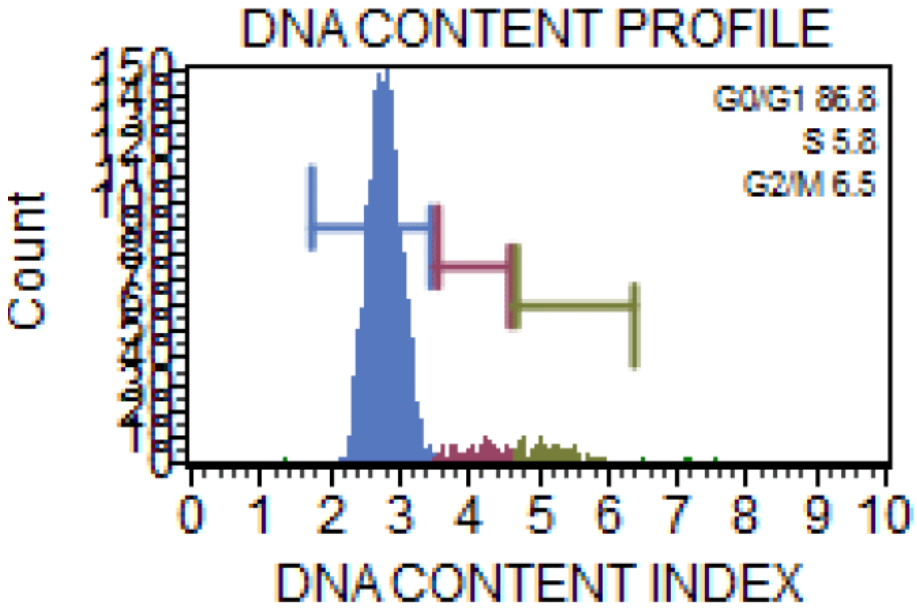
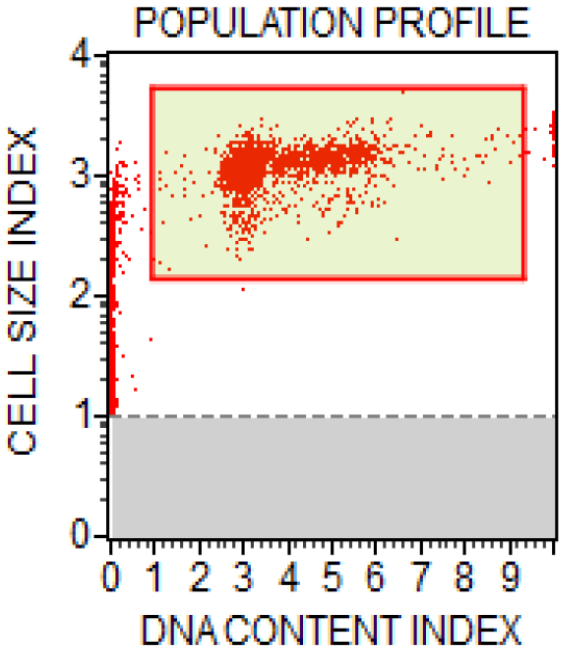


Fig. 25: Determination of cell cycle arrest in MCF-7 cells using flow cytometry-DNA content profile; **A**: negative control, **B**: MCF-7 cells treated with *F. drupacea* fruit extract.

A)



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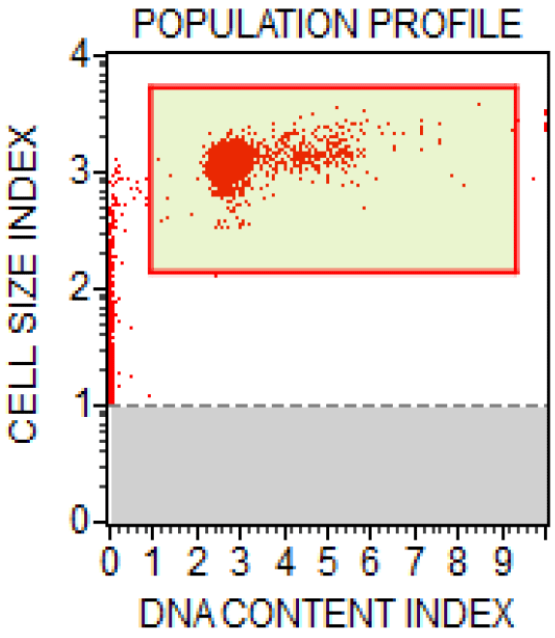


Fig. 26: Determination of cell cycle arrest in mcf-7 cells using flow cytometry-population profile; **A**: negative control, **B**: MCF-7 cells treated with *F. drupacea* fruit extract. The rectangle represents cells of interest without cellular debris.

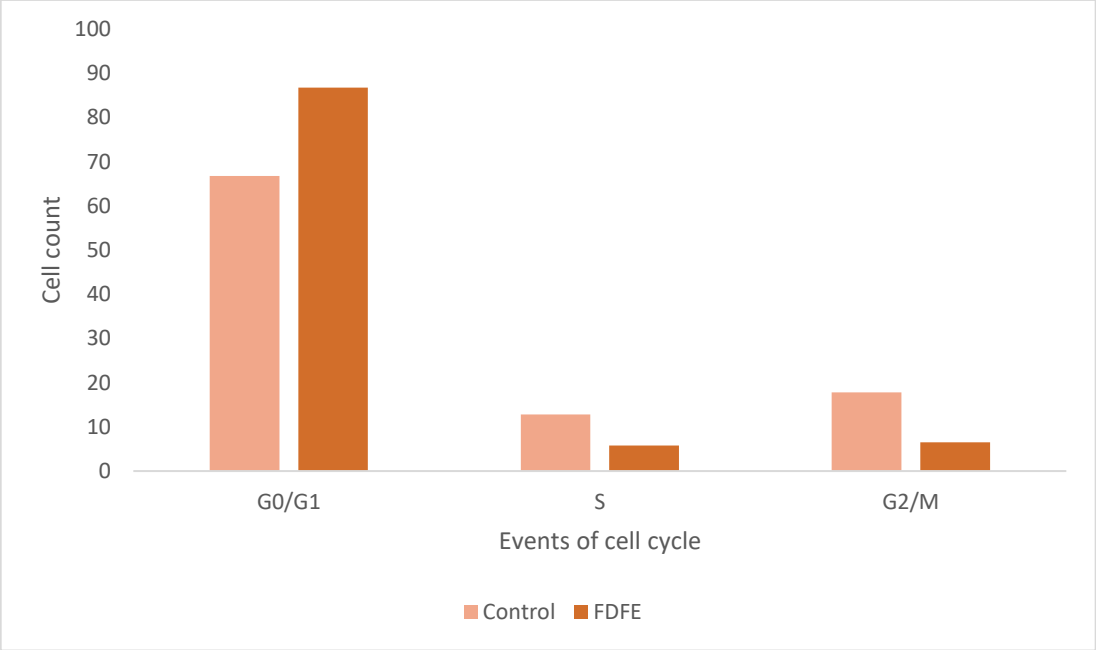


Fig. 27: DNA percentage of MCF-7 cells treated with fruit extract of *F. drupacea* (FDFE)

Gene expression study using RT-qPCR

This ensures an error-free cycle and accurate reproduction. When cells finish the cell cycle, they enter G0, a quiescent state mediated by cyclin C/CDK3. The molecular basis of cell cycle arrest, apoptosis, and the antiproliferative mechanism is further investigated by studying the expression variations of two essential genes involved in the cellular cycle. Using housekeeping gene GAPDH as internal reference control, the expression fold variations of both P53 and STAT 3 are investigated following a 24-hour treatment of MCF-7 cells with the IC50 concentration of FDFE extract. P53 expression is enhanced and STAT3 expression is significantly lowered in FDFE-treated cells, based upon the electrophoretic pictures. Activation of P53 genes supports the extract's potential to aid in cell death. When compared to the control, the expression fold of P53 is increased to -1.729, while STAT 3 was reduced to -0.1647. STAT 3 expression reduction also contributes to cell cycle arrest at G0/G1 phase and apoptosis initiation, as its activation is required for cell cycle progression and apoptosis inhibition. FDFE extract treatment activated apoptotic genes such as P53 while downregulating anti-apoptotic genes such as STAT 3 (**Fig. 28-29)**. The

findings support the extract's capacity to inhibit cancer cell growth by driving cancer cells into apoptosis, which kills and suppresses cancer cell proliferation.

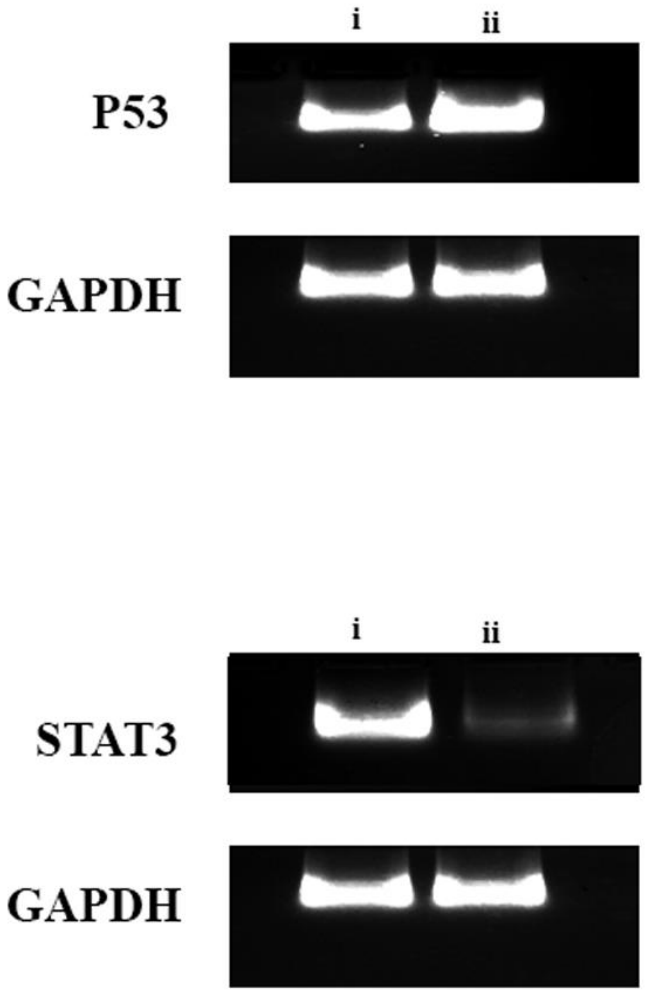
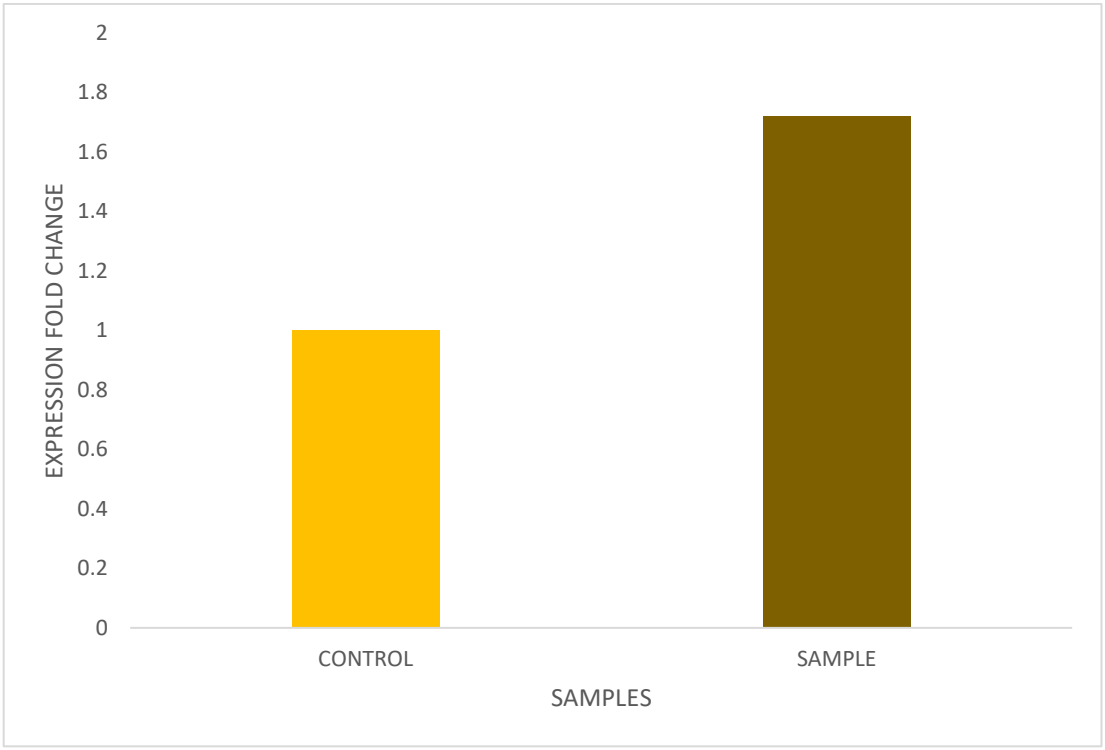


Fig. 28: Gene expression analysis of P53, STAT3 and GAPDH using real time PCR (i: control, ii: sample)



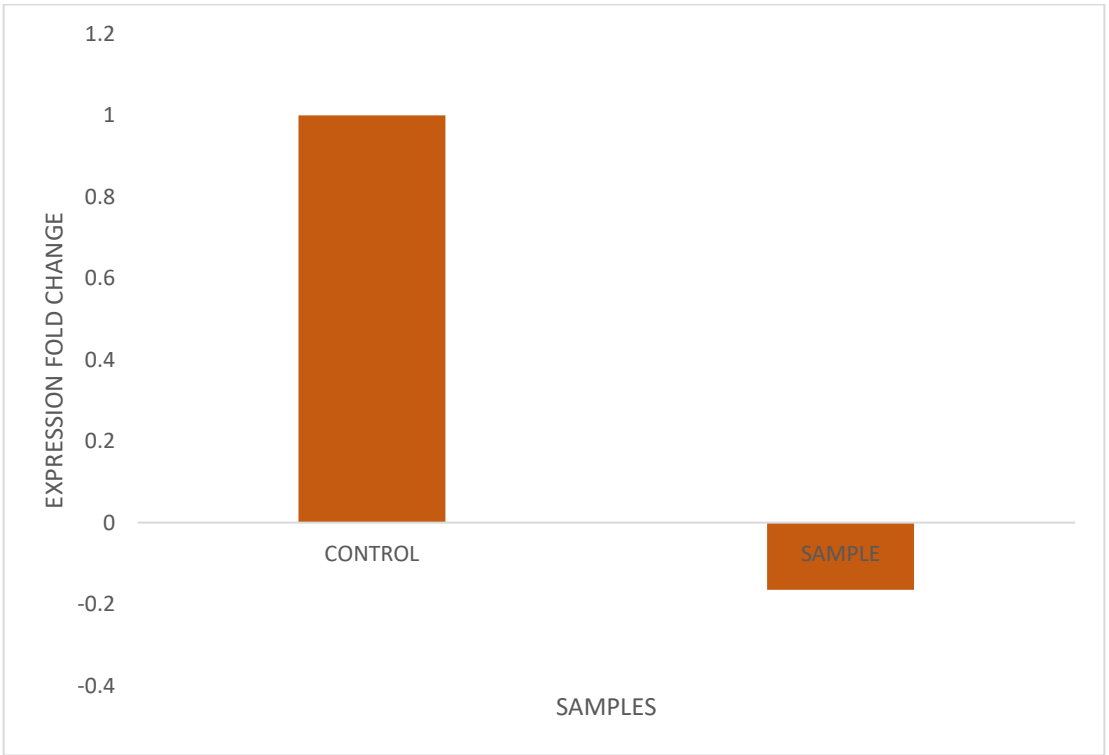


Fig. 29: Expression fold changes in the apoptosis related genes (P53, STAT3) in MCF-7 cells after treatment with *F. drupacea* fruit extract

PART III

Phytofabrication of Silver Nano Particles

The fruit and leaf extracts of the chosen plants, *Ficus exasperata* and *Ficus drupacea*, were employed to produce silver nanoparticles. Aqueous extracts of leaves and fruits were used in the study. The extracts were deep yellow in colour. When exposed to a 10 mM silver nitrate solution at 60^0C, these extracts rapidly changed the colour of the reaction mixture to brick-red, indicating the presence of silver nanoparticle formation (**Fig. 30)**.

The synthesis of silver nanoparticles was verified through additional characterisation tests, including SEM examination and UV-vis spectroscopy. When exposed to silver nitrate, *F. drupacea* leaf extract changed colour rapidly, whereas *F. drupacea* fruit extract and FELE both required 10 minutes to heat the solution to 60^0C before showing any colour change. The colour change was produced by FELE after a 15-minute treatment.

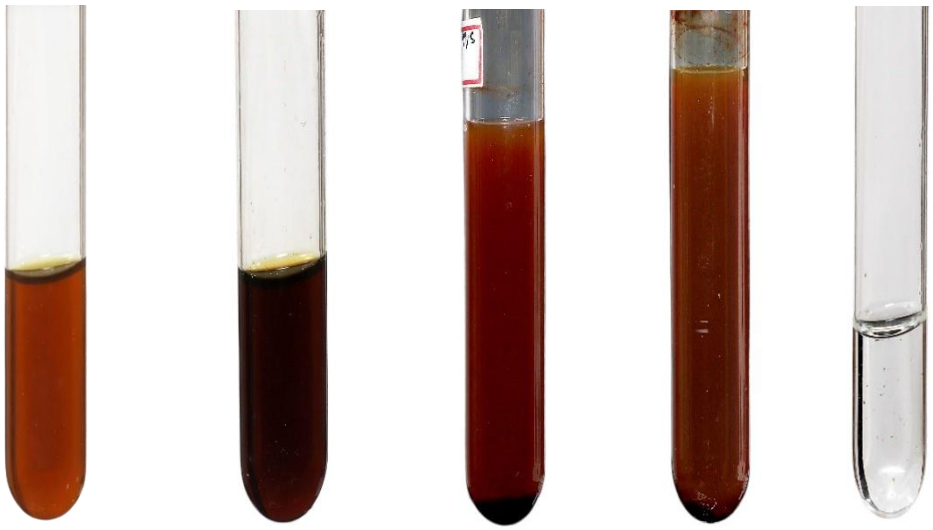


Fig.30: Visual color indication of nanoparticle formation in the order FDFE, FEFE, FDLE, FELE respectively, along with the color less silver nitrate solution

UV-Vis Analysis

Following the confirmation of the visual cue, these phyto-fabricated particles were characterized using two different techniques. Analyzing the absorbance of the

color-changed solution for the particular peak corresponding to silver nanoparticle was the first and easiest step. For silver nanoparticles, a peak that is formed between 350 and 460 nm is regarded as constitutive peak. *F. drupacea* fruit extract produced a peak at 455 nm with an absorption wave length of 2.05395AU, whereas FEFE produced a peak at 413 nm with an absorption range of 3.670 AU, *F. drupacea* leaf extract was able to produce a peak at the wavelength of 440 nm in an absorbance of 4.7304 AU and FELE produced a peak at 447 nm with 1.2 AU absorbance unit (**Fig. 31)**.

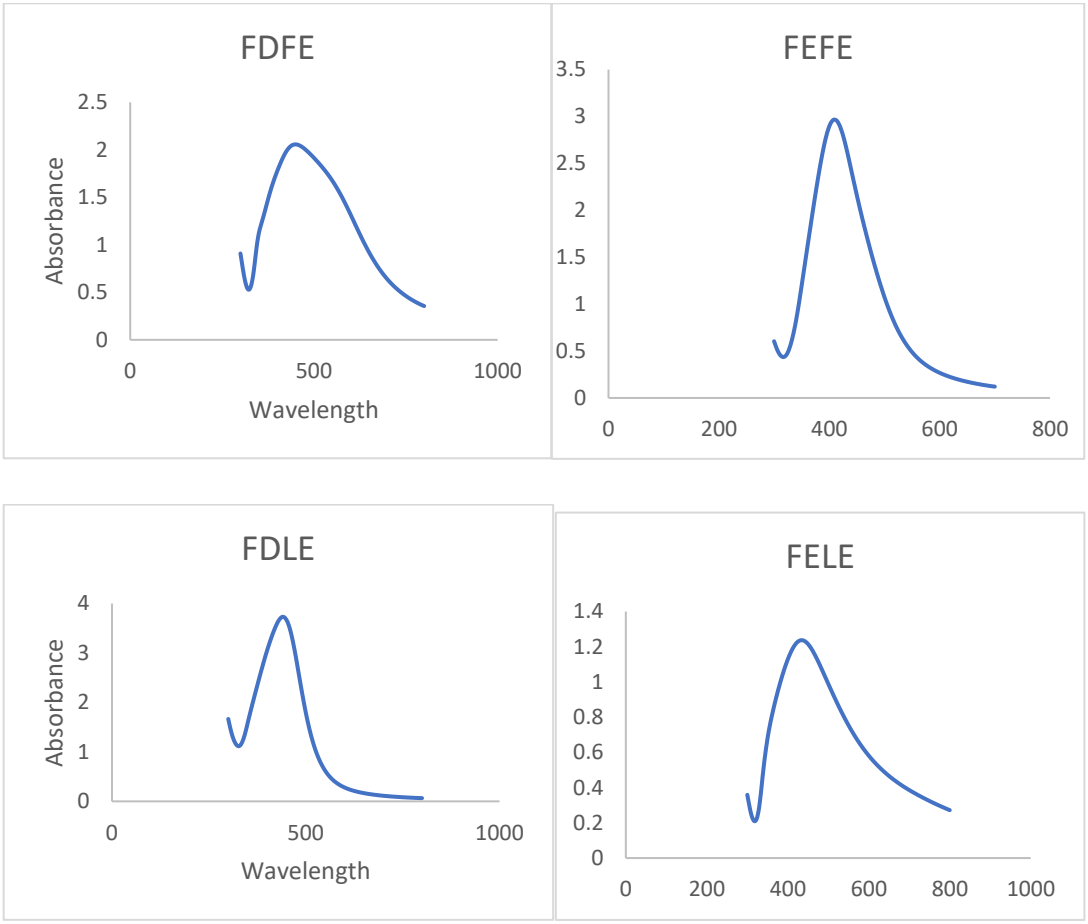


Fig. 31: UV-Vis analysis results of silver nanoparticle formation from the four types of extract used in the study (FDFE, FDLE *F. drupacea* leaf and fruit aqueous extract; FEFE, FELE *F. exasperata* leaf and fruit extract)

FE SEM analysis

Scanning electron microscopic image analysis of green synthesized silver nanoparticles revealed the surface structure of phytofabricated green nanoparticle. FE

SEM images could also reveal the shape, diameter and approximate size of biosynthesized silver nanoparticles. All the four extracts could effectively produce particles within a size range of 100 nm, and all of them were more or less spherical in shape. *F. drupacea* fruit extract produced spherical silver nanoparticle with a diameter of 28-49 nm, whereas the leaf extract produced silver nanoparticles with a size of 25-26 nm **Plate 13 (i-ii)**. *F. exasperata* leaf and fruit extract were also good in synthesizing silver nanoparticles. Leaves of *F. exasperata* produced nanoparticles of size range of 28-37 nm whereas *F. exasperata* fruits produced nanoparticles with a size range of 25-32 nm **Plate 14 (i-ii)**.

Antimicrobial Activity

Antimicrobial activity of silver nanoparticles was studied for better analyzing the bioactivities of the green nanoparticles derived from *F. exasperata* and *F. drupacea*. Two fungi, *Aspergillus niger* (ATCC 16404*)* and *Candida albicans* (ATCC 10231) *along* with four bacteria were taken for the study. *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 29212) were the bacteria. Antimicrobial activity was conducted with varying concentration of silver nanoparticles. Test concentrations were decided as 250, 500, 1000 µg/ml. Positive controls for antibacterial and antifungal studies were taken as Streptomycin (1000 µg) and Clotrimazole (1000 µg) respectively.

Antifungal Activity

The antifungal studies against *Aspergillus niger* were conducted using silver nanoparticles synthesized from the four extracts. The results showed that *F. exasperata* fruit nanoparticle and *F. drupacea* leaf nanoparticles produced the highest zone of inhibition in 1000 µg/ml, measuring 14 ± 0.318357 mm and 13 ± 0.279934 mm, respectively. At 500 µg/ml, *F. drupacea* leaf extract nanoparticles created an inhibitory zone of 12 ± 0.279934 mm. At the highest test concentrations, FELE and *F. drupacea* fruit extract-mediated nanoparticles produced an inhibitory zone measuring 11 ± 0.290401 and 11 ± 0.305703 mm, respectively. The lowest test dose revealed no zone of inhibition. A 26 mm zone of inhibition was generated by applying

1000 µg/ml of positive control clotrimazole in this instance. **Fig 32 and Plate 15** depicts the zone of inhibition formed by silver nanoparticles against the fungal strains under investigation.

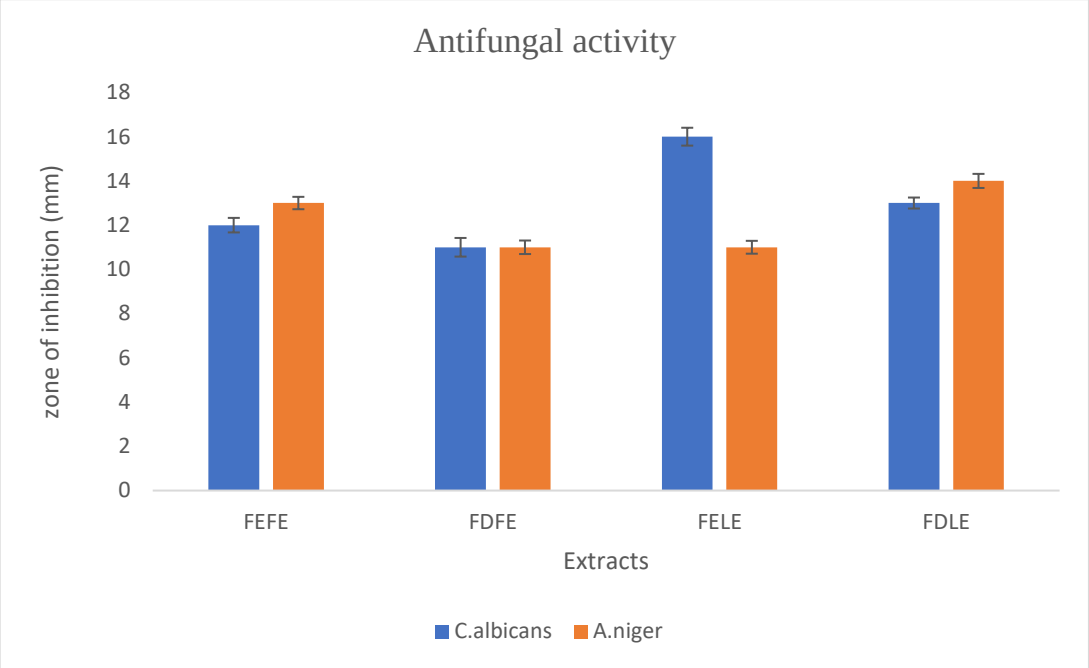


Fig. 32: Graph showing zone of inhibition of the fungal strains at the highest test concentration of silver nanoparticles synthesized from four different extracts.

When *Candida albicans* was treated with green nanoparticles derived from four type of extracts, FELE couldn't produce any zone of inhibition. *F. drupacea* leaf extract produced highest zone of inhibition with 13 ± 0.248151 mm in the highest test concentration. FEFE produced a zone of inhibition in 500 and 1000 µg/ml concentration silver nanoparticles, with a diameter of 11 ± 0.328837 and 12 ± 0.549386 mm respectively.

F. drupacea fruit extract could also produce a zone of inhibition of 11 ± 0.421662 mm in the highest test concentration. No zone of inhibition was produced in the lowest test concentration for any of the four types of biosynthesised silver nanoparticles. From the antifungal studies it is clear that *F. drupacea* leaf-mediated nanoparticles had a greater influence on the selected fungal strains. *Candida albicans* appeared to be more resistant than *Aspergillus niger*, as shown by the latter's lower inhibitory zone when exposed to *F. drupacea* leaf-mediated nanoparticles.

Antibacterial Studies

Nanoparticle biosynthesized from *F. drupacea* fruit extract, FEFE, *F. drupacea* leaf extract and FELE were used to test the antibacterial activity against selected organisms. Gram positive organisms such as *Enterococcus faecalis*, *Streptococcus mutans* and gram-negative organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* where the organisms used. *F. drupacea* leaf extract nanoparticles inhibited gram-negative bacteria *E. coli* at a dose of 1000 µg/ml, resulting in a 14 ± 0.485463 mm zone of inhibition.

Lower concentrations had no discernible influence on *E. coli* growth. FEFE, *F. drupacea* fruit extract, and FELE extracts had a zone of inhibition of 12 ± 0.351605, 11 ± 0.421424, and 11 ± 0.089611 mm respectively at the maximum concentration. Lower concentrations failed to create any zone of inhibition. *F. drupacea* fruit extract and *F. drupacea* leaf extract inhibited *Pseudomonas* with maximal zones of 15 ± 0.381461 and 15 ± 0.472434 mm, respectively. The zones of inhibition induced by FEFE and FELE were 11 ± 0.351605 and 12 ± 0.089611 mm respectively. Zone of inhibition created during the treatment with highest test concentration is represented in **Fig. 33, Plate 16-17**.

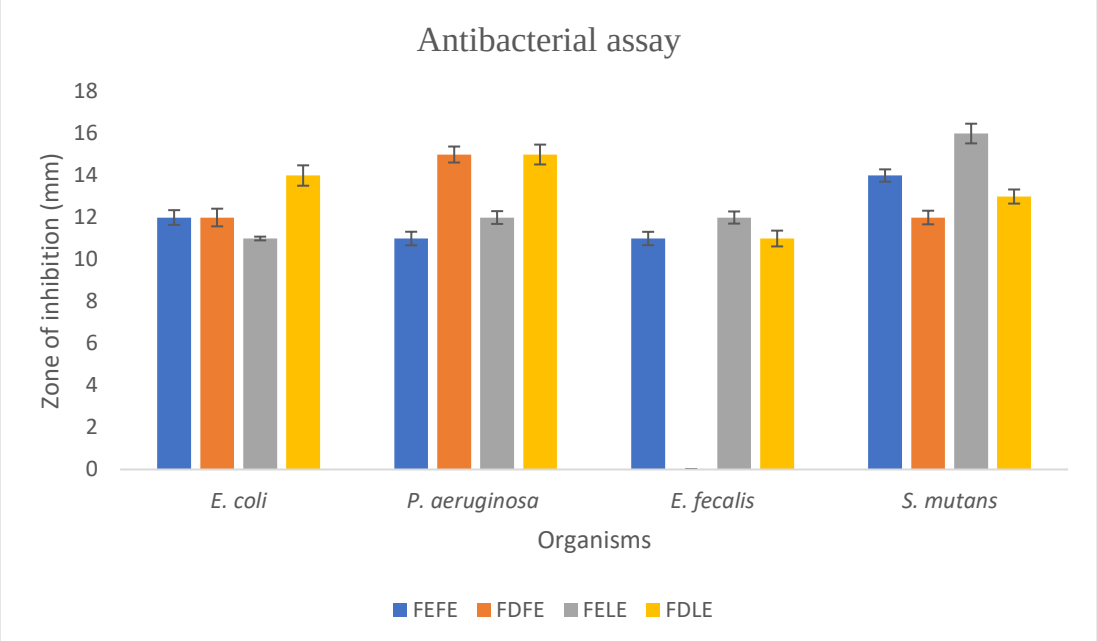


Fig. 33: Graph showing zone of inhibition for the bacterial strains at the highest test concentration of silver nanoparticles synthesized from four different extracts

Among the two gram-negative bacteria studied, *Pseudomonas aeruginosa* appeared to be more susceptible to the green silver nanoparticle, producing the largest zone of inhibition in the experiments. *Enterococcus faecalis* was inhibited by producing a zone of inhibition of 11 ± 0.376741 and 11 ± 0.318778 mm with silver nanoparticles derived from *F. drupacea* leaf extract and FEFE. Silver nanoparticles from FELE extract exhibited the highest zone of inhibition, measuring 12 ± 0.289325 mm at 1000 µg/ml and 11 ± 0.089611 mm at 500 µg/ml. *F. drupacea* fruit extractbased nanoparticles had no adverse effect on *Enterococcus faecalis* species.

FELE and FEFE nanoparticles showed the maximum zone of inhibition with the organism *Streptococcus mutans* (16 ± 0.469159mm, 14 ± 0.293788mm respectively). The *F. drupacea* fruit extract nanoparticles showed the lowest zone of inhibition at 12 ± 0.324572. The *F. drupacea* leaf extract produced a zone of inhibition of 13 ± 0.335955 mm. The concentration of 250 µg/ml did not induce a zone of inhibition for all the four types of nanoparticles synthesized. At a concentration of 500 µg/ml, both fruit-mediated nanoparticles (*F. drupacea* fruit extract and FEFE) produced a zone of inhibition measuring 11 ± 0.52221 mm and 12 ± 0.21555 mm, respectively. The study's findings revealed the importance of silver nanoparticles in the fight against bacteria, as they demonstrated a defined zone of inhibition and significant antibacterial activity against every tested organism.

PHASE II

PART I

Fungal Endophytes Isolation

Fungal endophytes were isolated from the fruits and leaves of both *F. exasperata* and *F. drupacea*. Surface sterilised healthy fruit and leaf parts were used for the isolation of endophytes. After three days of incubation at room temperature, extensive fungal growths were found on the cut ends of the plant portions (**Plate 18**). Eleven endophytes were identified and pure cultured based on their exterior morphology. To isolate pure colonies, streak plate method was used. DNA isolated from all the eleven pure strains were amplified and sequenced using PCR. Using ITS, LSU DNA sequencing, these eleven isolates were identified to the genus level. Primers used were given in the **Table 8**. Two of these were fruit endophytes, whereas others were leaf endophytes.

FDLE I, FDLE II, FDLE III, FDLE IV, and FDLE V were isolated from the leaves of *F. drupacea*. FELE I, FELE II, FELE III, and FELE IV were isolated from the leaves of *F. exasperata*. FDFE I and FEFE I were the fruit isolates of *F. drupacea* and *F. exasperata*. All of the fungal endophytes were from two fungus divisions, Ascomycota and Basidiomycota, based on ITS sequencing. The majority of endophytes were discovered from Ascomycota (**Table 9**). Looking at the overall number of isolates from both plants, the leaves had a larger number of colonization than the fruits. More than one organism was found to be identical in the leaves, and the fruit endophytes was also same in both plants (**Plate 19-20).**

Table 8: Forward and reverse primers used in the ITS sequencing of endophytic fungal isolates

Target	Primer name	Direction	Sequence 5'→3'
ITS	ITS 1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS 4R	Reverse	TCCTCCGCTTATTGATATGC

The result obtained by the sequencing were analysed by BLAST and the result obtained is tabulated in **Table 9.**

Table 9: BLAST analysis results of fungal isolates from the leaf and fruit of *F. exasperata* and *F. drupacea*

Sl No	Name of the isolate	Similar organism	Percentage of similarity (%)	Division
1	FDLE I	*Phanerochaete* spc.	99.62	Basidiomycota
2	FDLE II	*Aspergillus nomiae*	87.29	Ascomycota
3	FDLE III	*Daldinia eschscholtzii*	99.80	Ascomycota
4	FDLE IV	*Endomelanconiopsis endophytica*	99.77	Ascomycota
5	FDLE V	*Alternaria alternata*	90.06	Ascomycota
6	FDFE I	*Phlebia sp.*	99.82	Basidiomycota
7	FELE I	*Endomelanconiopsis endophytica*	99.77	Ascomycota
8	FELE II	*Alternaria alternata*	90.06.	Ascomycota
9	FELE III	*Lasiodiploida pseudotheobromae*	100	Ascomycota
10	FELE IV	*Malassezia restricta*	98.71	Basidiomycota
11	FEFE I	*Phlebia* sp.	99.82	Basidiomycota

From the results it is clear that most of the members were from ascomycota division and few organisms were found to be similar in both the plants, such as *Alternaria alternata, Endomelenoconiopsis endophytica* and *Phlebia* sp. One of them from the fruit and two of them were from the leaves. 100 percent similarity was obtained only with one organism that is FELE III which was found to be 100% similar to *Lasiodiploida pseudotheobromae,* and the least similar organism was *Aspergillus nomiae* with a similarity of 87.29 %. FDLE I, FDFE I, and FEFE I was only identified to the genus level, and rest of them were identified till species level.

PART II

Mycochemical Analysis of *Phlebia* sp.

As the bioactivity studies were concentrated on the fruit extracts of *F. drupacea* phytochemical analysis and further studies of endophytes were concentrated to the endophyte *Phlebia* species isolated from the fruits of *F. drupacea. Phlebia* species, an endophytic fungus found in *Ficus drupacea* fruits, were mass-cultured (**Plate 21)**. After 20 days of culture, the extract was collected by separating the hyphae from the culture medium. This fungal extract was employed in investigations involving biochemical analysis, gold and silver nanoparticle biosynthesis, and nanoparticle-mediated antibacterial tests.

Preliminary phytochemical analysis revealed the presence of terpenoids, glycosides, steroids and saponins. Phenols were found to be absent in *Phlebia sp*.

Table 10: Preliminary phytochemical analysis of fungal endophyte *Phlebia* species

Sl No	Class of compound	Chemical test	Results
1	Alkaloids	Wagners test	+
		Hagers test	+
2	Flavonoids	Alkaline reagent test	-
		Lead acetate test	-
3	Phenols	Ferric chloride test	-
4	Tannins	Braymers test	-
5	Terpenoids	Salkowski test	+
6	Steroids	Leibermann Burchards test	+
7	Saponins	Foam test	+
8	Glycosides	Keller Killiani test	+
9	Phlobatannins	Precipitation test	-
10	Anthraquinones	Borntragers test	+
11	Proteins and amino acids	Ninhydrin test	+
12	Coumarins	Alcoholic NaOH test	-

HR-LCMS analysis of the mass cultured pure *Phlebia* extract revealed the presence of 34 mycochemicals. These were found to be glycosides, terpenoids, isoquinoline, steroids, saponins and proteins. Compounds such as phytosulfokine b,

ophiopogonin B, dihydrocaffeic acid 3-O-glucuronide, boviquinone etc. were reported. Details of HR-LCMS results with retention time and class of compounds are explained in the **Table 11**. Structure of each compound with its MS spectrum are given from **Fig.34 (i)-34 (ix).**

Table 11: HR-LCMS analysis results of mycochemical components of endophytic *Phlebia* sp

Sl. No.	Class of compound	Chemical test	Results
1	5-Methylcytidine	Nucleoside	1.137
2	Dihydrocaffeic acid 3-O-glucuronide	Glycoside	1.198
3	Phytosulfokine b	Peptide growth factors	1.212
4	Ophiopogonone B	Steroid saponin	1.402
5	Isoplumbagin	Organic compound	1.419
6	4-hydroxy-L-threonine	Hydroxy-amino acid	1.478
7	Cynometrine	Imidazole	1.608
8	N-Acetyl-D-fucosamine	Amino sugar	1.744
9	Lotaustralin	Cyanogenic glycoside	1.804
10	Lys-Gly	Dipeptide	1.891
11	Isoleucyl-Proline	Dipeptide	1.937
12	6-Deoxyfagomine	Piperidine	1.981
13	2-(4-Methyl-5-thiazolyl) ethyl octanoate	Thiazole	2.117
14	N (6)-hydroxy-L-lysine	Amino-acid	2.231
15	Coccinin	Peptide	4.675
16	11-Hydroxyiridodial glucoside pentaacetate	Terpene glycoside	5.024
17	Glutaminyl-Arginine	Dipeptide	5.535
18	Tributyrin	Ester	5.604
19	Maculosin	Dipeptide	5.674
20	Tryptophyl-Alanine	Peptide	5.859
21	Phenethylamine glucuronide	N-Glycoside	5.886
22	7,8-Dihydrovomifoliol 9-[apiosyl-(1->6)-glucosid	Carbohydrate	5.937
23	Rodiasine	Isoquinoline	6.311
24	Adouetine Y	Cyclopeptide alkaloid	6.388
25	Physapubescin	Steroidal lactone	6.474
26	Glutaminyl-Glycine	Dipeptide	6.623
27	N-Heptanoylhomoserine lactone	N-acyl-amino acid	6.646
28	Hydroxy citrulline	Alpha amino acid	6.684
29	Harzianopyridone	Aromatic ketone	6.64
30	Mycinamicin IV	Macrolide	6.906
31	Sorbitan laurate	Ester	8.077
32	Manumycin A	Polyketide	9.811
33	Boviquinone 4	Diterpenoid	19.489
34	Goyaglycoside c	Glycoside	19.965

PART III

Biosynthesis of Silver and Gold Nanoparticles

Fungal *Phlebia* extract mediated silver and gold nanoparticles were biosynthesised through the present study. When the *Phelbia* extracts were challenged with the respective reagents for both silver and gold nanoparticle biosynthesis (silver nitrate and aurochloric acid) they readily produced silver and gold nanoparticles. Visual indication was a colour change from yellow to brick red in case of silver nanoparticles and yellow to purple in case of gold nanoparticles.

Characterization of Silver and Gold Nanoparticles

UV-Vis Spectroscopy

The combination of silver nitrate and fungal extract caused the pale yellow reaction liquid to turn brick red. A peak in the wave length of 450 nm for silver nanoparticles was observed when this solution was subjected to UV-Vis spectroscopy **(Fig. 35)**. When the fungal extract was challenged with aurochloric acid, the reaction mixture changed its colour to purple-red, and a peak at 550 nm was produced, indicating the synthesis of gold nanoparticles (**Fig. 35)**. These changes demonstrated the potential of this fungal extract to generate gold and silver nanoparticles from their corresponding substrates.

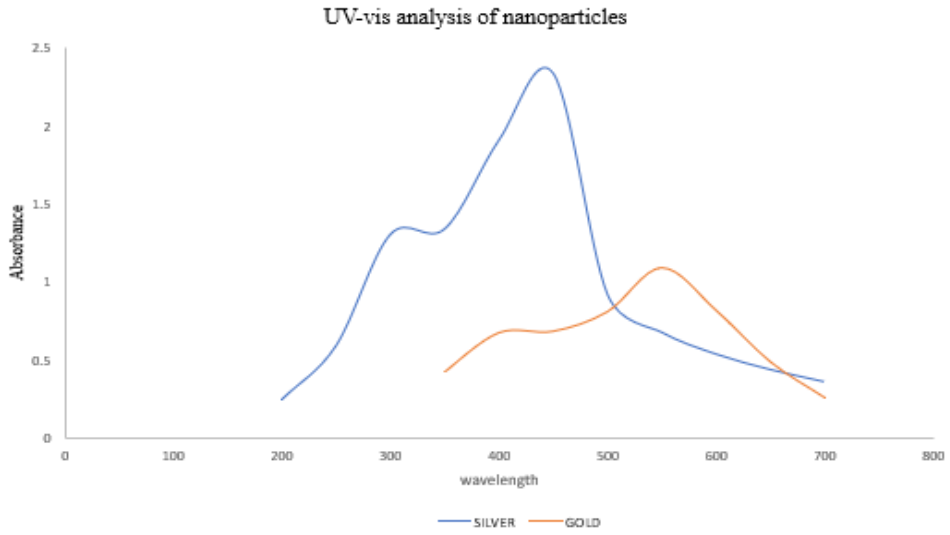


Fig. 35: UV-Vis spectrum of silver and gold nanoparticles produced using fungal endophyte *Phlebia* sp.

FE SEM Analysis

Scanning electron microscopy was used for identifying the surface morphology as well as the structure of silver and gold nanoparticles biosynthesised from the aqueous extracts of the endophytic fungus *Phlebia* sp. SEM analysis revealed the nearly spherical nature of silver and gold nanoparticles biosynthesised. The diameter of the gold and silver nanoparticles were 20-28 nm and 30-35 nm, respectively. SEM examination confirmed that the *Phlebia* extract could produce poly dispersed nanoparticles.

Antimicrobial Activities

The antibacterial abilities of silver and gold nanoparticles were studied at several concentrations, including 100 µg/ml, 500 µg/ml, and 1mg/ml. silver and gold nanoparticles produced by the *Phlebia* species were found to have strong antibacterial activity against the tested microorganisms. The analysis revealed a concentration-dependent increase in the zone of inhibition (**Plate 22, Fig.36)**. In all three test doses, gold nanoparticles inhibited *E. coli* with a narrower zone than silver nanoparticles. *S. paratyphi* appears to be more sensitive to both nanoparticles, generating a greater zone of inhibition even at the lowest test doses of gold and silver nanoparticles which is shown in the **Table 12**.

Table 12: Zone of inhibition produced by the organisms according to various concentrations of nanoparticles produced from the endophytic fungus *Phlebia* sp.

Pathogens	Zone of inhibition (in mm) according to test concentrations					
	Gold nanoparticles			Silver nanoparticles		
	100 µg/ml	500 µg/ml	1000 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
E. coli	0.4±0.111	2±0.559	16±0.5574	15±0.455	20±0.573	26±0.512
S. paratyphi	2±0.0826	8.5±0.148	18.6±0.417	20±0.474	24±0.384	29.9±0.754

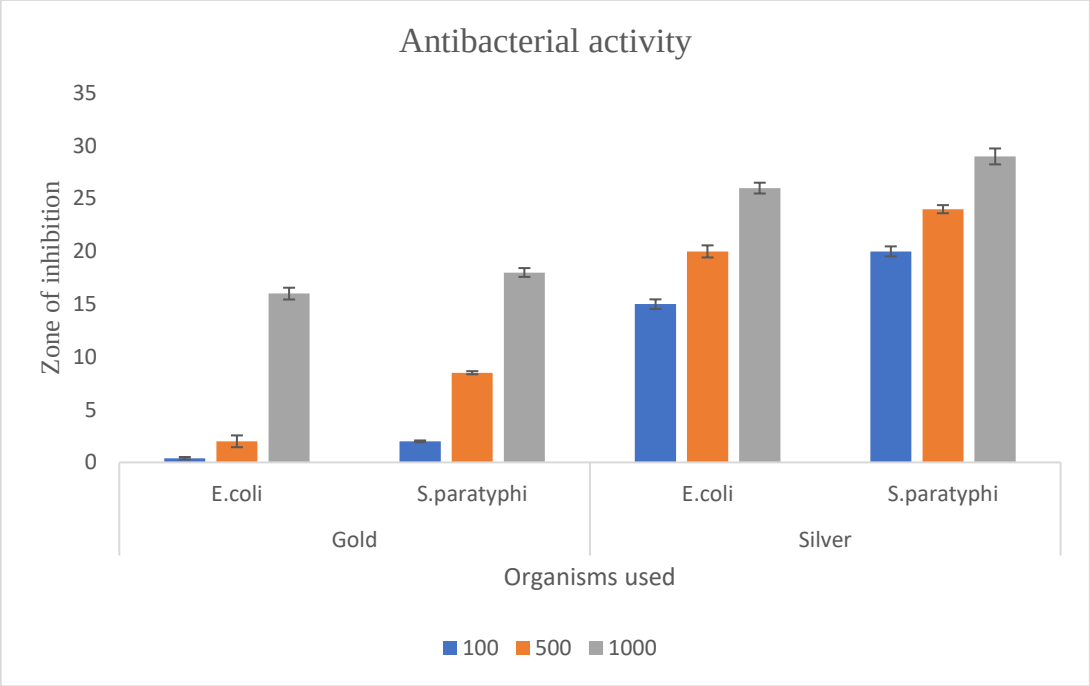


Fig. 36: Zone of inhibition produced by silver and gold nanoparticles biosynthesised from endophytic *Phlebia* sp. against the bacterial strains

DISCUSSIONS

PHASE I

PART I

Preliminary Phytochemical Analysis

Understanding the specific secondary metabolites present in a plant extract is critical because they have enormous potential in a variety of areas, including biomedical field, therapeutic field, food and nutraceuticals, crop protection, pesticides, fodder etc. Preliminary phytochemical study aids in understanding the primary and secondary metabolites found in a specific plant extract. Using an appropriate solvent, we can understand the presence of various secondary metabolites. In the present study, three distinct solvents were used to perform a preliminary phytochemical analysis of the fruit and leaf extracts of *F. drupacea* and *F. exasperata*. The polarity index values were used to select the solvents. One of the solvents, extremely nonpolar hexane, was chosen, along with water, the polar solvent, and methanol, a medium polar solvent. Findings of the study showed that polar solvents like water and methanol contain more phytochemical groups. Except for *F. exasperata* leaf extract, all three extracts were found to have a similar number of phytochemical groups. However, the number of phytochemical groups in the *F. exasperata* leaf methanolic extract was higher in number when compared to the aqueous extract fraction. The three phytochemical groups reported in the hexane extract of all four types of extracts used in the study were alkaloids, steroids, and flavonoids. The principal phytochemical groups found in the methanolic and aqueous fractions were alkaloids, phenols, flavonoids, glycosides, proteins, coumarins, saponins, anthraquinones, phlobatannins, and terpenoids. Many other *Ficus* species have produced similar results with the preliminary phytochemical studies, with different plant parts in different solvents. Uma et al. (2009) conducted preliminary phytochemical investigation of methanolic extract of two *Ficus* species, *F. religiosa and F. benghalensis*, demonstrating the presence of carbohydrates, flavonoids, amino acids, steroids, tannins, and saponins in the methanolic extract fraction. Antimicrobial activity against an *E. coli* strain was discovered in these plants. Another study looked at the phytochemicals in ethanolic root extracts of *F. racemosa* and discovered a variety of secondary metabolites such as sugars, glycosides, phenols, gum, saponins,

and tannins. Among these phytochemicals tannins and saponins were found in greater quantity (Murti et al., 2014). Another study on the preliminary phytochemical examination of *Ficus infectoria* leaves and bark revealed glucose, alkaloid, protein, flavonoids, amino acids, phytosterol, tannin, and glycoside (Kumar et al., 2012). The primary phytochemical groups of *Ficus pumila* leaves were investigated using five different solvents, including petroleum ether, chloroform, ethyl acetate, methanol, and water. Petroleum ether fraction revealed the presence of just steroids and terpenoids. Flavonoids and glycosides were the two groups that represented phytochemicals in the chloroform extract fraction. When the solvent was changed from chloroform to ethyl acetate, the number of phytochemical classes increased; a total of four classes were found in this solvent, including flavonoids, saponins, glycosides, steroids, and terpenoids. Both methanolic and aqueous extracts yielded the greatest number of secondary metabolite groups such as alkaloids, sugars, tannins, flavonoids, glycosides, saponins, steroids, and terpenoids (Kaur, 2012). Another study revealed the preliminary phytochemistry of the fruits of *Ficus capensis*. Tannins, terpenoids, alkaloids, flavonoids, cardiac glycosides, phenol, phytate, oxalate, and haemagglutinin are all found in this plant (Okoroh., 2019).

GC-HRMS Analysis

GC-HRMS analysis of phytochemicals from the fruit and leaf extracts of *F. exasperata* as well as *F. drupacea* revealed the presence of 9 phytochemicals, with major compounds 2 hydroxy-3-pentanone, catechol, 2,3- dihydrobenzo furan, 2-methoxy-4-vinylphenol, hydroquinone and 2-tridecanol. 2 hydroxy-3-pentanone is a secondary alpha hydroxy ketone, with a molecular mass of 102.13 µg/mol. Catechol is a naturally occurring phenolic molecule that is frequently utilized as a pesticide precursor and in a variety of fragrances. This is chemically 1,2 dihydroxy benzene, commonly known as pyrocatechol, and which is widely reported from diverse plants. This is an allelochemical with possible antibacterial properties. This phytochemical was discovered to have effective antioxidant properties (Ao et al., 2009). Bark extract of *F. microcarpa* was also found to have catechol in its phytochemical screening, which is in accordance with the current investigation (Mamoucha et al., 2016). Another component identified through GC-MS study of *F. drupacea* extracts is 2,3-dihydrobenzofuran. This is documented in a variety of plants and serves as a metabolite. Chemically, it belongs to the benzofuran class, with a molecular weight

of 120.15g/mol. In a prior investigation, *F. sagittifolia* stem bark extract was found to contain 2,3- dihydrobenzofuran (Taiwo et al., 2023). 2-methoxy-4-vinylphenol is a phenolic molecule that contributes to the aroma of various fruits and certain pheromones. This phytochemical has a variety of biological actions, including its potential anticancer, antioxidant and antibacterial activities (Rubab et al., 2020). This chemical has been identified from a variety of plants, including coffee and various other species of *Ficus*. This is having a molecular weight of 150.17 g/mol. In a GC-MS analysis-based characterization of aromatic compounds in the dried fruits of *F. carica*, researchers reported the presence of 2-methoxy-4-vinylphenol as one of the key aromatic components (Yao et al., 2021). GC-MS examination of *F. religiosa* seed extracts revealed the presence of 2-methoxy-4-vinylphenol, which has efficient pharmacokinetics and drug-like characteristics (Pinipay et al., 2023). Previous GC-MS studies on several *Ficus* species revealed the presence of 2-methoxy-4-vinylphenol in various plant parts, which is consistent with the current findings. Hydroquinone which is chemically benzene -1,4-diol comes under the class of phenolic compounds. This is an aromatic benzene derivative with the chemical formula $C_6H_4(OH)_2$. Hydroquinone has been found in a wide range of plants and has antibacterial, antioxidant, and germination-promoting properties. This is also associated with its ability in anticancer actions through DNA damage, arylation of nucleophiles, tubulin assembly inhibition, protein kinase inhibition, and inhibition of the arachidonic cascade (Sladic & Gasic, 2006). In *F. pandurata*, HPLC/QTOF-MS/MS of n-butanol extract of aerial roots revealed the presence of hydroquinone, which is in accordance with the current work (Zhang et al., 2015). *F. exasperata* leaf extract, and fruit extract (FELE, FEFE) contain hydroquinone. The *F. drupacea* leaf aqueous extracts contain 2 methoxy vinyl phenol. Previous research on the biological activities of these phytochemicals supports the biological activities demonstrated by the four extracts considered for this study. Implying that the presence of these phytochemicals, either alone or in combination with other phytochemicals, could have been critical for the bioactivities expressed by these four extracts considered in the present study.

HR-LCMS Analysis

Phytochemicals from the aqueous extract of *F. drupacea* and *F. exasperata* fruits and leaves were analyzed using HR-LCMS to determine the nonvolatile

components present in the four extracts chosen for the study. The results revealed the presence of 90 distinct chemicals, several of which have significant medicinal and biological properties. The extracts contain substances such as chlorogenic acid, isocarbostyril alkaloids and different phenolic compounds such as caffeine, gallic acid etc. A few significant phytochemicals discovered throughout the investigation are presented below to comprehend the major bioactivities held by the four extracts chosen for the study.

Quinic acid found in various medicinal plants, is chemically known as cyclohexane carboxylic acid. It is a colorless substance that contributes to the perceived acidity of coffee. Quinic acid is a phytochemical that has been demonstrated to have a number of biological actions, including antioxidant, antidiabetic, anticancer, antibacterial, antiviral, anti-aging, anti-nociceptive, analgesic actions, as well as protective properties. This is reported to be present in various plants such as *Coffea arabica*, *Ziziphus lotus*, and *Artemisia annua* etc. The ability of this molecule to interfere with ribosomes and production of aminoacyl-tRNAs has been found to hinder the oxidative phosphorylation pathway and disrupting membrane fluidity, which is the basis of its antibacterial activity. The antidiabetic activity is accomplished by stimulating insulin production via Ca^{2+} mobilization. Anticancer properties were expressed through apoptosis and downregulation of matrix metallopeptidase 9 (MMP-9) (Benali et al., 2022). This phytochemical also been shown to generate antibiofilm against clinically resistant *Pseudomonas aeruginosa* (Lu et al., 2021). Quinic acid has been found in several different *Ficus* species, including *Ficus cyathistipula* leaf extracts, *Ficus nitida* bark extract, *Ficus dubia* sap extract (El Sakhawy et al., 2016; Embaby et al., 2021; Chansriniyom et al., 2021). The presence of quinic acid in the fruit and leaves of *F. drupacea* is consistent with prior findings. In the current investigation, this component, along with other phytochemicals identified through GC-HRMS and HR-LCMS analysis, may have contributed significantly to the biological activities of these extracts.

Phenolic acids, which have been discovered to be excellent antioxidants, have gained attention due to the opposing association between their consumption through

diet and the incidence of harmful diseases such as cancer or other neurological disorders. Gallic acid (GA) is a well-known naturally occurring low molecular weight triphenolic molecule found throughout the plant kingdom, possibly paired with or without tannins. This is 3,4,5-trihydroxybenzoic acid, which is found practically in every part of the plant ranging from seed to bark. Gallic acids, along with their esters and salts, comprise a broad family of polyphenols known as gallates (Al zahrani et al., 2020).

Hydroxyl groups of gallic acid is readily available for ROS scavenging, which reduces free radical formation and checks the free radical chain reactions. Carboxyl groups in the GA that are easily ionizable can serve as effective proton donors, which transforms GA into a powerful antioxidant in the system. This antioxidant ability of GA helps in inhibiting the oxidation of key macromolecules such as DNA, lipids, proteins and enzymes. The importance of gallic acid is not restricted with antioxidant properties; It has a wide range of applications in numerous biomedical fields, as illustrated in **Fig. 37.**

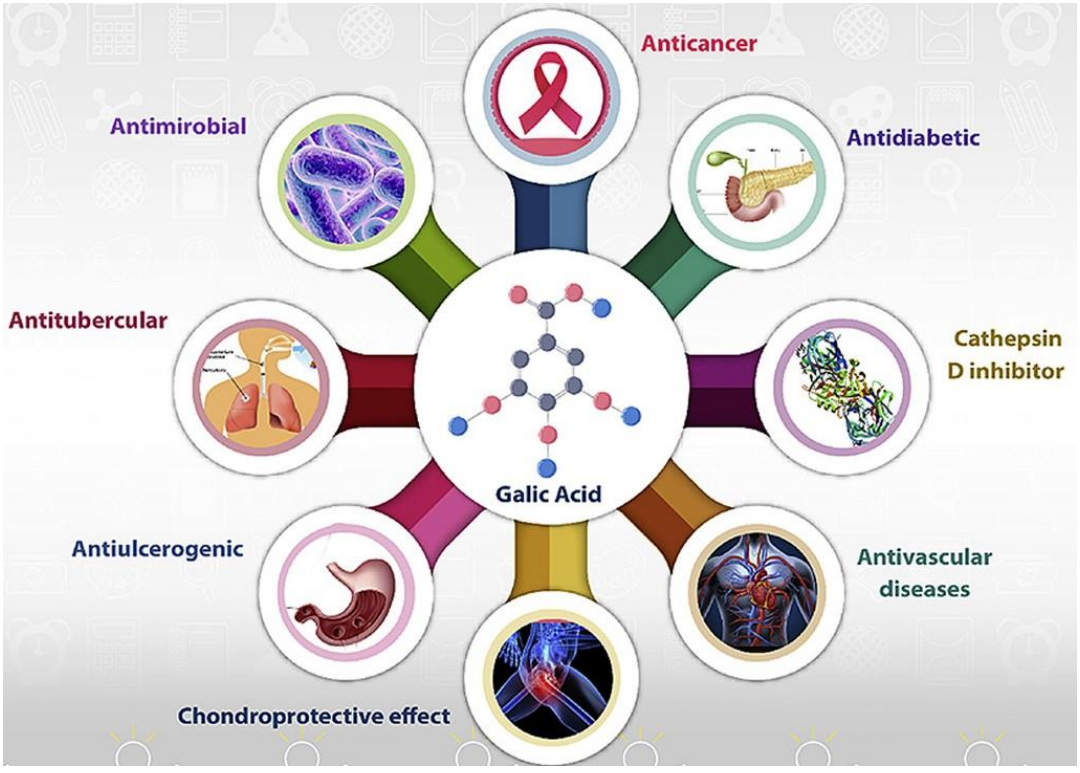


Fig. 37: **Role of gallic acid in biological system** (Al zahrani et al., 2020).

There have also been indications that GA and its derivatives are effective in inducing apoptosis within cancer cells while leaving healthy cells protected (Chen et al., 2009). Gallic acid has antibacterial, antiviral, anti-inflammatory, and antidiabetic properties (Kubo et al., 2003; Wang et al., 2009; Dhingra et al., 2014; Adefegha et al., 2015).

Numerous reports have previously been made regarding the presence of gallic acid in different parts of *Ficus* plants. GA and numerous other phytochemicals were found in the ultra sound aided leaf extract of *F. auriculata* (Baite et al., 2021). Gallic acid was contributing to a variety of bioactivities of *F. racemosa* leaves and fruits, *F. asperifolia* leaves and fruit extracts, *F. carica* fruit extracts, and *F. sycomorus* bark extracts. (Veberic et al., 2008; Ojo & akintayo, 2014; Sumi et al., 2016; Sulaiman et al., 2021). The current study is one among such works that highlights the gallic acid content of *F. drupacea* leaves and fruits.

Isocarbostyril is a class of alkaloids with potent antineoplastic and antimitotic properties. They are a small group of alkaloids that lack basic nitrogen atoms and have structures similar to hydroxylated benzophenanthridone or isoquinolinone. These chemicals were first identified in the amaryllidaceae family, with three well-known compounds being narciclasine 1, lycoricidine 2, and pancratistatin. This category has remarkable biological activity, including their superior *in vitro* and *in vivo* cytotoxicity against numerous tumor cell lines (Ingrassia et al., 2008). This has prompted various studies to find novel compounds of isocarbostyril alkaloids for preclinical development. The current study is the first to describe the existence of the isocarbostyril group of alkaloids from the genus *Ficus*. Fruits from both plants used in the study, *F. exasperata* and *F. drupacea*, showed the presence of these alkaloid groups in the HR-LCMS analysis. Vulgarin, a eudesmanolide sesquiterpene, is a phytochemical found in several *Artemisia judaica* plants that has bioactivities like anticancer properties and *in vivo* and *in vitro* antidiabetic properties (Althurwi, 2022). This is reported from both the fruits under in the current investigation.

Chlorogenic acid is a water-soluble phenyl acrylate phenolic acid synthesized by the plants through shikmic acid pathway during aerobic respiration (Huang et al.,

2023). One of the most available and biologically active dietary polyphenols which is commonly present in both tea and coffee green extracts. This is formed by the esterification reaction between caffeic acid and quinic acid and it is also named 5-O-caffeoylquinic acid (5-CQA). Chlorogenic acid is a broad term used to describe the whole set of hydroxyl cinnamic esters along with quinic acid such as coumaroyl quinic acids, feruloyl-, caffeoyl- and dicaffeoyl quinic acids. Major functional groups such as hydroxyl, carboxyl and o-diphenol are the backbone of its bioactivities. Chlorogenic acid isomerizes to create a more stable structure with six primary isomers and a number of isochlorogenic acids. The chlorogenic acid structure and its major isomers are shown in **Fig. 38.**

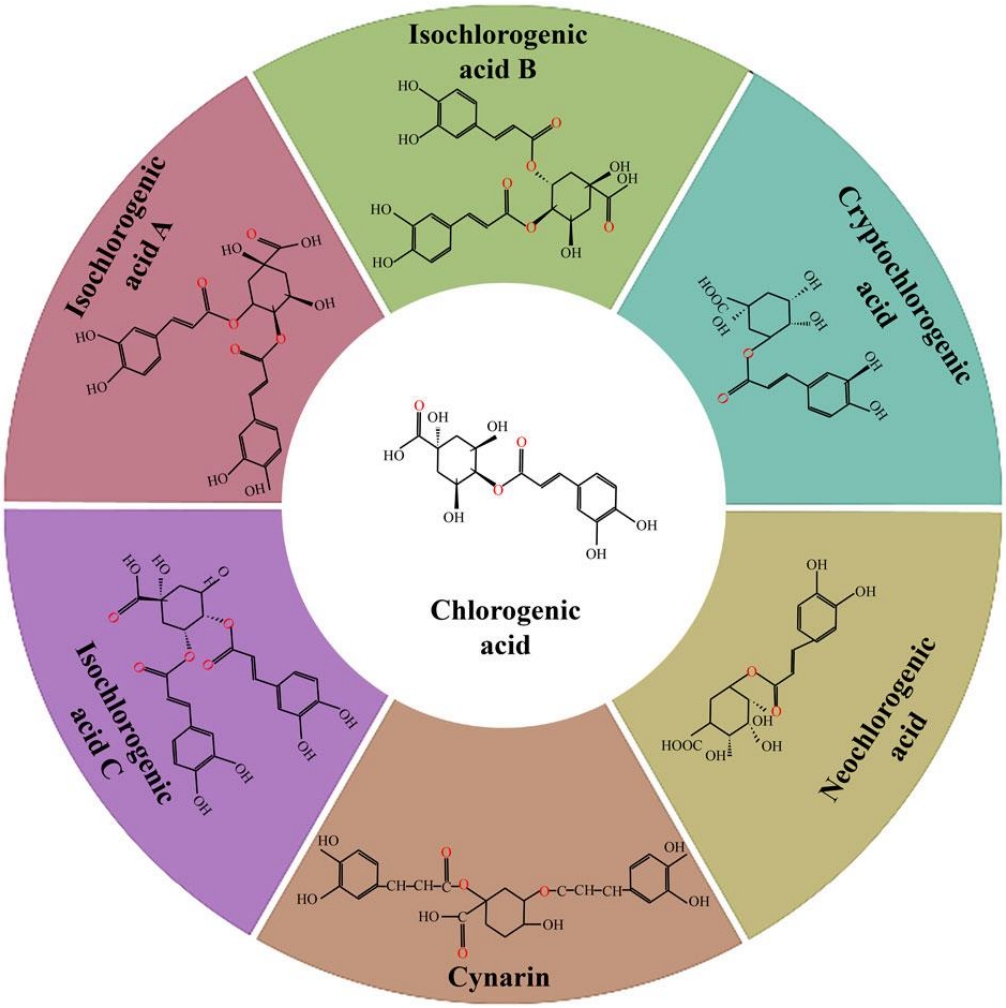


Fig. 38: Chlorogenic acid and its major isomers (Huang et al., 2023)

Antibacterial, antioxidant, cardioprotective, anti-inflammatory, antipyretic, and neuroprotective actions are some of the bioactivities of chlorogenic acid. It can activate the central nervous system, protect the liver from lipopolysaccharide damage, control the expression of the GLUT gene to avoid diabetes, and stop obesity by blocking the absorption of sugar. This phytochemical also influences lipid metabolism and hypocholesterolemia via altered metabolic pathways (Naveed et al., 2018). Various *Ficus* species including *F. racemosa, F. benjamina, F. carica, F. macrocarpa, F. religiosa* etc. were already reported to have the presence of chlorogenic acid (Ao et al., 2010; Vallejo et al., 2012; Imran et al., 2014; Sharma et al., 2021; Kar et al., 2023). The current research revealed the presence of chlorogenic acid in the fruits of *F. drupacea* and the leaves of *F. exasperata*. This finding is fully corroborated by the earlier report on the phytochemical analysis of other plants from the same genus.

Another polyphenolic molecule that is widely present in plants and is commonly included in the diet of humans is caffeic acid (CA), which has both medicinal and recognized health benefits. The biosynthesis of caffeic acid also happens through the shikimic acid pathway, which is involved in the generation of aromatic amino acids. This substance is an organic compound and belongs to the broad class of polyphenols. One of the main members of the hydroxycinnamic acid class with a phenyl propanoid structure is caffeic acid, also known as 3,4-dihydroxy-cinnamic acid. This is frequently esterified with quinic acid, which results in the formation of chlorogenic acid. This is incredibly high in fruits, including grains, carrots, plums, and blueberries (Espindola et al., 2019). This phytochemical has a variety of biological functions. One of the most important bioactivity is its antioxidant activity, which is caused by the presence of free hydroxyl groups in the aromatic ring, as well as the double bond in the carbon chain and its metal chelation properties (Birkova et al., 2020). ROS regulation also helps the compound's anti-inflammatory and immunoprotective properties. CA also has enormous potential for anticancer activity *in vitro* and *in vivo*, through DNA oxidation and angiogenesis inhibition (Espindola et al., 2019). Phytochemical analysis of *F. carica* leaves and fruits, *F. beecheyana* root extracts, *F. benjamina* leaf extract are reported to have the presence

of caffeic acid. This is in accordance with the present study as it reports caffeic acid from the fruits of *F. drupacea* and leaves of *F. exasperata* (Petruccelli et al., 2018; Yen et al., 2018; Ashraf et al., 2020).

Quercetin (chemical formula: 3, 3′, 4′, 5, 7-pentahydroxy-2-phenylchromen-4-one) belongs to the flavanol class, one of the six sub-classes of flavonoids (Li et al., 2016). This is a yellow, crystalline substance that is insoluble in water. It has a flavone skeleton made up of two benzene rings joined by a pyron ring. Quercetin glycoside, quercetin sulphate, quercetin glucuronide and methylated quercetin are the major forms of quercetin. This is frequent in fruits, seeds, onions, and tomatoes. There are numerous natural derivatives of quercetin. They all have a multitude of biological actions. Quercetin has numerous health benefits which include improving cardiovascular health, lowering cancer risk, treating eye ailments, allergies and arthritis (Lakhanpal & Rai, 2007). HR-LCMS analysis of *F. drupacea* leaf extract revealed the presence of quercetin 3,7-dirhamnoside, a quercetin -O-glycoside, which is consistent with the earlier reports showing the existence of several quercetin forms from the *Ficus* genus. *F. carica* leaves, *F. virens* extract, and *F. benjamina* leaf extracts were all reported to contain quercetin and its derivatives (Yarmolinsky et al., 2012; Orabi & Orabi, 2016; Alshaal et al., 2019).

Vanillic acid is a phenolic acid with the chemical formula 4-hydroxy-3-methoxy benzoic acid, derived from the oxidation of vanillin (Kim et al., 2010). This is highly common in foods including cereals, whole grains, fruits, and green tea (Sharma et al., 2020). This can also occur as a byproduct of caffeic acid. Vanillic acid is recognized as an endogenous biochemical derived from catecholamines. They are employed as flavouring agents in a variety of food products. Vanillic acid and its compounds were shown to have a variety of biological activities. Potential therapeutic activities include altered p53 expression to promote apoptosis in cancer cells, inhibition of biological targets involved in disease pathophysiology, ROS regulation-mediated antioxidant and anti inflammatory effects, etc. (Kaur et al., 2022). Presence of vanillic acid in the genus *Ficus* is already reported through phytochemical

screening of species like *F. carica* by Palmeira et al. (2019), which is similar to the results obtained from the fruits of *Ficus exasperata* in the present study.

The phytochemical screening of aqueous extracts of *F. drupacea* and *F. exasperata* fruit and leaves aided in the identification of the principal phytoconstituents. Looking at the important qualities of these biochemicals, it is clear that the extracts bioactivities, such as antioxidant, anticancer, and apoptotic capabilities, are inextricably linked to the existence of these biochemicals. Despite the fact that the leaf extract of *F. exasperata* contained the greatest number of phytochemical components (53 compounds), the fruit extract of *F. drupacea* exhibited the highest activity in the current study. This demonstrates that bioactivity is not dependent on the number of phytochemicals, but rather on the nature of the compounds and possibly, the quality. *F. drupacea* fruit extract displayed the maximum activity, as it contains the major biochemicals discussed here, including quinic acid, chlorogenic acid, caffeic acid, gallic acid, and isocarbostyril alkaloid. Except for the fruit extract of *F. drupacea*, no other extract (FEFE, FELE, FDLE) contained all of these components in a single extract. In this study, phytochemistry reflects bioactivity by combining the most active phytochemicals, which contribute significantly to the extract's characteristics

PART II

Antioxidant Activity

Free radicals are substances documented for a dual role in the living system with both deleterious as well as beneficial roles. The cellular mechanisms to yield energy by both aerobic and anaerobic oxidation of various biomolecules results in the production of free radicals. The amplitude of oxidant production in normal cells is well balanced by the elimination or sequestration of them by antioxidants present in the system. When this equilibrium is thrown off by an increase in free radical production or a decrease in antioxidant levels, the system experiences oxidative stress, which eventually leads to oxidative damage. Free radicals are atoms, molecules, or compounds containing one or more unpaired electrons that produce ROS when they try to pair with other molecules or compounds. Therefore, there is a strong connection

between the free radicals present in molecular systems and their degenerative processes.

Table 13: Common free radicals of the biological system (Phaniendra et al., 2015)

Reactive oxygen species	**Symbol**
Radicals	
Superoxide	$O_2^{\bullet}$-
Hydroxyl	$OH^{\bullet}$
Alkoxyl radical	$RO^{\bullet}$
Peroxyl Radical	$ROO^{\bullet}$
Non radicals	
Hydrogen peroxide	H_2O_2
Singlet oxygen	O_2
Ozone	O_3
Organic peroxide	ROOH
Hypochlorous acid	HOCl
Hypobromous acid	HOBr
Reactive nitrogen species-RNS	
Nitric oxide	$NO^{\bullet}$
Nitrogen dioxide	$NO_2^{\bullet}$
Non radicals	
Peroxynitrite	$ONOO^-$
Nitrosyl cation	NO^+
Nitroxyl anion	NO^-
Dinitrogen trioxide	N_2O_3
Dinitrogen tetraoxide	N_2O_4
Nitrous acid	HNO_2
Peroxynitrous acid	ONOOH
Nitryl chloride	NO_2CL

This could result in aging and diseases including cancer and auto immune diseases. Since ROS and RNS are primarily produced by the cellular metabolism of oxygen, chloroplast, mitochondria, apoplast, and peroxisome all play a significant role in their synthesis. The **Table 13** lists the major free radicals in the biological system.

Due to its capacity to induce DNA damage, lipid peroxidation, and protein oxidation, these ROS can be harmful to cells and may even result in cell death (Garcia et al., 2021). The redox equilibrium is represented in the **Fig. 39.**

Studies on antioxidants become increasingly significant and pertinent when we are aware of the harmful effects of free radicals and the capacity of antioxidants to scavenge them. Natural antioxidants are always preferred to synthetic ones. Using four different antioxidant potential determining assays, the current study aims to understand the antioxidant capacity of two locally available *Ficus* species.

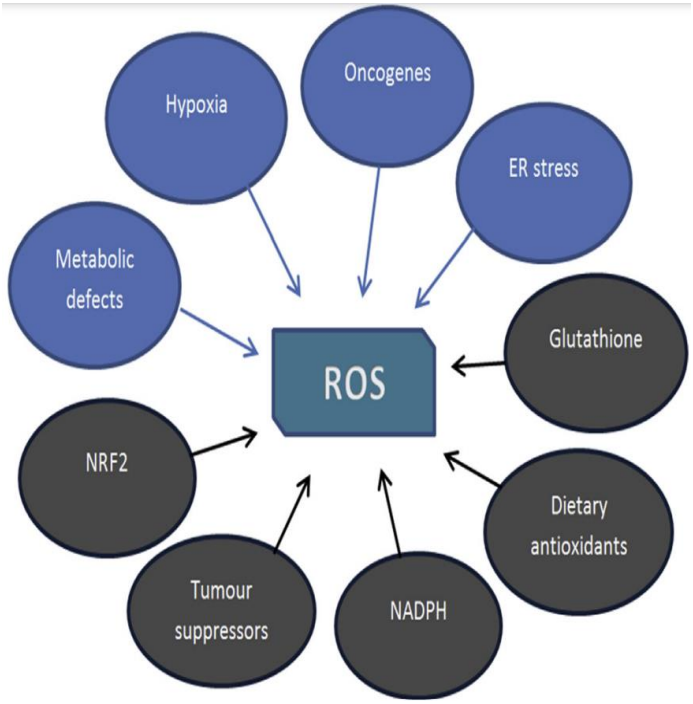


Fig. 39: The redox equilibrium of ROS scavengers and inducers. ROS inducers are represented by the blue circle. Scavengers of ROS are represented by the black circle (Neha et al., 2019).

DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazil) scavenging assay is the most commonly used assay to determine the antioxidant ability. Delocalization of electron within the molecule make it a stable free radical. DPPH makes it an easy but reliable method in assessing the antioxidant ability. DPPH assay is based on the spectrophotometric reading produced when antioxidants scavenge DPPH. When the

structure of DPPH is analysed (**Fig. 40**) there is a single electron with a nitrogen atom which can be reduced to hydrazine by the addition of hydrogen atoms from the antioxidant used. This DPPHH is having a lesser absorbance when compared to DPPH radical (Gulcin & Alwasel, 2023). This will lead to the change in colour from violet/purple to pale yellow which can be determined by spectrophotometer (**Fig. 41)**.

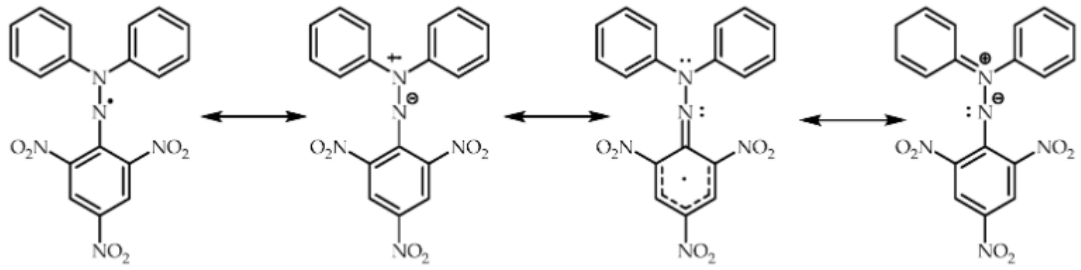


Fig. 40: Chemical structure of 1,1-diphenyl-2-picrylhydrazil

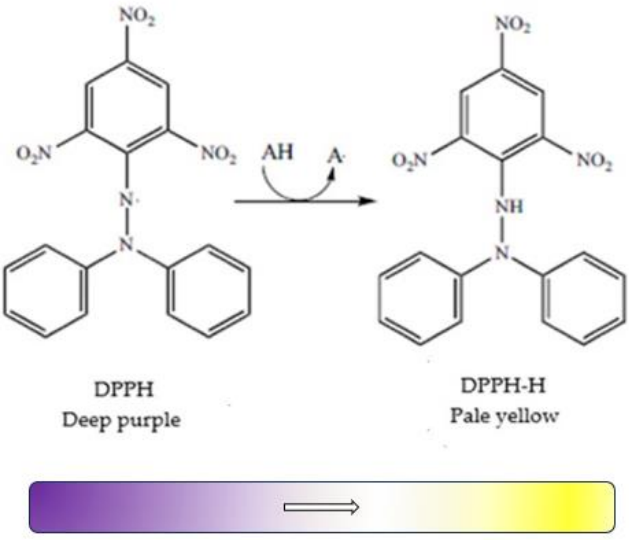


Fig. 41: Colour change indication of DPPH

The present study focuses on aqueous extracts of the fruits and leaves of *F. exasperata* and *F. drupacea*. The leaf extract of *F. drupacea* had the highest activity, with a value of 57.84 ± 0.453% and an IC 50 of 93.882 µg/mL. This is roughly equivalent to the ascorbic acid IC 50 value, which was used as a standard and has an IC 50 of 32.17 µg/mL. In a related study, the antioxidant activity of 18 different types of *F. carica* latex was assessed using the DPPH, ABTS, and FRAP assays

(Shahinuzzaman et al., 2020). With an inhibition percentage of 65.91 % ± 1.73%, the White Genoa variety was discovered to have the best antioxidant activity. These results are roughly identical to the values of FDLE extract in the current investigation. The same cultivar also contained a high amount of total phenolic compounds. This could explain its greater antioxidant capacity. Baliyan et al. (2022) discovered that *F. religiosa* leaf extract can quench DPPH radicals with an inhibition percentage of 43.415%. Which is less than the value achieved in the current investigation for the least effective extract FELE (53.766%). The findings suggest that the presence of polyphenols and flavonoids aided *F. religiosa*'s antioxidant activity.

Hydroxyl Radical Scavenging Activity

When molecular oxygen is incompletely reduced, it forms super oxide anion ($O_2^{-\cdot}$) which is dismutated by super oxide dismutase to generate H_2O_2, which then reacts with transition metals like Fe or Cu to form hydroxyl radicals in the system. These radicals can remove hydrogen from lipids, causing it to combine with oxygen, resulting in the creation of peroxyl radicals ($ROO^{\cdot}$) and the onset of lipid peroxidation. They can also contribute to mutations by disintegrating basic structure of DNA (Herraiz & Galisteo, 2015). The method of Kunchandy & Rao. (1990) with a slight modification is used in the current study to carry out the hydroxyl radical scavenging assay. This involves the degradation of the deoxyribose by hydroxyl radicals produced by the Fenton reaction (Eq1) and the measurement of the degradation product through malonaldehyde formation using spectrophotometry. The amount of the degradation product in the system decreases when an antioxidant is present as it scavenges HO radicals, which slows down the degradation of 2 deoxyribose.

$$Fe_2^{+} + H_2O_2 \longrightarrow Fe_3^{+} + HO\cdot + HO^{-} \qquad \text{(Eq1: Ahmed et al., 2022)}$$

When *F. racemosa* (stem bark) and *F. benghalensis* (arial roots) were tested for antioxidant ability *in vitro*, both extracts showed a concentration-dependent increase in free radical scavenging activity, as reported in the current study. The highest percentage of inhibition was observed in the stem bark methanolic extract of *F. racemosa*, with a value of 37.2 ± 0.9% in a concentration of 250g/mL (Manian et al., 2008). *F. glomerata* fruit extracts were evaluated for antioxidant potential using

hydroxyl radical scavenging assay. The result revealed moderate antioxidant activity of the ethyl acetate fraction of *F. glomerata* with highest inhibition percentage (0.282 ± 0.021 mg/ml) (Verma et al., 2010). Current investigation of *F. exasperata* and *F. drupacea* revealed a maximum value of 64.412 % using FDFE extract in 2000 µg/mL concentration, which is almost equal to the earlier reports.

Nitric Oxide Radical Scavenging Assay

Nitric oxides are created when the protein arginine is converted into citrulline in the presence of a specific nitric oxide synthase through a series of oxidation processes. Nitric oxides are also created by macrophages as a first line of defence against pathogens. A cytotoxic amount of ($NO^{\cdot}$) can interact with oxygen or cellular byproducts to generate peroxynitrites (ONOO-), which can start the oxidation of lipids (Gulcin, 2020). In the nitric oxide scavenging experiment, $NO^{\cdot}$ is produced when sodium nitroprusside breaks down at a physiological pH. The resulting interaction with oxygen then produces stable nitrate or nitrite. Griess reagent is used to estimate this stable molecule. The nitric oxide scavenging assay utilizing *F. exasperata* and *F. drupacea* fruit and leaf aqueous extracts revealed the modest scavenging activity towards NO in a concentration dependant manner. With an inhibition percentage of 58.477%, the leaf aqueous extract of *F. drupacea* had the highest activity among the four extracts. All three extracts had values that were essentially the same. An earlier study by Eshwarappa et al. (2015) on the leaf gall extracts of *F. glomerata* revealed significant quantity of phenols and flavonoids. Methanolic extract was found to be having higher capacity to scavenge nitric oxide radicals when compared to that of the aqueous extract. The IC50 value for the assay was found to be 172.02 µg/ml which when compared to the present study reveals the efficiency of *Ficus glomerata* over *F. exasperata* and *F. drupacea* fruit and leaf aqueous extracts. In a nitric oxide radical scavenging assay, ethanol fruit extracts of *F. bangalensis* demonstrated potential antioxidant scavenging activity with an inhibition percentage of 51.963 ± 64% (Tharini et al., 2018). The results of the previously discussed studies are completely consistent with the current investigation with comparable free radical scavenging activities. Another study on the ability of three plants (*F. exasperata*, *Moringa*

oleifera, and *Jatropha tanjorensis*) to scavenge nitric oxide; although *F. exasperata* was found to have the greatest phenolic estimate value, it had the lowest antioxidant potential, falling behind the other two plants studied (Anigboro et al., 2019).

Super Oxide Radical Scavenging Assay

In aerobic organisms, when energy is produced by oxidising biomolecules containing carbon and hydrogen it results in the production of heat. This process also helps in the reduction of molecular oxygen into a number of intermediate species such as superoxide anion radicals, hydroperoxyl radical, hydrogen peroxide, hydroxyl anion and hydroxyl radical etc. (Pisoschi & Pop, 2015). Super oxides ($O_2^{\cdot-}$) can be produced by various oxidases such as xanthine oxidase and cyclooxygenase (Pisoschi & Pop, 2015). They are also developed by the immune system to kill the invading microorganism. Phagocytes use NADPH oxidase to produce superoxide radicals which helps in the oxygen dependent killing of invading microorganism (Gulcin, 2020). They are relatively weak oxidants, but they generate stronger free radicals such as hydroxyl free radicals (Gulcin, 2020). According to valenato et al. (2002), the oxidation of NADH in the riboflavin-NADH system produces super oxide anions. Free radical scavenging ability of these anions will be calculated by measuring the colour change of nitro blue tetrazolium (NBT) as a result of its reduction into corresponding formazan crystals of purple colour. An earlier study evaluated the antioxidant activity of *F. microcarpa*'s bark, leaves, and fruits. The results revealed that the bark extract had stronger antioxidant activity, with an EC 50 value of 63.2 µg/ml. They estimated more phenolic components in the bark extract, which could explain the greater antioxidant activity of the bark extract (Ao et al., 2008). The super oxide radical scavenging activity of *F. auriculata* fruit extracts was investigated using figs dried in various ways, such as sun dried, hot air oven dried, and microwave oven dried samples. Hot air oven dried samples were shown to have better antioxidant activity in the super oxide radical scavenging assay, with an inhibition percentage of 40.85% (Paramanandam et al., 2021). The current study reports on the moderate superoxide radical scavenging effects of *F. drupacea* and *F. exasperata* fruit and leaf aqueous extracts. *F. drupacea* leaf extracts were shown to have the strongest

superoxide radical scavenging capacity, with an inhibition percentage of 63.157 %. With this value for the highest inhibition percentage, the present study is also comparable with the earlier researches. The HR-LCMS analysis of the four extracts in the current study revealed phytochemicals, including polyphenols like gallic acid, chlorogenic acid, caffeic acid, and ferulic acid as well as flavonoids like quercetin 3,7-dirhamnoside, sophorole, biorobin and others. These substances may have made a significant contribution to the antioxidant properties of the fruit and leaf extracts of *F. exasperata* and *F. drupacea***.**

MTT Assay

Cancer is characterized by dysregulated cell proliferation, where the equilibrium between cell division and cell death, typically maintained through cellular communication, is disrupted. This uncontrolled growth from genetic damage is triggered by diverse factors. Genetic abnormalities induce aberrant behaviour in various regulators responsible for governing normal cell division, apoptosis, and proliferation rate. These malfunctioning regulators subsequently promote the activation of numerous genes that facilitate unbridled cellular proliferation. However, uncontrolled cellular division is not the only characteristic trait of cancer. If not combated, replicative senescence would limit the growth of any tumour. Malignant cells circumvent this regulatory mechanism either by senescence pathways ceasing to operate or by inappropriately activating senescence-suppressing genes, telomerase being the most notable of them. Metastases are produced as a result of abnormal expression of certain homing receptors, their associated ligands, and released proteinases. In most of the cases, the initial tumour has an atypical expression of genes related to invasiveness. The choice of organs that metastases prefer to invade is determined by this unusual gene expression pattern. There are few numbers of drugs and treatments available in the field of breast cancer treatment. Still the resistance to chemotherapy by cancer cells make it difficult to treat and this is why antiproliferative study is important in current scenario. Large number of reports are available on various aspects of cancer, including the molecular aspects. These studies are increasing the possibility to find a cure for this deadly disease. The present study is

an *in vitro* study dealing with the breast cancer cell line. Aqueous fruit and leaf extract of the plants *F. drupacea* and *F. exasperata* are used here to analyse its antiproliferative effects on the MCF-7 cell line. Breast cancer is reported to be the most frequently diagnosed cancer type with 2.3 million new cases worldwide (Sung et al., 2021). Understanding the root causes of breast cancer and creating novel treatments have received a lot of attention. In recent decades, government organizations from all over the world, private foundations, and commercial corporations have committed billions of dollars in breast cancer research, which demonstrates the importance of breast cancer research. The current study of *in vitro* antiproliferative research is therefore essentially limited to breast cancer cell lines. This research makes an effort to comprehend both peripheral and molecular basis of the antiproliferative effect brought by the chosen plant extracts. The four extracts (both leaf and fruit) from *F. exasperata* and *F. drupacea* were used to test their cytotoxicity using MTT assay. MTT is an assay used to distinguish non-living cells from live cells by checking the change of MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) into formazan crystals by the action of live mitochondrial enzyme succinate dehydrogenase. Live mitochondrial succinate dehydrogenase reduces yellow coloured MTT salt into formazan crystals which will lead to a colour change in the cell mixture from yellow to purple and this can be determined by colorimetric assay. This colour change is a direct representation of the live cells present in the cell mixture. So higher the purple colour intensity, higher will be the number of viable cells. MTT test conducted in the present research resulted in finding the concentration-dependent antiproliferative activity with the leaves and fruits of both *F. exasperata* and *F. drupacea*. MTT assay was conducted on both L929 as well as MCF-7 breast cancer cell lines. Adherent type mouse fibroblast cell line L929 is a well-known alternative test system that is frequently utilized in *in vitro* experiments (Ray et al., 2008). Studies on L929 revealed the cytotoxic effect of various extracts on the cell line with its IC50 values. *F. exasperata* fruit extract seems to have created extensive damage and lowest percentage of cell viability with L929 cells in the highest test concentration. Highest IC50 (207 ± 0.5284) value was put forth by *F. drupacea* leaf extract indicating its lowest cytotoxic nature towards L929.

F. drupacea fruit and *F. exasperata* leaf extracts were also tested for cytotoxicity on L929 cell lines; they were comparably less toxic towards L929 cell lines even in the highest test concentration. Comparing the IC50 values of the MTT assay on MCF cell lines by four extracts used in the present study, *F. drupacea* fruit extract was reported with lowest IC50 value. Due to the greater IC50 value for the cytotoxicity studies on normal cell lines L929 and lower IC50 concentration for MCF-7 cell lines, the fruit extract of *F. drupacea* was chosen for the remaining research including cell cycle and gene expression studies.

Studies on local *Ficus* species and their fruits are less explored compared to the amount of literature available on the bioactivities of *Ficus* species such as *F. carica, F. racemosa* etc. According to earlier studies, *F. sycomorus* fruits and leaves, *F. religiosa* and *F. benghalensis* latex extracts, *F. racemosa* leaves, and *F. carica* fruit extract were all shown to effectively kill cancer cell lines in a concentration-dependent manner (El-Beltagi et al., 2019; Tulasi et al., 2018; Khan et al., 2017; Jasmine et al., 2015). *F. drupacea* bark extract was discovered to be safe to use on the normal cell line HEK-293 while also being reported to have effective anticancer effects against cell lines such as HeLa, MCF-7, Jurkat, HT-29, and T24. When the activities of the bark extract and a few isolated components from *F. drupacea* were investigated; the isolated compounds were shown to be more active than the crude extract. The outcome is in accordance with the present study, which highlights the concentration dependent antiproliferative activity of *F. drupacea* bark extracts (Yessoufou et al., 2015). Concentration dependent antiproliferative activity of *F. exasperata* against Pc-3 cell lines were already reported by Deeh et al. (2022) which is in accordance with the current study. A similar study was conducted on multiple extracts made from *F. exasperata* using different solvents. All extracts other than methanolic extract displayed antiproliferative effects on the ovarian cancer cell line A2780 with reference to increasing concentration, which is again consistent with the current investigation. However, the methanolic extract analysis conducted on the same cell line was in conflict with the current study since it led to elevated proliferation in the *in vitro* test. These researchers were able to isolate different fatty acids from the extracts, and they claim that these fatty acids are primarily responsible for the antiproliferative

properties of the various extracts (Bafor et al., 2017). Four fatty acids, including corchorifatty acid D, 16-hydroxy-10-oxohexadecanoic acid, and two others found in the leaf aqueous extract of *F. exasperata*, may have contributed to the antiproliferative activity of MCF-7 cell lines in the current study. Anticancer ability of *F. benghalensis* root extract on various cell lines such as MDA-MB-231, A549 (lung cancer) and HeLa (cervical cancer) was reported by Saloni & Sakthivel (2019). The extract revealed concentration dependent potential activity which was comparable to the positive control drug doxorubicin used in the study. Presence of hydroquinone in all extracts other than *F. drupacea* leaf extract, is reported to have efficient antiproliferative action via DNA damage, Tubulin assembly inhibition, inhibition of arachidonic cascade and protein kinase inhibition. Phenolic acids which are having efficient therapeutic potential such as caffeic acid and gallic acid are also reported from the aqueous extracts of *F. drupacea* and *F. exasperata*. Quinic acid is another bioactive phytochemical which is present in *F. drupacea* leaf and fruit extracts; this was found to be having antiproliferative activity towards various cancer cell lines. Antiproliferative action detected by MTT assay in the four types of extracts from fruits and leaves of *F. exasperata* and *F. drupacea* might be completely indebted to the phytochemicals it posses.

Apoptosis Detection

The MTT assay despite being an effective tool for antiproliferative activity, it cannot differentiate between necrosis and apoptosis. An anticancer agent's capacity to inhibit proliferation through apoptosis indicates its drug candidacy, while necrosis indicates toxicity (Liu et al., 2015). So, when a test sample is discovered to be antiproliferative, it should be verified whether the antiproliferation is caused by necrosis or apoptosis. For understanding whether the action of *F. drupacea* fruit extract lead to apoptosis or necrosis, comet assay and double staining assays were conducted. DNA fragmentation is one of the keys to understand apoptosis as this is one step in the starting stages of apoptosis. This DNA fragmentation can be detected by comet assay, and the double staining assay can distinguish living cells from dead cells and apoptotic cells from necrotic cells, even the early and late apoptotic cells

were also distinguished through double staining. Even though flow cytometry is used in apoptosis detection, the results obtained from both the methods do not have much difference (Liu et al., 2015). So, using cheap and reliable double staining method in detection of apoptosis makes sense.

Comet Assay

Comet assay or single cell gel electrophoresis is a simple rapid reliable and widely used assay for detecting DNA damage at cellular level. This is considered as a major tool in genotoxicity detection. This was first introduced in 1984 by Ostling and Johanson. This assay helps in direct determination of DNA damage, it can also find the difference in damage in a cell population (Fairbairn et al., 1995). The assay relies on the relaxation of supercoiled DNA (due to strand breaks) in an agarose gel forming comet like appearance while the electric field draws the DNA in the direction of the anode (Azqueta & Collins, 2013). Length of the tail and the DNA distribution present in each tail is called as an olive tail moment. Shape, size and amount of DNA of each comet have direct relation to the extent of DNA damage (Kumaravel et al., 2009). The present study reported comet formation of MCF-7 DNA, when treated with *F. drupacea* fruit extract. DNA strand breaks and fragmentation might have results from both necrosis and apoptosis. A comet with a shorter tail and most of its DNA concentrated in its head portion is the characteristic of a necrotic cell, but most of the DNA will be displaced to the tail causing a broadened and long tail in case of apoptosis (Majtnerová & Roušar, 2018). Here in the image provided for Comet assay it is visible that most of the DNA is present in the tail and the DNA damage is from apoptosis.

The comet length was higher in the treated cells when compared to the control cells with a value of 188.2 ± 2.192, whereas the control produced a comet length of 48.17± 0241. Tail length of the control was 1.38 micron and that of the treatment was 38.8 micron indicating huge DNA damages within the treated cell. Genotoxicity inducing ability of *F. drupacea* fruit extracts were visible from the values obtained from the results of the present study. In a similar study using *F. carica* leaf extracts on MCF 10A breast cancer cell line revealed genotoxicity of the extract using comet

assay with an olive tail moment of 175, 186, 165 for the three concentrations tested (Lightbourn & Thomas, 2019). *F. deltoida* var. *kunstleri* aqueous extract imparted genotoxicity and DNA degradation on V79B cells with significant olive tail moment in the comet assay conducted (Muhammad et al., 2021).

Double Staining

Double staining with ethidium bromide (EtBr) and acridine orange (AO) is a versatile technique used in cell biology to categorize cells based on their fluorescence emission and nuclear morphology. Viable cells exhibit bright green nuclei with a well-organized structure, as AO intercalates into their intact double-stranded DNA. Early apoptotic cells display green nuclei with vivid green patches or pieces, indicating perinuclear chromatin condensation while retaining intact membranes. Late apoptotic cells, further along in programmed cell death, exhibit both green and red fluorescence in their nuclei due to EtBr intercalation and may display fragmented nuclei. Necrotic cells, nonviable due to injury, show red fluorescence from both EB and AO, often without intact cell membranes (Liu et al., 2015). Late apoptotic cells feature constricted or broken chromatin and orange to red nuclei. The nuclei of necrotic cells are consistently orange to red and have a well-organized structure. This staining method provides a valuable tool for distinguishing cell states and assessing cellular health in experimental samples. Comparing the *Ficus drupacea* fruit aqueous extract to the control, AO/EB staining for the detection of apoptosis revealed that roughly 70 % of the cells were classified into the category of late apoptotic cells with both green and red fluorescence. Only few cells seem to have intact nuclei with red fluorescence showing that the cells were leading to death through apoptosis but not via necrosis. Nearly all of the cells in control appeared to be alive and fluoresced green. Liu et al. (2015) used AO/EB double staining for the detection of apoptosis in osteosarcoma and found similar results when compared with that of the current study. Cellular morphology analysis is very important in the detection of apoptosis as it have special characters which can distinguish normal live cells from the necrotic or apoptotic cells. The use of *Ficus hispida* leaf extract in the treatment of HT-29 cell lines indicated its ability to induce apoptosis in the cancer cells. Using acridine orange and ethidium

bromide labelling (AO/EB staining), the researchers discovered significant differences in cellular responses between the treatment and control groups. In particular, there was a distinct change in coloring, with the majority of control cells having a primarily green hue in contrast to the test sample (Sathiyamoorthy & Sudhakar, 2018). The current study supports previous research findings by demonstrating a consistent and predictable pattern of apoptosis in MCF-7 cell lines used in this study.

The antiproliferative effects of *F. carica* and *F. salicifolia* latex on the MDA-MB-231 breast cancer cell line were investigated, and it was discovered that these effects were concentration dependant. The experimental treatments also caused microscopic visible cellular abnormalities in these cell lines, including vacuolation in cytoplasm, shrinking of spindle shape, shrunken sphere shape, cell blebbing and uneven cell shape (Alghalban et al., 2021). This previous study's findings are intriguingly similar to those of the present study because the MCF-7 cell lines were treated with four test extract samples for 24 hours, and the results revealed identical morphological changes in the treated cell line, including membrane blebbing, nuclear fragmentation, cell shrinkage, budding, echinoid spikes, condensed nuclei, apoptotic bodies, etc. In the present study, HR-LCMS analysis of the fruit extract of *F. drupacea* revealed phytochemicals such as gallic acid, caffeic acid, quinic acid etc. which comes under the polyphenol group. The presence of polyphenols has been discovered to affect genes that inhibit carcinogenesis by causing cells to undergo apoptosis via both intrinsic and extrinsic pathways (Curti et al., 2017). These phytochemicals individually or in combination with other phytochemicals in the crude extract might have contributed to the antiproliferative action and triggering cell death via apoptosis rather than necrosis.

Cell Cycle Regulation

Cell cycle is a complex process consisting of four distinct phases termed G0/G1, S, G2 and M with multiple checkpoints and closely monitored by many CDKs with its associated cyclin partners. This will ensure the cycle to progress in an error free manner with faithful replication. The cells once exit cell cycle enter in to the

quiescent state named G0 which is regulated by cyclin C/CDK3. The cell enters into cell cycle from this stage only after receiving specific signals such as mitogens, which stimulate synthesis of cyclin D and CDK4/6, which results in the entry to the cell cycle. Once this CDK4/6 complex become active it will phosphorylate RB Protein which in turn unleash E2F transcription factors resulting in the expression of genes for S phase transition along with Cyclin A, E, and cyclin B. CDK2 is activated by this cyclin E which in turn again activates RB and this will lead to the entry into S phase. Cyclin A/CDK2 complex is formed by the end of S phase by the removal of cyclin E by cyclin A. S phase get terminated once CDC6 and E2F1 are phosphorylated by Cyclin A/CDK2. These cells reach the M phase after transitioning from S to G2 and activating CDK1 via cyclin A. PLK1 and aurora proteins are also involved in the progression from G2 to M phase through S phase. Cyclin B/CDK1 will maintain the CDK1 activity throughout mitosis. Once the CDK1 gets deregulated chromosome separation and completion of mitosis and cytokinesis will happen. Furthermore, DNA damage checkpoints protect genomic integrity and initiate cell-cycle arrest via checkpoint kinase 2 (CHK2) and p53 in the G1 phase or by CHK1 in the S or G2 phase. **Fig. 42** depicts the cell cycle regulation by CDKs and check points (Ding et al., 2020). This type of a controlled cell cycle happens in normal cells, which is altered and will bypass one or many check points to gain uncontrolled division in cancer cells. Understanding the mechanism behind antiproliferative effect is critical, particularly in relation to the efficacy in modifying the cell cycle, as it may lead to new discoveries in cancer treatment. The FDA's preliminary approval of palbociclib, a CDK4/CDK6-selective inhibitor, for the treatment of breast cancer marks the first effective clinical translation in this field (Otto & Sicinski, 2017). In the current study, the ability of *F. drupacea* fruit extract in altering the cell cycle is studied. This revealed the ability of the plant extract in arresting the cell cycle at G1 phase.

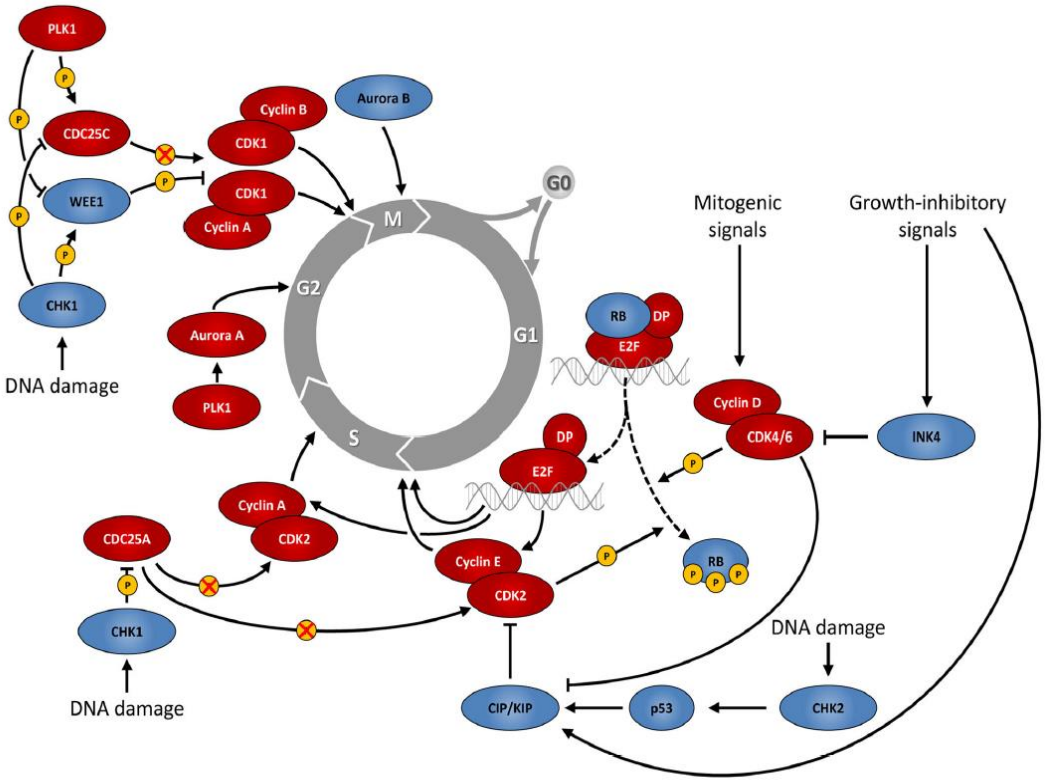


Fig. 42: Cell cycle progression and regulation by CDKs and checkpoints (Otto & Sicinski, 2017).

Close examination of the cell cycle regulation of *F. drupacea* treated MCF-7cell lines revealed that the cell cycle was not progressed from its G1 phase. In comparison to the control cells used in the study, the results showed the DNA content of the treated cells at each stage of the cell cycle. The percentage of DNA in the G1 phase was 86.8 % in the treated cells after 24 hours. The control had a lower DNA content of 66.8 %. Treatment with *F. drupacea* fruit extract caused a similar drop in DNA concentration in following phases of the cell cycle, such as S and G2/M. When the population profile for the control is compared to that of the treatment, the cells seems more scattered in the control. Whereas the cells that seems much aggregated on the first phase is visible with the treatment. From both population profile and DNA content measures it is clear that the cell cycle got arrested in the first stage of cell cycle and the DNA content as well as the cell number in the population profile was found to be accumulated towards the G0/G1 phase of the cell cycle.

In a previous report by Choudhari et al. (2013) after treating cervical cancer SiHa cell lines with *F. religiosa* leaf aqueous extract, cell cycle arrest at G1/S phase was observed. According to the study, this is attributable to increased expression of G1 checkpoint proteins such as P53 and P21, as well as decreased phosphorylation of the pRb tumour suppressor protein. Breast cancer cell lines MDA-MB 231 proliferation is greatly reduced when treated with *F. carica* leaf extract, which is attributable to the extract's ability to cause cell cycle arrest. Flow cytometry results show that S phase of the cell cycle is with the highest number of cells in comparison to all other stages of the cell cycle which support cell cycle arrest in the S phase. The results showed that *F. carica* extract was able to down regulate the transcription of CDK family proteins, resulting in decreased or no synthesis of CDK family mRNA in the MDA-MB 231 breast cancer cell line (Zhang et al., 2018).

In a similar study Saida et al. (2021) revealed the ability of *F. religiosa* latex extracts to impose cell cycle arrest on MDA-MB 231 cell lines at G1 stage of cell cycle by regulating the activity of p53 and by the activation of apoptosis genes including caspases. The studied extract was capable of stopping the cell cycle and directing the cancer cells towards death by activating the genes involved in apoptosis. *F. drupacea* fruit extract is also having the ability to regulate the cell cycle of MCF-7 cell lines and induce cell cycle arrest. *F. dubia* latex has been shown to have antiproliferative action in colorectal cancer cell lines HCT 116 and HT 29. According to the research, the latex extract was able to control cell cycle progression from G1 phase, which uncovered results similar to the current research. This result was attributable to *F. dubia* latex's ability to upregulate p21 and downregulate p-NF-B, cyclin D1, and CDK4 (Hu et al., 2022). The molecular interactions of different proteins and genes found in the MCF-7 breast cancer cell line with various phytochemicals found in the *F. drupacea* fruit extract might have contributed to the cell cycle arrest at G1 phase. Major phytochemicals reported from the fruit extracts of *F. drupacea* include chlorogenic acid, caffeic acid, gallic acid, gravolenic acid, etc. and are highly effective polyphenols. Polyphenols were found to be very effective against cancer in *in vitro* studies. They have multiple mechanism in regulating the uncontrolled cell division by arresting cell cycle, and induce apoptosis by the

upregulation of caspase 3, 8 and 9, releasing cytochrome c by disrupting mitochondrial membrane. They are also reported to have the ability to suppress transcription of STAT 3 as well as NF-kB which will again help in the reduction of cellular proliferation (Alaswad et al., 2021). Chlorogenic acid is one among them with numeral pharmacological properties including anticancer and immune modulatory activities, this particular compound is capable of regulating the cell cycle and also able in directing the cancer cells to undergo apoptosis (Gupta et al., 2022). Gallic acid is a potential substance in containing the uncontrolled growth by halting the cell cycle in G0/G1 phase with accumulation of cells in these phases (Lee et al., 2017). Gallic acid's capacity for activating apoptosis genes were also reported through previous studies. Anticancer properties of gallic acid come from its ability to regulate the genes that control apoptosis, angiogenesis, cell cycle, and metastasis. In order to inhibit the development of cancer, this compound can also activate the ATM kinase signalling pathway (Verma et al., 2013). Caffeic acid, another polyphenol found in *F. drupacea* fruit extract and a common ingredient in many fruits, is capable of inhibiting the growth of various cancers, including breast, lung, liver, cervical, colorectal, prostate, and oral cancers. Caffeic acid can imitate antioestrogen activity, causing disrupted cell cycles and inhibiting cell proliferation (Alam et al., 2022).

Gene expression studies

The antiproliferative activity of *F. drupacea* on the MCF-7 cell line demonstrated the potential ability of the fruit extract to govern and direct cancer cell apoptosis. The results could potentially demonstrate the extract's usefulness in controlling the cell cycle by stopping it at the G1 stage. Gene expression investigations were carried out to uncover the molecular basis of cell cycle arrest induction. The study discovered amplified expression of p53 genes, as well as a suppressed production of STAT 3 proteins when compared to the internal reference control GAPDH which explains why MCF-7 cells treated with *F. drupacea* extract were arrested in the G1 phase of the cell cycle. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a housekeeping gene commonly used in the gene expression studies of various *in vitro* cancer cell lines (Wang et al., 2023). Gene

expression analysis employing RTPCR requires an internal reference control that is expressed consistently in all cells, regardless of their state, stage, therapies, or even disease situations (Aithal & Rajeswari, 2015). The reliability of the gene expression outcomes is completely dependent on the reference gene employed in the investigation. One of the housekeeping genes that is utilized as a reference is GAPDH since it expresses itself constitutively in cells. The stability of housekeeping gene expression under the experimental conditions aids improved comprehension of other genes under research. Seven transcription factors make up the Signal Transduction and Activator of Transcription (STAT) family, the most prevalent of which is the oncogene STAT3 (STAT1, 2, 3, 4, 5a, 5b, 6). When they are not activated, they remain as homodimers, but when they are phosphorylated in response to particular signals, they dimerize, enter the nucleus, and operate as the transcription factor for target genes involved in cell growth, division, death, immune responses, etc. **Fig. 43** represent the functions and regulation of STAT3. Recent research has demonstrated that STAT is inevitably involved in the development of many malignant tumours. Due to genetic mutations or the inactivation of crucial phosphor tyrosine regulators, STAT is constitutively activated in many tumours (Kim et al., 2018). Regulating STAT3 may have a major impact on cancer cell lines, making it a molecular target that is still being studied for cancer therapy. Even if negative regulators of STAT 3 (PIAS, SOCS, PTP) is discovered their mechanism of action during carcinogenesis and in anticancer therapy is still unknown (Wu et al., 2019; Kim et al., 2018). In an experiment where STAT 3 expression in oesophageal cancer cell lines ECA 109 was knocked down, the cells showed the down regulation of STAT3 is inducing apoptosis in the studied cell line with visible microscopic changes including membrane blebbing, apoptosis vacuole formation etc. in a time-dependent manner. The same procedure, according to the study, resulted in cell cycle arrest at the G1 phase of the cell cycle. This work suggests that STAT3 downregulation can induce apoptosis as well as cell cycle arrest in the G1 phase (Zhou et al., 2018).

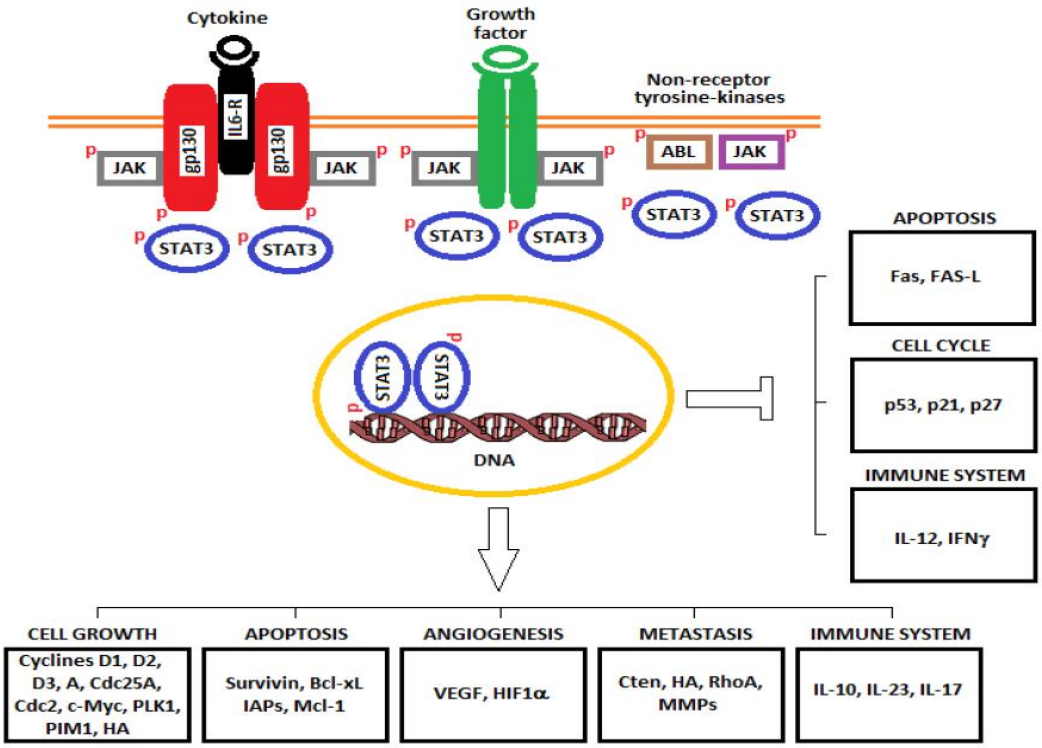


Fig. 43: STAT3 regulation in cell growth, apoptosis, angiogenesis, invasion, metastasis and in the immune system

The antiproliferative effect of *F. drupacea* fruit extract is thus supported by the downregulation of STAT 3 by the extract. More research is required to determine which negative regulators were activated as a result of the treatment and are accountable for STAT3's downregulation. Similar outcomes against human colorectal cancer cells were reported using *Hedyotis diffusa* extracts. This was owing to apoptosis activation via IL-6-inducible STAT3 pathway, which is accomplished by decreasing the expression of cyclin D1, Bcl-2, and by increasing the expression of the Bcl2 related X protein (Lin et al., 2015). In a previous report on the triple negative MDA-MB-231 breast cancer cell lines the antiproliferative action of the cancer cells were attained by the down regulation of STAT3 as well as AKT, NF kB signalling. These results were also in accordance with the present study representing the importance of STAT3 in cancer therapy (Lee et al., 2020). An et al. (2020) reported the antiproliferative action of *Oxalis obtriangulata* in pancreatic cancer cell lines due to regulatory effect of the extract on ERK/Src/STAT3 pathways.

The gene TP53 encodes p53, a transcription factor. This plays a crucial function in the regulation of cellular growth and is a prominent tumour suppressor; it is a critical regulator of tumour suppressor pathways that play roles in cell cycle arrest, DNA repair, senescence, and apoptosis. This is why p53 is known as the guardian of the genome or molecular policeman (Lane, 1992). P53 can prevent carcinogenesis caused by DNA damage by directing cells with DNA damage to perform DNA repair, cell cycle arrest or, in the case of irreparable damage, apoptosis. P53 mutations are quite prevalent in wide range of cancers. P53 is a homo tetramer that contributes to normal cellular activity. This is activated as part of the cell's stress response to things like DNA damage and oncogenic stress. P53 is typically expressed at lower level in normal cells due to the feedback regulation of the E3 ubiquitin-protein ligase MDM2, which destroys p53 via proteasome degradation. Phosphorylation of both proteins caused by stress prevents p53 from being degraded, which causes it to undergo acylation, accumulate in the cell, and become active (**Fig. 44**) (Bykov et al., 2018). The accumulation of p53 in the cell activates its proapoptotic properties. Activated p53 in the cell, on the other hand, aids in cell cycle arrest and directs the cell to either DNA repair or apoptosis (Ozaki & Nakagawara, 2011). The potential cause of the P53 accumulation in MCF-7 cell lines could be attributed to the phenolic chemicals found in the fruit extract. Specifically, there have been studies of gallic acid's capacity to upregulate P53 in MCF-7 cell lines and subsequently trigger apoptosis (Rezaei-Seresht et al., 2019). Chlorogenic acid is another phenolic molecule that can limit metastasis while also contributing significantly to anticancer action by upregulating a number of master genes, including P53 and P21 (Huang et al., 2019).

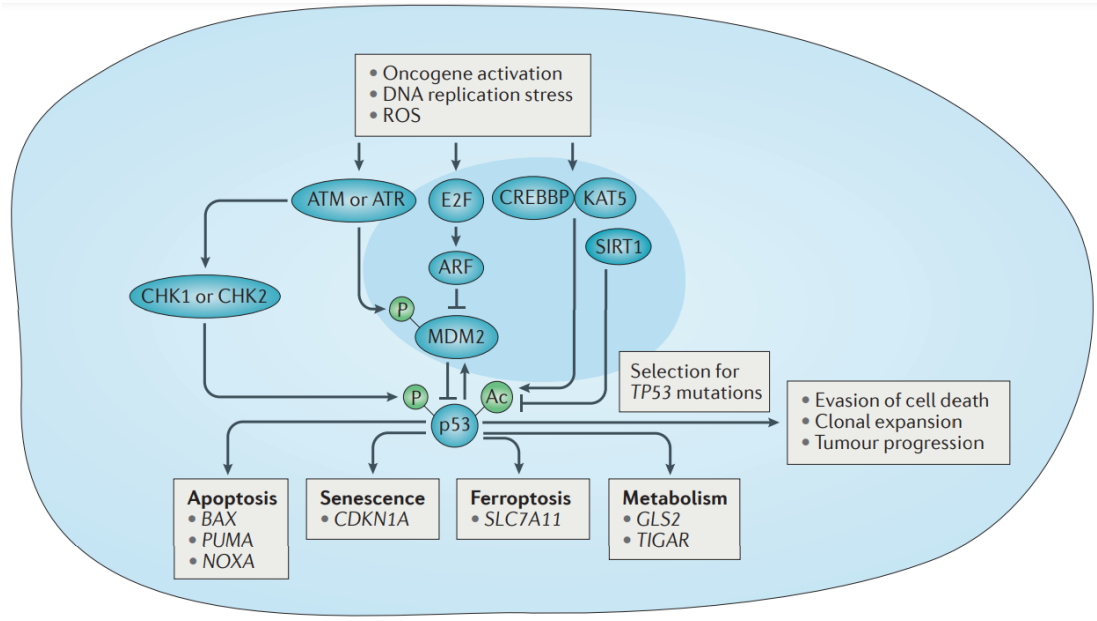


Fig. 44: p53 response to oncogenic stress at early stages of tumour development (Bykov et al., 2018)

In a previous report, the ethanolic extract of *Scutellaria baicalensis* were able to induce cell cycle arrest in G1 phase and induced apoptosis due to the upregulation of the activity of p53 and Bax protein in human lung cancer cell lines (Gao et al., 2011). *F. religiosa* bark aqueous extract was reported to have shown antiproliferative activity against human cervical cancer cell lines. This was due to the cell cycle arrest in the G1 stage which directed the cancer cells to apoptosis via upregulation of p53, p21 and Rb (Choudhari et al., 2013). Lung cancer cell lines H1573, H1437 were treated with methanolic and water extracts of *Drimia calcarata*. The extract caused the H1573 cells to undergo cell cycle arrest in the G0/G1 phase of the cell cycle independently of p53, whereas the H1437 cells had cell cycle arrest at the S phase in a pathway that is dependent on p53 (Laka & Mbita, 2022). Because of their respective biological functions—one is a tumour suppressor and the other is an oncogene—p53 and STAT3 interact negatively with one another. All of these earlier studies provide support for the present research findings, which show that overexpression of p53 and downregulation of STAT 3 have contributed largely to the cell cycle's arrest in the G1 phase and the induction of apoptosis.

PART III

Silver nanoparticle biosynthesis

The four extracts selected for the study were all used to biosynthesize silver nanoparticles. Plant extracts, such as those from *F. drupacea* and *F. exasperata*, contain a variety of phytochemicals that belong to the class of glycosides, phenols, terpenoids, and alkaloids. These phytochemicals may have served as a capping and reducing agent during the process of silver nanoparticles bio fabrication using the plant extract and silver nitrate solution.

Primary indication and confirmation for silver nanoparticle biosynthesis is the colour change that happens with the reaction mixture. When *F. drupacea and F. exasperata* were treated with silver nitrate solution, *F. drupacea* leaf extract readily produced a colour change to the constitutive brick red coloration. The rest of the three extracts made colour change in the order FDFE, FEFE and FELE. Colour change in the solution is mediated by an optical phenomenon called localised surface plasmon resonance. This is an optical phenomenon created by light, when the light is trapped between the nanoparticles which interacts with the conductive electrons band in the surface of nanoparticles (Petryayeva & Krull, 2011). This interaction produces local plasmon oscillation which is strongly dependent on the various characters such as the size, dielectric moment, geometry etc. of the particles. Energy levels of d-d transition gives local plasmon resonance of silver nanoparticles under the visible spectrum which leads to the colour change of the reaction mixture to brick red colour when silver nanoparticles are formed (Liz Marzan, 2006).

UV-vis spectroscopy was used to further characterize the green nanoparticles derived from *F. exasperata* and *F. drupacea*. Since UV-vis spectroscopy is straightforward, quick, easy to apply, sensitive, selective for various forms of nanoparticles, and trustworthy for comprehending the production and stability of nanoparticles, it can be utilized as the ideal technique in the fundamental characterisation process. Peaks were formed by the surface plasmon resonance in the visible light region, which is easily measured using UV-vis spectroscopy. The four extracts used in the current study produced UV-vis peaks that falls below the inherent peaks of the silver nanoparticle which corresponds to 430 nm. Shift in the peak from 430 nm is due to the polydisperse heterogenous nature of silver nanoparticles formed

in the solution (Patil & Chougale, 2021). The extracts formed peaks from 413 nm to 455 nm which shows the polydispersed and heterogenous nature of biosynthesised silver nanoparticles from the current study. This is consistent with the earlier research on the biosynthesis of silver nanoparticles from *Ficus sycomorus* (Salem et al., 2014). Similar results were also reported from *F. ingens* leaf mediated silver nanoparticles which were able to produce a characteristic peak at 425 nm.

In the present research, SEM analysis was used to confirm the formation of silver nanoparticles. Scanning electron microscope was utilized to examine the oven-dried sample of the four nanoparticles biosynthesised in the present study. SEM is a standard instrument used for assisting the analysis of surface morphology, forms, and size distribution of the biosynthesised nanoparticles since it can provide complete details about the surface of the particles. When high-energy electron beams scan the surface of nanoparticles, they interact with atoms on the surface, producing signals that provide details about the surface topography and composition of the particles being studied. Silver nanoparticles biosynthesised from *F. exasperata* and *F. drupacea* were having a particle size which comes under 100 nm. Four extracts used in the study could provide nanoparticles of spherical shape. Highest particle size obtained from the present study was with *F. drupacea* fruit mediated nanoparticle which was having a size range from 28-49 nm. *F. drupacea* leaf extract was able to produce nanoparticles of size 25-26 nm. *F. exasperata* leaf mediated nanoparticles were of size 28-37 nm and its fruit was able to produce a size of 25-32 nm. Small particle size with spherical shape might have contributed largely to the antimicrobial ability of these green nanoparticles from *F. exasperata* and *F. drupacea*. The findings from these two *Ficus* species about spherical silver nanoparticles in the size range below 100 nm are generally consistent with earlier publications by Riaz et al. (2022), and Jacob et al. (2017).

Antimicrobial activities

Antimicrobial properties of nanoparticles are well known, as they are a component of modern mechanisms for combating multidrug resistant organisms. As silver is naturally antimicrobial, it has been used in bacterial combat since antiquity. Nanoparticles become so effective due to high surface area to volume ratio of these particles. The current study revealed the production of silver nanoparticles by

changing the colour of the reaction mixture from pale yellow to brick red. The presence of a conspicuous peak at 450 nm, and its SEM imaging confirmed the particles' spherical shape, which is consistent with previous reports. Mechanism of action of these nanoparticles have been a part of discussion. Nanoparticles start their action by binding to the cell wall and penetrating the cell membranes due to the convenient size and shape as well as the perfect surface area to volume ratio. This step act as the initial step in the silver nanoparticle mediated disruption of microbial system, as this will lead to membrane lesions and leakage of cellular components to the cell exterior. Once the molecules enter into the cell, they readily interact with the cellular components like ribosome and almost all biomolecules which are open and vulnerable to the action of nanoparticles. This includes proteins, lipid, DNA, RNA etc. Interaction of the nanoparticle with cell machinery impairs the function of the cell, which might also lead to the production of ROS. ROS production was observed to be high when the microbial cells were treated with nanoparticle. This will also contribute to the destruction of the cell (Dakal et al., 2016). ROS mediated oxidative damage to the cell membrane and biomolecules in turn decreases the chances for survival. Ag NP can also cause a reduction in ATP production and modify cellular signal system which ultimately lead to the death of the cell (Yin et al., 2020). Damages caused by silver nanoparticle in a bacterial cell is schematically represented in **Fig. 45.**

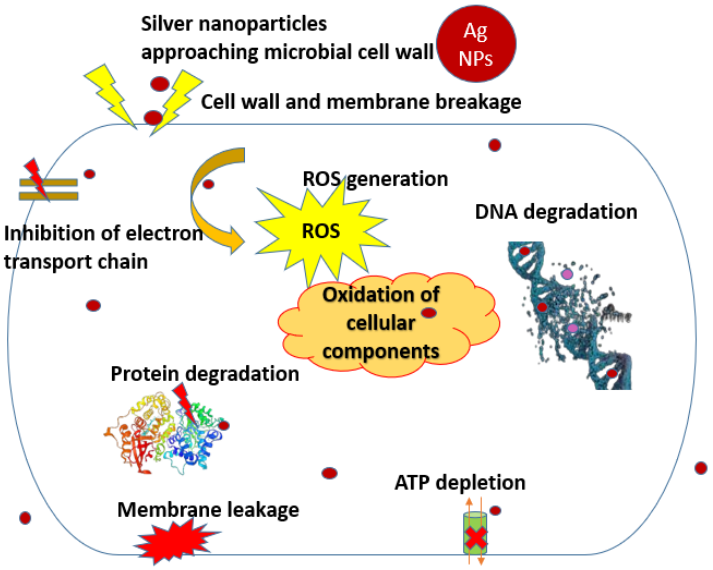


Fig. 45: Schematic representation of silver nanoparticle mediated damage in bacterial cell

Antimicrobial studies were conducted on a group of microorganisms including two fungal strains and 4 bacterial strains. Among the four extracts used in bacterial combat, silver nanoparticles formed out of *F. drupacea* leaf extract was found to be very effective against two fungal strains and two bacterial strains, *Aspergillus niger*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* were the susceptible organisms. Interestingly *Streptococcus mutans*, a gram positive bacteria was able to produce highest inhibition zone (16 ± 0.4691mm) with nanoparticles from the *F. exasperata* leaf extract mediated silver nanoparticles. This finding contradicts the findings of Meikle et al. (2020), who found that silver nanoparticles are more effective against gram-negative bacteria than gram-positive bacteria, which have a thick peptidoglycan covering that act as a barrier to nanoparticle penetration.

Antifungal activity of the silver nanoparticles was visible with the treatments from the four types of nanoparticles of two *Ficus* species. The mechanism of action for silver nanoparticle with fungal strains was previously reported in various studies. AgNps are reported to cause extensive damage to the cell wall and cell membrane components causing the disruption of structural integrity of the hyphae and spores. The respiratory mechanisms gets dysregulated by the inactivation of key enzymes leading to cell lysis and cell death. AgNps could also increase the water permeability of the hyphae, resulting in the swelling and exudation of the cell contents to the exterior. In some other cases, treatment with silver nanoparticles were reported to cause the shriveling, heavy vacuolation and distortion of the fungal hyphae (Li et al., 2022).

Previous reports on the antimicrobial activity of *Ficus sycomorus*, *Ficus benghalensis*, *F. microcarpa*, etc. are all in accordance with this research, demonstrating the effectiveness of silver nanoparticles biosynthesised from the genus *Ficus* as an effective tool in the combat against various types of pathogenic organisms (Salem et al., 2014; Praba et al., 2015; Sudhakar et al., 2017).

PHASE II

PART I

Endophyte Discussion

Even though study on endophytes extends back almost 100 years, this group of organisms remains underexplored. This is why screening the fruit and leaves of *F. drupacea and F. exasperata* for the presence of endophytic fungi become relevant. The current study revealed the presence of endophytes from both the plants. Endophytes from *F. exasperata* and *F. drupacea* were found to possess eleven different fungal species. ITS sequencing of the isolated DNA of pure cultured strains helped in the identification to genus and species level for most of the fungal strains. Fungal endophytes are extremely common, and can be found in all plants in the natural ecosystem. Endophytes are a diverse and dynamic community that may live in complex habitats and have a significant impact on the growth and metabolism of their hosts. They are particularly efficient plant health improvers because they raise plant immunity in resisting infections and improve stress tolerance, making the plant resistant to diverse biotic and abiotic stresses. They also assist the host plant collect biomass by boosting its development and reducing the need for water for the plant's survival (Rudgers et al., 2004). They play roles in the creation of phytohormones, which aid in the uptake of nutrients. They may also aid in the plant's recovery and mitigation of pathogen damage through antibiosis. Moreover, they play a part in the synthesis of lytic enzymes and secondary metabolites (Chaudhary et al., 2022). The function of endophytes in their host plant is depicted in **Fig. 46**.

Selecting an endophytic community is not a random process, but rather one that includes the interaction of multiple complicated components specific to each host. Both the host genotype and abiotic variables influence the formation of a plant's microbial population. Plant microbial community structure is closely monitored. A small number of particular taxa with effective microbial interactions convey their influence to affect the relationship between the microbiome and the plants. Fungal endophytes have the ability to adapt to the genetic and phenotypic characteristics of the host in order to resist infections, allowing the endophytes to survive in harmony

with the host by meeting its needs (Yan et al., 2019). Most of these species reported in the present study have already been described from other plants and demonstrated considerable bioactivities. The isolated species *Phlebia* and *Lasiodiploida psuedotheobromae* support the findings of Schulz et al. (2002). The majority of previous investigations support the current research findings. Do nascimento et al. (2020) discovered *Phanerocheate* species in *Androanthus impetigenosis* that exhibit strong trypanocydal activity. Endophytic *Aspergillus nomiae* from Aloe vera was discovered to have anticancer action against breast cancer cell lines (Mane & Vedamurthy, 2020).

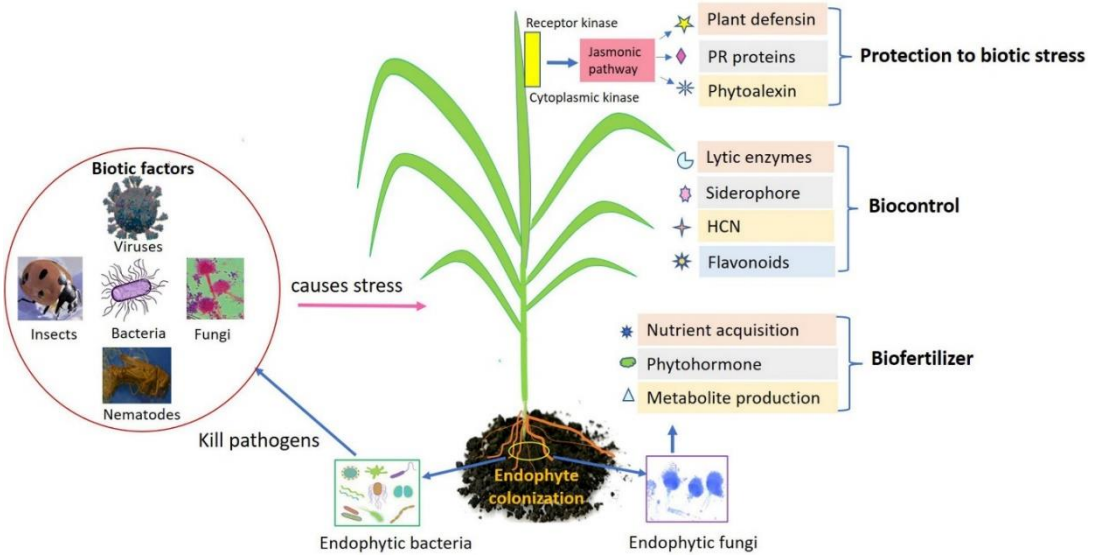


Fig. 46: Role of endophytes in its host plant (Chaudhary et al., 2022).

Daldinia eschscholtzii was also isolated and identified with herbicidal properties against monocotyledonous and dicotyledonous weeds (Flores Reséndiz et al., 2021). In a study on *F. hirta*, researchers discovered *Endomelanconiopsis endophytica*, which contributed two new xyloketals to science (Sun et al., 2016). *Alternaria alternata*, which was isolated from *Camellia sinensis*, produced three novel chemicals. This fungus was also discovered to exhibit antibacterial and cytotoxic properties (Wang et al., 2014). Endophytic *Trichoderma asperellum* was discovered to be particularly effective against the vascular streak dieback disease of cacao (Rosmana et al., 2015). A study on endophytic *Lasiodiploida psuedotheobromae* found that it can inhibit xanthine oxidase (Kapoor & Saxena,

2014). *Malassezia restricta* was also identified as an endophyte in a previous study (Harrison et al., 2016). Endophytic *Phlebia* species were reported to be effective in textile dye decolorization and degradation (Bulla et al., 2017).

Previous reports on this endophytic species have highlighted its usefulness in a variety of sectors, including medicines, biopesticides, and environmental protection. So, all of the isolated endophytes of both plants are critical for revealing their capacities. As the host plants' biological activities vary, so will their biochemical condition. Among the isolates, two leaf endophytes and one fruit endophyte were found to be frequent in both species. Thus it supports the report of Solis et al. (2016) using the leaves of three *Ficus* species, *F. religiosa, F. benjamina*, and *F. elastica.* Phylogenetic distance is inversely related to the frequency of comparable endophytic organisms inside a host species (Solis et al., 2016). The presence of a greater number of ascomycetes and less basidiomycetes in the current study is consistent with previous research findings by the same team. Endophytes were also isolated from the genus *F. religiosa* with high antioxidant in *in vitro* DPPH radical scavenging assay and antidiabetic activity in alpha amylase assay (Jayant & Vijaykumar, 2021). *F. pumila* stem was also reported to have endophytic fungus with antimicrobial activities (Rakshith et al., 2013). Screening for fungal endophytes from *F. exasperata* and *F. drupacea* revealed that there are a number of organisms, which live inside these plants in symbiosis. From the current study using leaves and fruits, higher number of organisms were found to be present in leaves when compared to fruits and the identification of these organisms revealed the presence of common organisms in both plants studied. Further studies are needed for understanding the role of these endophytes within these particular plants.

PART II

Mycochemical Characterization

Endophytes are organism which are dwelling in a quiet unique space as they are adapted to live within the cellular environment of other organisms. Novel biochemical reports are only possible from organisms living in a challenging environment. Endophytes are organism directly coming in contact with the internal

environment of the host species which might contribute a lot in their ability to synthesize novel biochemicals. Moreover, endophyte is a community which seems to be underexplored till date. This is why the biochemical analysis of the endophytic fungus from the fruits of *F. drupacea* shows its importance. Presence of 34 mycochemicals were revealed from this species with HR-LCMS studies conducted. Major compounds such as phenolic acids, alkaloids, terpenoids and protein derivatives were revealed through the study. The mycochemical analysis of *Phlebia*, an endophyte of *F. drupacea* fruits were helpful in understanding the contribution of endophytes in the phytochemistry of the host plant. When the bioactivity of *F. drupacea* fruits were analysed they seems to be having effective potential activity than other three extracts used in the study. So, it should be clear weather the phytochemicals from the plant is shared with that of its endophytic partner or not. Interestingly when the phytochemical constituents of this plant were compared with that of the mycochemical components of its fungal endophyte there were no similar biochemicals reported other than one caffeic acid derivative and some protein derivatives. Number of compounds were also found to be high in the *Phlebia* extract when compared to the phytochemicals reported from the *F. drupacea* fruit extracts.

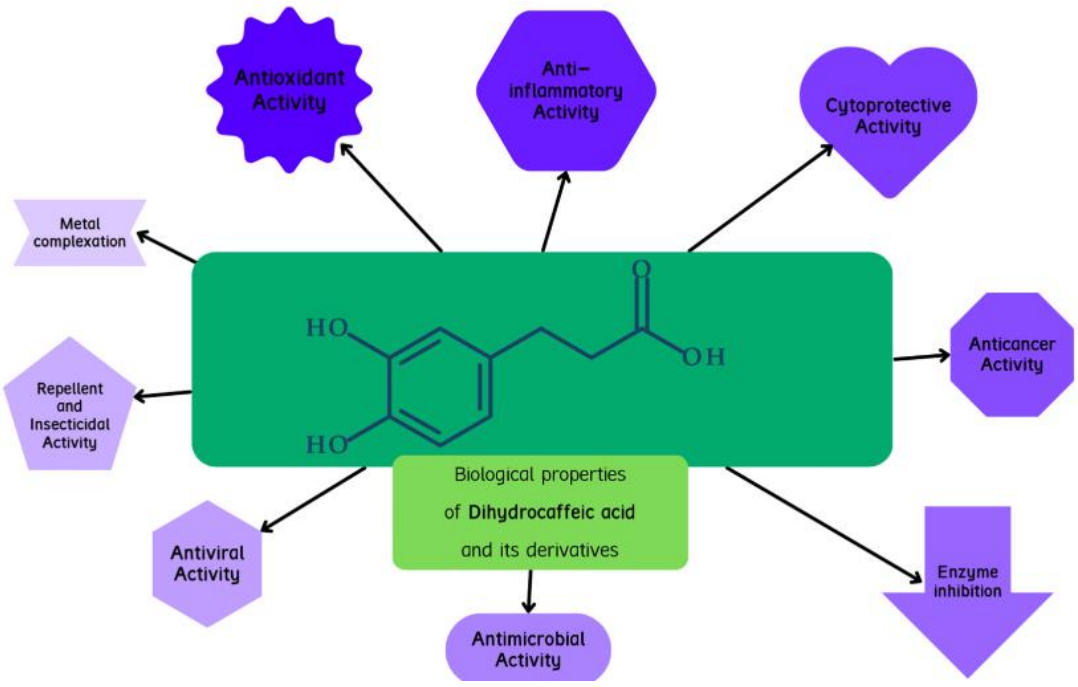


Fig. 47: Activities of dihydrocaffeic acid and its derivatives

When the plant extract revealed caffeic acid a phenolic compound, the mycochemical extract found to be having dihydro caffeic acid 3-O glucuronide which was found to be a phenolic glycoside. Dihydrocaffeic acid, is also chemically known as 3-(3,4-dihydroxyphenyl) propanoic acid. This, comes under phenylpropanoic acid, which is a member of the phenolic acid group. It is distinguished by having a three-carbon side chain with a carboxyl group and a six-carbon aromatic ring. Major bioactivities of dihydro caffeic acid include its antioxidant potential, cytoprotection, anti-inflammatory action, anticancer activities and antimicrobial activity. **Fig.47** explains the activities of dihydrocaffeic acid and its derivatives (Zieniuk, 2023).

Phytosulfokine B is the first identified sulfated penta peptide with two sulfated tyrosine residues, reported in many plants and it is identified as a peptide plant hormone with many growth promoting efficiencies. It can help in somatic embryogenesis in carrots, helps in the cell proliferation of *Oryza sativa*, and also contribute greatly to the adventitious root formation in the hypocotyle of cucumbers (Ding et al., 2023). Isoplumbagin, which is chemically 5-hydroxy-3-methyl-1,4-naphthoquinone is reported from plants like *Lawsonia inermis* and *Plumbago europaea*. This compound is reported to have anti-inflammatory, anticancer and antimicrobial activities (Tsao et al., 2020). Tributyrin is another mycochemical revealed from the extract of endophytic *Phlebia* sp. This is a triglyceride and butyric acid precursor with antiinflammation activities and contribute largely to the intestine health. This chemical is also having role in immunoprotection (Yang et al., 2023). Maculosin is another biochemical with strong antioxidant property and reported from *Streptomyces* sp. (Paudel et al., 2021). Hydroxy citrulline is another compound reported from the present study. Citrulline comes under the group of amino acids which are not proteins. This is synthesized during the arginine pathway. This compound has effective antioxidant activities and also have a major impact on the draught stress regulation in plants (Song et al., 2020). Manumycin A, which is reported from *Streptomyces parvulus* is an antibiotic group with antimicrobial action against various gram-positive bacteria and some fungus. This can also inhibit the development and maturation of some insect groups (Zeeck et al., 1987).

PART III

Bio-Fabrication of Nanoparticles

Nanotechnology is a field which have immense importance in the research field as it has enumerable applications in various fields including in the field of medicine, catalysts, optics, therapeutics etc. Biofabricated nanoparticles seem to be much safe and environmentally friendly than its chemical or physical counterparts as the process itself uses biological agents such as microbial or plant biochemicals with reducing properties. Once these biochemicals help in the reduction of the substrates they can produce nanoparticles which might have these chemicals as their capping agent. Among the biosynthetic methods metal nanoparticle using fungal extracellular extract is having much importance as this fungus can secrete many mycochemicals to its surroundings. The secretome which contain all the secreted biochemicals of the fungi are often rich in phenols, flavonoids etc. When the fungus is an endophyte the possibility to have various biochemicals is increased as its environment is more challenging. In the present study, *Phlebia* is such a species which revealed the presence of 34 mycochemicals through HR-LCMS which falls under various classes such as phenolic acids, alkaloids, terpenes etc. These mycochemicals might have contributed largely in the reduction of silver nitrate as well as aurochloric acid into nanoparticles.

Visual representation is the first way to understand the biofabrication of nanoparticles. This is due to an optical phenomenon called surface plasmon resonance in which the electrons in the conductive band of this biofabricated nanoparticles upon production will interact with the light trapped between them. When this oscillation falls under the visible range region, this will lead to produce a visible colour change. In case of both gold and silver, the surface plasmon resonance have values, which comes under the visible spectrum, giving visible colour change during the reaction. This surface plasmon resonance is totally dependent on the characteristic properties of the nanoparticles produced here, like its size, shape, dielectric moment, geometry etc. (Liz Marzan, 2006).

Once the visual observation made the presence of nanoparticle in the reaction mixture, it should be characterised to know whether the nanoparticle formed here is having the characteristics of the nanoparticles produced from the respective metal, and this can be done by characterisation using UV-vis spectrometry. UV-vis analysis is a reliable cheap and powerful method in the characterisation of nanoparticles as it will produce specific peak for each nanoparticle. UV-Vis data can also produce information about the nature of the nanoparticles formed such as if they are monodispersed or ploy dispersed in nature i.e. the heterogenicity of the particles. When the curve became broad as here in the gold nanoparticles, the nature of particle produced is totally hetero genic. Silver nanoparticle from *Phlebia* didn't produce a peak of monodispersed particles, which is narrow when compared to that of the gold nanoparticle produced here. Gold and silver nanoparticles within the size range of 5-50 nm once formed will be able to produce a peak in UV-vis spectroscopy with values from 520-560 nm and 390-450 nm respectively (Shukla & Iravani, 2017).

SEM anlysis were used to characterise the surface morphology and shape of the bio fabricated nanoparticles. When the SEM analysis of bio fabricated silver and gold nanoparticles were conducted, both silver and gold nanoparticles were found to be more or less spherical in nature. Size of the particles were found to be 20-28 nm and 30-35 nm in nature for both silver and gold respectively.

Antimicrobial Activity

Antimicrobial activities were tested using two gram-negative bacteria *E. coli* and *S. paratyphi.* The ability of silver and gold nanoparticle fabricated from the endophytic fungus *Phlebia* sp. in bacterial combat seems to be effective against the studied gram-negative organisms. *E. coli* is the most extensively researched and well-understood bacterium on earth due to its resilience, flexibility, broad palette, and simplicity of handling. All of these characteristics make *E. coli* a model organism in a variety of scientific fields including antimicrobial activity studies. *Salmonella paratyphi* belongs to the typhoidal *Salmonella* bacteria, which cause bacteraemic paratyphoid fever. This bacterium is extremely adapted to exist only in human systems. Both *Salmonella* and *E. coli* seems to be susceptible to the nanoparticles

produced from the fungal endophyte. Increase in concentration had contributed to the increased zone of inhibition in all the test concentrations studied. At all three test dosages, gold nanoparticles generated comparatively smaller zone of inhibition than silver nanoparticles. In both silver and gold nanoparticles *Salmonella paratyphy* seems to be more susceptible in comparison with the *E. coli* strains.

Silver has been employed in bacterial combat since ancient times due to its intrinsic antibacterial properties. The current study demonstrated the creation of silver nanoparticles by changing the colour of the reaction mixture from pale yellow to brick red. The appearance of a prominent peak at 450 nm, and its SEM imaging, corroborated the particles' spherical shape, which is consistent with prior observations (Aswathi & Thoppil, 2023). Despite the fact that the same extract was used to synthesize both nanoparticles, the biochemicals that cause each particle's reduction may differ, which could account for the silver nanoparticle's superior activity throughout the trial (Ramamurthy et al., 2013). When silver and gold nanoparticles were biosynthesized using cell free *Candida albicans* extracts, silver nanoparticles were found to have better antibacterial power than gold nanoparticles against *E. coli* and *S. aureus*, which are found to be consistent with the current work (Ahmad et al., 2013).

Talaromyces purpureogenus, an endophytic fungus, produced silver nanoparticles with significant antibacterial activity against *S. aureus, B. cereus, S. enterica, Pseudomonas aeruginosa*, and *E. coli*, which is consistent with the current findings (Xiaowen et al., 2019). A similar investigation found that endophyte *Lesiodiplodia theobromae* mediated silver nanoparticles efficiently inhibited the growth of *Pseudomonas aeruginosa* (Adnan, 2020). The antibacterial properties of gold nanoparticles from the endophytic fungus *Phoma* sp. were investigated, and it was discovered to be an efficient antimicrobial agent against phytopathogens *Xanthomonas oryzae* and *Rhizoctonia solani* (Sharma et al., 2022).

There have been reports that green gold nanoparticles can operate as efficient antibacterial agents, despite the fact that ionic gold and gold nanoparticles produced through physiochemical synthesis are not bactericidal. The ability of capping agents,

technique of manufacture, size, shape, concentration, and other parameters all influence the bioactivity of nanoparticles (Zhang et al., 2015). Positively charged gold nanoparticles bind to the negatively charged cell membrane. Their small size and ease of entry into the cell contribute in cellular disintegration, resulting in cellular breakdown and microorganism death (Zhang et al., 2015). Proteins found in the cell play an important role in the normal, healthy functioning of any bacterial cell. Interactions between nanoparticles and protein molecules have a significant impact on the organism's survival. Gold nanoparticles can react with the soft bases containing sulphur and phosphorus, impairing proteins that contain these elements because GNPs actively target them. Gold nanoparticles may bind to thiol groups in respiratory enzymes like NADH dehydrogenase, interrupting the respiratory chain and producing ROS, ultimately leading to the death of the organism (Ahmad et al., 2013). When the particles are nanosized, the surface area in contact with the microbes rises, and a larger surface area gives stronger binding strength to the nanoparticles, which has a significant impact on antibacterial activity.

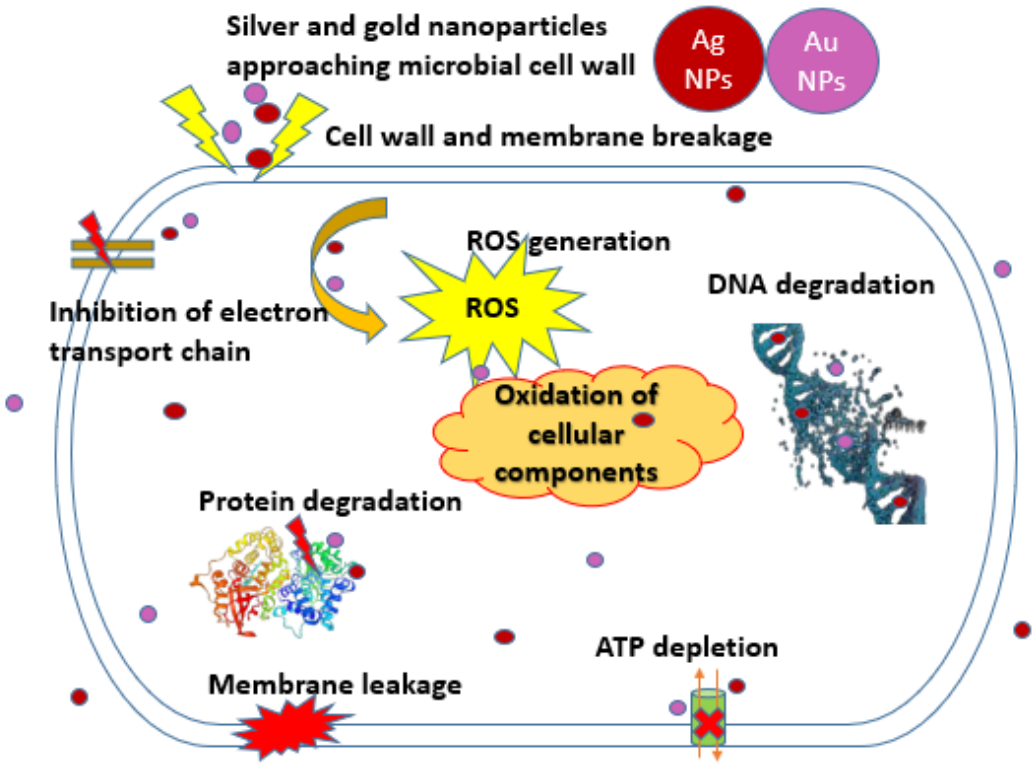


Fig. 48: General antimicrobial mechanism of biofabricated gold and silver nanoparticles (Nushiba & Thoppil, 2023).

Silver nanoparticles have antibacterial effects on gram-negative bacteria by causing cell wall pits. This increases the permeability of the cell wall, leading to the loss of cell content. The mechanism of action of these nanoparticles could also be attributed to their capacity to produce reactive oxygen species (ROS), which lead to oxidative damage, cellular disintegration, and leaking of contents to the cell exterior. Additionally, ROS often result in a decrease in ATP generation and damage to DNA after entering the cell. **Fig 48** represents the general antimicrobial mechanism of gold and silver nanoparticles. The endophyte isolated from the fruits of *F. drupacea* seems to be an effective organism to produce nanoparticles as well as mycochemicals of therapeutic importance as well as biosynthesis of nanoparticles.

SUMMARY AND CONCLUSIONS

Ficus is a genus with strong traditional roots in South Asia, where it has been utilized for food, medicinal, and other reasons. Ayurvedic, Siddha, Unani, and homoeopathic medicine have all made use of medicinal plants like *Ficus carica. Ficus carica* is one of the oldest tree crops, with over 11,000 years of cultivation. It has been used to treat a variety of ailments and conditions, including diabetes, liver disorders, diarrhoea, inflammatory conditions, haemorrhoids, respiratory and urinary problems. *Ficus* is a reliable source of conventional medicine for inflammatory, infectious, epileptic, diabetic, and diarrhoeal conditions. The bark, fruits, leaves, adventitious roots, latex, and seeds are all used for medicinal purposes. Due to its significance and richness, India is a hotspot for *Ficus* species; nonetheless, relatively few of these species have had their active ingredients fully investigated by science. Understanding the phytochemicals and bioactivities of two commonly accessible but little-studied *Ficus* species, such as *F. exasperata* and *F. drupacea*, is the aim of this study. This study also considered the isolation and identification of fungal endophytes from these two plants.

Phytochemicals, found in plants, are divided into primary and secondary metabolites. Primary metabolites, including carbohydrates, proteins, fats, sugars, and esters, directly impact cellular processes and growth. Secondary metabolites, produced from primary metabolites, have been used since 2600 BC for medicine, food, and poisoning. Secondary metabolites are crucial in plant systems for fruit set, abscission, flowering stimulation, perennial growth, signal transduction, defence, and protection. Produced by complex biochemical processes, they accumulate in specialized tissues. secondary metabolites, which are synthesised in response to environmental conditions like pests or illnesses. These compounds have therapeutic properties and are used in traditional medicine for treating and preventing chronic disorders. Phytochemicals, such as flavonoids and antioxidants, contribute to the nutritional value of plant-based foods and can help develop dietary guidelines and functional foods. They also play a key role in agriculture, reducing the use of synthetic

pesticides and promoting sustainable practices. Researchers are studying the therapeutic uses of plant chemicals like alkaloids, phenols, terpenoids, phytosterols, and glycosides, which have gained significant attention in recent years. In the present study, two therapeutically and traditionally important *Ficus species, Ficus exasperata and Ficus drupacea* is analysed for the presence of important phytochemicals they produce and the bioactivities of these particular phytochemicals. The preliminary phytochemicals with a number of biochemical assays and a detailed metabolite profiling using HR-GC/MS for the volatile components and, HR-LC/MS for non-volatile components were conducted. Ninety-two compounds were revealed in the LC/MS and nine compounds were revealed in the GC/MS. The compounds reported in the study were all very important phytochemicals with number of reported bioactivities from the earlier literature. Phytochemicals such as gallic acid, chlorogenic acid, catechol etc. were the important phytochemicals revealed. And they might have been the reason of the specific bioactivities determined through the study.

Antioxidants are compounds that inhibit oxidation and are found in both endogenous and exogenous forms. Exogenous antioxidants, such as vitamins and carotenoids, enter the system through food or supplements. Endogenous antioxidants, produced by metabolism, can be enzymatic or nonenzymatic. They protect cells by preventing free radical reactions, repairing damage, and inactivating harmful byproducts of various cellular reactions. Free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in aerobic organisms during mitochondrial respiration. When balanced, they play different roles in cell metabolism, growth, differentiation, and migration. Polyphenols and flavonoids found in plants have antioxidant properties. *Ficus exasperata* and *Ficus drupacea* when analysed for the antioxidant activities using various *in vitro* biochemical assays such as DPPH assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay, superoxide scavenging assay, the four plant extracts were found to be moderative in action when they were compared to that of the controls considered for the study. Even though the antioxidant action was nearly similar with the four types of extracts considered for the study, *F. drupacea* leaves and fruits were bit better antioxidant

when compared to the *F. exasperata* extracts. This is mainly due to the presence of various polyphenols in the *F. drupacea* extract.

Cancer is a disorder in which a cell lacks the capacity to respond to normal cell growth and division signals, resulting in tumours that infiltrate healthy tissues and spread throughout the body. This loss of control is frequently caused by dynamic genetic changes, such as mutations in oncogenes and tumour suppressor genes. Cancer can arise from a variety of tissues or cells, resulting in more than 100 distinct forms. Tumours can be benign or malignant, with benign tumours remaining localized and easily eliminated via surgery or radiation. Common causes of tumour growth include mutations, chemical compounds, smoking, viral and bacterial infections, and epigenetics. Breast cancer is a frequent malignancy in women, accounting for an estimated 2.3 million new occurrences yearly. It is among the most common cancers and the sixth largest cause of cancer-related mortality. Several non-modifiable and modifiable risk factors contribute to breast cancer development. Despite the fact that women in high-income nations have a higher incidence of breast cancer, low-income countries have the highest death rate. Medical research is currently focused on developing a low-risk, cost-effective natural product derived from biochemicals that can successfully battle many types of cancer. The current study primarily focuses on the *in vitro* anticancer activity of the selected *Ficus* species in breast cancer.

The study revealed that *F. drupacea* fruit extract was the most effective anticancer agent among the two plants. Additional investigation and identification of the primary phytochemical in this extract may make it a viable medication candidate for cancer treatment. This extract was shown to selectively destroy the breast cancer cell line MCF without significantly harming normal cells. Since fig fruits are edible *F. drupacea* fruits can be added to food as such or as modified products in the future so that people can benefit from the antioxidant and anticancer potential of the fruit extract through their diet. This plant and its fruits are fairly widespread, but people aren't consuming them because of the bland taste and a lack of awareness about its therapeutic potential.

Nanoparticles have been used for medical purposes since antiquity. Plant extracts have been transforming them into nanoforms since the early twentieth century. Nanotechnology is gaining popularity for its potential applications in electronics, chemistry, energy, and medicine. Nanoparticles have a high surface area to volume ratio, which improves heat conductivity, catalytic activity, and chemical stability. Spray pyrolysis, vapour deposition, mechanical grinding, chemical precipitation, electromagnetic levitation, ball milling, and chemical reduction are some of the techniques utilized for producing them. Green synthesis, a technology derived from plants, bacteria, fungi, and algae, is both environmentally friendly and superior to traditional nanoparticle production methods. The current study attempted the biosynthesis of silver nanoparticles from four extracts, which were characterized by UV-vis spectroscopy and SEM analysis, demonstrating the presence of roughly spherical silver nanoparticles. Nanoparticles were tested for antibacterial activity against several organisms, including fungal and bacterial species. Although numerous extracts have shown antibacterial action, it is impossible to select a single nanoparticle as the best effective as each organism reacts differently.

Endophytes are endosymbiotic organisms that live within plants without causing disease symptoms. They consist of viruses, bacteria, archaea, fungi, oomycetes etc. Endophytes are gaining popularity for their ability to synthesize new natural compounds for medical purposes and mimic host biochemicals, giving them an eco-friendly option for phytochemical biosynthesis. The connection between hosts and endophytes is complex and interdependent, and even little changes in host gene expression can affect endophyte gene expression. Endophytes help the host in a variety of ways, including reducing herbivory, priming host plant defensive responses, resolving abiotic and biotic stress, and protecting against ROS. The current work aimed to isolate fungal endophytes from both plants, and 11 were successfully isolated and identified by ITS sequencing and BLAST analysis. Fruit appears to contain less endophytes than leaves. Mycochemical analysis of fruit endophytes revealed thirty-two biochemicals that were almost wholly distinct from those found in the host fruit. These phytochemicals could produce both silver and gold nanoparticles with antibacterial capabilities.

Deliverables

- The phytochemical analysis demonstrated that two figs are (*F. exasperata* and *F. drupacea*) stores of biochemicals with medicinal significance.
- Evaluating the antioxidant capacity provided insight into their moderate ability to scavenge free radicals.
- The extract displayed antitumor efficacy against breast cancer cell lines.
- The fruit extract of *F. drupacea* triggered both cell cycle arrest and apoptosis on MCF breast cancer cell lines.
- Plant extracts shown exceptional efficacy in the synthesis of silver nanoparticles possessing antibacterial properties.
- Research on the endophytic fungal community has shown that these plants harbour a variety of endophytes.
- The isolates of endophytes were also highly successful in generating a wide range of myco-chemicals that can be used to create antibacterial silver and gold nanoparticles.

RECOMMENDATIONS

- ✓ Comprehend the nutrient profile and acquaint the public with fruit-derived nutraceuticals
- ✓ *In vivo* investigations employing animal models to enhance comprehension of these phytochemicals on living organisms
- ✓ Molecular docking analysis to get more insight into a potential therapeutic candidate derived from the phytochemicals obtained through this investigation
- ✓ Comprehending and utilizing green nanoparticles in diverse biological domains
- ✓ Determine endophytes that are not fungal colonists
- ✓ Characterizing the biochemical similarities between host plant and its endophytic organisms by analysing the biochemicals of the entire endophytic community.
- ✓ Explore the bioactivities of endophytic biochemicals to better grasp their potential in diverse fields

REFERENCES

Abbooda, K. W., Ibrahim, N. A., Yaseen, N. Y., Wang, Z., &Chowd, M. S. (2014). Cytotoxic effect *of Ficus religiosa* leaves chloroform extract on cancer cell lines. *The Journal of Applied Biochemistry*, *107*(2), 181-188.

Abdel Aziz, H. M., Farag, R. S., & Abdel-Gawad, S. A. (2019). Carbamazepine removal from aqueous solution by green synthesis zero-valent iron/Cu nanoparticles with *Ficus benjamina* leaves' extract. *International Journal of Environmental Research*, *13*, 843-852. https://doi.org/10.1007/s41742-019-00220-w

Abdel Rahman, R., Ghoneimy, E., Abdel-Wahab, A., Eldeeb, N., Salem, M., Salama, E., & Ahmed, T. (2021). The therapeutic effects of *Ficus carica* extract as antioxidant and anticancer agent. *South African Journal of Botany*, *141*, 273-277.https://doi.org/10.1016/j.sajb.2021

Abdou, R., Alqahtani, A. M., & Attia, G. H. (2021). Bioactive metabolites of *Aspergillus neoniger*, an endophyte of the medicinal plant *Ficus carica*. *Indian Journal of Pharmaceutical Sciences*, *83*(1), 101-109. 10.36468/pharmaceutical-sciences.755

Abdou, R., Attia, G. H., Mojally, M., Dawoud, M., & Rateb, M. E. (2022). Bioguided isolation of alternariol derivatives from *Ficus*-derived endophyte *Alternaria alternata*. *Indian Journal of Pharmaceutical Education and Research*, *56*(2), 497-502.

Abdou, R., Mojally, M., & Attia, G. H. (2021). Investigation of bioactivities of endophytes of *Ficus carica* L. Family Moraceae. *Bulletin of the National Research Centre*, *45*, 1-7. 10.1186/s42269-021-00505-1

Abotsi, W. M., Woode, E., Ainooson, G. K., Amo-Barimah, A. K., & Boakye-Gyasi, E. (2010). Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv. (Moraceae). *Pharmacognosy Research*, *2*(2), 89–97. https://doi.org/10.4103/0974-8490.62958.

Abubakar, U. S., Danmalam, U. H., Ahmed, A., Abdullahi, S., Abba, A., & Rukayya, N. (2016). GC-MS Analysis of ethyl acetate extract of *Ficus sycomorus* root Linn. *Journal of Pure and Applied Sciences*, *9*(2), 30-34. http://dx.doi.org/10.4314/bajopas.v9i2.6

Adefegha, S. A., Oboh, G., Ejakpovi, I. I., & Oyeleye, S. I. (2015). Antioxidant and antidiabetic effects of gallic and protocatechuic acids: a structure–function perspective. *Comparative Clinical Pathology*, *24*, 1579-1585. 10.1007/s00580-015-2119-7

Adegoke, A.A., Iberi, P. A., Akinpelu, D. A., Aiyegoro, O. A., &Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potential of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, *3*(3), 6-12

Adnan, M. (2020). Synthesis, characterization and applications of endophytic fungal nanoparticles. *Inorganic and Nano-Metal Chemistry*, *51*(2), 280-287. https://doi.org/10.1080/24701556.2020.1784231

Ahmad, T., Wani, I. A., Manzoor, N., Ahmed, J., & Asiri, A. M. (2013). Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. *Colloids and surfaces. B: Biointerfaces*, *107*, 227–234. https://doi.org/10.1016/j.colsurfb.2013.02.004

Ahmad, V., Jamal, Q. M. S., Shukla, A. K., Alam, J., Imran, A., & Abaza, U. M. (2017). *Bacilli* as biological nano-factories intended for synthesis of silver nanoparticles and its application in human welfare. *Journal of Cluster Science*, *28*, 1775-1802. https://doi.org/10.1007/s10876-017-1206-0

Ahmed, F., & Urooj, A. (2010). Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: A review. *Pharmaceutical Biology*, *48*(6), 672–681. https://doi.org/10.3109/13880200903241861

Ahmed, F., Mueen Ahmed, K. K., Abedin, Z., & Karim, A. A. (2012). Traditional uses and pharmacological potential of *Ficus exasperata* Vahl. *Systematic Reviews in Pharmacy*, *3*(1), 15-23. DOI: 10.4103/0975-8453.107131

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, *7*(1), 17-28. http://dx.doi.org/10.1016/j.jare.2015.02.007

Ahmed, Y., Zhong, J., Yuan, Z., & Guo, J. (2022). Roles of reactive oxygen species in antibiotic resistant bacteria inactivation and micropollutant degradation in Fenton and photo-Fenton processes. *Journal of Hazardous Materials*, *430*, 128408. https://doi.org/10.1016/j.jhazmat.2022.128408.

Aithal, M. G., & Rajeswari, N. (2015). Validation of housekeeping genes for gene expression analysis in glioblastoma using quantitative real-time polymerase chain reaction. *Brain Tumour Research and Treatment*, *3*(1), 24–29. https://doi.org/10.14791/btrt.2015.3.1.24

Aja, P. M., Uguru, C., Nweke, O. L., Enechi, O. C., Ozougwu, V. E., & Okoro, C. O. (2017). Gas chromatography-Mass spectrometric (GC-MS) analysis of *Ficus capensis* leaf from Abakaliki, Ebonyi state, Nigeria. *Journal of Pharmaceutical Sciences and Research, 9*(9), 1562-1567. https://doi.org/10.5829/idosi.ijpms.2016.6.1.1119

Akanni, O. O., Owumi, S. E., & Adaramoye, O. A. (2014). *In vitro* studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperate* and *Kigelia africana*. *Asian Pacific Journal of Tropical Biomedicine*, *4*(1), 492–499. https://doi.org/10.12980/APJTB.4.2014C581

Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., & Wilkinson, G. S. (2015). Cancer across the tree of life: Cooperation and cheating in multicellularity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1673), 1-21. https://doi.org/10.1098/rstb.2014.0219

Al Zahrani, N. A., El-Shishtawy, R. M., & Asiri, A. M. (2020). Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. *European Journal of Medicinal Chemistry*, *204*, 1-37. https://doi.org/10.1016/j.ejmech.2020.112609

Alam, M., Ahmed, S., Elasbali, A. M., Adnan, M., Alam, S., Hassan, M. I., & Pasupuleti, V. R. (2022). Therapeutic implications of caffeic acid in cancer and neurological diseases. *Frontiers in Oncology*, *12*, 1-18. https://doi.org/10.3389/fonc.2022.860508

Alaswad, H. A., Mahbub, A. A., Le Maitre, C. L., & Jordan-Mahy, N. (2021). Molecular action of polyphenols in leukaemia and their therapeutic potential. *International Journal of Molecular Sciences*, *22*(6), 30-85. https://doi.org/10.3390/ijms22063085

AlGhalban, F. M., Khan, A. A., & Khattak, M. N. K. (2021). Comparative anticancer activities of *Ficus carica* and *Ficus salicifolia* latex in MDA-MB-231 cells. *Saudi Journal of Biological Sciences*, *28*(6), 3225-3234.

Ali, B., Mujeeb, M., Aeri, V., Mir, S. R., Faiyazuddin, M., & Shakeel, F. (2012). Anti-inflammatory and antioxidant activity of *Ficus carica* Linn. leaves. *Natural Product Research*, *26*(5), 460–465. https://doi.org/10.1080/14786419.2010.488236.

Ali, M., Qadir, M. A., & Saleem, M. (2017). Medicinal uses of eugenol (a phytochemical from essential oils). *The Journal of Essential Oil Research*, *29*(4), 269-277. 10.1155/2021/2497354

AlMasoud, N., Alhaik, H., Almutairi, M., Houjak, A., Hazazi, K., Alhayek, F, & Awad, M. A. (2021). Green nanotechnology synthesized silver nanoparticles: Characterization and testing its antibacterial activity. *Green Processing and Synthesis*, *10*(1), 518-528. https://doi.org/10.1515/gps-2021-0048

Al-Qahtani, K. M. (2017). Cadmium removal from aqueous solution by green synthesis zero valent silver nanoparticles with *Ficus Benjamina* leaves extract. *Egyptian Journal of Aquatic Research*, *43*(4), 269–274. https://doi.org/10.1016/j.ejar.2017.10.003

AlShaal, S., Daghestani, M., & Karabet, F. (2019). Determination of the isolated rutin and quercetin contents in Syrian *Ficus carica* L. leaves extracts. *Journal of the Turkish Chemical Society*, *7*(1), 197-206. https://doi.org/10.18596/jotcsa.622442

Althurwi, H. N., Soliman, G. A., Abdel-Rahman, R. F., Abd-Elsalam, R. M., Ogaly, H. A., Alqarni, M. H., Albaqami, F. F., & Abdel-Kader, M. S. (2022). Vulgarin, a sesquiterpene lactone from *Artemisia judaica*, improves the antidiabetic effectiveness of glibenclamide in streptozotocin-induced diabetic rats *via* modulation of PEPCK and G6Pase genes expression. *International Journal of Molecular Sciences*, *23*(24), 1-16. https://doi.org/10.3390/ijms232415856

Alvarado Marchena, L., Schmidt Duran, A., Alvarado Ulloa, C., Chacón Cerdas, R., & Flores Mora, D. (2016). Molecular characterization of the endophytic bacteria found in the fig crops (*Ficus carica* var. Brown Turkey) in Costa Rica. *American Journal of Agricultural and Biological Science*, *11*(7), 290-297. DOI: 10.3322/ajbs.2016.110702

Aly, H. M., El-Sayed, A. B., Gohar, Y. M., & Salem, M. Z. (2013). The Value-added uses of *Ficus retusa* and *Dalbergia sissoo* grown in Egypt: GC/MS analysis of extracts. *Journal of Forest Products and Industries*, *2*(3), 34-41.

Amponsah, I. K., Fleischer, T. C., Annan, K., Dickson, R. A., & Sarpong, F. M. (2013). Anti-inflammatory, antioxidant and antimicrobial activity of the stem bark extract and fractions of *Ficus exasperata* Vahl. (Moraceae). *Journal of Pharmacognosy and Phytochemistry*, *2*(3), 38-44.

An, E. J., Kim, Y., Lee, S. H., Ko, H. M., Chung, W. S., & Jang, H. J. (2020). Anti-cancer potential of *Oxialis obtriangulata* in pancreatic cancer cell through regulation of the ERK/Src/STAT3-mediated pathway. *Molecules*, *25*(10), 2301. https://doi.org/10.3390/molecules25102301

Anand, U., Pal, T., Yadav, N., Singh, V. K., Tripathi, V., Choudhary, K. K., & Singh, A. K. (2023). Current scenario and future prospects of endophytic microbes: Promising candidates for abiotic and biotic stress management for agricultural and environmental sustainability. *Microbial Ecology*, *86*, 1455–1486. https://doi.org/10.1007/s00248-023-02190-1

Andrade, C., Ferreres, F., Gomes, N. G. M., Duangsrisai, S., Srisombat, N., & Vajrodaya, S. (2019). Phenolic profiling and biological potential of *Ficus curtipes* Corner leaves and stem bark: 5-Lipoxygenase inhibition and interference with NO Levels in LPS-Stimulated RAW264.7 Macrophages. *Biomolecules*, *9*(9), 400, 1-17. https://doi.org/10.3390/biom9090400

Anigboro, A. A., Avwioroko, O. J., Ohwokevwo, O. A., & Pessu, B. (2019). Bioactive components of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts and evaluation of their antioxidant properties. *EurAsian Journal of BioSciences*, *13*(2), 1763-1769.

Antony, J. J., Sithika, M. A., Joseph, T. A., Suriyakalaa, U., Sankarganesh, A., Siva, D., Kalaiselvi, S., & Achiraman, S. (2013). *In vivo* antitumor activity of biosynthesized silver nanoparticles using *Ficus religiosa* as a nanofactory in DAL induced mice model. *Colloids and Surfaces. B, Biointerfaces*, *108*, 185–190. https://doi.org/10.1016/j.colsurfb.2013.02.041.

Ao, C., Deba, F., Tako, M., & Tawata, S. (2009). Biological activity and composition of extract from aerial root of *Ficus microcarpa* L. *International Journal of Food Science & Technology*, *44*(2), 349-358. https://doi.org/10.1111/j.1365-2621.2008.01732.

Ao, C., Higa, T., Ming, H., Ding, Y. T., & Tawata, S. (2010). Isolation and identification of antioxidant and hyaluronidase inhibitory compounds from *Ficus microcarpa* L. fil. bark. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *25*(3), 406–413. https://doi.org/10.3109/14756360903213473.

Ao, C., Li, A., Elzaawely, A. A., Xuan, T. D., & Tawata, S. (2008). Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. *Food Control*, *19*(10), 940-948. https://doi.org/10.1016/j.foodcont.2007.09.007

Ashraf, A., Zafar, S. A., Ashraf, M. Y., Ijaz, M. U., Muzammal, S., Asad, F., & Shahid, M. (2020). Phytochemical composition and potent biological activities of *Ficus benjamina* var. comosa leaves extract. *Pakistan Journal of Botany*, *52*(2), 531-535. https://api.semanticscholar.org

Ashraf, M. Y. (2018). Preparation of eco-friendly antibacterial silver nanoparticles from leaf extract of *Ficus benjamina*. *Biomedical Journal of Scientific & Technical Research*, *9*(4), 7260-7264. https://doi.org/10.26717/bjstr.2018.09.001829

Aswathi, P., & Thoppil, J. E. (2023). Synthesis and physicochemical characterization of silver nanoparticles from the dye-yielding plants, *Terminalia paniculata* and *Mallotus philippensis*. *Nanotechnology for Environmental Engineering*, *8*(1), 99-108. https://doi.org/10.1007/s41204-022-00263-z

Awad, A. B., Chinnam, M., Fink, C. S., & Bradford, P. G. (2007). beta-Sitosterol activates Fas signaling in human breast cancer cells. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *14*(11), 747–754. https://doi.org/10.1016/j.phymed.2007.01.003

Azqueta, A., & Collins, A. R. (2013). The essential comet assay: A comprehensive guide to measuring DNA damage and repair. *Archives of Toxicology*, *87*(6), 949–968. https://doi.org/10.1007/s00204-013-1070-0

Babu, A., Anand, D., & Saravanan, P. (2017). Phytochemical analysis of *Ficus arnottiana* (Miq.) leaf extract using GC-MS analysis. *International Journal ofPharmacognosy and Phytochemical Research*, *9*(6), 775-779.

Badgujar, S. B., Patel, V. V., Bandivdekar, A. H., & Mahajan, R. T. (2014). Traditional uses, phytochemistry and pharmacology of *Ficus carica*: a review. *Pharmaceutical Biology*, *52*(11), 1487–1503. https://doi.org/10.3109/13880209.2014.892515

Bafor, E. E., McKenna, J., Rowan, E. G., & Edrada-Ebel, R. (2017). Characterisation of the antiproliferative constituents and activity of *Ficus exasperata* (Vahl) on ovarian cancer cells -a preliminary investigation. *Natural Product Research*, *31*(18), 2164–2168. https://doi.org/10.1080/14786419.2016.1277348

Baite, T. N., Mandal, B., & Purkait, M. K. (2021). Ultrasound assisted extraction of gallic acid from *Ficus auriculata* leaves using green solvent. *Food and Bioproducts Processing*, *128*, 1-11. DOI: 10.1016/j.fbp.2021.04.008

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, *27*(4), 1326, 1-19. https://doi.org/10.3390/molecules27041326

Banne, S. V., Patil, M. S., Kulkarni, R. M., & Patil, S. J. (2017). Synthesis and characterization of silver nano particles for EDM applications. *Materials Today: Proceedings*, *4*(11), 12054-12060. https://doi.org/10.1016/j.matpr.2017.09.130

Bao, Z., & Lan, C. Q. (2019). Advances in biosynthesis of noble metal nanoparticles mediated by photosynthetic organisms-A review. *Colloids and Surfaces. B, Biointerfaces*, *184*, 1-8. https://doi.org/10.1016/j.colsurfb.2019.110519.

Basista, R. S., Kumar, V., Kumar, S., & Panesar, P. S. (2020). Microwave assisted extraction of phytochemicals from *Ficus racemosa. Journal of Food Science and Technology*, *52*(7), 4426–4434. https://doi.org/10.1007/s13197-014-1473-3

Beach, E. S., Cui, Z., & Anastas, P. T. (2009). Green chemistry: A design framework for sustainability. *Energy & Environmental Science*, *2*(10), 1038-1049. https://doi.org/10.1039/b904997p

Bello, M. O., Abdulhammed, M., & Ogunbeku, P. (2014). Nutrient and anti-nutrient phytochemicals in *Ficus exasperata* Vahl Leaves. *International Journal of Scientific & Engineering Research*, *5*(1), 2177-2181.

Bello, O. M., Agbendeh, Z. M., & Adikwu, J. G. (2013). Comparative studies of phytochemical screening of *Ficus sycomorus* Linn stem bark extract and *Piliostigma thonningii* roots extract. *Journal of Plant Science and Research*, *3*(6), 69-73.

Benali, T., Bakrim, S., Ghchime, R., Benkhaira, N., El Omari, N., Balahbib, A., Taha, D., Zengin, G., Hasan, M. M., Bibi, S., & Bouyahya, A. (2022). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology & Genetic Engineering Reviews*, 1–30. https://doi.org/10.1080/02648725.2022.2122303

Bhakat, C., Chetal, G., Sarkar, P., Singh, P., Babu, S., & Reddy, A. (2012). Effects of silver nanoparticles synthesized from *Ficus benjamina* on normal cells and cancer cells. *Journal of Pharmaceutical and Biological Science, 1*, 33-36. https://doi.org/10.9790/3008-0143336

Bhat, P. R., Gadiga, L. P., & Silva, V. B. (2019). A study on the phytochemical screening, antibacterial activity and silver nanoparticle synthesis from *Ficus racemosa* L. *International Journal of Research in Pharmacy and Chemistry*, *9*(3), 131-141. DOI: 10.33289/IJRPC.9.3.2019.948

Birková, A., Hubková, B., Bolerázska, B., Mareková, M., & Čižmárová, B. (2020). Caffeic acid: A brief overview of its presence, metabolism, and bioactivity. *Bioactive Compounds in Health and Disease*, *3*(4), 74-81. https://doi.org/10.31989/bchd.v3i4.692

Borase, H. P., Patil, C. D., Suryawanshi, R. K., & Patil, S. V. (2013). *Ficus carica* latex-mediated synthesis of silver nanoparticles and its application as a chemophotoprotective agent. *Applied Biochemistry and Biotechnology*, *171*(3), 676–688. https://doi.org/10.1007/s12010-013-0385-x.

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. *A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/10.3322/caac.21492

Bulla, L. M. C., Polonio, J. C., Portela-Castro, A. L., Kava, V., Azevedo, J. L., & Pamphile, J. A. (2017). Activity of the endophytic fungi *Phlebia* sp. and *Paecilomyces formosus* in decolourisation and the reduction of reactive dyes' cytotoxicity in fish erythrocytes. *Environmental Monitoring and Assessment*, *189*, 1-11. https://doi.org/10.1007/s10661-017-5790-0

Bykov, V. J. N., Eriksson, S. E., Bianchi, J., & Wiman, K. G. (2018). Targeting mutant p53 for efficient cancer therapy. *Nature Reviews Cancer*, *18*(2), 89–102. https://doi.org/10.1038/nrc.2017.109

Çalışkan, O., & Polat, A. A. (2011). Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Scientia Horticulturae*, *128*(4), 473-478. https://doi.org/10.1016/j.scienta.2011.02.023

Chandran, K. C., Jat, R. K., & Gopalan, R. K. (2022). Phytochemical and bioactive compounds identification of *Ficus auriculata* fig methanolic extraction and its antioxidant activity. *GSC Biological and Pharmaceutical Sciences*, *21*(02), 246-254. https://doi.org/10.30574/gscbps.2022.21.2.0453

Chang, S. T., Wu, J. H., Wang, SY., Kang, P. L., Yang, N. S., & Shyur, L. F. (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heart wood. *Journal of Agricultural and Food Chemistry*, *49* (7), 3420-3424.

Chansriniyom, C., Nooin, R., Nuengchamnong, N., Wongwanakul, R., Petpiroon, N., Srinuanchai, W., Chantarasuwan, B., Pitchakarn, P., Temviriyanukul, P., & Nuchuchua, O. (2021). Tandem mass spectrometry of aqueous extract from *Ficus dubia* sap and its cell-based assessments for use as a skin antioxidant. *Scientific Reports, 11*(1), 1-13. https://doi.org/10.1038/s41598-021-96261-3

Chaudhary, L. B., Sudhakar, J. V., Kumar, A., Bajpai, O., Tiwari, R., & Murthy, G. V. S. (2012). Synopsis of the genus *Ficus* L. (Moraceae) in India. *Taiwania*, *57*(2), 193-216.

Chaudhary, N., Husain, S. S., & Ali, M. (2014). Phytochemical investigation of the stem bark of *Ficus hispida* L. *Journal of Scientific and Innovative Research*, *3*(4), 409-413.

Chaudhary, P., Agri, U., Chaudhary, A., Kumar, A., & Kumar, G. (2022). Endophytes and their potential in biotic stress management and crop production. *Frontiers in Microbiology*, *13*, 1-22. https://doi.org/10.3389/fmicb.2022.933017

Chen, C., Peng, X., Chen, J., & Wan, C. (2020). Antioxidant and antifungal activities of ethnobotanical *Ficus hirta* Vahl. and analysis of main constituents by HPLC-MS. *Biomedicines*, *8*, 15-22. https://doi.org/10.3390/biomedicines8010015

Chen, H. M., Wu, Y. C., Chia, Y. C., Chang, F. R., Hsu, H. K., Hsieh, Y. C., Chen, C. C., & Yuan, S. S. (2009). Gallic acid, a major component of *Toona sinensis* leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. *Cancer letters*, *286*(2), 161–171. https://doi.org/10.1016/j.canlet.2009.05.040.

Choudhari, A. S., Suryavanshi, S. A., & Kaul-Ghanekar, R. (2013). The aqueous extract of *Ficus religiosa* induces cell cycle arrest in human cervical cancer cell lines SiHa (HPV-16 Positive) and apoptosis in HeLa (HPV-18 positive). *PloS One*, *8*(7), 1-10. https://doi.org/10.1371/journal.pone.0070127

Choudhari, A. S., Suryavanshi, S. A., Ingle, H., & Kaul-Ghanekar, R. (2011). Evaluating the antioxidant potential of aqueous and alcoholic extracts of *Ficus religiosa* using ORAC assay and assessing their cytotoxic activity in cervical cancer cell lines. *Biotechnology Bioinformatics and Bioengineering*, *1*(4), 443-450.

Cooper, G. M. (2000). The development and causes of cancer. *The cell: A molecular approach* 2nd ed. (pp. 57-70). Sunderland, MA: Sinauer Associates.

Corso, G., Intra, M., Trentin, C., Veronesi, P., & Galimberti, V. (2016). CDH1 germline mutations and hereditary lobular breast cancer. *Familial Cancer*, *15*, 215-219. https://doi.org/10.1007/s10689-016-9869-5

Cravotto, G., Boffa, L., Genzini, L., & Garella, D. (2010). Phytotherapeutics: An evaluation of the potential of 1000 plants. *Journal of Clinical Pharmacy and Therapeutics*, *35*(1), 11-48. DOI: 10.1111/j.1365-2710.2009.01096.x

Curti, V., Di Lorenzo, A., Dacrema, M., Xiao, J., Nabavi, S. M., & Daglia, M. (2017). *In vitro* polyphenol effects on apoptosis: An update of literature data. *Seminars in Cancer Biology*, *46*, 119–131. https://doi.org/10.1016/j.semcancer.2017.08.005

Dahl, T. W., & Arens, S. K. (2020). The impacts of land plant evolution on Earth's climate and oxygenation state–An interdisciplinary review. *Chemical Geology*, *547*, 1-25. https://doi.org/10.1016/j.chemgeo.2020.119665

Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, *7*, 1-17. https://doi.org/10.3389/fmicb.2016.01831.

De Las Llagas, M. C., Santiago, L., & Ramos, J. D. (2014). Cytotoxicity and apoptotic activity of *Ficus pseudopalma* Blanco leaf extracts against human prostate cancer cell

lines. *Tropical Journal of Pharmaceutical Research*, *13*(1), 93-100. https://doi.org/10.4314/tjpr.v13i1.14

Deeh, P. B. D., Arumugam, M., Alagarsamy, K., Karanam, G., Natesh, N. S., Watcho, P., & Vishwakarma, V. (2022). *Phyllanthus muellerianus* and *Ficus exasperata* exhibit anti-proliferative and pro-apoptotic activities in human prostate cancer PC-3 cells by modulating calcium influx and activating caspases. *Biologia*, *77*(7), 1981-1994. https://doi.org/10.21203/rs.3.rs-1063694/v1

Dhingra, M. S., Dhingra, S., Chadha, R., Singh, T., & Karan, M. (2014). Design, synthesis, physicochemical, and pharmacological evaluation of gallic acid esters as non-ulcerogenic and gastroprotective anti-inflammatory agents. *Medicinal Chemistry Research*, *23*, 4771-4788. DOI: 10.1007/s00044-014-1041-x

Din, S. M., Malek, N., Shamsuddin, M., Matmin, J., Hadi, A. A., & Asraf, M. H. (2022). Antibacterial silver nanoparticles using different organs of *Ficus deltoidea* Jack var. *kunstleri* (King) Corner. *Biocatalysis and Agricultural Biotechnology*, *44*, 1-14. http://dx.doi.org/10.1016/j.bcab.2022.102473

Ding, L., Cao, J., Lin, W., Chen, H., Xiong, X., Ao, H., Yu, M., Lin, J., & Cui, Q. (2020). The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. *International Journal of Molecular Sciences*, *21*(6), 19-60. https://doi.org/10.3390/ijms21061960

Ding, S., Lv, J., Hu, Z., Wang, J., Wang, P., Yu, J., & Shi, K. (2023). Phytosulfokine peptide optimizes plant growth and defence via glutamine synthetase GS2 phosphorylation in tomato. *The EMBO Journal*, *42*(6), 1-17. DOI: 10.15252/embj.2022111858

Ding, Z., Tao, T., Wang, L., Zhao, Y., Huang, H., Zhang, D., Liu, M., Wang, Z., & Han, J. (2019). Bioprospecting of novel and bioactive metabolites from endophytic fungi isolated from rubber tree *Ficus elastica* leaves. *Journal of Microbiology and Biotechnology*, *29*(5), 731-738. DOI: 10.4014/jmb.1901.01015

Do Nascimento, J. S., Silva, F. M., Magallanes-Noguera, C. A., Kurina-Sanz, M., Dos Santos, E. G., Caldas, I. S., & Silva, E. D. (2020). Natural trypanocidal product produced by endophytic fungi through co-culturing. *Folia Microbiologica*, *65*, 323-328. https://doi.org/10.1007/s12223-019-00727-x

Dongfack, M. D., Lallemand, M. C., Kuete, V., Mbazoa, C. D., Wansi, J. D., Trinh-van-Dufat, H., Michel, S., & Wandji, J. (2012). A new sphingolipid and furanocoumarins with antimicrobial activity from *Ficus exasperata*. *Chemical & Pharmaceutical Bulletin*, *60*(8), 1072–1075. https://doi.org/10.1248/cpb.c12-00279

Dykman, L. A., & Khlebtsov, N. G. (2011). Gold nanoparticles in biology and medicine: Recent advances and prospects. *Acta Naturae*, *3*(2), 34–55.

Dzolin, S., Ahmad, R., Zain, M. M., & Ismail, M. I. (2014). Flavonoid distribution in four varieties of *Ficus deltoida* (JACK). *Journal of Advances in Chemistry*, *11*(1), 3314-3324. https://doi.org/10.24297/jac.v11i1.2221

Edeoga, H. O., Okwu, D.E. & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, *4*(7), 685-688.

El-Beltagi, H. S., Mohamed, H. I., Abdelazeem, A. S., Youssef, R., & Safwat, G. (2019). GC-MS analysis, antioxidant, antimicrobial and anticancer activities of extracts from

Ficus sycomorus fruits and leaves. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47*(2), 493-505. https://doi.org/10.15835/nbha47211405

Elhawary, S. S., Younis, I. Y., Elbishbishy, M. H., & Khattab, A. R. (2018). LC–MS/MS-based chemometric analysis of phytochemical diversity in 13 *Ficus* spp. (Moraceae): Correlation to their *in vitros* antimicrobial and *in silico* quorum sensing inhibitory activities. *Industrial Crops and Products*, *152*, 261-271. https://doi.org/10.1016/j.indcrop.2020.112506

Elizabeth, K, & Rao, M. N. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, 58, 237-240.

El-Rafie, H. M., & Sleem, A. A. (2016). Phytochemical studies of *Ficus binnendijkii* leaf extracts: Fractionation and bioactivities of its petroleum ether extract. *Journal of Natural Remedies, 16*(4), 110-118. https://doi.org/10.18311/jnr/2016/15294

El-Sakhawy, F., Kassem, H., Abou-Hussein, D., El-Gayed, S., Mostafa, M., & Ahmed, R. (2016). Phytochemical investigation of the bioactive extracts of the leaves of *Ficus cyathistipula* Warb. *Zeitschrift fur Naturforschung.* C, *Journal of Biosciences*, *71*(5-6), 141–154. https://doi.org/10.1515/znc-2015-0274.

Embaby, M. A., El-Raey, M. A., Zaineldain, M., Almaghrabi, O., & Marrez, D. A. (2021). Synergistic effect and efflux pump inhibitory activity of *Ficus nitida* phenolic extract with tetracycline against some pathogenic bacteria. *Toxin Reviews*, *40*(4), 1187-1197. DOI: 10.1080/15569543.2019.1659370

Ercisli, S., Tosun, M., Karlidag, H., Dzubur, A., Hadziabulic, S., & Aliman, Y. (2012). Color and antioxidant characteristics of some fresh fig (*Ficus carica* L.) genotypes from northeastern Turkey. *Plant Foods for Human Nutrition*, *67*(3), 271–276. https://doi.org/10.1007/s11130-012-0292-2.

Eshwarappa, R. S., Iyer, S., Subaramaihha, S. R., Richard, S. A., & Dhananjaya, B. L. (2015). Antioxidant activities of *Ficus glomerata* (Moraceae) leaf gall extracts. *Pharmacognosy Research*, *7*(1), 114–120. https://doi.org/10.4103/0974-8490.147225.

Espíndola, K. M., Ferreira, R. G., Narvaez, L. E., Silva Rosario, A. C., da Silva, A. H., Silva, A. G., Vieira, A. P., & Monteiro, M. C. (2019). Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Frontiers in Oncology*, *9*, 1-10. https://doi.org/10.3389/fonc.2019.00541.

Essien, E. E., Newby, J. M., Walker, T. M., Ogunwande, I. A., Setzer, W. N., & Ekundayo, O. (2016). Essential oil constituents, anticancer and antimicrobial activity of *Ficus mucoso* and *Casuarina equisetifolia* leaves. *American Journal of Essential Oils and Natural Products*, *4*(1), 1-6.

Evans, W. C., & Evans, D. (2002). *Trease and evans's pharmacognosy*. 15th edition, Elsevier Health sciences, New york (pp. 21-24)

Fairbairn, D. W., Olive, P. L., & O'Neill, K. L. (1995). The comet assay: A comprehensive review. *Mutation Research*, *339*(1), 37–59. https://doi.org/10.1016/0165-1110(94)00013-3

Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Piñeros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. *International Journal of Cancer*, *149*(4), 778-789. DOI: 10.1002/ijc.33588

Firdhouse, M. J., & Lalitha, P. (2022). Biogenic green synthesis of gold nanoparticles and their applications: A review of promising properties. *Inorganic Chemistry Communications*, *143*, 109800. https://doi.org/10.1016/j.inoche.2022.109800.

Flieger, J., Flieger, W., Baj, J., & Maciejewski, R. (2021). Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. *Materials*, *14*(15), 1-54. https://doi.org/10.3390/ma14154135

Flores Reséndiz, M., Lappe-Oliveras, P., & Macías-Rubalcava, M. L. (2021). Mitochondrial damage produced by phytotoxic chromenone and chromanone derivatives from endophytic fungus *Daldinia eschscholtzii* strain GsE13. *Applied Microbiology and Biotechnology*, *105*(10), 4225-4239. https://doi.org/10.1007/s00253-021-11318-7

Gao, J., Morgan, W. A., Sanchez-Medina, A., & Corcoran, O. (2011). The ethanol extract of *Scutellaria baicalensis* and the active compounds induce cell cycle arrest and apoptosis including upregulation of p53 and Bax in human lung cancer cells. *Toxicology and Applied Pharmacology*, *254*(3), 221–228. https://doi.org/10.1016/j.taap.2011.03.016

Garcia Caparros, P., De Filippis, L., Gul, A., Hasanuzzaman, M., Ozturk, M., Altay, V., & Lao, M. T. (2021). Oxidative stress and antioxidant metabolism under adverse environmental conditions: A review. *The Botanical Review*, *87*, 421-466. 10.1007/s12229-020-09231-1

Gherghi, I. C.h, Girousi, S. T., Voulgaropoulos, A. N., & Tzimou-Tsitouridou, R. (2003). Study of interactions between DNA-ethidium bromide (EB) and DNA-acridine orange (AO), in solution, using hanging mercury drop electrode. *Talanta*, *61*(2), 103–112. https://doi.org/10.1016/S0039-9140(03)00238-8

Ginsburg, O., Bray, F., Coleman, M. P., Vanderpuye, V., Eniu, A., Kotha, S. R., & Conteh, L. (2017). The global burden of women's cancers: A grand challenge in global health. *The Lancet*, *389*(10071), 847-860. https://doi.org/10.1016/S0140-6736(16)31392-7

Giordano, S. H. (2018). Breast cancer in men. *New England Journal of Medicine*, *378*(24), 2311-2320. https://doi.org/10.1056/NEJMra1707939

Govindappa, M., Prathap, S., Vinay, V., & Channabasava, R. (2014). Chemical composition of methanol extract of endophytic fungi, *Alternaria* sp. of *Tebebuia argentea* and their antimicrobial and antioxidant activity. *International Journal of Biological and Pharmaceutical Research*, *5*(11), 861-869.

Greenwell, M., & Rahman, P. K. (2015). Medicinal plants: Their use in anticancer treatment. *International Journal of Pharmaceutical Sciences and Research*, *6*(10), 1-13. https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12

Gulcin İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651–715. https://doi.org/10.1007/s00204-020-02689-3.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 1-20. https://doi.org/10.3390/pr11082248

Gulecha, V., & Sivakuma, T. (2011). Anticancer activity of *Tephrosia purpurea* and *Ficus religiosa* using MCF 7 cell lines. *Asian Pacific Journal of Tropical Medicine*, *4*(7), 526-529. https://doi.org/10.1016/S1995-7645(11)60139-9

Gupta, A., Atanasov, A. G., Li, Y., Kumar, N., &Bishayee, A. (2022). Chlorogenic acid for cancer prevention and therapy: Current status on efficacy and mechanisms of action. *Pharmacological Research*, *186*, 106505. https://doi.org/10.1016/j.phrs.2022.106505

Halali, M., & Malekzadeh, M. (2013). An investigation on the effects of experimental variables on silver nano particles produced by electromagnetic levitation technique. *Journal of Cluster Science*, *24*, 635-642. https://doi.org/10.1007/s10876-012-0535-2

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, *100*(1), 57-70. DOI: 10.1016/s0092-8674(00)81683-9

Harborne, A. J. (1998) *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd edition. Springer Pvt. Ltd., New Delhi

Hardoim, P. R., van Overbeek, L. S., & Elsas, J. D. (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*, *16*(10), 463–471. https://doi.org/10.1016/j.tim.2008.07.008.

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews: MMBR*, *79*(3), 293–320. https://doi.org/10.1128/MMBR.00050-14.

Harrison, J. G., Urruty, D. M., & Forister, M. L. (2016). An exploration of the fungal assemblage in each life history stage of the butterfly, *Lycaeides melissa* (Lycaenidae), as well as its host plant *Astragalus canadensis* (Fabaceae). *Fungal Ecology*, *22*, 10-16. https://doi.org/10.1016/j.funeco.2016.02.001

Harzallah, A., Bhouri, A. M., Amri, Z., Soltana, H., & Hammami, M. (2016). Phytochemical content and antioxidant activity of different fruit parts/juices of three figs (*Ficus carica* L.) varieties grown in Tunisia. *Journal of Food Science and Technology*, *53*(1), 147-156. https://doi.org/10.1016/j.scienta.2012.09.032.

Hashemi, S. A., Abediankenari, S., Ghasemi, M., Azadbakht, M., Yousefzadeh, Y., & Dehpour, A. A. (2011). The effect of fig tree latex (*Ficus carica*) on stomach cancer line. *Iranian Red Crescent Medical Journal*, *13*(4), 272-275.

Hassan, M., Bala, S. Z., Bashir, M., Waziri, P. M., Adam, R. M., Umar, M. A., & Kini, P. (2022). LC-MS and GC-MS profiling of different fractions of *Ficus platyphylla* stem bark ethanolic extract. *Journal of Analytical Methods in Chemistry*, *2022*, 1-14. https://doi.org/10.1155/2022/6349332

Hassanpour, S. H., & Dehghani, M. (2017). Review of cancer from perspective of molecular. *Journal of Cancer Research and Practice*, *4*(4), 127-129. https://doi.org/10.1016/j.jcrpr.2017.07.001

Hengartner, M. O. (2001). Apoptosis: Corralling the corpses. *Cell*, *104*(3), 325-328. https://doi.org/10.1016/s0092-8674(01)00219-7

Herraiz, T., & Galisteo, J. (2015). Hydroxyl radical reactions and the radical scavenging activity of β-carboline alkaloids. *Food Chemistry*, *172*, 640–649. https://doi.org/10.1016/j.foodchem.2014.09.091.

Herschkowitz, J. I., Simin, K., Weigman, V. J., Mikaelian, I., Usary, J., Hu, Z., & Perou, C. M. (2007). Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biology*, *8*, 1-17. https://doi.org/10.1186/gb-2007-8-5-r76

Hoskins, L. M., Roy, K., Peters, J. A., Loud, J. T., & Greene, M. H. (2008). Disclosure of positive BRCA1/2-mutation status in young couples: The journey from uncertainty to bonding through partner support. *Families, Systems, & Health*, *26*(3), 296-316. 10.1037/a0012914

Hu, R., Chantana, W., Pitchakarn, P., Subhawa, S., Chantarasuwan, B., Temviriyanukul, P., & Chewonarin, T. (2022). *Ficus dubia* latex extract induces cell cycle arrest and apoptosis by regulating the NF-κB pathway in inflammatory human colorectal cancer cell lines. *Cancers*, *14*(11), 1-18. https://doi.org/10.3390/cancers14112665.

Hu, Y., Dong, Z., & Liu, K. (2024). Unraveling the complexity of STAT3 in cancer: Molecular understanding and drug discovery. *Journal of Experimental & Clinical Cancer Research: CR*, *43*(1), 23, 1-29. https://doi.org/10.1186/s13046-024-02949-5

Huang, J., Xie, M., He, L., Song, X., & Cao, T. (2023). Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. *Frontiers in Pharmacology*, *14*, 1-10. https://doi.org/10.3389/fphar.2023.1218015.

Huang, S., Wang, L. L., Xue, N. N., Li, C., Guo, H. H., Ren, T. K., Zhan, Y., Li, W. B., Zhang, J., Chen, X. G., Han, Y. X., Zhang, J. L., & Jiang, J. D. (2019). Chlorogenic acid effectively treats cancers through induction of cancer cell differentiation. *Theranostics*, *9*(23), 6745–6763.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, *15*(1), 1-23.

Hulkoti, N. I., & Taranath, T. C. (2014). Biosynthesis of nanoparticles using microbes—a review. *Colloids and Surfaces B: Biointerfaces*, *121*, 474-483. https://doi.org/10.1016/j.colsurfb.2014.05.027

Ibrahim, M., Oyebanji, E., Fowora, M., Aiyeolemi, A., Orabuchi, C., Akinnawo, B., & Adekunle, A. A. (2021). Extracts of endophytic fungi from leaves of selected Nigerian ethnomedicinal plants exhibited antioxidant activity. *Complementary Medicine and Therapies*, *21*(1), 98, 1-13.

Imran, M., Rasool, N., Rizwan, K., Zubair, M., Riaz, M., Zia-Ul-Haq, M., Rana, U. A., Nafady, A., & Jaafar, H. Z. (2014). Chemical composition and biological studies of *Ficus benjamina*. *Chemistry Central Journal*, *8*(1), 1-10. https://doi.org/10.1186/1752-153X-8-12

Ingrassia, L., Lefranc, F., Mathieu, V., Darro, F., & Kiss, R. (2008). Amaryllidaceae isocarbostyril alkaloids and their derivatives as promising antitumor agents. *Translational Oncology*, *1*(1), 1–13. https://doi.org/10.1593/tlo.08100.

Ivanov, I., Dincheva, I., Badjakov, I., Petkova, N., Denev, P., & Pavlov, A. (2018). GC-MS analysis of unpolar fraction from *Ficus carica* L. (fig) leaves. *International Food Research Journal*, *25*(1), 282-286.

Jacob, S. J. P., Prasad, V. L. S., Sivasankar, S., & Muralidharan, P. (2017). Biosynthesis of silver nanoparticles using dried fruit extract of *Ficus carica* - Screening for its anticancer activity and toxicity in animal models. *Food and Chemical Toxicology*, *109*(2), 951–956. https://doi.org/10.1016/j.fct.2017.03.066.

Jain, A., Ojha, V., Kumar, G., Karthik, L., & Rao, K. V. (2013). Phytochemical composition and antioxidant activity of methanolic extract of *Ficus benjamina* (Moraceae) leaves. *Research Journal of Pharmacy and Technology*, *6*(11), 1184-1189.

Jasmine, R., Manikandan, K., & Karthikeyan, K. (2015). Evaluating the antioxidant and anticancer property of *Ficus carica* fruits. *African Journal of Biotechnology*, *14*(7), 634-641. https://doi.org/10.5897/AJB2014.13742

Jassal, P. S., & Sharma, M. O. (2019). Evaluation of antioxidant, antibacterial, antihemolytic, and phytochemical properties of *Ficus benjamina*, *Ficus infectoria*, and *Ficus krishnae*. *Evaluation*, *12*(3), 68-73. DOI: 10.22159/ajpcr.2019.v12i3.25351

Jayant, K. K., & Vijayakumar, B. S. (2021). In-vitro anti-oxidant and anti-diabetic potential of endophytic fungi associated with *Ficus religiosa*. *Italian Journal of Mycology*, *50*, 10-20. https://doi.org/10.6092/issn.2531-7342/12104

Jiang, Z., Li, L., Huang, H., He, W., & Ming, W. (2022). Progress in laser ablation and biological synthesis processes: "Top-Down" and "Bottom-Up" approaches for the green synthesis of Au/Ag nanoparticles. *International Journal of Molecular Sciences*, *23*(23), 1-23. https://doi.org/10.3390/ijms232314658

Johnson, I., & Prabu, H. J. (2015). Green synthesis and characterization of silver nanoparticles by leaf extracts of *Cycas circinalis*, *Ficus amplissima*, *Commelina benghalensis* and *Lippia nodiflora*. *International Nano Letters*, *5*(1), 43-51. https://doi.org/10.1007/s40089-014-0136-1

Kambli, J. A. Y., Patil, A., & Keshava, R. (2014). Phytochemical screening and evaluation of antibacterial, antioxidant and cytotoxic activity of *Ficus racemosa* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(4), 464-468.

Kanjikar, A. P., Aruna, L. H., & Londonkar, R. (2017). A novel investigation of in-vitro anti-inflammatory and antioxidant activity of *Ficus krishnae*. *European Journal of Biomedical and Pharmaceutical sciences*, *4*(10), 313-317.

Kanjikar, A. P., Hugar, A. L., & Londonkar, R. L. (2018). Characterization of phyto-nanoparticles from *Ficus krishnae* for their antibacterial and anticancer activities. *Drug Development and Industrial Pharmacy*, *44*(3), 377–384. https://doi.org/10.1080/03639045.2017.1386205

Kapoor, N., & Saxena, S. (2014). Potential xanthine oxidase inhibitory activity of endophytic *Lasiodiplodia pseudotheobromae*. *Applied Biochemistry and Biotechnology*, *173*, 1360-1374. https://doi.org/10.1007/s12010-014-0927-x

Kar, A., Mahar, D., Biswas, S., Chakraborty, D., Efferth, T., & Panda, S. (2023). Phytochemical profiling of polyphenols and thyroid stimulatory activity of *Ficus*

religiosa leaf extract in 6-propyl-thiouracil-induced hypothyroid rats. *Journal of Ethnopharmacology*, *313*, 116479. https://doi.org/10.1016/j.jep.2023.116479.

Karp, G. (2009). *Cell and molecular biology: Concepts and experiments*. John Wiley & Sons.

Kaul, S., Sharma, T., & K Dhar, M. (2016). "Omics" tools for better understanding the plant-endophyte interactions. *Frontiers in Plant Science*, *7*, 1-9. https://doi.org/10.3389/fpls.2016.00955.

Kaur, J. (2012). Pharmacognostical and preliminary phytochemical studies on the leaf extract of *Ficus pumila* Linn. *Journal of Pharmacognosy and Phytochemistry*, *1*(4), 105-111.

Kaur, J., Gulati, M., Singh, S. K., Kuppusamy, G., Kapoor, B., Mishra, V., & Corrie, L. (2022). Discovering multifaceted role of vanillic acid beyond flavours: Nutraceutical and therapeutic potential. *Trends in Food Science & Technology*, *122*, 187-200. https://doi.org/10.1016/j.tifs.2022.02.023 ·

Kechagioglou, P., Papi, R. M., Provatopoulou, X., Kalogera, E., Papadimitriou, E., Grigoropoulos, P., & Gounaris, A. (2014). Tumor suppressor PTEN in breast cancer: Heterozygosity, mutations and protein expression. *Anticancer Research*, *34*(3), 1387-1400.

Khadhraoui, M., Bagues, M., Artés, F., & Ferchichi, A. (2019). Phytochemical content, antioxidant potential, and fatty acid composition of dried Tunisian fig (*Ficus carica* L.) cultivars. *Journal of Applied Botany and Food Quality*, *92*, 143-150. https://doi.org/10.5073/JABFQ.2019.092.020

Khan, A., Anand, V., Badrinarayanan, V., Thirunethiran, K., & Natarajan, P. (2017). *In vitro* antioxidant and cytotoxicity analysis of leaves of *Ficus racemosa*. *Free Radicals and Antioxidants*, *7*(1), 8-12. https://doi.org/10.5530/fra.2017.1.2

Khan, A., El-Toni, A. M., Alrokayan, S., Alsalhi, M., Alhoshan, M., & Aldwayyan, A. S. (2011). Microwave-assisted synthesis of silver nanoparticles using poly-N isopropylacrylamide/acrylic acid microgel particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *377*(1-3), 356-360.

Khanal, P., & Patil, B. M. (2020). *In vitro* and *in silico* anti-oxidant, cytotoxicity and biological activities of *Ficus benghalensis* and *Duranta repens*. *Chinese Herbal Medicines*, *12*(4), 406–413.

Khare, E., Mishra, J., & Arora, N. K. (2018). Multifaceted interactions between endophytes and plant: Developments and prospects. *Frontiers in Microbiology*, *9*, 1-12. https://doi.org/10.3389/fmicb.2018.02732.

Khodarahmi, G. A., Ghasemi, N., Hassanzadeh, F., & Safaie, M. (2011). Cytotoxic effects of different extracts and latex of *Ficus carica* L. on HeLa cell line. *Iranian Journal of Pharmaceutical Research*, *10*(2), 273-277.

Kim, M., Morales, L. D., Jang, I. S., Cho, Y. Y., & Kim, D. J. (2018). Protein tyrosine phosphatases as potential regulators of STAT3 signalling. *International Journal of Molecular Sciences*, *19*(9), 1-19. https://doi.org/10.3390/ijms19092708

Kim, S. J., Kim, M. C., Um, J. Y., & Hong, S. H. (2010). The beneficial effect of vanillic acid on ulcerative colitis. *Molecules (Basel, Switzerland)*, *15*(10), 7208–7217. https://doi.org/10.3390/molecules15107208

Kislev, M. E., Hartmann, A., & Bar-Yosef, O. (2006). Early domesticated fig in the Jordan Valley. *Science*, *312*(5778), 1372–1374. https://doi.org/10.1126/science.1125910.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, *26*(6), 459–516. https://doi.org/10.1016/j.mam.2005.10.001

Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, *87*(1), 99-163. https://doi.org/10.1152/physrev.00013.2006

Kubo, I., Fujita, K.I., Nihei, K.J., & Masuoka, N. (2003). Non-antibiotic antibacterial activity of dodecyl gallate. *Bioorganic & Medicinal Chemistry*, *11*(4), 573–580. https://doi.org/10.1016/s0968-0896(02)00436-4

Kumar, A., Jha, K. K., Kumar, D., Agrawal, A., & Gupta, A. (2012). Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficus infectoria* plant. *The Pharma Innovation Journal*, *1*(5), 71-74.

Kumar, B., Smita, K., Cumbal, L., & Debut, A. (2016). *Ficus carica* (Fig) fruit mediated green synthesis of silver nanoparticles and its antioxidant activity: A comparison of thermal and ultrasonication approach. *BioNanoScience*, *6*, 15-21. https://doi.org/10.1007/s12668-016-0193-1

Kumar, G. S., Jayaveera, K. N., Kumar, C. K., Sanjay, U. P., Swamy, B. M., & Kumar, D. V. (2007). Antimicrobial effects of Indian medicinal plants against acne inducing bacteria. *Tropical Journal of Pharmaceutical research*, *6*(2), 717-723.

Kumar, M. A., Sonal, U., Akshi, G., Kumar Ebenezer, J., Lawrence, R., & Nidhi, M. (2021). Microwave assisted green synthesis of silver nanoparticles with leaf of *Ficus racemosa* and its *in vitro* antibacterial analysis and dye catalytic activity. *Research Journal of Chemical and Environmental Science*, *25*, 111-119.

Kumar, S., Singh, A., Kushwaha, A. K., Tiwari, R., Chaudhary, L. B., Srivastava, M., & Kumar, B. (2018). The UPLC–ESI–QqQLIT–MS/MS method for quantitative determination of phytochemicals in ethanolic extracts of different parts of eight *Ficus* species: Development and validation. *International Journal of Food Properties*, *21*(1), 328–344. https://doi.org/10.1080/10942912.2017.1414838

Kumaran, A. & Karunakaran, J. R. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chemistry, *97* (1), 109-114

Kumaravel, T. S., Vilhar, B., Faux, S. P., & Jha, A. N. (2009). Comet assay measurements: a perspective. *Cell Biology and Toxicology*, *25*(1), 53–64. https://doi.org/10.1007/s10565-007-9043-9

Kumaresan, S., Ramasamy, R., & Jayachandran, P. R. (2018). Antioxidant and cytotoxic activity of combined extracts prepared using *Ficus religiosa* and *Ficus benghalensis* leaves against cervical cancer cell line (HeLa). *Asian Journal of Pharmaceuticals and Clinical Research*, *11*, 407-410.

Kunchandy, E., & Rao, M. N. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, *58*(3), 237-240. https://doi.org/10.1016/0378-5173(90)90201-E

Kurihara, L. K., Chow, G. M., & Schoen, P. E. (1995). Nanocrystalline metallic powders and films produced by the polyol method. *Nanostructured Materials*, *5*(6), 607-613. https://doi.org/10.1016/0965-9773(95)00275-J

Laka, K., & Mbita, Z. (2022). *p53*- Related anticancer activities of *Drimia calcarata* bulb extracts against lung cancer. *Frontiers in Molecular Biosciences*, *9*, 1-29. https://doi.org/10.3389/fmolb.2022.876213

Lakhanpal, P., & Rai, D. K. (2007). Quercetin: A versatile flavonoid. *Internet Journal of Medical Update*, *2*(2), 22-37. https://doi.org/10.4314/ijmu.v2i2.39851

Lane, D. P. (1992). Cancer. p53, guardian of the genome. *Nature*, *358*(6381), 15–16. https://doi.org/10.1038/358015a0

Lee, H. L., Lin, C. S., Kao, S. H., & Chou, M. C. (2017). Gallic acid induces G1 phase arrest and apoptosis of triple-negative breast cancer cell MDA-MB-231 via p38 mitogen-activated protein kinase/p21/p27 axis. *Anti-cancer Drugs*, *28*(10), 1150–1156. https://doi.org/10.1097/CAD.0000000000000565

Lee, M. M., Chan, B. D., Wong, W. Y., Qu, Z., Chan, M. S., Leung, T. W., Lin, Y., Mok, D. K., Chen, S., & Tai, W. C. (2020). Anti-cancer activity of *Centipeda minima* extract in triple negative breast cancer *via* inhibition of AKT, NF-κB, and STAT3 signalling pathways. *Frontiers in Oncology*, *10*, 1-15. https://doi.org/10.3389/fonc.2020.00491

Leh, H. E., & Lee, L. K. (2022). Lycopene: A potent antioxidant for the amelioration of type II diabetes mellitus. *Molecules*, *27*(7), 1-20. https://doi.org/10.3390/molecules27072335

Leonita, S., Bintang, M., & Pasaribu, F. H. (2016). Isolation and identification of endophytic bacteria from *Ficus variegata* Blume as antibacterial compounds producer. *Current Biochemistry*, 2(3), 116-128.10.29244/cb.2.3.116-128.

Li, L., Pan, H., Deng, L., Qian, G., Wang, Z., Li, W., & Zhong, C. (2022). The antifungal activity and mechanism of silver nanoparticles against four pathogens causing kiwifruit post-harvest rot. *Frontiers in Microbiology*, *13*, 1-16. https://doi.org/10.3389/fmicb.2022.988633

Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M. T., Wang, S., Liu, H., & Yin, Y. (2016). Quercetin, inflammation and immunity. *Nutrients*, *8*(3), 1-14. https://doi.org/10.3390/nu8030167.

Liang, X. A., Ma, Y. M., Zhang, H. C., & Liu, R. (2016). A new helvolic acid derivative from an endophytic *Fusarium* sp. of *Ficus carica*. *Natural Product Research*, 30(21), 2407-2412. DOI: 10.1080/14786419.2016.1190722

Lightbourn, A. V., & Thomas, R. D. (2019). Crude edible fig (*Ficus carica*) leaf extract prevents diethylstilbestrol (DES)-induced DNA strand breaks in single-cell gel electrophoresis (SCGE)/comet assay: Literature review and pilot study. *Journal of Bioequivalence & Bioavailability*, *11*(2), 19–28. https://doi.org/10.35248/0975-0851.19.11.389

Lin, J., Li, Q., Chen, H., Lin, H., Lai, Z., & Peng, J. (2015). *Hedyotis diffusa* Willd. extract suppresses proliferation and induces apoptosis *via* IL-6-inducible STAT3 pathway inactivation in human colorectal cancer cells. *Oncology Letters*, *9*(4), 1962–1970. https://doi.org/10.3892/ol.2015.2956

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

Liz-Marzán, L. M. (2006). Tailoring surface plasmons through the morphology and assembly of metal nanoparticles. *Langmuir: The ACS Journal of Surfaces and Colloids*, *22*(1), 32–41. https://doi.org/10.1021/la0513353.

Logaranjan, K., Devi, S., & Pandian, K. (2012). Biogenic synthesis of silver nanoparticles using fruit extract of *Ficus carica* and study its antimicrobial activity. *Nano Biomedicine & Engineering*, *4*(4), 177-182. https://doi.org/10.5101/nbe.v4i4.p

Logesh, R., Sathasivampillai, S. V., Varatharasan, S., Rajan, S., Das, N., Pandey, J., & Devkota, H. P. (2023). *Ficus benghalensis* L. (Moraceae): A review on ethnomedicinal uses, phytochemistry and pharmacological activities. *Current Research in Biotechnology*, *6*, 1-9. https://doi.org/10.1016/j.crbiot.2023.100134

Lu, L., Zhao, Y., Yi, G., Li, M., Liao, L., Yang, C., Cho, C., Zhang, B., Zhu, J., Zou, K., & Cheng, Q. (2021). Quinic acid: A potential antibiofilm agent against clinical resistant *Pseudomonas aeruginosa*. *Chinese Medicine*, *16*(1), 1-17. https://doi.org/10.1186/s13020-021-00481-8

Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast cancer: Epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—An updated review. *Cancers*, *13*(17), 1-30. https://doi.org/10.3390/cancers13174287

Ma, Y. M., Liang, X. A., Zhang, H. C., & Liu, R. (2016). Cytotoxic and antibiotic cyclic pentapeptide from an endophytic *Aspergillus tamarii* of *Ficus carica*. *Journal of Agricultural and Food Chemistry*, *64*(19), 3789-3793. DOI: 10.1021/acs.jafc.6b01051

Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., & Baiti, I. (2016). Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. *Asian Pacific Journal of Tropical Biomedicine*, *6*(3), 239-245. https://doi.org/10.1016/j.apjtb.2015.12.010

Maity, A., Bisoi, P. C., Das, A. B., Maiti, P., & Senapati, M. R. (2017). Cytotoxicity study of *Ficus religiosa* leaf extract against jurkat cell line. *Exploratory Animal and Medical Research*, *7*(1), 18-21.

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

Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. *Acta Naturae*, *6*(1), 35–44.

Mamoucha, S., Fokialakis, N., & Christodoulakis, N. S. (2016). Leaf structure and histochemistry of *Ficus carica* (Moraceae), the fig tree. *Flora-Morphology, Distribution, Functional Ecology of Plants, 218*, 24-34. DOI: 10.13140/RG.2.1.4580.8723

Mane, R. S., & Vedamurthy, A. B. (2020). Mycochemical analysis and anticancer activity of endophytic *Aspergillus nomius* against MCF7 human breast cancer cell line. *International Journal of Pharmaceutical Science*, *11*(2), 1-6.

Manian, R., Anusuya, N., Siddhuraju, P., & Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) Kuntz, *Ficus bengalensis* L.and *Ficus racemosa* L. *Food Chemistry*, *107*(3), 1000-1007. https://doi.org/10.1016/j.foodchem.2007.09.008

Marslin, G., Siram, K., Maqbool, Q., Selvakesavan, R. K., Kruszka, D., Kachlicki, P., & Franklin, G. (2018). Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials*, *11*(6), 1-25. https://doi.org/10.3390/ma11060940.

Meikle, T. G., Dyett, B. P., Strachan, J. B., White, J., Drummond, C. J., & Conn, C. E. (2020). Preparation, characterization, and antimicrobial activity of cubosome encapsulated metal nanocrystals. *Applied Materials and Interfaces*, *12*(6), 6944–6954. https://doi.org/10.1021/acsami.9b21783.

Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, *31*(2), 346–356. https://doi.org/10.1016/j.biotechadv.2013.01.003.

Mladěnka, P., Applová, L., Patočka, J., Costa, V. M., Remiao, F., Pourová, J., Mladěnka, A., Karlíčková, J., Jahodář, L., Vopršalová, M., Varner, K. J., Štěrba, M., & Hradec, K. (2018). Comprehensive review of cardiovascular toxicity of drugs and related agents. *Medicinal Research Reviews*, *38*(4), 1332–1403. https://doi.org/10.1002/med.21476

Mohammed, R. S., Kntapo, F. M., Yagana, B. A., Saidu, G., & Abah, J. O. (2022). GC-MS and spectrophotometric quantitative phytochemical analysis of bioactive phytochemicals in ethyl acetate extract of leaves of *Ficus exasperata* Vahl: Further evidence for its medicinal diversity. *Nigerian Journal of Chemical Research*, *27*(1), 35-42. https://doi.org/10.4314/njcr. v27i1.4

Mondal, A. K., Mondal, S., Samanta, S., & Mallick, S. (2011). Synthesis of ecofriendly silver nanoparticle from plant latex used as an important taxonomic tool for phylogenetic interrelationship. *Advances in Bioresearch*, *2*(1), 122-133.

Moricca, S., & Ragazzi, A. (2008). Fungal endophytes in Mediterranean oak forests: A lesson from *Disculaquercina*. *Phytopathology*, *98*(4), 380–386. https://doi.org/10.1094/PHYTO-98-4-0380.

Morris, J. L., Puttick, M. N., Clark, J. W., Edwards, D., Kenrick, P., Pressel, S., Wellman, C. H., Yang, Z., Schneider, H., & Donoghue, P. C. J. (2018). The timescale of early land plant evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), 2274-2283. https://doi.org/10.1073/pnas.1719588115

Muhammad, A. A., Garba, Y., Bashir, M., Bello, N. U., & Mohammed, S. Y. (2022). Free radical scavenging potential of methanol extract of *Ficus thonningii* leaves and liquid chromatography-mass spectrometer (LC/MS) profiling. *Sokoto Journal of Veterinary Sciences*, *20*(3), 186-196.

Muhammad, H., Omar, M. H., Rasid, E. N. I., Suhaimi, S. N., Mohkiar, F. H., Siu, L. M., & Awang, N. (2021). Phytochemical and *in vitro* genotoxicity studies of

standardized *Ficus deltoidea* var. *kunstleri* aqueous extract. *Plants*, *10*(2), 1-11. https://doi.org/10.3390/plants10020343

Mukhtar, H. M., Singh, A., & Kaur, H. (2018). Bioassay Guided Fractionation and *in vitro* Anti-plasmodial Activity of *Ficus deltoidea* and *Ficus benjamina*. *Pharmacognosy Journal*, *10*(2), 235-240. 10.5530/pj.2018.2.41.

Murti, K., Kumar, U., Panchal, M., & Shah, M. (2014). Exploration of preliminary phytochemical studies of roots of *Ficus racemosa*. *Marmara Pharmaceutical Journal*, *15*(2), 80-83. https://doi.org/10.12991/mpj.74832

Murugan, R., Arunachalam, K., & Parimelazhagan, T. (2012). Antioxidant, anti-inflammatory activity, and phytochemical constituents of *Ficus* (*Ficus amplissima* Smith) bark. *Food Science and Biotechnology*, *21*(1), 59-67. https://doi.org/10.1007/s10068-012-0008-2

Nakkala, J. R., Mata, R., & Sadras, S. R. (2017). Green synthesized nano silver: Synthesis, physicochemical profiling, antibacterial, anticancer activities and biological *in vivo* toxicity. *Journal of Colloid and Interface Science*, *499*, 33–45. https://doi.org/10.1016/j.jcis.2017.03.090.

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A. A., Khan, G. J., Shumzaid, M., Ahmad, F., Babazadeh, D., FangFang, X., Modarresi-Ghazani, F., WenHua, L., & XiaoHui, Z. (2018). Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine & Pharmacotherapy*, *97*, 67–74. https://doi.org/10.1016/j.biopha.2017.10.064

Neha, K., Haider, M. R., Pathak, A., & Yar, M. S. (2019). Medicinal prospects of antioxidants: A review. *European Journal of Medicinal Chemistry*, *178*, 687–704. https://doi.org/10.1016/j.ejmech.2019.06.010.

Nushiba Naser, P. T., & Thoppil, J. E. (2023). Biochemical screening, fabrication of green nanoparticles and its antimicrobial, and antioxidant studies of endophytic fungus *Phlebia* species. *Indian journal of microbiology*, *63*(4), 447–460. https://doi.org/10.1007/s12088-023-01094-5

Nworu, C. S., Nwuke, H. C., Akah, P. A., Okoye, F. B., & Esimone, C. O. (2013). Extracts of *Ficus exasperata* leaf inhibit topical and systemic inflammation in rodents and suppress LPS-induced expression of mediators of inflammation in macrophages. *Journal of Immunotoxicology*, *10*(3), 302–310. https://doi.org/10.3109/1547691X.2012.732121

Odoemenam, V. U., & Ujam, T. N. (2017). Phytochemical, proximate analysis, vitamin and mineral composition of aqueous extract of *Ficus capensis* leaves in South Eastern Nigeria. *Journal of Applied Pharmaceutical Science*, *7*(3), 117-122. https://doi.org/10.7324/JAPS.2017.70319

Ojo, O. A., & Akintayo, C. O. (2014). Assessment of antioxidant activity of *Ficus asperifolia*Miq aqueous extract- in vitro studies. *The Journal of Phytopharmacology*, *3*(1), 1-6. 10.31254/phyto.2014.3103

Okoroh, P. N., Duru, M. K., Onuoha, S. C., & Amadi, B. A. (2019). Proximate composition, phytochemical and mineral analysis of the fruits of *Ficus capensis*. *International Journal of Innovative Research and Development*, *8*(8), 84-88. https://doi.org/10.24940/ijird/2019/v8/i9/SEP19021

Oladiipo, O. T., Akinpelu, B. A., Folorunso, A. E., Godwin, A., Omotoso, S. E., Dosunmu, O. A., & Joseph, W. A. (2014). Chemotaxonomic study of six Nigerian *Ficus*species (Moraceae). *Natural Products Chemistry & Research*, *2*(2), 1-5. https://doi.org/10.4172/2329-6836.1000126

Olaokun, O. O., Mcgaw, L. J., Eloff, J. N., & Naidoo, V. (2013). Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African *Ficus* species (Moraceae) used traditionally to treat diabetes. *BMC Complementary and Alternative Medicine*, *13*(1), 1-10. https://doi.org/10.1186/1472-6882-13-94.

Oliveira, A. P., Silva, L. R., Ferreres, F., de Pinho, P. G., Valentão, P., Silva, B. M., Pereira, J. A., & Andrade, P. B. (2010). Chemical assessment and *in vitro* antioxidant capacity of *Ficus carica* latex. *Journal of Agricultural and Food Chemistry*, *58*(6), 3393-3398. https://doi.org/10.1021/jf9039759

Orabi, M. A., & Orabi, E. A. (2016). Antiviral and antioxidant activities of flavonoids of *Ficus virens*: Experimental and theoretical investigations. *Journal of Pharmacognosy and Phytochemistry*, *5*(3), 120-128.

Otto, T., & Sicinski, P. (2017). Cell cycle proteins as promising targets in cancer therapy. *Nature Reviews Cancer*, *17*(2), 93–115.

Ozaki, T., & Nakagawara, A. (2011). Role of p53 in cell death and human cancers. *Cancers*, *3*(1), 994–1013. https://doi.org/10.3390/cancers3010994

Palmeira, L., Pereira, C., Dias, M. I., Abreu, R. M., Corrêa, R. C., Pires, T. C., & Ferreira, I. C. (2019). Nutritional, chemical and bioactive profiles of different parts of a Portuguese common fig (*Ficus carica* L.) variety. *Food Research International*, *126*, 1-10. https://doi.org/10.1016/j.foodres.2019.108572

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, *2*(5), 270-278. https://doi.org/10.4161/oxim.2.5.9498

Paramanandam, V., Jagadeesan, G., Muniyandi, K., Manoharan, A. L., Nataraj, G., Sathyanarayanan, S., & Thangaraj, P. (2021). Comparative and variability analysis of different drying methods on phytochemical, antioxidant and phenolic contents of *Ficus auriculata* Lour. Fruit. *Phytomedicine Plus*, *1*(3), 1-9.https://doi.org/10.1016/j.phyplu.2021.100075

Pathak, K., Keharia, H., & Kharkwal, A. C. (2009). Isolation, cultivation and in planta visualization of bacterial endophytes in hanging roots of banyan tree (*Ficus bengalensis)*. In *Soil Biology* (pp. 211-225). https://doi.org/10.1007/978-3-540-95894-9_13

Patil, R. B., & Chougale, A. D. (2021). Analytical methods for the identification and characterization of silver nanoparticles: A brief review. *Materials Today: Proceedings*, *47*, 5520-5532. https://doi.org/10.1016/j.matpr.2021.03.384

Paudel, B., Maharjan, R., Rajbhandari, P., Aryal, N., Aziz, S., Bhattarai, K., Baral, B., Malla, R., & Bhattarai, H. D. (2021). Maculosin, a non-toxic antioxidant compound isolated from *Streptomyces* sp. KTM18. *Pharmaceutical Biology*, *59*(1), 933–936. https://doi.org/10.1080/13880209.2021.1946091

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, *6*(11), 1–5. https://doi.org/10.4103/0973-7847.95849

Petruccelli, R., Ieri, F., Ciaccheri, L., & Bonetti, A. (2018). Polyphenolic profiling and chemometric analysis of leaves from Italian *Ficus carica* L. varieties. Polyphenol compounds in common fig. *Europenan Journal of Horticultural Science*, *83*(2), 94-103. https://doi.org/10.17660/eJHS.2018/83.2.5

Petryayeva, E., & Krull, U. J. (2011). Localized surface plasmon resonance: Nanostructures, bioassays and biosensing- A review. *Analytica Chimica Acta*, *706*(1), 8–24. https://doi.org/10.1016/j.aca.2011.08.020.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, *4*(2), 89-96.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0

Pinipay, F., Rokkam, R., Botcha, S., &Tamanam, R. R. (2023). Evaluating the anti-urolithiasis potential of *Ficus religiosa* seed GC/MS evaluated phytoconstituents based on their in vitro antioxidant properties and in-silico ADMET and molecular docking studies. *Clinical Phytoscience*, *9*(1), 1-17. https://doi.org/10.1186/s40816-023-00359-2

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, *97*, 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040

Praba, P. S., Vasantha, V. S., Jeyasundari, J., & Jacob, Y. B. A. (2015). Synthesis of plant-mediated silver nanoparticles using *Ficus microcarpa* leaf extract and evaluation of their antibacterial activities. *European Chemical Bulletin*, *4*(3), 116-120. https://doi.org/10.5958/0974-360X.2017.00287.6

Prabavathy, D., &Nachiyar, C. V. (2011). Screening and characterization of antimicrobial compound from endophytic *Aspergillus* sp. isolated from *Ficus carica. Journal of Pharmacy Research*, *4*(6), 1935-1936.

Pranitha, V., & Krishna, G. (2017). Silver nanoparticles biosynthesized using *Ficus hispida* aqueous extract and their antibacterial studies. *International Journal of Pharmaceutical Science Invention*, *6*(7), 44–47.

Prasad, T. N., & Elumalai, E. K. (2011). Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(6), 439–442. https://doi.org/10.1016/S2221-1691(11)60096-8.

Priya, V., & Abinaya, G. (2018). GC-MS analysis of bioactive components of *Ficus mollis* Vahl leaves. *Journal of Medicinal Plants Studies*, *6*(2), 98-100.

Purushotham, E., & Krishna, N. G. (2014). Preparation and characterization of silver nano particles. *Indian Journal of Physics*, *88*, 157-163.

Raheel, R., Saddiqe, Z., Iram, M., & Afzal, S. (2017). *In vitro* antimitotic, antiproliferative and antioxidant activity of stem bark extracts of *Ficus benghalensis* L. *South African Journal of Botany*, *111*, 248-257.https://doi.org/10.1016/j.sajb.2017.03.037

Rakshith, D., Santosh, P., & Satish, S. (2013). Isolation and characterization of antimicrobial metabolite producing endophytic *Phomopsis* sp. from *Ficus pumila* Linn. (Moraceae). *International Journal of Chemical and Analytical Science*, *4*(3), 156-160. https://doi.org/10.1016/j.ijcas.2013.08.006

Ramamurthy, C. H., Padma, M., Mareeswaran, R., Suyavaran, A., Kumar, M. S., Premkumar, K., & Thirunavukkarasu, C. (2013). The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. *Colloids and Surfaces B: Biointerfaces*, *102*, 808-815. https://doi.org/10.1016/j.colsurfb.2012.09.025

Ramesh, A. V., Devi, D. R., Battu, G., & Basavaiah, K. (2018). A facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of *Ficus hispida* Linn. f. for catalytic, antioxidant and antibacterial applications. *South African Journal of Chemical Engineering*, *26*, 25-34. https://doi.org/10.1016/j.sajce.2018.07.001

Ray, R. S., Agrawal, N., Sharma, A., & Hans, R. K. (2008). Use of L-929 cell line for phototoxicity assessment. *Toxicology In Vitro*, *22*(7), 1775–1781. https://doi.org/10.1016/j.tiv.2008.06.015

Reed, J. C. (1997). Bcl-2 family proteins: Regulators of apoptosis and chemoresistance in hematologic malignancies. *Seminars in Hematology*, *34* (5), 9-19.

Rezaei-Seresht, H., Cheshomi, H., Falanji, F., Movahedi-Motlagh, F., Hashemian, M., & Mireskandari, E. (2019). Cytotoxic activity of caffeic acid and gallic acid against MCF-7 human breast cancer cells: An *in silico* and *in vitro* study. *Avicenna Journal of Phytomedicine*, *9*(6), 574–586. https://doi.org/10.22038/AJP.2019.13475

Riaz, A., Nosheen, S., & Mughal, T. A. (2022). Fabrication of biogenic silver nanoparticles from *Ficus religiosa* bark extract and their application for chromium removal. *Microscopy Research and Technique*, *85*(11), 3618–3622. https://doi.org/10.1002/jemt.24214.

Riss, T. L., Moravec, R.A., Niels, A. L., Duellman, S., Benink, H. A., Worzella, T. J. & Minor, L. (2013). Cell viability assays. *Assay Guidance Manual.* Eli Lilly &Company and the National Centre for Advancing Translational Sciences, Bethesda, Madison, USA.

Rohmah, N. S., Suharjono, Jatmiko, Y. D., Siswanto, D., & Mustafa, I. (2020). The potency of endophytic bacteria isolated from *Ficus septica* as phytoremediation promoting agent of Cr (VI) contaminated soil. *Biodiversitas*, *21*(12), 1920-1927. DOI: 10.13057/biodiv/d210519

Romeh, A. A. (2013). Phytochemicals from *Ficus sycomorus* L. leaves act as insecticides and acaricides. *Journal of Biopesticides*, *6*(2), 118-125. https://doi.org/10.5897/AJAR2013.7243

Rosli, N. M., Ashari, K. I. A. H., & Azmi, N. S. A. (2020). Isolation and preliminary screening of endophytic fungi from *Ficus carica* for biocontrol and phosphate solubilization. *Environment and Ecosystem Science*, *4*(2), 77-84. DOI: 10.26480/ees.02.2020.77.84.

Rosmana, A., Samuels, G. J., Ismaiel, A., Ibrahim, E. S., Chaverri, P., Herawati, Y., & Asman, A. (2015). *Trichoderma asperellum*: A dominant endophyte species in *Cacao* grown in Sulawesi with potential for controlling vascular streak dieback disease. *Tropical Plant Pathology*, *40*, 19-25. https://doi.org/10.1007/s40858-015-0004-1

Rubab, M., Chelliah, R., Saravanakumar, K., Barathikannan, K., Wei, S., Kim, J. R., Yoo, D., Wang, M. H., & Oh, D. H. (2020). Bioactive potential of 2-methoxy-4-vinylphenol and benzofuran from *Brassica oleracea* L. var. *capitate f. rubra* (Red Cabbage) on oxidative and microbiological stability of beef meat. *Foods*, *9*(5), 1-21. https://doi.org/10.3390/foods9050568

Ruddaraju, L. K., Pammi, S. V., Guntuku, G. S., Padavala, V. S., & Kolapalli, V. R. (2020). A review on anti-bacterials to combat resistance: from ancient era of plants and metals to present and future perspectives of green nano technological combinations. *Asian Journal of Pharmaceutical Sciences*, *15*(1), 42–59. https://doi.org/10.1016/j.ajps.2019.03.002.

Rudgers, J. A., Koslow, J. M., & Clay, K. (2004). Endophytic fungi alter relationships between diversity and ecosystem properties. *Ecology Letters*, *7*(1), 42-51. https://doi.org/10.1046/j.1461-0248.2003.00543

Saheed, Y., Nasir, M. U., Abbas, B., & Bello, R. Y. (2020). GC-MS analysis and antimicrobial spectrum of stem bark extracts of *Ficus sycomorus*. *Microbiology Research Journal International*, *30*(8), 118-128. https://doi.org/10.9734/mrji/2020/v30i830257

Saida, L., Tulasi, C. D., & Narasu, M. L. (2021). Evaluation of chemo-preventive efficacy of *Ficus religiosa* latex extract by flow cytometry analysis and gene expression studies performed by RT-PCR in various cell lines. *Future Journal of Pharmaceutical Sciences*, *7*, 1-21. https://doi.org/10.1186/s43094-021-00182-x

Salar, R. K., Sharma, P., & Kumar, N. (2015). Enhanced antibacterial activity of streptomycin against some human pathogens using green synthesized silver nanoparticles. *Resource-Efficient Technologies*, *1*(2), 106-115. https://doi.org/10.1016/j.reffit.2015.11.004

Salem, W. M., Haridy, M., Sayed, W. F., & Hassan, N. H. (2014). Antibacterial activity of silver nanoparticles synthesized from latex and leaf extract of *Ficus sycomorus*. *Industrial Crops and Products*, *62*, 228-234. https://doi.org/10.1016/j.indcrop.2014.08.030

Saloni, J., & Sakthivel, J. (2019). Evaluation of antioxidant and anticancer potential of flavonoids from aerial roots of *Ficus benghalensis* Linn. *International Journal of Pharmaceutical Research, 11*(1), 1-12. https://doi.org/10.31838/ijpr/2019.11.01.006

Salvi, P., Mahawar, H., Agarrwal, R., Kajal, Gautam, V., & Deshmukh, R. (2022). Advancement in the molecular perspective of plant-endophytic interaction to mitigate drought stress in plants. *Frontiers in Microbiology*, *13*, 1-14. https://doi.org/10.3389/fmicb.2022.981355.

Sankar, R., Maheswari, R., Karthik, S., Shivashangari, K. S., & Ravikumar, V. (2014). Anticancer activity of *Ficus religiosa* engineered copper oxide nanoparticles. *Materials Science & Engineering. C, Materials for Biological Applications*, *44*, 234–239. https://doi.org/10.1016/j.msec.2014.08.030.

Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., & Mathur, P. (2022). Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. *The Indian Journal of Medical Research*, *156*(4&5), 598–607. https://doi.org/10.4103/ijmr.ijmr_1821_22

Sathiyamoorthy, J., & Sudhakar, N. (2018). *In vitro* cytotoxicity and apoptotic assay in HT-29 cell line using *Ficus hispida* Linn: Leaves extract. *Pharmacognosy Magazine, 13*(4), 756–761. https://doi.org/10.4103/pm.pm_319_17

Satish, A., Kumar, R. P., Rakshith, D., Satish, S., & Ahmed, F. (2013). Antimutagenic and antioxidant activity of *Ficus benghalensis* stem bark and *Moringa oleifera* root extract. *International Journal of Chemical and Analytical Science*, *4*(2), 45-48. DOI: 10.1016/j.ijcas.2013.03.008

Saware, K., & Venkataraman, A. (2014). Biosynthesis and characterization of stable silver nanoparticles using *Ficus religiosa* leaf extract: a mechanism perspective. *Journal of Cluster Science*, *25*, 1157-1171. https://doi.org/10.1007/s10876-014-0697-1

Saware, K., Sawle, B., Salimath, B., Jayanthi, K., & Abbaraju, V. (2014). Biosynthesis and characterization of silver nanoparticles using *Ficus benghalensis* leaf extract. *International Journal of Engineering Research and Technology*, *3*, 867-874.

Saxena, A., Tripathi, R. M., Zafar, F., & Singh, P. (2012). Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity. *Materials Letters, 67*(1), 91-94. https://doi.org/10.1016/j.matlet.2011.09.038

Schneider, P., & Tschopp, J. (2000). Apoptosis induced by death receptors. *Pharmacochemistry Library*, *31*, 281-286. https://doi.org/10.1016/s0031-6865(99)00038-2

Schulz, B., Boyle, C., Draeger, S., Römmert, A. K., & Krohn, K. (2002). Endophytic fungi: A source of novel biologically active secondary metabolites. *Mycological Research*, *106*(9), 996-1004. https://doi.org/10.1017/S0953756202006342

Shahbandi, A., Nguyen, H. D., & Jackson, J. G. (2020). TP53 mutations and outcomes in breast cancer: Reading beyond the headlines. *Trends in Cancer*, *6*(2), 98-110. https://doi.org/10.1016/j.trecan.2020.01.007

Shahinuzzaman, M., Yaakob, Z., Anuar, F. H., Akhtar, P., Kadir, N. H. A., Hasan, A. K. M., Sobayel, K., Nour, M., Sindi, H., Amin, N., Sopian, K., & Akhtaruzzaman, M. (2020). *In vitro* antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Scientific Reports*, *10*(1), 1-14. https://doi.org/10.1038/s41598-020-67765-1

Shaikh, A., Ibrahim, M., & Khan, M. (2020). Effect of *Ficus glomerata* leaf extract in Streptozotocin-induced early diabetic complications and its characterization by LC-MS. *Experimental and Clinical Science*, *19*, 33-47. https://doi.org/10.17179/excli2019-1441

Shanmugam, S., Kumar, T. S., & Panneer Selvam, K. (2019). Laboratory handbook on Biochemistry. PHI Learning Pvt. Ltd. Delhi.

Sharma, A. K., Kumar, A., Kumar, S., Mukherjee, S., Nagpal, D., Nagaich, U., & Rajput, S. K. (2017). Preparation and therapeutic evolution of *Ficus benjamina* solid lipid nanoparticles against alcohol abuse/anti-abuse induced hepatotoxicity and cardio-renal injury. *Royal Society of Chemistry Advances*, *7*(57), 35938-35949. https://doi.org/10.1039/C7RA04866A

Sharma, A., Sagar, A., Rana, J., & Rani, R. (2022). Green synthesis of silver nanoparticles and its antibacterial activity using fungus *Talaromyces purpureogenus* isolated from *Taxus baccata* Linn. *Micro and Nano System Letters*, *10* (2), 1-12. https://doi.org/10.1186/s40486-022-00144-9

Sharma, B. R., Kumar, V., Kumar, S., Singh, A., & Pinakin, D. J. (2021). Process optimization for extraction of phytochemicals from *Ficus racemosa*: Phytochemical extraction. *The Natural Products Journal*, *11*(5), 682-689. DOI: 10.2174/2210315510999200728125644

Sharma, N., Tiwari, N., Vyas, M., Khurana, N., Muthuraman, A., & Utreja, P. (2020). An overview of therapeutic effects of vanillic acid. *Plant Archives*, *20*(2), 3053-3059.

Shiovitz, S., & Korde, L. A. (2015). Genetics of breast cancer: A topic in evolution. *Annals of Oncology*, *26*(7), 1291-1299. https://doi.org/10.1093/annonc/mdv022

Shukla, A. K., & Iravani, S. (2017). Metallic nanoparticles: Green synthesis and spectroscopic characterization. *Environmental Chemistry Letters*, *15*, 223–231 https://doi.org/10.1007/s10311-017-0618-2

Si, S., & Mandal, T. K. (2007). Tryptophan-based peptides to synthesize gold and silver nanoparticles: A mechanistic and kinetic study. *Chemistry (Weinheim an der Bergstrasse, Germany)*, *13*(11), 3160–3168. https://doi.org/10.1002/chem.200601492.

Siddiqi, K. S., Husen, A., & Rao, R. A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology*, *16*(1), 1-28. https://doi.org/10.1186/s12951-018-0334-5.

Siddiqui, A. A. & Ali, M. (1997). *Practical pharmaceutical chemistry*. CBS publishers & Distributors, New Delhi, (pp. 126-131).

Singh, D., Singh, B., & Goel, R. K. (2011). Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. *Journal of Ethnopharmacology*, *134*(3), 565–583.

Singh, P. P., & Bhakat, C. (2012). Green synthesis of gold nanoparticles and silver nanoparticles from leaves and bark of *Ficus carica* for nanotechnological applications. *International Journal of Scientific and Research Publication*, *2*(5), 1-4.

Singhal, A., Singhal, N., Bhattacharya, A., & Gupta, A. (2017). Synthesis of silver nanoparticles (AgNPs) using *Ficus retusa* leaf extract for potential application as antibacterial and dye decolourising agents. *Inorganic and Nano-Metal Chemistry*, *47*(11), 1520-1529.

Sinha,D., Odoh, U.E., Ganguly, S., Muhammad, M., Chikeokwu, I. & Egbuna, C., (2023). Phytochemistry, computational tools, and databases in drug discovery. In *Elsevier eBooks* (pp.1-26). https://doi.org/10.1016/B978-0-323-90593-0.00001-0

Škubník, J., Pavlíčková, V. S., Ruml, T., & Rimpelová, S. (2021). Vincristine in combination therapy of cancer: Emerging trends in clinics. *Biology*, *10*(9), 1-25. https://doi.org/10.3390/biology10090849

Sladić, D., & Gasić, M. J. (2006). Reactivity and biological activity of the marine sesquiterpene hydroquinone avarol and related compounds from sponges of the order Dictyoceratida. *Molecules*, *11*(1), 1–33. https://doi.org/10.3390/11010001

Solis, M. J. L., Cruz, T. E. D., Schnittler, M., & Unterseher, M. (2016). The diverse community of leaf-inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina, F. Elastic* and *F. religiosa*. *Mycoscience*, *57*(2), 96-106. https://doi.org/10.1016/j.myc.2015.10.002

Solis, M. J. L., Yurkov, A., Dela cruz, T. E., & Untersher, M. (2014). Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three *Ficus* species from botanical garden greenhouses in Germany. *Mycological Progress*, *14*(1), 1-10. https://doi.org/10.1007/s11557-014-1019-6.

Solomon, A., Golubowicz, S., Yablowicz, Z., Grossman, S., Bergman, M., Gottlieb, H. E., Altman, A., Kerem, Z., & Flaishman, M. A. (2006). Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* L.). *Journal of Agricultural and Food Chemistry*, *54*(20), 7717–7723. https://doi.org/10.1021/jf060497h.

Somashekhar, M., Nayeem, N., & Sonnad, B. (2013). A review on family Moraceae (Mulberry) with a focus on *Artocarpus* species. *World Journal of Pharmacy and Pharmaceutical Sciences*, *2*(5), 2614-2626.

Song, Q., Joshi, M., DiPiazza, J., & Joshi, V. (2020). Functional relevance of citrulline in the vegetative tissues of watermelon during abiotic stresses. *Frontiers in Plant Science*, *11*, 1-13. https://doi.org/10.3389/fpls.2020.00512

Sudhakar, T., Premkumar, J., Sapkota, R., & Rijal, S. (2017). Antimicrobial activity of silver nanoparticles synthesized from *Ficus benghalensis* against human pathogens. *Research Journal of Pharmacy and Technology*, *10*(6), 1635-1640.

Suliman, S., Yagi, S., Elbashir, A. A., Mohammed, I., Hussein, A., Ak, G., Zengin, G., Orlando, G., & Ferrante, C. (2021). Phenolic profile, enzyme inhibition and antioxidant activities and bioinformatics analysis of leaf and stem bark of *Ficus sycomorus* L. *Process Biochemistry*, *101*, 169–178. https://doi.org/10.1016/j.procbio.2020.11.003

Sumi, S. A., Siraj, M. A., Hossain, A., Mia, M. S., Afrin, S., & Rahman, M. M. (2016). Investigation of the key pharmacological activities of *Ficus racemosa* and analysis of its major bioactive polyphenols by HPLC-DAD. *Evidence-Based Complementary and Alternative Medicine*, *2016*(1), 1-9. https://doi.org/10.1155/2016/3874516.

Sun, L., Zhang, Z., & Dang, H. (2003). A novel method for preparation of silver nanoparticles. *Materials Letters*, *57*(24-25), 3874-3879. https://doi.org/10.1016/S0167-577X(03)00232-5

Sun, Z. H., Liang, F. L., Chen, Y. C., Liu, H. X., Li, H. H., & Zhang, W. M. (2016). Two new xyloketals from the endophytic fungus *Endomelanconiopsis endophytica* derived from medicinal plant *Ficus hirta*. *Journal of Asian Natural Products Research*, *18*(11), 1036–1041. https://doi.org/10.1080/10286020.2016.1188084

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *A Cancer Journal for Clinicians*, *71*(3), 209-249. https://doi.org/10.3322/caac.21660

Taiwo, B. J., & Igbeneghu, O. A. (2014). Antioxidant and antibacterial activities of flavonoid glycosides from *Ficus exasperata* Vahl-Holl (Moraceae) leaves. *African Journal of Traditional, Complementary, and Alternative Medicines*, *11*(3), 97–101. https://doi.org/10.4314/ajtcam.v11i3.14

Taiwo, O. M., Olaoluwa, O. O., Aiyelaagbe, O. O., & Schmidt, T. J. (2023). Chemical constituents from *Ficus sagittifolia* stem bark and their antimicrobial activities. *Plants*, *12*(15), 1-12. https://doi.org/10.3390/plants12152801

Takahashi, T., Okiura, A., Saito, K., & Kohno, M. (2014). Identification of phenylpropanoids in fig (*Ficus carica* L.) leaves. *Journal of Agricultural and Food Chemistry*, *62*(41), 10076-10083. https://doi.org/10.1021/jf5025938

Tallei, T. E., Linelejan, Y. T., Umboh, S. D., Adam, A. A., & Idroes, R. (2020). Endophytic bacteria isolated from the leaf of Langusei (*Ficus minahassae*Tesym. & De Vr.) and their antibacterial activities. *Materials Science and Engineering*, *796*(1), 1-13, DOI: 10.1088/1757-899X/796/1/012047

Tang, W., Chen, J., Du, M., Chen, L., Yang, Y., Wu, S., Zhang, B., & Yu, C. (2020). Analysis of chemical constituents in *Ficus hirta* Vahl. by LCMS-IT-TOF and GC-MS. *International Conference on Materials Engineering and Applications*,*4*, 12-27. https://doi.org/10.1088/1757-899X/730/1/012027

Teoh, E. S., & Teoh, E. S. (2016). Secondary metabolites of plants. In *Medicinal orchids of Asia* (pp. 59-73). https://doi.org/10.1007/978-3-319-24274-3_5

Tetgure, S. R., Borse, A. U., Sankapal, B. R., Garole, V. J., & Garole, D. J. (2015). Green biochemistry approach for synthesis of silver and gold nanoparticles using *Ficus racemosa* latex and their pH-dependent binding study with different amino acids using UV/Vis absorption spectroscopy. *Amino Acids*, *47*(4), 757–765. https://doi.org/10.1007/s00726-014-1906-9.

Tharini, P., Sivaraj, C., Arumugam, P., & Manimaran, A. (2018). Antioxidant activities and GCMS analysis of fruits of *Ficus benghalensis* L. *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 518-523.

Timi, D., Gopalakrishnan, S., & Maino, M. (2019). Antimicrobial application and assessment of green synthesized silver nanoparticles using aqueous leaf extract of *Ficus copiosa. Journal of Biomedical Engineering and Medical Imaging*, *6*(3), 06–15. https://doi.org/10.14738/jbemi.63.7641

Tsao, Y. C., Chang, Y. J., Wang, C. H., & Chen, L. (2020). Discovery of isoplumbagin as a novel NQO1 substrate and anti-cancer quinone. *International Journal of Molecular Sciences*, *21*(12), 1-16. https://doi.org/10.3390/ijms21124378

Tulasi, C. D., Lakshmi, N. M., & Saida, L. (2018). Cell viability assay of *Ficus benghalensis* latex solvent extracts on different cell lines. *Asian Journal of Pharmaceuticals and Clinical Reseaerch*, *11*, 335-339. https://doi.org/10.22159/ajpcr.2018.v11i10.26552

Tulasi, C. D., Narasu, M. L., & Saida, L. (2018). Cytotoxic effect of *Ficus religiosa* and *Ficus benghalensis* latex extracts on MCF-7 cell line. *International Journal of Scientific Research in Biological Sciences*, *5*(6), 96-100. https://doi.org/10.26438/ijsrbs/v5i6.96100

Tuo, L., Yan, X.-R., & Xiao, J.-H. (2019). *Jiella endophytica* sp. a novel endophytic bacterium isolated from root of *Ficus microcarpa* Linn. *Antonie van Leeuwenhoek, 112*(10), 1457-1463. https://doi.org/10.1007/s10482-019-01274-8

Twaij, B. M., & Hasan, M. N. (2022). Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, *13*(1), 4-14. https://doi.org/10.3390/ijpb13010003

Ulug, B., Haluk Turkdemir, M., Cicek, A., & Mete, A. (2015). Role of irradiation in the green synthesis of silver nanoparticles mediated by fig (*Ficus carica*) leaf extract. *Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy*, *135*, 153–161. https://doi.org/10.1016/j.saa.2014.06.142.

Uma, B., Prabhakar, K., & Rajendran, S. (2009). *In vitro* antimicrobial activity and phytochemical analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against diarrhoeal enterotoxigenic *E. coli*. *Ethnobotanical Leaflets*, *2009*(4), 472-474.

Usman, M., Ahmed, A., Yu, B., Peng, Q., Shen, Y., & Cong, H. (2019). Photocatalytic potential of bio-engineered copper nanoparticles synthesized from *Ficus carica* extract for the degradation of toxic organic dye from waste water: Growth mechanism and study of parameter affecting the degradation performance. *Materials Research Bulletin*, *120*, 1-11. https://doi.org/10.1016/j.materresbull.2019.110583

Valentão, P, Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M., & Bastos, M. L. (2002). Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. Journal of food and agricultural Chemistry, *50*(17), 4989-4993. https://doi.org10.1021/jf20225o

Vallejo, F., Marín, J. G., & Tomás-Barberán, F. A. (2012). Phenolic compound content of fresh and dried figs (*Ficus carica* L.). *Food Chemistry*, *130*(3), 485-492. DOI: 10.1016/j.foodchem.2011.07.032

Veberic, R., Colaric, M., & Stampar, F. (2008). Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the Northern Mediterranean region. *Food Chemistry*, *106*(1), 153-157. https://doi.org/10.1016/j.foodchem.2007.05.061

Veerapur, V. P., Prabhakar, K. R., Parihar, V. K., Kandadi, M. R., Ramakrishana, S., Mishra, B., Satish Rao, B. S., Srinivasan, K. K., Priyadarsini, K. I., & Unnikrishnan, M. K. (2009). *Ficus racemosa* stem bark extract: A potent antioxidant and a probable natural radioprotector. *Evidence-Based Complementary and Alternative Medicine*, *6*(3), 317–324. https://doi.org/10.1093/ecam/nem119

Velayutham, K., Rahuman, A. A., Rajakumar, G., Roopan, S. M., Elango, G., Kamaraj, C., Marimuthu, S., Santhoshkumar, T., Iyappan, M., & Siva, C. (2013). Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against *Culex quinquefasciatus* and *Culex gelidus*. *Asian Pacific Journal of Tropical Medicine*, *6*(2), 95–101. https://doi.org/10.1016/S1995-7645(13)60002-4.

Verma, A. R., Vijayakumar, M., Rao, C. V., & Mathela, C. S. (2010). *In vitro* and *in vivo* antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*. *Food and Chemical Toxicology*, *48*(2), 704–709. https://doi.org/10.1016/j.fct.2009.11.052.

Verma, S., Singh, A., & Mishra, A. (2013). Gallic acid: Molecular rival of cancer. *Environmental Toxicology and Pharmacology*, *35*(3), 473–485. https://doi.org/10.1016/j.etap.2013.02.011

Vital, P. G., Velasco, R. N., Demigillo, J. M., & Rivera, W. L. (2010). Antimicrobial activity, cytotoxicity and phytochemical screening of *Ficus septica* Burm and *Sterculia foetida* L. leaf extracts. *Journal of Medicinal Plants Research*, *4*(1), 58-63. https://doi.org/10.5897/JMPR09.400

Wang, J., Yu, X., Cao, X., Tan, L., Jia, B., Chen, R., & Li, J. (2023). GAPDH: A common housekeeping gene with an oncogenic role in pan-cancer. *Computational and Structural Biotechnology Journal*, *21*, 4056-4069. https://doi.org/10.1016/j.csbj.2023.07.034

Wang, P., Liu, C., Sanches, T., Zhong, Y., Liu, B., Xiong, J., Neamati, N., & Zhao, G. (2009). Design and synthesis of novel nitrogen-containing polyhydroxylated aromatics as HIV-1 integrase inhibitors from caffeic acid phenethyl ester. *Bioorganic & Medicinal Chemistry Letters*, *19*(16), 4574–4578. https://doi.org/10.1016/j.bmcl.2009.06.100

Wang, Y., Yang, M. H., Wang, X. B., Li, T. X., & Kong, L. Y. (2014). Bioactive metabolites from the endophytic fungus *Alternaria alternata*. *Fitoterapia*, *99*, 153–158. https://doi.org/10.1016/j.fitote.2014.09.015

Wattimena, S. C., Ririmasse, V., Killay, A., & Patty, P. J. (2022). Kinetics of formation and characterization of green silver nanoparticles of *Ficus variegata* leaf extract. *Journal Kimia Sains Dan Aplikasi*, *25*(1), 34-40. https://doi.org/10.14710/jksa.25.1.34-40

Weathers, P. J., Towler, M. J., & Xu, J. (2010). Bench to batch: Advances in plant cell culture for producing useful products. *Applied Microbiology and Biotechnology*, *85*(5), 1339–1351. https://doi.org/10.1007/s00253-009-2354-4

Wong, R. S. (2011). Apoptosis in cancer: From pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, *30*(1), 1-14. https://doi.org/10.1186/1756-9966-30-87

Wu, M., Song, D., Li, H., Yang, Y., Ma, X., Deng, S., Ren, C., & Shu, X. (2019). Negative regulators of STAT3 signalling pathway in cancers. *Cancer Management and Research*, *11*, 4957–4969. https://doi.org/10.2147/CMAR.S206175

Xiaowen, H., Saravanakumar, K., Jin, T., & Wang, M. (2019). Mycosynthesis, characterization, anticancer and antibacterial activity of silver nanoparticles from endophytic fungus *Talaromyces purpureogenus*. *International Journal of Nanomedicine*, *14*, 3427-3438. DOI: 10.2147/IJN.S200817

Yadav, A., Kumari, R., Yadav, A., Mishra, J. P., Srivatva, S., & Prabha, S. (2016). Antioxidants and its functions in human body-A Review. *Research in Environment and Life Science*, *9*(11), 1328-1331.

Yadav, Y. C., Srivastava, D. N., Saini, V., Singhal, S., Seth, A. K., & Kumar, S. (2011). In-vitro antioxidant activity of methanolic extraction of *Ficus benghalensis* L. latex. *Pharmacology Online*, *1*, 140-148.

Yan, L., Zhu, J., Zhao, X., Shi, J., Jiang, C., & Shao, D. (2019). Beneficial effects of endophytic fungi colonization on plants. *Applied Microbiology and Biotechnology*, *103*, 3327-3340. https://doi.org/10.1007/s00253-019-09713-2

Yang, N., Lan, T., Han, Y., Zhao, H., Wang, C., Xu, Z., Chen, Z., Tao, M., Li, H., Song, Y., & Ma, X. (2023). Tributyrin alleviates gut microbiota dysbiosis to repair intestinal damage in antibiotic-treated mice. *PloS One*, *18*(7), 1-16. https://doi.org/10.1371/journal.pone.0289364

Yang, X. M., Yu, W., Ou, Z. P., Ma, H. L., Liu, W. M., & Ji, X. L. (2009). Antioxidant and immunity activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. *Plant Foods for Human Nutrition*, *64*(2), 167–173. https://doi.org/10.1007/s11130-009-0120-5.

Yao, L., Mo, Y., Chen, D., Feng, T., Song, S., Wang, H., & Sun, M. (2021). Characterization of key aroma compounds in Xinjiang dried figs (*Ficus carica* L.) by GC–MS, GC–olfactometry, odor activity values, and sensory analyses. *Food Science and Technology*, *150*, 1-9. DOI: 10.1016/j.lwt.2021.111982

Yarmolinsky, L., Huleihel, M., Zaccai, M., & Ben-Shabat, S. (2012). Potent antiviral flavone glycosides from *Ficus benjamina* leaves. *Fitoterapia*, *83*(2), 362–367. https://doi.org/10.1016/j.fitote.2011.11.014

Yen, G. C., Chen, C. S., Chang, W. T., Wu, M. F., Cheng, F. T., Shiau, D. K., & Hsu, C. L. (2018). Antioxidant activity and anticancer effect of ethanolic and aqueous extracts of the roots of *Ficus beecheyana* and their phenolic components. *Journal of Food and Drug Analysis*, *26*(1), 182–192. https://doi.org/10.1016/j.jfda.2017.02.002.

Yessoufou, K., Elansary, H. O., Mahmoud, E. A., & Skalicka-Woźniak, K. (2015). Antifungal, antibacterial and anticancer activities of *Ficus drupacea* L. stem bark extract and biologically active isolated compounds. *Industrial Crops and Products*, *74*, 752-758. https://doi.org/10.1016/j.indcrop.2015.06.011

Yin, I. X., Zhang, J., Zhao, I. S., Mei, M. L., Li, Q., & Chu, C. H. (2020). The antibacterial mechanism of silver nanoparticles and its application in dentistry. *International Journal of Nanomedicine*, *15*, 2555–2562. https://doi.org/10.2147/IJN.S246764

Zeeck, A., Schröder, K., Frobel, K., Grote, R., &Thiericke, R. (1987). The structure of manumycin I characterization, structure elucidation and biological activity. *The Journal of Antibiotics*, *40*(11), 1530–1540. https://doi.org/10.7164/antibiotics.40.1530

Zhang, H. C., Ma, Y. M., & Liu, R. (2012). Antimicrobial additives from endophytic fungus *Fusarium solani of Ficus carica*. *Applied Mechanics and Materials*, *178*, 783-786. DOI: 10.4028/www.scientific.net/AMM.

Zhang, H.C., Ma, Y.M., Liu, R., & Zhou, F. (2012). Endophytic fungus *Aspergillus tamarii* from *Ficus carica* L., a new source of indolyl diketopiperazines. *Journal of Natural Products*, *75*(1), 156-161. DOI: 10.1016/j.bse.2012.07.020

Zhang, X., Lv, H., Li, Z., Jiang, K., & Lee, M. R. (2015). HPLC/QTOF-MS/MS application to investigate phenolic constituents from *Ficus pandurata* aerial roots. *Biomedical Chromatography*, *29*(6), 860–868. https://doi.org/10.1002/bmc.3366.

Zhang, Y., Shareena Dasari, T. P., Deng, H., & Yu, H. (2015). Antimicrobial activity of gold nanoparticles and ionic gold. *Journal of Environmental Science and Health, 33*(3), 286-327. https://doi.org/10.1080/10590501.2015.1055161

Zhang, Y., Wan, Y., Huo, B., Li, B., Jin, Y., & Hu, X. (2018). Extracts and components of *Ficus carica* leaves suppress survival, cell cycle, and migration of triple-negative breast cancer MDA-MB-231 cells. *Onco Targets and Therapy*, *11*, 4377–4386. https://doi.org/10.2147/OTT.S171601

Zhou, C., Ma, J., Su, M., Shao, D., Zhao, J., Zhao, T., Song, Z., Meng, Y., & Jiao, P. (2018). Down-regulation of STAT3 induces the apoptosis and G1 cell cycle arrest in oesophageal carcinoma ECA109 cells. *Cancer Cell International*, *18*, 1-12. https://doi.org/10.1186/s12935-018-0549-4

Zhou, X., Zhu, H., Liu, L., Lin, J., & Tang, K. (2010). A review: Recent advances and future prospects of taxol-producing endophytic fungi. *Applied Microbiology and Biotechnology*, *86*(6), 1707–1717. https://doi.org/10.1007/s00253-010-2546-y.

Zieniuk, B. (2023). Dihydrocaffeic acid - Is it the less known but equally valuable phenolic acid?. *Biomolecules*, *13*(5), 1-41. https://doi.org/10.3390/ biom13050859

APPENDICES

Wagner's reagent **Appendix 1**

Iodine : 1.27 g

KI : 2 g

Dissolve the above chemicals in 5 ml H_2SO_4 and make upto 100 mL.

Griess reagent **Appendix 2**

Naphthyl ethylenediamine HCl: 0.1% in distilled water

Sulfanilamide: 1% in 5% H_3PO_4

Both solutions are mixed in 1:1 ratio

Phosphate buffer saline (PBS) **Appendix 3**

NaCl : 8 g

KCl : 0.2 g

Na_2HPO_4 : 1.44 g

KH_2PO_4 : 0.24 g

Dissolve in 1 L double distilled water and adjust pH to 7.4.

DMEM (Dulbecco's Modified Eagle's medium) **Appendix 4**

Sodium bicarbonate : 1.85 g

HEPES : 2.95 g

DMEM powder : 1 packet

Distilled water : 1 L

Vacuum sterilized and stored at 4^{o}C

Modified Carnoy's fluid **Appendix 5**

Acetic acid : 10 mL

Ethanol : 30 mL

Acetocarmine **Appendix 6**

Carmine : 2 g

Acetic acid : 100 mL of 45% acetic acid

The solution is heated to dissolve carmine and is filtered to remove the undissolved carmine powder.

Ethidium bromide **Appendix 7**

Ethidium bromide: 20 µg/ mL

Add 10 mg to 50 mL distilled water and store at room temperature (10X).

For making 1X stock, mix 1 mL with 9 mL of distilled water. Handle ethidium bromide with caution as it is a known carcinogen.

TE (Tris-EDTA) buffer **Appendix 8**

Tris HCl: 10 mM, pH 8

EDTA: 0.1 mM, pH 8

Ringer's salt solution **Appendix 9**

NaCl : 8.6 g

$CaCl_2$. $2H_2O$: 330 mg

KCl : 300 mg

pH 8

Dissolve the above chemicals in 900 mL of distilled water

Sterilized the solution by filtration and stored at 4°C

Moscona solution **Appendix 10**

NaCl : 8 g

KCl : 0.3 g

Na_2HPO_4. H_2O : 0.05 g

KH_2PO_4 : 0.025 g

$NaHCO_3$: 1.0 g

D (+) – glucose : 2 g

pH 7.4

Dissolve the above chemicals in 900 mL of distilled water

Sterilized by filtration and stored at 20°C.

Calcium- and magnesium-free Hank's balanced salt solution Appendix 11

NaCl	:	8 g
KCl	:	0.4 g
KH_2PO_4	:	0.06 g
$NaHCO_3$	:	0.35 g
Na_2HPO_4. $12H_2O$	:	0.112 g

pH 7.4

Dissolve the above chemicals in 900 mL of distilled water

Sterilized by filtration and stored at 4°C.