

**Bioactivity screening and phytochemical profiling of  
selected species of *Euphorbia* L.  
(Euphorbiaceae)**

*Thesis  
submitted to the University of Calicut  
for the award of the degree of*

**DOCTOR OF PHILOSOPHY IN BOTANY**

by

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**CERTIFICATE**

I certify that no corrections were pointed out by the adjudicators in thesis entitled  
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## **DECLARATION**

I, Sameena V. P., hereby declare that the thesis entitled “**Bioactivity screening and phytochemical profiling of selected species of *Euphorbia* L. (Euphorbiaceae)**” 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

### Bioactivity screening and phytochemical profiling of selected species of *Euphorbia* L. (Euphorbiaceae)

*Euphorbia* is one of the three groups of flowering plants with the highest species richness. *Euphorbia* plants have been used in traditional medicine for centuries in various parts of the World, including China, Europe, India, Africa, Iran, and Pakistan. Traditional medicine has utilised various species of *Euphorbia* to treat a range of ailments. The distribution of phytochemicals and potential biological activities of three *Euphorbia* species (*E. deccanensis*, *E. rothiana*, and *E. thymifolia*) are the focus of the current study.

The phytoconstituents found in the chosen *Euphorbia* species were identified using GC-MS and HR LC-MS analyses, quantitative estimation of major phytoconstituents, and qualitative phytochemical analyses. It was observed that there were more non-volatile chemicals than volatile ones in all three selected *Euphorbia* species. In the HRLC-MS analysis, the methanolic extract of *E. deccanensis* contained the highest concentration of phytoconstituents. The phytochemical profiling revealed the presence of numerous bioactive secondary metabolites with synergistic activity that can have significant therapeutic effects. DPPH, hydroxyl, nitric oxide, ABTS, and superoxide radical scavenging assays were employed to evaluate the ability of the selected *Euphorbia* species to scavenge free radicals. The *E. deccanensis* extract demonstrated strong antioxidant activity, according to the IC<sub>50</sub> values. The potential of the selected *Euphorbia* species to reduce inflammation was first assessed using two *in vitro* biochemical assays, and the most effective plant extract (*E. deccanensis*) was chosen for further investigation of its activity on the LPS-induced RAW 264.7 macrophage cell line. ELISA was used to measure COX-2, one of the main inflammatory mediators. From the results, the plant extract of *E. deccanensis* can be considered a good candidate for anti-inflammatory experiments. The methanolic extracts of the three selected *Euphorbia* plants were tested for cytotoxicity using the *Allium cepa* root tip assay. The percentage of aberration increased, and the mitotic index decreased as a result of the cytotoxic effects of all three plant extracts in relation to dosage. All three extracts showed both clastogenic and non-clastogenic aberrations. To evaluate the antiproliferative potential of the selected *Euphorbia* plants, the MCF-7 (human breast cancer) cell line and the normal L929 cell line were chosen. On analysing the cell viability percentages and the LC<sub>50</sub> values of all three extracts in both the cell lines through MTT assay, *E. deccanensis* was found to

significantly suppress the proliferation of MCF-7 breast cancer cells. This extract was chosen for further studies. The double staining method revealed that the cause of the antiproliferative effect was due to apoptosis rather than necrosis. Flow cytometry analysis revealed that the stoppage of the cell cycle occurred at the G0/G1 phase of the cell cycle. The upregulation of p53 and downregulation of STAT-3 further confirmed the anticancer effect of *E. deccanensis* methanolic extract on the breast cancer cells under investigation.

Silver nanoparticles were effectively synthesized from the three *Euphorbia* species under consideration. They are characterised using FTIR, FE-SEM, and UV-visible spectroscopy. Silver nitrate was considerably reduced to silver nanoparticles in every plant extract that was examined. A reddish-brown colour developed when the plant extract and 2 mM silver nitrate solution were mixed, signifying the production of silver nanoparticles. The development of silver nanoparticles was confirmed by UV-visible spectroscopy. FE-SEM analysis was used to determine the morphology of the synthesised nanoparticles, which showed that they were roughly spherical and ranged in size from 23 to 44 nm. FTIR analysis was used to identify the different phytochemicals that contributed to the reduction of the silver nitrate solution to Ag NPs. In antibacterial tests, green-synthesised silver nanoparticles have proven to be effective against both Gram-positive and Gram-negative bacteria. The biofabricated Ag NPs of all three *Euphorbia* species also demonstrated significant antifungal properties. Thus it is confirmed that all three *Euphorbia* plant species contain components that inhibit the growth of deadly pathogenic microorganisms.

Keywords: *Euphorbia*, Antioxidant, Cytotoxicity, Breast cancer, Silver nano Particles



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## സംഗ്രഹം

### Bioactivity screening and phytochemical profiling of selected species of *Euphorbia* L. (Euphorbiaceae)

ഏറ്റവും കൂടുതൽ സ്പീഷീസ് സമ്പന്നതയുള്ള മൂന്ന് പൂച്ചെടികളിൽ ഒന്നാണ് യൂഫോർബിയ. ചൈന, യൂറോപ്പ്, അമേരിക്ക, തുർക്കി, ഇന്ത്യ, ആഫ്രിക്ക, ഇറാൻ, പാകിസ്ഥാൻ എന്നിവയുൾപ്പെടെ ലോകത്തിന്റെ വിവിധ ഭാഗങ്ങളിൽ പരമ്പരാഗത വൈദ്യശാസ്ത്രത്തിൽ യൂഫോർബിയ സസ്യങ്ങൾ വളരെക്കാലമായി ഉപയോഗിച്ചുവരുന്നു. വിവിധരോഗങ്ങൾ ചികിത്സിക്കാൻ പരമ്പരാഗതവൈദ്യശാസ്ത്രം യൂഫോർബിയയുടെ വിവിധ ഇനങ്ങൾ ഉപയോഗിച്ചിട്ടുണ്ട്. മൂന്ന് യൂഫോർബിയ ഇനങ്ങളുടെ (ഇ. ഡെക്കാനെൻസിസ്, ഇ. റോത്തിയാന, ഇ. തൈമിഫോളിയ) ഫൈറ്റോകെമിക്കലുകളുടെ വിതരണവും, അതിന്റെ സാധ്യതയുള്ള ജൈവപ്രവർത്തനങ്ങളുമാണ് നിലവിലെ പഠനത്തിന്റെ കേന്ദ്രബിന്ദു.

തിരഞ്ഞെടുത്ത യൂഫോർബിയ സ്പീഷീസുകളിൽ കാണപ്പെടുന്ന ഫൈറ്റോകെമിക്കലുകളുടെ ജി.സി.എം.എസ്. (GC-MS), എച്ച്.ആർ.-എൽ.സി.എം.എസ്. (HR-LCMS) വിശകലനങ്ങൾ, പ്രധാന ഫൈറ്റോകെമിക്കലുകളുടെ അളവ് കണക്കാക്കൽ, അവയുടെ ഗുണപരമായ വിശകലനങ്ങൾ എന്നിവ ഉപയോഗിച്ചാണ് തിരിച്ചറിഞ്ഞത്. തിരഞ്ഞെടുത്ത മൂന്ന് യൂഫോർബിയ സ്പീഷീസുകളിലും ബാഷ്പശീലമല്ലാത്ത രാസവസ്തുക്കളേക്കാൾ കൂടുതൽ ബാഷ്പശീലമല്ലാത്ത രാസവസ്തുക്കളുണ്ടെന്ന് നിരീക്ഷിക്കപ്പെട്ടു. എച്ച്.ആർ.-എൽ.സി.എം.എസ്. വിശകലനത്തിൽ, ഇ. ഡെക്കാനെൻസിസിന്റെ മെഥനോളിക് സത്തിൽ പരമാവധി ഫൈറ്റോകെമിക്കലുകൾ അടങ്ങിയിരിക്കുന്നതായി കണ്ടു. ഗണ്യമായ ചികിത്സാഫലങ്ങൾ ഉണ്ടാക്കാൻ കഴിയുന്ന സിനർജിസ്റ്റിക് പ്രവർത്തനമുള്ള നിരവധി ബയോ ആക്ടിവ് സെക്കൻഡറി മെറ്റബോളൈറ്റുകളുടെ സാന്നിധ്യം ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിംഗ് വെളിപ്പെടുത്തി.

തിരഞ്ഞെടുത്ത യൂഫോർബിയ സ്പീഷീസുകൾക്ക് ഫ്രീ റാഡിക്കലുകളെ നീക്കം ചെയ്യാനുള്ള കഴിവ് വിലയിരുത്താൻ ഡി.പി.പി.എച്ച്. (DPPH), ഹൈഡ്രോക്സിൽ, നൈട്രിക് ഓക്സൈഡ്, എ.ബി.ടി.എസ്. (ABTS), സൂപ്പർഓക്സൈഡ് റാഡിക്കൽ സ്ക്വാവഞ്ചിംഗ് അസ്സേയ്സ് ഉപയോഗിച്ചു. ഐ.സി. 50 (IC50) മൂല്യങ്ങൾ അനുസരിച്ച്, ഇ. ഡെക്കാനെൻസിസിൻ്റെ സത്ത് ശക്തമായ ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം പ്രകടമാക്കി. തിരഞ്ഞെടുത്ത യൂഫോർബിയ സ്പീഷീസുകളുടെ വീക്കം കുറയ്ക്കാനുള്ള കഴിവ് ആദ്യം രണ്ട് ഇൻ വിടോ ബയോകെമിക്കൽ അസ്സേയ്സ് ഉപയോഗിച്ച് വിലയിരുത്തി, LPS-ഇൻഡ്യൂസ്ഡ് റോ 264.7 (RAW 264.7 മാക്രോഫേജ് സെൽ ലൈനിൽ അതിന്റെ പ്രവർത്തനത്തെക്കുറിച്ചുള്ള കൂടുതൽ അന്വേഷണത്തിനായി ഏറ്റവും ഫലപ്രദമായ സസ്യ സത്തായ ഇ. ഡെക്കാനെൻസിസ് തിരഞ്ഞെടുത്തു. പ്രധാന വീക്കം മധ്യസ്ഥരിൽ ഒന്നായ കോക്സ്-2 (COX-2) അളക്കാൻ എലിസ (ELISA) ഉപയോഗിച്ചു. ഫലങ്ങളിൽ നിന്ന്, ഇ. ഡെക്കാനെൻസിസിൻ്റെ സസ്യ സത്ത് ആന്റി-ഇൻഫ്ലമേറ്ററി പരീക്ഷണങ്ങൾക്ക് ഫലപ്രദമായി കണക്കാക്കാം.

തിരഞ്ഞെടുത്ത മൂന്ന് യൂഫോർബിയ സസ്യങ്ങളുടെ മെഥനോളിക് സത്തുകൾ അല്ലിയം സെപ (*Allium cepa*) റൂട്ട് ടിപ്പ് അസ്സേയ്സ് ഉപയോഗിച്ച് സൈറ്റോടോക്സിസിറ്റിക്കായി പരിശോധിച്ചു. മൂന്ന് സസ്യ

സത്തകളുടെയും ഡോസേജമായി ബന്ധപ്പെട്ട് സൈറ്റോടോക്സിക് ഇഫക്റ്റുകളുടെ ഫലമായി വ്യതിയാനത്തിന്റെ ശതമാനം വർദ്ധിച്ചു, മൈറ്റോട്ടിക് സൂചിക കുറഞ്ഞു. മൂന്ന് സത്തകളും ക്ലസ്റ്റോജെനിക്, നോൺ-ക്ലസ്റ്റോജെനിക് വ്യതിയാനങ്ങൾ കാണിച്ചു. തിരഞ്ഞെടുത്ത യൂഫോർബിയ സസ്യങ്ങളുടെ ആന്റിപ്രൊലിഫറേറ്റീവ് സാധ്യത വിലയിരുത്തുന്നതിന്, എം.സി.എഫ്-7 (MCF-7) (മനുഷ്യ സ്തനാർബുദം) സെൽ ലൈനും സാധാരണ എൽ929 (L929) സെൽ ലൈനും തിരഞ്ഞെടുത്തു. എം. ടി. ടി. (MTT) അസ്സേയിലൂടെ രണ്ട് സെൽ ലൈനുകളിലെയും സെൽ എബിലിറ്റി ശതമാനങ്ങളും മൂന്ന് സത്തകളുടെയും എൽ.സി. 50 (LC50) മൂല്യങ്ങളും വിശകലനം ചെയ്തപ്പോൾ, ഇ. ഡെക്കാനെൻസിസ് എം.സി.എഫ്-7 സ്തനാർബുദ കോശങ്ങളുടെ വ്യാപനത്തെ ഗണ്യമായി അടിച്ചമർത്തുന്നതായി കണ്ടെത്തി. കൂടുതൽ പഠനങ്ങൾക്കായി ഈ സത്ത് തിരഞ്ഞെടുത്തു. ഇരു സ്റ്റേയിനിംഗ് രീതി ആന്റിപ്രൊലിഫറേറ്റീവ് ഇഫക്റ്റിന്റെ കാരണം നെക്രോസിസിനേക്കാൾ അപ്റ്റോപ്റ്റോസിസ് മൂലമാണെന്ന് വെളിപ്പെടുത്തി. ഫ്ലോ സൈറ്റോമെട്രി വിശകലനം സെൽ സൈക്കിളിന്റെ ജി0/ജി1 (G0/G1) ഘട്ടത്തിൽ സെൽ സൈക്കിൾ നിലച്ചതായി വെളിപ്പെടുത്തി. പി53 (p53) ന്റെ വർദ്ധിച്ച നിയന്ത്രണവും സ്റ്റാറ്റ്-3 (STAT-3) ന്റെ കുറഞ്ഞ നിയന്ത്രണവും, അന്വേഷണത്തിലിരിക്കുന്ന സ്തനാർബുദ കോശങ്ങളിൽ ഇ. ഡെക്കാനെൻസിസ് മെഥനോളിക് സത്തിന്റെ കാൻസർ വിരുദ്ധ പ്രഭാവം കൂടുതൽ സ്ഥിരീകരിച്ചു.

പരിഗണനയിലുള്ള മൂന്ന് യൂഫോർബിയ സ്പീഷീസുകളിൽ നിന്ന് സിൽവർ നാനോകണങ്ങൾ ഫലപ്രദമായി സമന്വയിപ്പിച്ചു. എഫ്.ടി.ഐ.ആർ. (FTIR), എഫ്.ഇ.-എസ്.ഇ.എം. (FE-SEM), യു.വി.-ദൃശ്യ സ്പെക്ട്രോസ്കോപ്പി (UV-VIS Spectroscopy) എന്നിവ ഉപയോഗിച്ചാണ് ഇവയുടെ സ്വഭാവം പരിശോധിച്ചത്. നിരീക്ഷണത്തിലുള്ള മൂന്ന് സസ്യ സത്തകളിലും സിൽവർ നൈട്രേറ്റ് സിൽവർ നാനോകണങ്ങളായി ഗണ്യമായി കുറഞ്ഞു. സസ്യ സത്തും 2 mM സിൽവർ നൈട്രേറ്റ് ലായനിയും കലർത്തിയപ്പോൾ ചുവപ്പ് കലർന്ന തവിട്ട് നിറം രൂപപ്പെട്ടു. ഇത് സിൽവർ നാനോകണങ്ങളുടെ ഉത്പാദനത്തെ സൂചിപ്പിക്കുന്നു. യു.വി.-ദൃശ്യ സ്പെക്ട്രോസ്കോപ്പി വഴി സിൽവർ നാനോകണങ്ങളുടെ വികസനം സ്ഥിരീകരിച്ചു. സിന്തസൈസ് ചെയ്ത നാനോകണങ്ങളുടെ രൂപഘടന നിർണ്ണയിക്കാൻ എഫ്.ഇ.-എസ്.ഇ.എം. വിശകലനം ഉപയോഗിച്ചു, അവ ഏകദേശം ഗോളാകൃതിയിലുള്ളതാണെന്നും 23 മുതൽ 44 nm വരെ വലുപ്പമുള്ളതാണെന്നും ഇത് കാണിച്ചു. സിൽവർ നൈട്രേറ്റ് ലായനി കുറയ്ക്കുന്നതിന് കാരണമായ വ്യത്യസ്ത ഫൈറ്റോകോമ്പൗണ്ടുകളെ തിരിച്ചറിയാൻ എഫ്.ടി.ഐ.ആർ. വിശകലനം ഉപയോഗിച്ചു. ആൻറി ബാക്ടീരിയൽ പരിശോധനകളിൽ, ഗ്രാം-പോസിറ്റീവ്, ഗ്രാം-നെഗറ്റീവ് ബാക്ടീരിയകൾക്കെതിരെ ബയോ സിന്തസൈസ് ചെയ്ത സിൽവർ നാനോകണങ്ങൾ ഫലപ്രദമാണെന്ന് തെളിയിക്കപ്പെട്ടിട്ടുണ്ട്. മൂന്ന് യൂഫോർബിയ സ്പീഷീസുകളുടെയും ബയോഫാബ്രിക്കേറ്റഡ് എ.ജി.എൻ.പി. (Ag NP)-കൾ ഗണ്യമായ ആന്റിഫംഗൽ ഗുണങ്ങളും പ്രകടമാക്കി. മൂന്ന് യൂഫോർബിയ സസ്യ ഇനങ്ങളിലും മാരകമായ രോഗകാരിയായ സൂക്ഷ്മാണുക്കളുടെ വളർച്ചയെ തടയുന്ന ഘടകങ്ങൾ അടങ്ങിയിട്ടുണ്ടെന്നതിന്റെ വ്യക്തമായ തെളിവാണ്.

സൂചകപദങ്ങൾ: യൂഫോർബിയ, ആന്റിഓക്സിഡന്റ്, സൈറ്റോടോക്സിസിറ്റി, സ്തനാർബുദം, സിൽവർ നാനോ കണികകൾ

  
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## **DECLARATION**

I, Sameena V. P., hereby declare that the thesis entitled “**Bioactivity screening and phytochemical profiling of selected species of *Euphorbia* L. (Euphorbiaceae)**” 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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***Sameena V. P.***

*Dedicated to*  
*Owner of the Day of Judgement*

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## ABBREVIATIONS

|                    |   |                                          |
|--------------------|---|------------------------------------------|
| µg/ml              | : | Microgram per millilitre                 |
| µg                 | : | Microgram                                |
| µl                 | : | Microlitre                               |
| µm                 | : | Micrometre                               |
| µM                 | : | Micromolar                               |
| Ag                 | : | Silver                                   |
| AgNP               | : | Silver nanoparticle                      |
| AlCl <sub>3</sub>  | : | Aluminium chloride                       |
| amu                | : | Atomic mass unit                         |
| ANOVA              | : | Analysis of variance                     |
| AO                 | : | Acridine orange                          |
| Au Cl <sub>4</sub> | : | Tetrachloroaurate                        |
| Bcl-2              | : | B-cell lymphoma 2                        |
| BLAST              | : | Basic local alignment search tool        |
| CaCl <sub>2</sub>  | : | Calcium chloride                         |
| cDNA               | : | Complementary DNA                        |
| cm                 | : | Centimetre                               |
| CO <sub>2</sub>    | : | Carbon dioxide                           |
| COX                | : | Cyclooxygenase                           |
| CUPRAC             | : | Cupric ion reducing antioxidant capacity |
| DLS                | : | Dynamic light scattering                 |
| DMEM               | : | Dulbecco's Modified Eagle's Medium       |
| DMSO               | : | Dimethyl sulphoxide                      |
| dNTP               | : | Deoxyribonucleotide Triphosphate         |
| DPPH               | : | 2, 2-Diphenyl-1- Picrylhydrazyl          |
| ED                 | : | <i>E. deccanensis</i>                    |



|                                |   |                                                           |
|--------------------------------|---|-----------------------------------------------------------|
| EDTA                           | : | Ethylenediaminetetraacetic acid                           |
| ELISA                          | : | Enzyme-linked immunosorbent assay                         |
| ER                             | : | <i>E. rothiana</i>                                        |
| ER                             | : | Estrogen receptor                                         |
| ET                             | : | <i>E. thymifolia</i>                                      |
| EtBr                           | : | Ethidium bromide                                          |
| eV                             | : | Electron volt                                             |
| FeCl <sub>3</sub>              | : | Ferric chloride                                           |
| FE-SEM                         | : | Field Emission Scanning Electron Microscope               |
| Fig.                           | : | Figure                                                    |
| FRAP Assay                     | : | Ferric reducing antioxidant power Assay                   |
| FTIR                           | : | Fourier Transform Infrared Spectroscopy                   |
| FWHM                           | : | Full Width at Half Maximum Intensity                      |
| G                              | : | Gram                                                      |
| GC-MS                          | : | Gas Chromatography - Mass Spectrometry                    |
| H                              | : | Hour                                                      |
| H <sub>2</sub> O <sub>2</sub>  | : | Hydrogen peroxide                                         |
| H <sub>2</sub> SO <sub>4</sub> | : | Sulphuric acid                                            |
| HCl                            | : | Hydrochloric acid                                         |
| HE Agar                        | : | Hektoen Enteric Agar                                      |
| HEPES                          | : | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid        |
| HPLC                           | : | High Performance Liquid Chromatography                    |
| HRLC-MS                        | : | High Resolution Liquid Chromatography - Mass Spectrometry |
| IC <sub>50</sub>               | : | Inhibition Concentration 50%                              |
| iNOS                           | : | Inducible nitric oxide synthase                           |
| ITS                            | : | Internal transcribed spacer                               |
| KCl                            | : | Potassium chloride                                        |

|                                 |   |                                                                         |
|---------------------------------|---|-------------------------------------------------------------------------|
| KOH                             | : | Potassium hydroxide                                                     |
| L                               | : | Litre                                                                   |
| L929                            | : | Continuous mouse fibroblast cell line (NCTC clone 929)                  |
| LC <sub>50</sub>                | : | Lethal concentration 50%                                                |
| LDH                             | : | Lactate Dehydrogenase                                                   |
| LDLs                            | : | Low-density lipoproteins                                                |
| LOX                             | : | Lipoxygenase                                                            |
| LPS                             | : | Lipopolysaccharide                                                      |
| m/z                             | : | Mass-to-charge                                                          |
| M                               | : | Molar                                                                   |
| MCF-7                           | : | Michigan Cancer Foundation-7<br>(Human breast cancer cell line)         |
| MDA-MB 231                      | : | M. D. Anderson-Metastatic Breast 231<br>(Human breast cancer cell line) |
| mg                              | : | Milligram                                                               |
| MHI agar                        | : | Mueller-Hinton Agar Medium                                              |
| min                             | : | Minute                                                                  |
| ml                              | : | Millilitre                                                              |
| mm                              | : | Millimetre                                                              |
| mM                              | : | Millimolar                                                              |
| MTT                             | : | 3-(4,5-dimethylthiazol-2-yl),2-5-diphenyltetrazolium<br>bromide         |
| N                               | : | Normal                                                                  |
| Na <sub>2</sub> CO <sub>3</sub> | : | Sodium carbonate                                                        |
| NaCl                            | : | Sodium chloride                                                         |
| NADH                            | : | Nicotinamide Adenine Dinucleotide (reduced)                             |
| NaNO <sub>2</sub>               | : | 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                                                    |
| NOS       | : | Nitric oxide synthase                                           |
| NSAIDs    | : | Non-steroidal anti-inflammatory drugs                           |
| °C        | : | Degree Celsius                                                  |
| ORAC      | : | Oxygen Radical Absorbance Capacity                              |
| PBS       | : | Phosphate-Buffered Saline                                       |
| PR        | : | Progesterone Receptor                                           |
| RAW 264.7 | : | Mouse leukemic monocyte/macrophage cell line                    |
| RNS       | : | Reactive nitrogen species                                       |
| ROS       | : | Reactive oxygen species                                         |
| rpm       | : | Rotations per minute                                            |
| RT        | : | Retention Time                                                  |
| RT-qPCR   | : | Reverse Transcription-quantitative Polymerase Chain<br>Reaction |
| s         | : | Seconds                                                         |
| SE        | : | Standard error                                                  |
| SEM       | : | Scanning Electron Microscopy                                    |
| SPR       | : | Surface Plasmon Resonance                                       |
| SPSS      | : | Statistical package for social sciences                         |
| STAT 3    | : | Signal transducer and activator of transcription 3              |
| TBA       | : | Thiobarbituric acid                                             |
| TCA       | : | Trichloroacetic acid                                            |
| TE        | : | Tris EDTA                                                       |
| TEAC      | : | Trolox equivalent antioxidant capacity                          |

|              |   |                                     |
|--------------|---|-------------------------------------|
| TEM          | : | Transmission Electron Microscopy    |
| TGF- $\beta$ | : | Transforming growth factor- $\beta$ |
| TNF          | : | Tumour necrosis factor              |
| UV           | : | Ultraviolet                         |
| UV-Vis       | : | Ultraviolet-visible                 |
| v/v          | : | Volume per volume                   |
| V            | : | Volt                                |
| w/v          | : | Weight per volume                   |
| WHO          | : | World Health Organisation           |
| XRD          | : | X-ray Diffraction                   |



# INTRODUCTION

From the dawn of recorded human history, there has been an association between humans and plants. The surrounding plants have been utilized by humans for various purposes, including clothing, fuel, food, and shelter. Life on Earth first appeared a few years after the formation of our planet, approximately 4.87 billion years ago. Approximately three billion years ago, the first photosynthetic organisms, known as Cyanobacteria, emerged. Angiosperms had evolved through multiple successive forms of life, and during the Cretaceous period, several families, including the Onagraceae, Myrtaceae, and Fabaceae, had developed and underwent continuous differentiation and multiplication. Following that, Asteraceae, one of the largest families based on recent estimates, evolved (Raven, 2021).

Mankind has relied on nature's healing powers for thousands of years for medical purposes. The medicinal plants exhibited great potential for healing because they contain secondary metabolites like glycosides, alkaloids, phenols, phytosterols, and diterpenes. Regardless of part, the plant was beneficial to all life on Earth. Plant parts that may be useful for medicinal purposes include the leaf, fruit, seed, flower, and sometimes the entire plant. Medical plants have very few side effects and can be used safely to treat a variety of illnesses. In addition, this herbal remedy is suitable for all age groups and genders. Thus, Medicinal plants are used as the main ingredient in many drugs, and the majority of modern medications are derived from plants. To date, a large number of practitioners of traditional medicine from around the globe have investigated medicinally valued plants to develop medical treatments (Hassan, 2015).

In ancient times, the various forms of medicinal plants, such as crude extracts or pure molecules isolated from the plants, were the mode of medication. People were aware of the curative properties of medicinal plants in prehistoric times. There is a wealth of evidence in the literature supporting human use of medicinal plants. One such ancient written record is found on a Sumerian clay slab from Nagpur that describes about 250 plants, including the alkaloid-containing plants like poppy, mandrake, and henbane, which are used to make a variety of medicines.

Chinese and Egyptians used plants as medicine for more than 27 centuries BC, making them among the first people to do so. One of the most famous Chinese book, “Pen T’Sao” written by Emperor Shen Nung circa, is said to contain 365 medicines prepared from the dried parts of medicinal plants. Ephedra, podophyllum, and cinnamon bark are just a few of the plants he included in his book that are still significant and have therapeutic uses. Greeks were also highly knowledgeable about important plant species for medicine. Hippocrates, the father of Greek medicine, employed a variety of highly valued species as remedies for a wide range of ailments. Theophrastus then wrote books on plants, including “De Causis Plantarum”, which describes plant etiology, and “De Historia Plantarum”, which explores plant history. In his book, he provided information on almost 500 plants with therapeutic value. Subsequently, a military physician named Dioscorides—regarded as the father of pharmacognosy—wrote a book titled “De Materia Medica” in which he elucidates the importance and necessity of the therapeutic potential of roughly 600 plants (Jamshidi-Kia et al., 2017; Petrovska, 2012).

Phytomedicines, also known as plant-based medicines and herbal drugs have been used for centuries in India and are an essential part of the country's healthcare systems. Approximately 80% of the rural population in this area relies primarily on traditional medical systems like Ayurveda, Unani, and Siddha. As per the government of India, around 65% of the Indian population is solely dependent on traditional medicines for their healthcare needs. The Indian subcontinent harbours an extensive collection of diverse medicinal plants, primarily found in areas such as the Eastern Himalayas, Western Ghats, and the Andaman and Nicobar Islands. India is referred to as the world's botanical garden because of its extraordinary capacity to produce a large number of medicinal plants. In India, there are reportedly about 45,000 plants with therapeutic qualities. Out of these, traditional healers use between 7000 and 7500 plant species to treat a range of illnesses (Singh, 2023; Kadam & Pawar, 2020).

Synthetically derived medications developed in laboratories are being produced and used more frequently to treat a variety of ailments. But in some ways, using it damages the body. Furthermore, a variety of unfavourable side effects were brought on by the use of synthetic drugs like steroids, antibiotics, and painkillers.

This has led to a surge in the public's desire for medicinally valuable plants and their products, as well as an alarming strengthening of their use. Additionally, the role of traditional and medicinal plants in the contemporary drug industries has grown due to the compounds' effective synergistic action and minimal negative effects (Dar et al., 2017; Rohilla & Gupta, 2023).

Phytochemicals are low molecular weight, bioactive, nutritional, or non-nutritional metabolites that are naturally occurring in plants. They can be found in a wide range of foods, such as whole grains, fruits, vegetables, nuts, legumes, and herbs. Countless varieties of phytocompounds have been identified up to this point. The principal phytocompounds found in plants are polyphenols, polysaccharides, isoprenoids, saponins, flavonoids, phytosterols, etc. Primary metabolites such as fats, proteins, and carbohydrates are primarily involved in energy metabolism and aids in the construction of plant cell structure. They also support the upkeep of cellular functions such as respiration, photosynthesis, and translocation. Conversely, the byproducts of the primary metabolites, known as secondary metabolites, enable plants to fend off insects and other predators and are fundamental to how plants interact with their surroundings. Phytochemicals are primarily produced by plants to protect themselves against environmental stresses and enemies, but when consumed by humans, these metabolites can also have pronounced bioactive effects (Kumar et al., 2023).

Secondary metabolites have numerous uses in dyes, crop protection, medications, flavours, and fragrances, which have connected them to the medical and biological domains. The antifungal, antibacterial, and antiviral properties of secondary metabolites provide a scientific basis for their use in traditional medicine. The three types of metabolites that drew the most attention were flavonoids, phenolic compounds, and anthocyanins. The phytochemical compound polyphenol, which is produced by the shikimate pathway in higher groups of plants, is very beneficial to human health and has anti-inflammatory, anti-cancer, antimicrobial, and anti-allergic properties in addition to its important role in maintaining plant physiology (Khade et al., 2023). Moreover, plants with high concentrations of



flavonoids and phenolic compounds have been shown to have antioxidant properties; as a result, they are used to treat Parkinson's disease, depression, and anxiety. Another significant class of metabolites is alkaloids, which have a wide range of pharmacological characteristics and recreational effects (Abubakar & Haque, 2020).

Typically, the term medicinal plant refers to a plant that contains either secondary metabolites or active components with significant bioactivity. Phytochemicals present in medicinal plants work in a synergistic or additive manner to exert their medicinal effects; this combined action of phytochemicals eliminates the side effects associated with the dominance of a single xenobiotic compound. This also aids in giving herbal medications a wide range of action and prevents infections from developing resistance (Njeru et al., 2013). The active components of medicinal plants have a wide range of medical uses. Moreover, these bioactive substances can be extracted from therapeutic plants without compromising their effectiveness. Therefore, in order to carry out the required research and identify the important primary and secondary metabolic compounds, it is imperative to understand the biochemical composition of medicinal plants and their potential therapeutic uses.

Because of their therapeutic qualities, which can aid in preventing and fending off a variety of diseases, phytocompounds have a significant impact on human health. In addition, these substances demonstrated a wide range of pharmacological actions on humans, acting as neuroprotective, hepatoprotective, anticancer, and inflammatory agents. It has also been demonstrated that they must prevent the onset of obesity, diabetes, cancer, and cardiovascular diseases. Therefore, phytocompounds hold the greatest promise for the fields of medicine and health sciences. Additionally, nutraceuticals, which have a high concentration of phytocompounds, and functional foods, which include phytocompounds as constituents, are used as preventive measures to ward off numerous illnesses (Gupta, 2023). Antibacterial activity is a significant characteristic displayed by phytochemicals. It demonstrated this activity in a number of ways, including the

inhibition of the development of bacterial films, suppressing enzymes, and causing damage to the bacterial membrane. Additionally, polyphenols work against bacteria by breaking down the cell membrane or by preventing the synthesis of amino acids, which are necessary for bacterial growth (Rodríguez-Negrete et al., 2024).

Plants can heal due to the actions of their phytochemicals, either separately or in combination. Phytochemical screening plays a major role in the identification of possible medicinal properties in plants. Thus, phytochemical analysis is an essential tool for figuring out the composition, metabolism, and biological functions of different organic compounds found in plants. This will enable the isolation and assessment of the qualitative and quantitative status of potent compounds. Though there are a plethora of techniques for analysing phytoconstituents, traditional qualitative tests remain widely used for the initial identification of phytochemicals present in plants (Shaikh & Patil, 2020). Quantitative analysis is a more thorough and useful approach than qualitative testing. In this instance, the results show promise for drug discovery and standardisation pertaining to herbal drugs. They also shed light on the toxicity levels of various plants and provide an explanation for their medicinal activity (Egbuna et al., 2018). The most important stage in separating the phytoconstituents is extraction. During extraction, caution must be taken to prevent any loss or disruption of phytochemicals. Even though there are three different kinds of extraction processes, solvent extraction is the most common. The choice of solvent is crucial in this situation. The main factors affecting extraction procedures are temperature, time, solvent pH, sample-to-solvent ratio, and sample dimensions. The process of extracting phytochemicals primarily involved the solutes dissolving in the solvent after the solvent permeated the solute matrix. Subsequently, the solute diffuses from the solid and is eventually extracted (Sinha et al., 2022).

Many efforts have been made to properly identify active compounds using a variety of identification and quantification techniques. One of the most important is GC-MS, which is the fastest, most effective, and most accurate way to find compounds like organic acids, amino acids, nitro compounds, hydrocarbons, and long-chain hydrocarbons that are volatile in nature. In this instance, two analytical

techniques are combined into a single method for the analysis of chemicals found in mixtures. LC-MS is another widely used analytical method that can identify a large number of chemicals quickly and is highly capable of separating phytochemicals. By giving mass measurements of each compound, this method also guarantees the simple and accurate identification of metabolites (Abdulhafiz et al., 2022). The ultimate goal of all of these phytochemical analytical techniques is to determine the exact concentration of active compounds present in the obtained plant or sample.

Free radicals, which are extremely reactive and unstable due to the presence of unpaired electrons, are involved in both human physiology and human pathology. Cell signalling pathways that regulate immune responses, apoptosis, and other normal cell functions depend on them. The production of ROS by immune system cells is essential for eliminating all invasive infections. Nitric oxide ( $\bullet\text{NO}$ ), superoxide ( $\text{O}_2\cdot^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), alkoxy ( $-\text{OR}$ ), peroxy ( $\text{ROO}\cdot$ ), and hydroxyl ( $\cdot\text{OH}$ ) are some examples of reactive species. It is produced by the mitochondria as a byproduct of metabolism, as well as by phagocytosis, xanthine oxidase activity, peroxisome activity, and arachidonic acid pathways. External factors that contribute to the production of free radicals include radiation, contaminants in the environment, medicines, ozone, heavy metals, poisons, pesticides, and smoking (Chandimali et al., 2025; Chaudhary et al., 2023). Oxidative stress results from an imbalance between the production of free radicals and the body's defensive antioxidant system. Antioxidants protect and maintain the normal metabolism and cell function by safeguarding against oxidative damage. There are two ways that antioxidant action is accomplished: either by scavenging radicals or by reducing the generation of free radicals (Gulcin, 2025).

ROS are necessary for many cellular functions under normal physiological conditions, but when they are produced in excess, they damage mitochondria, proteins, lipids, carbohydrates, and nucleic acids, as well as cause oxidative damage, which destroys DNA, RNA, lipids, and proteins, disrupting cell functions and ultimately resulting in cell death. An established endogenous antioxidant defence system protects the organisms from the damaging effects of free radicals. Both the

enzyme and non-enzyme antioxidant systems played a significant role in this. The ROS are neutralised by enzymatic antioxidants like superoxide dismutase and glutathione peroxidase, while they are scavenged by non-enzymatic antioxidants, including vitamin C, vitamin E, and glutathione. Exogenous antioxidant systems are necessary to maintain the redox status if the endogenous antioxidant system is unable to balance the pro-oxidant and antioxidant reaction (Chandimali et al., 2025; Gulcin, 2025).

Exogenous antioxidants can be classified as either natural or synthetic. The two synthetic antioxidants that are most frequently used are BHA and BHT. Although they are safe, occasionally they are discovered to be toxic, harmful, carcinogenic, and hepatotoxic to the human body. In addition to their potential therapeutic and nutritional benefits for human health, natural antioxidants obtained from fruits, vegetables, and other plant sources play a significant role in maintaining the redox balance. Flavonoids are one of the special classes of secondary metabolites found in plants that act as antioxidants against free radicals, according to recent studies. The overall safety and positive effects of plant-based exogenous antioxidants make their consumption more relevant. These can prevent chronic metabolic disorders, age-related conditions, and other oxidative stress-related problems (Martemucci et al., 2022).

Natural antioxidants are found in every part of plants, including the leaves, bark, roots, seeds, fruits, and vegetables. Large doses of antioxidant supplements can have pro-antioxidant effects. Therefore, taking natural antioxidants from plants could be a safe way to prevent overdosing (Tumilaar et al., 2024). Common methods for evaluating the antioxidant capacity of plants and pharmaceutical ingredients include the following: DPPH• radical scavenging; 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS•+) scavenging; ferric ions (Fe<sup>3+</sup>) reducing assay; ferric reducing antioxidant power (FRAP) assay; superoxide radical anion (O<sub>2</sub>•<sup>-</sup>); hydroxyl radical (OH•) scavenging; peroxy radical (ROO•) removing; hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposing and nitric oxide radical (NO•) scavenging assay.

Flavonoids, lignans, phenolic acids, vitamins, and carotenoids are among the antioxidants found in plants. By blocking free radicals and lowering lipid peroxidation, naturally occurring antioxidants such as polyphenols, gallic acid, and ascorbic acid can potentially reduce oxidative stress in humans (Yashin et al., 2017). Phenolic compounds are among the secondary metabolites that have been shown to have significant and efficient antioxidant activity in addition to other health advantages. Because of their enormous capacity to scavenge free radicals, a variety of herbs and medicinal plants are used in a wide range of medical applications. An additional noteworthy and potent bioactivity is the anti-inflammatory effect. A vital biological defence system, inflammation protects the body from damage and aids in its defence against illness, pathogens, and other irritants. Pain, redness, heat, and swelling are the four main signs of inflammation. Two primary mechanisms underlie the process of inflammation: first, the removal of harmful chemicals, and second, the repair of damaged tissues. There are acute and chronic phases of inflammation. As soon as there is tissue damage or any other disturbance, the acute stage starts. This process involves inflammatory mediators such as serotonin, histamine, and Cox-2. Despite being a vital defence mechanism, improper management of acute inflammation can result in chronic inflammation. Prostaglandins 2, lipoxxygenase, and nitric oxide were among the inflammatory mediators that were involved in this process. Rheumatoid arthritis, autoimmune diseases, peptic ulcers, and cancer are just a few of the health issues that are brought on by chronic inflammation (Ansari et al., 2017; Alur, 2023).

An inflammatory response is brought on by the coordinated activation of signalling pathways that regulate the levels of inflammatory mediators in local tissue cells and inflammatory cells drawn from the blood (Chen et al., 2017). Even though the precise characteristic and location of the initial stimulus determine the inflammatory response processes, they all share a common mechanism that can be summed up as follows.

- Identification of harmful stimuli by cell surface pattern receptors
- Inflammation- related pathway activation

- Expression of inflammatory markers
- Recruitment of inflammatory cells

Inflammatory mediators are produced in response to an inflammatory stimulus, which in turn activates intracellular signalling pathways. Inflammation is largely regulated by three most common pathways, MAPK, JAK-STAT, and NF- $\kappa$ B, and any dysregulation of these pathways lead to diseases associated with inflammation (Oeckinghaus et al., 2011). Cytokines play a part in both inflammation and anti-inflammation, depending on the type of inflammatory response. Therefore, the pro-inflammatory cytokines IL-6, IL-8, and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) initiate and promote mechanism of inflammation. While anti-inflammatory cytokines such as transforming growth factor, IL-10, and anti-inflammatory receptor agonist (IRA) can negatively regulate it (Zhu & Hou, 2021; Muzamil et al., 2021).

When a microbial attack occurs, immune cells like mast cells, neutrophils, leukocytes, and macrophages identify the pathogen-associated molecular patterns or cellular damage. Accordingly, the immune cells release inflammatory mediators, such as cytokines, leukotrienes, histamine, and nitric oxide, to start the inflammatory process. TNF- $\alpha$  and interleukins are two cytokines released by macrophages that aid in the healing of local injuries. Histamines are released by mast cells to induce vasodilation, which raises vascular permeability. Endothelial cells generate leukotrienes and prostaglandins using the phospholipids of damaged cells and membranes, which encourages vessel dilation and permeability. The combination of these inflammatory mediators causes fever, pain, and redness and also draws immune cells to the site of the injury (Dvorakova & Landa, 2017).

One of the important enzymes that contributes significantly to the inflammatory response, specifically in the production of prostaglandins, is cyclooxygenase (COX). Arachidonic acid, which is released from the cell membrane by the enzyme phospholipase A2, is the source of both lipoxygenase and cyclooxygenase. These enzymes produce thromboxanes, leukotrienes, and prostaglandins, which are important inflammatory markers, through various series of reactions (Ribeiro et al., 2018). Cox has two distinct isoforms, Cox-1 and Cox-2,

respectively. The housekeeping gene Cox-1 regulates the flow of blood to the kidneys, the synthesis of prostaglandin E<sub>2</sub>, and the production of thromboxane in platelets. Cox-2, on the other hand, is an inducible response gene that is triggered by both extracellular and intracellular stimuli, such as lipopolysaccharide and epidermal growth factor.

Risky pathological conditions like metabolic syndrome, neurodegenerative diseases, diabetes, atherosclerosis, and even various cancers are frequently brought on by uncontrolled inflammation, especially chronic inflammation. Therefore, it is thought that lowering inflammation will aid in preventing several issues and maintain the tissues' normal state of health (An et al., 2019). The molecular target of herbal remedies, analgesics, and anti-inflammatory nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin has as its main molecular target the inhibition of Cox-2 activity, which is activated by inflammatory stimuli like bacterial endotoxin and cytokines. By blocking the arachidonic acid pathway and thereby reducing the production of prostaglandins, these drugs work to reduce inflammation and pain. The onset of severe conditions like gastrointestinal, cardiovascular, kidney, and cerebral injuries is, however, occasionally brought on by these medications. Additionally, it causes a variety of side effects as a result of the use of these medications (Hu et al., 2022; Desai et al., 2018; Gandhi et al., 2022).

Molecules with anti-inflammatory potential are abundant in the plant kingdom. By directly interacting with pathogens like bacteria and viruses, polyphenols can inhibit the anti-inflammatory process (Sangiovanni & Dell'Agli, 2021). Similarly, coumarins had the ability to reduce inflammation. By interacting with several pro-inflammatory cytokines and altering prostaglandin levels, coumarins exert anti-inflammatory effects (Ghosh et al., 2023). The planar ring structure of flavonoids, along with unsaturation at the C<sub>2</sub>-C<sub>3</sub> and hydroxyl group positions, makes them one of the best anti-inflammatory agents. By lowering inflammatory cytokines like IL-6, IL-8, and TNF- $\alpha$ , it demonstrated the ability to reduce inflammation. They are also effective at blocking enzymes like iNOS and Cox-2 (Al-Khayri et al., 2022). Different mechanisms are used by phenols to

produce their anti-inflammatory effects. To exert their anti-inflammatory effects on macrophages, phenolics can inhibit several essential inflammatory response regulators, including TNF- $\alpha$  and IL-6. In addition, they can act as an anti-inflammatory agent by deactivating inflammatory enzymes like LOX and COX (Liu et al., 2023). Inflammation-reducing properties of terpenoids are also significant (Prado-Audelo et al., 2021). The importance of medicinal plants, crude extracts, and pure compounds in treating diseases when inflammation arises has been emphasised in numerous reports. The development of anti-inflammatory drugs may therefore benefit greatly from the inclusion of plant species that contain these bioactive components.

Cytotoxicity is one of the important bioactivities that medicinal plants display, and it is connected to how well they fight cancer. Numerous phytoconstituents in the plant have a cytotoxic effect by interfering with the normal cell cycle and causing chromosome abnormalities of different types. One of the popular and trustworthy test methods for figuring out toxicity in lab settings is the Allium test. In addition to being easy to handle and store, onions make it simple to observe having both macroscopic and microscopic characteristics. Additionally, this system exhibits a strong correlation with the data gathered from prokaryotic and eukaryotic systems (Saxena et al., 2005). It is a legitimate technique to assess the type of cytotoxicity present in all living things.

Mitotic index (MI) is a feasible method for determining the degree of cytotoxic potential in living things. It involves quantitatively estimating an organism's mitotic activity. It determines the proportion of cells in the M-phase of the cell cycle, and its inhibition is known as cell death or a delay in the kinetics of cell division. The cell cycle arrest that occurs at the G2 phase or the interruption of mitosis may be the cause of the decrease in mitotic activity. Changes in the normal structure of chromosomes that result from chromosome exchange or breakage are known as aberrations. Some chromosomal abnormalities are viable and result in either somatic or inherited genetic effects, but many of the abnormalities that are analysed in the cells are found to be fatal. Plant extracts' cytotoxic efficacy is



demonstrated by a dose-dependent decrease in MI values and an increase in the percentage of abnormality. Various herbal extracts have been documented with the property to inhibit mitosis (Aşkin Çelik & Aslantürk, 2010; Akinboro & Bakare, 2007).

The significance of this test lies in the fact that the roots grow in direct contact with the plant extract or substance of interest, potentially causing damage to the DNA that could be linked to human DNA. Because plants and animals have similar chromosome morphologies, they seem to react to treatments containing mutagens similarly to how mammals and eukaryotes do (Owolarafe et al., 2020). The toxicity preference is low or non-existent if the test compound of interest is utilised in the pharmaceutical or cosmetics industries. Selective cytotoxicity against cancerous cells is important if the compounds are used as anticancer agents. Thus, in order to proceed with further analysis of the test compounds, substances, or extracts, a preliminary assay of cytotoxic determination is required.

Under normal circumstances, the processes of cell division and death are balanced to maintain a healthy living. This is done by creating and multiplying new cells to replenish or balance the ones that are lost as a result of ageing or damage. Any disruption to the normal homeostasis of cells leads to abnormal and uncontrollable cell proliferation, which ultimately results in tumours that impact and spread to neighbouring tissues. A chronic disease that is diverse and multifactorial, cancer is one of the leading causes of death worldwide, accounting for 10 million deaths in 2020. Of all the cancers, lung cancer is the one that kills patients the most, followed by breast and colorectal cancer. It is the second leading cause of death after cardiovascular disease. Cell growth abnormalities, angiogenesis, multiple replications, non-responsiveness to the signals of anti-growth, and the ability to spread and metastasize to other parts of the body are characteristics of cancer. These distinct characteristics give cancer cells a greater survival potential, rapid growth, and a great capacity to spread to nearby normal cells. Sarcoma, lymphoma, carcinoma, leukaemia, and myeloma are some of the more common cancer types. In men, the prostate, colon, rectum, lungs, and bladder are the primary sites where

cancer is seen. In females, the highest percentage of cancer cases is found in the thyroid, uterine corpus, and breast. Blood cancer was identified as the most common type in children's cases (Chandra et al., 2023).

A combination of external factors and internal genetic variation leads to the development of this genetic disease. This disease is caused by somatic DNA mutations that occur after exposure to any type of carcinogenic material. Gene mutation results from carcinogenic substances that either directly or indirectly disrupt the cytoplasm and nucleus of cells. Approximately 7% of cancers are caused by carcinogenesis factors, such as radiation, bacteria, and viruses. Oxidative stress, obesity, and hyperglycaemia all contribute to the rise in cancer cases. The disruption of cellular fates brought on by cancer results in abnormalities in the normal functioning of essential genes, and proto-oncogenes turn into oncogenes. The overproduction of proteins due to a mutated oncogene can lead to excessive cell proliferation. Similarly, the absence or aberrant activity of the tumour suppressor gene also plays a role and initiates unchecked cell division. The tumour suppressor gene codes for and regulates the proteins that cause cell division to stop. These proteins are affected by mutations during their formation, which results in their inactivation. Individuals with cancer receive different treatments. The type of cancer, the patient's circumstances, and their medical history are the primary determinants. Hormone therapy, chemotherapy, radiation therapy, and bone marrow transplants are a few of the frequently used treatments (Hassanpour & Dehghani, 2017; Schottenfeld & Fraumeni, 2006).

In women, more than 2 million cases of breast cancer were diagnosed globally in 2020. Breast cancer is primarily caused by mutations in the tumour suppressor genes BRCA1 and BRCA2. Similarly, this formation is influenced by specific reproductive and lifestyle changes. A diet high in sugar and fat, alcohol consumption, processed food consumption, smoking, chemical exposure, obesity, and variations in menstruation, such as early menarche and late menopause, delivery which happens late in life (typically after the age of thirty), are some of the factors that lead to breast cancer. Breastfeeding has the potential to mitigate the effects and

prevent breast cancer. Sarcomas and carcinomas are the two forms of breast cancer. Carcinomas include breast cancers that originate from components such as lobules and terminal ducts that are based on breast epithelial cells. On the other hand, sarcomas develop from connective tissues such as myofibroblasts, which support the lobules and ducts. Because of its highly invasive nature and poor response to treatment, triple-negative breast cancer accounts for 15 - 20% of all cases of the four types of breast cancer. Although chemotherapy and radiation therapy are commonly used to treat cancer, they are very costly and have negative side effects. Therefore, an alternative approach is required to address this issue, and medicinal plants may be the best choice due to their low risk of adverse effects and lower financial loss.

Natural compounds derived from aromatic and medicinal plants hold the most promise as anticancer agents because they contain powerful chemicals such as flavonoids and terpenes, which can induce apoptosis and alter gene expression. Epidemiological research has demonstrated that a diet rich in antioxidants and phytochemicals from fruits and vegetables can dramatically lower the incidence of a number of cancers. Plant-based antioxidant molecules, including flavonoids, terpenes, alkaloids, and tannins, have promising anticancer properties. Therapeutic benefits result from the synergistic action of these bioactive compounds. Studies conducted both *in vitro* and *in vivo* have demonstrated that antioxidants' capacity to quench free radicals accounts for their strong anticancer effects (Kamal et al., 2022). Phytochemicals that are isolated from plants are used to prevent the growth of cancer. One well-known natural polyphenolic antioxidant that comes from plant extract, gallic acid, demonstrated the capacity to induce apoptosis in lung cancer cells. Similarly, various compounds that are extracted from various plants, such as Vinca alkaloid, podophyllotoxin, and camptothecin, are used in cancer treatments (Khan et al., 2020).

Examining compounds that have anticancer properties and developing plant-based medications may be an important step in the treatment of breast cancer. Thus, the current study focusses on the antiproliferative effects of three *Euphorbia* species' methanolic extract on the MCF-7 breast cancer cell line *in vitro*. Common breast

cancer cell lines used in *in vitro* experiments include MCF-7, MDA-MB-231, MDA-MB-468, and T47B. The work takes into account MCF-7, a model cell line for breast cancer that has both oestrogen and progesterone receptors. Phytochemicals work against cancer by interfering with different signalling pathways. To gain a thorough understanding of the effects of phytocompounds found in selected plant extracts that exhibited anticancer potential, certain molecular pathways are considered. Variations in the expression of crucial genes such as STAT-3 and p53 were examined. The tumour suppressor gene p53 has a major role in regulating cellular processes like cell proliferation. It is known as a "gatekeeper gene" because of its enormous capacity to prevent the growth of tumours. It can encourage cellular repair processes and identify DNA damage. p53 gene mutations impair the gene's ability to inhibit tumour growth, which increases the risk of developing cancer, including breast cancer (Shomali et al., 2023). The gene signal transducer and activator of transcription 3 (STAT-3) contributes to breast cancer. According to recent clinical findings, consistently activated STAT-3 is linked to breast cancer growth, spread, and metastasis. The phosphorylation of STAT-3 by the IL-6 family of cytokines initiates the signalling cascade systems in breast cancer. Other interleukins, such as IL-35 and IL-8, have also been shown to activate STAT-3, which advances breast cancer. IL-35 inhibits conventional T cells while also accelerating the progression of breast cancer by activating STAT-3. On the other hand, IL-8 and growth-regulated oncogene (GRO) chemokines activate STAT-3, hastening the progression of inflammatory breast cancer (Ma et al., 2020). Therefore, using both morphological and molecular methods, the current study aims to analyse how phytocompounds found in methanolic extracts of specific *Euphorbia* species induce an antiproliferation effect against the MCF-7 breast cancer cell line.

Richard Feynman, an American physicist, introduced the idea of nanotechnology in his 1959 lecture "There's Plenty of Room at the Bottom." A multidisciplinary field of study, nanotechnology has made rapid and substantial contributions to all facets of life, including human health. It has numerous applications in a variety of fields, including bioimaging, environmental remediation, drug and gene delivery, and more. Its nanoscale effects created new opportunities for

the development of new innovations in various industrial fields. Organic, inorganic, and hybrid materials are all used in nanotechnology. The diameter of nanoparticles ranges from one to one hundred nanometres. Its size and surface area to volume ratio showed off its distinctive characteristics. In many different sectors, such as the food, agricultural, and biomedical industries, they play a significant role. Metallic nanoparticles, which have their own electronic, catalytic, and optical properties, are found to be highly effective in a variety of sectors among other nanoparticles, including lipid-based, semiconductor, and polymeric nanoparticles. There are primarily two methods for producing nanoparticles: top-down and bottom-up methods. In the top-down scenario, bulk materials are broken up or divided in order to synthesise nanoscale materials. This category includes physical method of nanoparticle formation like sonication, electron explosion, laser ablation, and mechanical milling. The bottom-up approach, on the other hand, creates nanoparticles by combining small molecules and atoms. This category includes biological methods that use microbes, plants, and biological wastes like egg shell, vegetable, and fruit peel extract, as well as chemical methods like the sol-gel process, co-precipitation, and molecular condensation (Altammar, 2023).

From 2019 to 2023, it was discovered that the production of metal nanoparticles using plant extracts advanced more. When the metal salt solution is mixed with plant extracts, nanoparticles are created at room temperature in a short duration of time. Through this process, nanoparticles of gold and silver are developed. The ability of the phytoconstituents—saponins, polyphenolics, alkaloids, and terpenoids—to act as both stabilising and reducing agents makes it easier for metal ions to be reduced smoothly to nanoparticles. For this reason, plants are a promising source for nanoparticle formation. The green synthesis of nanoparticles is inexpensive, widely accessible, and creates them without the use of dangerous chemicals by employing sustainable molecules in an eco-friendly manner. The formation of nanoparticles is influenced by a number of variables, which differ from plant to plant and include temperature, reaction mixture pH, metal ion concentration, and reaction time. A range of techniques, including FTIR, X-ray diffraction, transmission electron microscopy, scanning electron microscopy, UV-visible

spectroscopy, and dynamic light scattering (DLS), can be used to determine the properties of the formed nanoparticle, such as its morphology, size, shape, purity, and distribution of functional groups, which are crucial to understanding their effectiveness. The first and most fundamental technique, UV-visible spectroscopy, verifies the existence and formation of silver nanoparticles by generating a characteristic peak at a particular wavelength. The synthesised nanoparticles' morphology is determined using either TEM or SEM. Information about the different functional groups present in plant extracts that are specific to each extract and play a role in the stabilisation and capping process for the development of nanoparticles is provided by FTIR analysis (Hosseingholian et al., 2023).

Since ancient times, people have utilised metals like copper and silver due to their strong antimicrobial properties. Nowadays, these are used in contraceptives, textiles, and hygiene products. Ag NPs have been used extensively in a variety of fields due to their high surface-to-volume ratio and comparatively small size. It works well as a catalytic agent to break down hazardous contaminants such as organic dyes. Likewise, it was discovered that this is less harmful to mammalian cells than other metal nanoparticles (Dawadi et al., 2021). In addition, compared to other metallic nanoparticles, Ag NPs exhibited a wide range of antimicrobial activity, low toxicity, and high biocompatibility. Ag NPs' antimicrobial properties against fungi, viruses, and both gram-positive and gram-negative bacteria have been known since ancient times. They also work in concert with antibiotics to increase their effectiveness. There is ongoing research into the antibacterial and other bioactivities that silver nanoparticles exhibit, the explanation for these properties. One of the explanations is that Ag NPs can interact with the phospholipid bilayer of the bacterial cell membrane, causing the contents of the cell to escape and increasing the permeability of the membrane. Ag NPs have the ability to create reactive oxygen species, cause oxidative stress, and cause related damages when they enter a cell. It can interfere with and disrupt the process of replication and repair, as well as alter the normal structure of bacteria by interacting with their DNA. Additional bacterial cell damage from Ag NPs include disruption of the respiratory chain, change in protein expression, and inactivation of enzymes (Casals et al., 2025). Since the

emergence of antibiotic-resistant microorganisms and their enormous potential to spread illness are major global concerns these days, Ag NPs are seen as a promising antibacterial alternative. This has increased the significance of producing silver nanoparticles and other nanoparticles using environmentally friendly methods that use plant extracts. For this reason, the current study focuses on the ability of methanolic extracts of three *Euphorbia* species to form nanoparticles.

Among flowering plants, the Euphorbiaceae family is one of the largest and most important, with many medicinal plants. There are about 300 genera and 8000 species in the family. This family is known for its unique reproductive structure, the cyathium inflorescence, which is unique to this genus and not found in any other plant group worldwide. In reality, this structure consists of a single female flower encircled by numerous male flowers, all of which are encased in bracts that resemble the petiole of other plants' flowers. One of their most remarkable and fascinating characteristics is the presence of milky latex, which is toxic and poisonous and protects plants due to the presence of terpenes and phenolics. In their traditional medicine, indigenous people used various species to treat conditions like cancer, hepatitis, malaria, jaundice, snake bites, inflammation, and urinary tract infections.

In the genus *Euphorbia*, there are roughly 2000 recognised species with notable biological and medicinal properties. The members are spread throughout tropical and temperate regions, including Madagascar, Africa, America, and tropical Asia. The members displayed themselves in a variety of habitats, including xerophytes, succulents, canopy trees, herbs, shrubs, and geophytes. In India, the genus is represented by 93 species, 40 of which are endemic. Due to the presence of diterpenoids, the genus's members demonstrated strong pharmacological properties, including anti-inflammatory, anti-HIV, cytotoxic, and anti-bacterial effects. Prior to the time of Christ, the members were used in Chinese and Ayurvedic medicines to treat rheumatism, asthma, and liver disorders (Kemboi et al., 2021). According to reports, all parts of the plant, including root, stem, stem bark, latex, aerial parts, seed, leaves, and entire plants, have therapeutic uses. A vast array of natural compounds with a variety of pharmacological actions is abundant among *Euphorbia*

members, which can aid in the development of pharmaceutical agents and medications. Consequently, it is very important for research. Furthermore, the use of diterpenoids like Taxol for cancer treatment and Picato for actinic keratosis increased the importance of the members. Despite the fact that this genus contains numerous species, *E. rothiana* and *E. deccanensis* have not been studied. Thus, three species of *Euphorbia* plants are being examined phytochemically in this study in order to identify their potential bioactivities.

### **Major objectives of the study**

- To screen the phytochemicals found in the aerial parts of chosen plant species through qualitative and quantitative analysis by *in vitro* assays.
- To comprehend the antioxidant capacity of the chosen species.
- To reveal the anti-inflammatory potential of the aerial parts of the chosen species.
- To determine the cytotoxic potential of extracts using the *Allium cepa* assay.
- To examine the chosen extract's antitumor properties using MCF -7 breast cancer cell lines.
- To outline the chosen extract's capacity to induce apoptosis and cell cycle arrest in MCF -7 breast cancer cell lines.
- To elucidate the antiproliferative mechanism through gene expression studies.
- Bio-fabrication of silver nanoparticles from selected extracts and determining the antimicrobial activity exerted by the developed nanoparticles.





## REVIEW OF LITERATURE

The current study primarily focuses on the distribution of different phytometabolites and biological activities of chosen species of *Euphorbia*. *Euphorbia* is a significant genus that possesses a wide range of habits, including trees, xerophytes, succulents, and herbs. It has over 2000 species and can be found in tropics, subtropics, and Mediterranean regions. The members are aware of the existence of white sap, which is toxic but has medicinal and health advantages. Many species are utilised for cultural and commercial purposes, as well as for the treatment of various illnesses in traditional medicine. This chapter compiles and documents a summary of the literature survey conducted on the genus, which is crucial to the current work.

### Background in taxonomy

The genus *Euphorbia* is one of the three most species-rich flowering plant group in the family Euphorbiaceae, with 2046 species found worldwide, particularly in warm, arid, and semi-arid climates. The latex produced by some species is well-known and used as both an insecticide and a fishing poison (Frazão et al., 2020). It is distributed almost cosmopolitanly, with diverse hotspots in southern Africa, eastern and northeastern Africa, north and central Mexico, and western Asia. However, it is absent in subarctic regions and tropical rainforests (Smith & Carter, 1988). About 850 succulent species of *Euphorbia* are safeguarded by CITES regulations, which govern international trade in threatened and endangered species of wild fauna and flora (Carter et al., 1997). In India, the genus is represented by 84 native species. It is the largest genus of the family in India and has a high level of endemism with 31 endemic species and seven varieties (Binojkumar & Balakrishnan, 2010; Binojkumar, 1993). New species of *Euphorbia* have recently been added to the Indian flora, including *Euphorbia kadapensis* Sarojin & R.R.V. Raju, *Euphorbia gokakensis* S.R. Yadav, Malpure & Chandore, and *Euphorbia seshachalamensis* Prasad & Prasanna (Raja et al., 2020). They occupy a variety of habitats and display a wide range of growth forms. The species come in a variety of

sizes, including tiny ephemerals, different kinds of herbs, large shrubs, cushion-forming subshrubs, understory and canopy trees, and succulents that resemble cacti (Naidu, 2017). The genus is morphologically distinguished by having a cyathium inflorescence and a highly reduced inflorescence that resembles a single flower despite having a great deal of vegetative diversity (Fayed et al., 2020). The cyathium, which Linnaeus incorrectly identified as a flower when he first described the genus, was later revealed to be an inflorescence. The word "inflorescence" was used in place of "cyathium," which called for a wider, more modern perspective on the genus (Binojkumar, 1993). According to recent molecular studies, the genus *Euphorbia* has been divided into four monophyletic subgenera such as *Euphorbia* (800 species), *Chamaesyce* (600 species), *Esula* (500 species) and *Athymalus* (150 species) (Pahlevani, 2017). The taxonomy of the *Euphorbia* genus is a difficult task because of the abundance of species with global distribution, the extreme morphological plasticity of some species, and the convergent evolution of some morphological characters.

#### **Bentham & Hooker classification**

#### **APG Classification**

|           |   |                |         |   |               |
|-----------|---|----------------|---------|---|---------------|
| Kingdom   | : | Plantae        | Kingdom | : | Plantae       |
| Class     | : | Dicotyledon    | Clade   | : | Angiosperms   |
| Sub-class | : | Monochlamydeae | Clade   | : | Eudicots      |
| Series    | : | Unisexuales    | Clade   | : | Rosids        |
| Order     | : | Euphorbiales   | Order   | : | Malpighiales  |
| Family    | : | Euphorbiaceae  | Family  | : | Euphorbiaceae |

#### **Phytochemical profile**

Plant-based bioactive compounds are used in a vast array of biological processes. These substances are crucial to plants' defence mechanisms and have sparked a lot of interest due to their potential therapeutic applications. More than a

thousand phytochemicals have been identified to date, and they can be obtained from a variety of foods, including whole grains, fruits, vegetables, nuts, and herbs. Certain polysaccharides, dietary fibers, phytosterols, carotenoids, polyphenols, and isoprenoids are a few of the important phytochemicals. In addition to their potent antioxidant properties, these phytochemicals have antiviral, antibacterial, antidiarrheal, anthelmintic, and antiallergic properties. A great deal of research has been done to find out whether powerful phytometabolites such as alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, steroids, phlobatannins, and glycosides have the potential to be used as medications (Kumar et al., 2023).

Phenolic compounds are one of the most well-known secondary metabolites that plants produce; they are notable for having antimicrobial, antioxidant, and anti-inflammatory properties (Lagana et al., 2019). These substances have a lot of potential for preventing and treating cancer because they can inhibit the growth of tumours and encourage apoptosis, or the controlled death of cells. Their numerous bioactivities highlight their significance for health and wellness and establish them as strong candidates for therapeutic interventions in a variety of chronic diseases (Sinha et al., 2016; Gupta et al., 2011; Sharifi-Rad et al., 2022).

Because of the wide range of bioactivities and potential health benefits it demonstrates, a class of phenolic compounds known as flavonoids. They have anti-inflammatory, anti-cancer, anti-oxidant, cardiovascular, and neuroprotective properties. Their importance in many physiological and pathological processes has been highlighted in recent studies. Flavonoids serve as painkillers, tumour-growth inhibitors, cardiovascular health promoters, and provide defences against neurodegenerative diseases. The improvement of overall health and well-being as well as the prevention and treatment of chronic diseases may be made possible by these bioactive substances (Liu et al., 2014; Grosso et al., 2015).

Alkaloids are a broad class of substances that contain nitrogen. Their notable pharmacological traits include analgesic, anti-inflammatory, anti-malarial, and anti-cancer effects. The potential of these substances as medications has been thoroughly investigated (Ng et al., 2015). Terpenoids are another imperceptible class of

compounds that have been shown to possess antiviral, antifungal, antiparasitic, and anticancer characteristics. They have been investigated as possible therapeutic candidates for the management of a variety of conditions, such as infectious diseases, cancer, and neurological disorders (Paduch et al., 2007).

Developing novel manufacturing techniques for pharmaceuticals, nutraceuticals, and complementary medicine requires a thorough analysis of the bioactivities of phytochemicals. Examining the chemical makeup of phytochemicals is essential before determining the active ingredient responsible for their bioactivities (Agidew, 2022). Molecules' unique chemical structure and properties significantly influence their interaction with biological systems. Plant parts like leaves, stems, roots, and flowers have different phytochemical profiles. Researchers can analyse these profiles to identify specific plant parts with higher bioactive compounds, facilitating standardized extract production and efficient plant resource utilization.

Various techniques and technologies are utilized to determine and comprehend the bioactive molecules found in plants. Individual compound identification can be achieved using chromatographic techniques such as thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC). Likewise, several compounds in a sample can be analysed simultaneously with the LC-MS method, which also provides structural information about chemical compounds (Pitt, 2009).

In phytochemical analysis, a multi-technique integrated approach is used to perform a comprehensive analysis of the bioactive substances in plants. This integration makes it possible to understand plant extracts' chemical makeup and biological activities as well as the different ways that they can be combined and used in pharmaceutical analysis, phytochemical research, and drug quality control. Different phytochemicals can be separated and characterised simultaneously by combining spectroscopic techniques like mass spectrometry (MS) with chromatographic techniques like gas chromatography (GC), liquid chromatography (LC), and high-performance liquid chromatography (HPLC) (Parys et al., 2022).

However, metabolomics makes use of a variety of technologies to get around the inherent drawbacks of each analytical method, producing better outcomes.

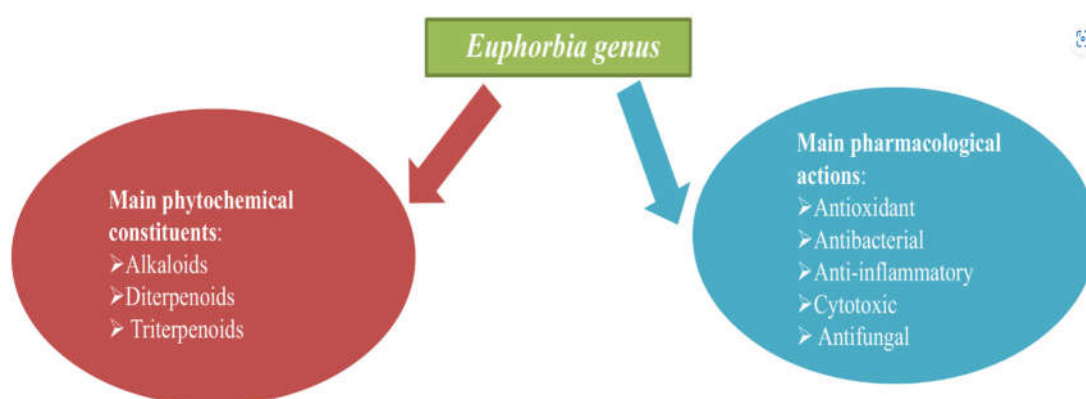
### **Phytochemical distribution in the genus *Euphorbia***

Because of their wide range of phytochemicals, which include lignans, diterpenoids, triterpenoids, flavonoids, alkaloids, and phorbol esters, *Euphorbia* species are valuable for natural products and drug discovery. Their effectiveness in conventional systems can be attributed to their high concentration of phytometabolites, which include essential oils, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, and macrocyclic diterpenoids (Kemboi et al., 2020). Due to the quantity and complexity of phytochemicals present, these compounds display a broad range of bioactivities, including cytotoxic, anti-inflammatory, antimicrobial, anticancer, and anti-inflammatory effects (**Fig. 1**). Some of the major phytometabolites found in the members of *Euphorbia* are listed below.

- i) Diterpenoids: With over 1500 isolated compounds belonging to over 30 different skeletal types, diterpenoids are the most significant chemical family found in *Euphorbia* plants. There is a great deal of interest in natural product chemistry because of the structural diversity of these diterpenoids, which are present in various species of *Euphorbia*. Due to the wide range of therapeutically relevant activities, they exhibit, including antitumor, multidrug resistance reversal, antiviral, and anti-inflammatory effects, they are advantageous candidates for drug discovery (Zhao et al., 2022).
- ii) Triterpenoids: Many species of *Euphorbia* contain the triterpenoids, one example includes, euphol. It has anti-inflammatory, antioxidant, and analgesic qualities.
- iii) Flavonoids: After macrocyclic diterpenes and triterpenoids, flavonoids are one of the main components of the *Euphorbia* species. Isoflavonoids, neoflavonoids, chalcones, etc. are the most common forms of flavonoids.

They have a wide range of pharmacological properties and show great promise as medicines.

- iv) It is known that several *Euphorbia* species produce specialised phytochemicals like phorbol esters. These chemicals have received a great deal of attention because of their potent skin irritant properties and potential as anticancer agents.
- v) Phenolic compounds (Magozwi et al., 2021).



**Fig. 1: Major phytochemicals found in the genus *Euphorbia* and associated pharmacological activity** (Amtaghri et al., 2022).

*Euphorbia* plants' ability to preserve food is enhanced by the secondary metabolites they produce. Candelilla wax (E 902) may be used as a food glazing agent, according to European Union (EU) regulations. In the EU, it is legal to use it as a component to give some food products a glossy or protective coating. Candelilla wax is made from the leaves of the plant *Euphorbia antisyphilitica*, which is adapted to semi-arid environments. The wax is extracted from the plant's leaves and converted into a form suitable for use in food applications. Its addition to food products enhances their texture, appearance, and preservation (EFSA., 2012). The Food and Drug Administration (FDA) has also recognised candelilla wax, which is derived from specific *Euphorbia* plant species found in Northern Mexico and the Southwest United States, as a food additive with gelling properties, according to Toro-Vazquez et al. (2007).

Metabolites from *Euphorbia* have received a lot of attention due to the presence of the diterpene ingenol mebutate. It has been found in several *Euphorbia* species, including *E. peplus*, *E. lathyris*, *E. nivulia*, *E. esula*, *E. antiquorum*, *E. serpens* and *E. fischeriana*. Ingenol mebutate is the active ingredient in Picato®, a medication used topically to treat actinic keratosis, a precancerous skin condition (Frezza et al., 2018). Resiniferatoxin, a potent daphnane orthoester from the *E. resinifera* plant and a potent capsaicin receptor agonist, is currently being tested in phase III clinical trials for the treatment of chronic pain and overactive bladder (Brown, 2016). Since metabolites have been discovered and used in medicine, there has been a great deal of interest in researching the therapeutic potential of other metabolites derived from *Euphorbia* plants.

### **Traditional significance of *Euphorbia* genus**

A variety of regions, including China, Europe, America, Turkey, India, Africa, Iran, and Pakistan, have a long history of using *Euphorbia* plants in traditional medicine. For their therapeutic qualities and health advantages, they are highly valued (Amtaghri et al., 2022). Various parts of *Euphorbia* plants such as leaves, stems, latex, seeds and roots have been utilized in traditional medicine to address a wide range of ailments. The medicinal applications of *Euphorbia* species include the treatment of respiratory conditions, infectious diseases, skin irritations, digestive issues, inflammatory infections, body pain, microbial diseases, snake or scorpion bites, and inflammatory infections. These plants have been employed to alleviate symptoms and promote healing in traditional healing systems (Shi et al., 2008). The name "*Euphorbia*" is connected to the Greek surgeon Euphorbus, who worked as a physician for Juba II, a Romanized king of a North African kingdom. It is believed that Euphorbus included milky latex from the species of *Euphorbia* in his medicine (Jasbi, 2006).

Various *Euphorbia* species have been used for medicinal purposes in various regions. In Chile, *E. thymifolia* was known to stimulate lactation but also to have abortifacient properties. Because of its emetic and purgative properties, *E. lathyris* was used to treat conditions such as hydropsy, ascites, schistosomiasis, and



snakebites in Europe. In Northern America and China, *E. maculata* was used to treat warts, corneal opacities, and blood disorders. *Euphorbia* preparations such as decoctions, ointments, and hot steam were used to treat inflammatory conditions. *E. corollata* was known for its effectiveness in treating dropsy, whereas *E. marginata* and *E. antiquorum* were traditionally used to treat swellings. In Vietnam, *E. antiquorum* was used to treat cancer, liver disease, cutaneous dropsy, infections, and toothaches (Salehi et al., 2019).

Three *Euphorbia* species—*E. hirta*, *E. thymifolia*, and *E. lathyris*—stand out among the 33 species that are mentioned in folk practices across the globe as being frequently used in traditional medicine (Ernst et al., 2015). Traditional uses of *Euphorbia hirta* for treating a range of medical conditions can be found in many nations. For the treatment of diarrhoea, it has been used in Burundi, China, the Philippines, and Nigeria. A decoction of *E. hirta* is also used to treat dysentery in Vietnam, India, and Mozambique. Additionally, it is used to treat bronchitis, asthma, and coughs in Nepal, Australia, the South Western United States, and Hawaii. Furthermore, its latex is used to treat infections such as ringworm, gonorrhea, candidiasis, and malaria (Osemeobo, 1991).

For a variety of skin conditions, preparations made from various *Euphorbia* species have been used. Itching skin, warts, eczema, hair loss, boils, sunburn, calluses, rashes, irritation, and pustules can all be effectively treated with these home remedies. They also have antiseptic, disinfectant, and emollient properties, which add to their value for their advantageous effects on the skin. Among the *Euphorbia* species that are frequently used to treat these infections are *E. maculata*, *E. hirta*, *E. peplus*, *E. sessiliflora*, *E. apios* and *E. macroclada* (Lai et al., 2004; Ernst et al., 2015).

The use of *Euphorbia* species for the treatment of respiratory ailments, such as asthma, coughs, bronchial complaints, and pneumonia, has been documented in the literature. Additionally, extracts from these species have been shown in laboratory studies to have a variety of pharmacological properties, including anti-arthritis, anti-inflammatory, antioxidant, antitumor, and antiproliferative effects. As

contemporary medicines, these extracts have been isolated and patented. The use of *Euphorbia* species for these purposes has been documented in a number of nations, with *E. hirta*, *E. pululifera*, *E. neriifolia*, and *E. rigida* being the most frequently mentioned species (Ernst et al., 2015).

The carminative and expectorant properties of *Euphorbia neriifolia*'s latex make it useful for treating a variety of ailments, such as tumours, stomach and skin issues, leprosy, asthma, and kidney stones. *E. neriifolia* roots are specifically used to treat snakebites and scorpion stings. Additionally, this plant's leaves have carminative properties and can be used to treat conditions like pain, inflammation, bronchial infections, and anorexia (Mali & Panchal, 2017). Another species utilised in traditional Chinese medicine is *Euphorbia helioscopia*. It is used to treat bacillary dysentery, osteomyelitis, and malaria. *Euphorbia resinifera* is frequently employed in Uyghur medicine to treat chronic pain, toothaches, and tuberculosis (Frezza et al., 2018).

Numerous *Euphorbia* species have been used for their medicinal benefits by the tribes living in the Tirunelveli hills of India's Western Ghats. To treat wounds, ulcers, deafness, cough, and inflammation, they have used the roots of *Euphorbia antiquorum*, in addition to treating digestive issues. Similarly, the leaves of both *Euphorbia rosea* and *E. rothiana* were utilized as vermifuges, indicating their use in expelling or eliminating intestinal worms. These traditional uses of *Euphorbia* species by the tribes in Tirunelveli Hills highlight the diverse medicinal applications of these plants within the local traditional medicinal practices (Jothi et al., 2008).

Through phytochemical screening, terpenes were discovered to be the main ingredients of the latex of various species of the genus *Euphorbia*. Latex extracts from *Euphorbia* species have a variety of pharmacological effects, such as antimicrobial, anticancer, anticholinesterase, anti-inflammatory, antioxidant, cytotoxic, anti-angiogenic, genotoxic/mutagenic, and wound-healing qualities (Benjamaa et al., 2022). Some *Euphorbia* species' latex has been used in traditional medicine to treat warts and cuts on the skin, as well as some nervous diseases, dropsy, paralysis, deafness, and amaurosis (Ozbilgin et al., 2019). In Spain, fresh

latex from the local *Euphorbia serrata* plant is used to hasten the coagulation of milk. On the other hand, Turkey uses the latex of *Euphorbia stricta* and *E. amygdaloides* to purify domestic water (Ernst et al., 2015). Some of the species of *Euphorbia* having medicinal value is given in **Table 1**.

**Table 1: Traditional uses of some *Euphorbia* species**

| Plant name                   | Uses                                                                              | Plant part used      | References              |
|------------------------------|-----------------------------------------------------------------------------------|----------------------|-------------------------|
| <i>Euphorbia hirta</i>       | Purifies blood, skin                                                              | Whole plant          | Jothi et al., 2008      |
| <i>Euphorbia indica</i>      | Treat diarrhoea, dysentery and leucorrhoea                                        | Whole plant          | Jothi et al., 2008      |
| <i>Euphorbia nivulia</i>     | Treat skin disorders, ear disorders, retention of urine, swelling, worm infection | Leaf, latex and root | Jothi et al., 2008      |
| <i>Euphorbia thymifolia</i>  | Treat ring worm, wounds, asthma, skin diseases                                    | Whole plant          | Jothi et al., 2008      |
| <i>Euphorbia tirucalli</i>   | Treat warts, toothache, cough asthma and earache                                  | Milky juice          | Wal et al., 2013        |
| <i>Euphorbia formosana</i>   | Cures snake bite, dermatoses                                                      | Aerial part          | Yu et al., 2012         |
| <i>Euphorbia triaculeata</i> | Treat asthma, leukemia, warts, as a laxative and diuretic                         | Aerial part          | Al-Faifi et al., 2017   |
| <i>Euphorbia officinarum</i> | Treat skin and ophthalmologic conditions                                          | Latex                | Chamkhi et al., 2022    |
| <i>E. coniosperma</i>        | Wound healing, cure snake-scorpion bites, warts                                   | Latex, aerial parts  | Özbilgin & Çitogl, 2012 |
| <i>E. armena</i>             | Wound healing, treat constipation                                                 | Latex                | Özbilgin & Çitogl, 2012 |
| <i>E. tirucalli</i>          | Treat warts, epilepsy, toothache, haemorrhoids, snake bites                       | Latex                | Wal et al., 2013        |
| <i>Euphorbia neriifolia</i>  | Treat abdominal troubles, bronchitis                                              | Whole plant          | Bigoniya & Rana, 2008   |
| <i>E. cotinifolia</i>        | Cure vertebrate poisoning                                                         | Latex, stems         | Kemboi et al., 2020     |
| <i>E. falcata</i>            | Cure skin irritation & eczema                                                     | Roots, latex         | Kemboi et al., 2020     |
| <i>E. macroclada</i>         | Treat warts, wounds, snake-scorpion bites, tumors, toothache, eczema              | Latex                | Kemboi et al., 2020     |
| <i>E. stracheyi</i>          | Treat hemostasis, analgesia                                                       | Roots, latex         | Kemboi et al., 2020     |

Members of the genus *Euphorbia* can be distinguished by their specialised cyathium inflorescence and white milky latex, which shields the plant from pathogens, insects and herbivores (Sameena & Thoppil, 2022). This genus has yielded approximately 650 triterpenes and diterpenes with potent bioactivities such as anticancer, antimicrobial, antioxidant, cytotoxic, and antiproliferative properties (Smeriglio et al., 2021). Researchers are becoming more interested in the *Euphorbia* species as a result of these promising bioactivities. Nevertheless, a few understudied species exist, including *E. deccanensis* and *E. rothiana*.

The "asthma plant," *Euphorbia hirta*, is renowned for its abundance of flavonoids including quercetin and quercitrin, as well as polyphenols, alkaloids, ascorbic acid, terpenoids, tannins, and volatile oils, fats, mucilage, cardiac glycosides, anthraquinones, coumarins, proteins, gums, saponins, glycosides (Khursheed & Jain, 2022). The highest total phenolic content was found in the leaf extract with 206.17 mg GAE/g dry weight. In addition, the leaves had the highest total flavonoid content value with 37.97 mg CE/g dry weight (Ghosh et al., 2019). These phytochemicals are highly effective in treating respiratory-related illnesses. Because of this, the plant *E. hirta* was used to determine the potential of phytochemicals to inhibit primary protease (SARS-CoV-2 Mpro), a corona virus disease 2019 (COVID-19-which also targets the respiratory system) therapeutic target that is essential to the SARS-CoV-2 infection process. It was discovered that there were approximately 170 potential COVID-19 inhibitors within this single plant through virtual screening of phytochemicals using molecular docking (Cayona & Creencia, 2022). Thus, the study established the presence of potent chemicals in *E. hirta* and validated its pharmacological value.

The early phytochemical analysis of *E. antiquorum* revealed the presence of flavonoids with a variety of functional classes, including jatrophone, tiglane, ingenol types, and myrsinol, as well as diterpenes, triterpenes, and other terpenes. Diterpenoids have been found to have anti-HIV, anti-inflammatory, cytotoxic, and antibacterial properties (Kumar & Saikia, 2016). From this plant, the active substances euphol, antiquol A, euphorbol, isohelinol, and camelliol were extracted

by Prashant et al. (2015). *Euphorbia heterophylla* phytometabolite screening revealed a prominent amount of medicinally active chemicals such as alkaloids, saponins, tannins and flavonoids. About 40 different types of essential oils were identified from this plant through high-performance liquid chromatography (HPLC) and gas chromatography (GC) techniques (Wellington et al., 2019). Aqueous and ethanolic extracts of *E. heterophylla* demonstrated significant wound-healing capacity. This property is primarily attributed to the plant's tannin and flavonoid content, which appears to be involved in wound contraction and epithelisation (James & Friday, 2010).

To screen the phytochemical composition of various leaf extracts of *Euphorbia neriifolia*, including hydro-ethanolic, petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and aqueous extracts, Mali and Panchal. (2017) used fluorescence analysis. The findings demonstrated the presence of proteins, glycosides, alkaloids, phenolics, flavonoids, saponins, and terpenoids in various compositions including significant, moderate and trace amounts. In tiny quantities, proteins and amino acids were discovered. In a similar way, Swamy et al. (2011) examined the phytochemical components and antimicrobial activity of *Euphorbia neriifolia* leaf extracts. The chloroform extracts exhibited the best antibacterial properties against *Proteus vulgaris*, followed by ethanol extracts against *Klebsiella pneumoniae*. The presence of tannins and flavonoids is primarily responsible for the antibacterial property.

Screening of chemical constituents and fatty acid profile of petroleum ether extracts of nine species of *Euphorbia* by using GC-MS and LC-IT-TOF-MS was conducted by Yener et al. (2018). GC-MS analysis revealed fatty acids such as palmitic acid, linoleic acid, hexatriacontane, and 17-tetra-triacontane. By using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, 11 compounds from the chosen species, including quinic acid, protocatechuic acid, rutin, hesperidin, eugenol, p-coumaric acid, piceatannol, scopoletin, DL-kavain, chrysophanic acid, and resiniferatoxin, were also quantitatively determined. The

findings demonstrated the diversity of compounds in the *Euphorbia* plants under investigation.

The antioxidant, GC/MS, and LC-MS/MS spectroscopic analyses of *E. macroclada* and *E. gaillardotii* revealed various types of essential oils and biologically active metabolites. Palmitic acid was found to be the most abundant fatty acid component in both species, while arachidic acid and tetracontane (essential oils) were found in *E. gaillardotii* and *E. macroclada*, respectively. Hesperidin, hyperoside, rutin, malic acid, quinic acid, tannic acid, and gallic acid were found to be prominent in two species among the 27 compounds identified (Ertas et al., 2015). Ten new terpenoids including five diterpenoids, three nortriterpenoids, and two triterpenoids were isolated by Gao and Aisa (2017) from acetone extracts of *E. soongarica*. Isolated compounds were cytotoxic to the human epidermoid carcinoma cell line KB.

The presence of methyl 11 octadecenoate, methyl octadecenoate, methyl palmitate, 5,9-heptadecadienoate, and 3,7,11,15-tetramethyl-2-hexadecene-1-ol was revealed in a study on GC-MS analysis and antimicrobial activity of *E. caducifolia* latex extracts. The plant's latex extract was antimicrobial against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. The antimicrobial potential is due to the diverse chemical composition of latex (Goyal et al., 1970).

In many tropical and subtropical Asian countries, snakebite is still a common medical concern. According to the World Health Organization, snakebites are a neglected tropical disease that demands increased attention from both national and international health authorities (WHO, 2007). Gopi et al. (2015) demonstrated the ability of *E. hirta* to inhibit the Indian cobra *N. naja* snake venom enzyme. The methanolic extract's LC-SRM/MS analysis revealed the presence of phenolics such as quercetin, kaempferol, ellagic acid, gallic acid, luteolin, and quinic acid. The presence of phenolic compounds is responsible for the anti-venom effect. Three species of *Euphorbia* from the flora of Algeria showed an immense amount of condensed tannin and anthocyanin. The anthocyanin and condensed tannin content of *E. terracina* is higher than that of *E. dendroides* and *E. biumbellata*. Ascorbic

acid, chlorogenic acid, and ellagic acid were the most prevalent polyphenolic chemicals in the leaf and seed extracts of three plant species, while linoleic acid was the most common unsaturated fatty acid (Toudert et al., 2021)

*Euphorbia thymifolia* is known for producing a vibrant green essential oil characterized by a potent aroma and a pungent flavour. This plant species contains a crystalline alkaloid compound that shares similarities with quercetin. Additionally, it contains a notable quantity of triterpenoid substances, including euphorbol, epitaraxerol, taraxerol, and campesterol (Mali & Panchal, 2013). Potent metabolites like alkaloids, flavonoids, and phenols were discovered in another study using *E. thymifolia* extracts of acetone and hexane. Steroids and saponins were not present in either extract (Kaur et al., 2017). Quantitative estimation of flavonoids and phenolic compounds in an aqueous extract of *E. thymifolia* was explored by Sisodiya and Shrivastava (2018). The flavonoid content was 2.12 mg/100 mg (in quercetin equivalents), and the phenolic content was 0.998 mg/100 mg (in gallic acid equivalents).

A study on the characterization of cytotoxic compounds found in the *E. clementei* extract identified and isolated a new diterpene ester, 4, 20-dideoxy-4 $\alpha$ -phorbol-12 $\beta$ -acetate-13 $\alpha$ -isobutyrate along with 11 known compounds. Their structures and configurations were determined using 1D and 2D-NMR as well as with mass spectrometry. When tested against human erythromyeloblastoid leukemia K562 and promyelocytic leukemia HL60 cell lines, isolated compounds exhibited weak cytotoxic potential (Benmerache et al., 2017).

Hujon and Saral (2022) identified several bioactive molecules found in the epicuticular wax of *E. milii* using GC-MS analysis and discovered that pentacyclic triterpenoids predominated over other compounds such as lupeol acetate, lupenone, glutinol and glutinyl acetate. The weed *Cenchrus echinatus* was significantly affected by the essential oil of *E. heterophylla* aerial parts, which inhibited its germination, root, and shoot growth. GC-MS analysis of essential oils revealed the distribution of potent allelochemicals like limonene, pinene, and camphor (Elshamy et al., 2019). Several compounds were isolated and identified as a result of

phytochemical analysis performed on the ethanolic extract of *Euphorbia peplus*. These substances consist of kaempferol, simarenol, chlorogenic acid, neo-chlorogenic acid, crypto-chlorogenic acid, 3-O-L-rhamnopyranosyl-L-rhamnose, pepluanone, euphopeplin A, pepluanins A and E, esulatin D, euphodendrophane E, and nicaaenin G (Frezza et al., 2018).

When the phytochemical makeup of *E. royleana* was examined using different solvents like methanol, hexane, and aqueous extracts, it was discovered that phenols, flavonoids, gallic acid, and quercetin were prominent. Hexane extract was found to have a high phenolic and flavonoid content when compared to other extracts (Ashraf et al., 2015). In a similar way, Ndam et al. (2016) assessed the phytochemical components of *Euphorbia golondrina* through GC-MS analysis. This invasive alien herb is used to treat diabetes, conjunctivitis, and gastroenteritis-related conditions. Furthermore, it demonstrated that the plant contains strong phytoconstituent classes like sesquiterpenes, monoterpenes, alkaloids, phenolics, and aromatic hydrocarbons. The three main compounds among the 30 substances found were phytol (5.75%), camphor (9.41%), and caryophyllene oxide (14.16%).

One of the earliest known medicinal plants of the Western tradition is *E. characias*, which is mentioned in many ancient treatises of Greek and Latin medicine. Terpenoids, sterol hydrocarbons, fatty acids, cerebrosides, and phenolic and carboxylic acid compounds were discovered by Fias et al. (2021) while attempting to examine various phytochemicals produced in the latex, seeds, stems, leaves, and flowers of this plant. In addition to these components, *E. characias* latex also contains a low-molecular-weight natural rubber. With the aid of HPLC PDA-MS/MS, Elgamal et al. (2021) discovered 21 secondary metabolites in the aerial parts of *Euphorbia retusa*, including phenolic acids, flavonoids, and their glycoside derivatives. Mass spectrometry,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectroscopy were used to determine the chemical structures of compounds like esculetin, quercetin 3-O-b-D-glucuronide, kaempferol 3-O-b-D-glucuronide, and kaempferol 3-O-b-D-glucoside. Additionally, compounds with a high potential to bind to the active sites of enzymes that are essential for the aging and skin remodelling processes were discovered in



the extract. Phytometabolites isolated from various *Euphorbia* species were summarised in **Table 2** and biological activities exhibited by different secondary metabolites were tabulated in **Table 3**.

**Table 2: Phytochemical compounds isolated from the genus *Euphorbia***

| Sl. No. | Plant                        | Plant part   | Compound isolated                                                                                                                                                                              | Class of compound                                    | References              |
|---------|------------------------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------|
| 1       | <i>Euphorbia pekinensis</i>  | Roots        | Euphopanones A, B, C, cembrane                                                                                                                                                                 | Diterpenoid                                          | Yan et al., 2021        |
| 2       | <i>Euphorbia helioscopia</i> | Whole plant  | Euphorhelipanes A, B                                                                                                                                                                           | Diterpenoid                                          | Li et al., 2019         |
| 3       | <i>Euphorbia stracheyi</i>   | Roots        | Euphstrachenols A, B, C                                                                                                                                                                        | Diterpenoid                                          | Ye et al., 2020         |
| 4       | <i>Euphorbia marginata</i>   | Seeds        | 3-de-O-acetyeuphornan R                                                                                                                                                                        | Diterpenoid                                          | Liu et al., 2021        |
| 5       | <i>Euphorbia lathyris</i>    | Seeds        | Euphohyrisnoids A, B                                                                                                                                                                           | Lathyrane diterpenoid                                | Huang et al., 2021      |
| 6       | <i>Euphorbia helioscopia</i> | Latex        | Euphoheliosnoid A, epieuphoscopin B, euphohelioscopin A, euphoscopin C                                                                                                                         | Macrocyclic Diterpenoid                              | Hua et al., 2015        |
| 7       | <i>Euphorbia milii</i>       | Flowers      | Taraxerol, 28-hydroxyfriedelan-1,3-dione-29-oic acid, quercetin 3-O-(2"-O-galloyl)-a-Larabinofuranoside, 7 7'- dihydroxy, 8, 6'-bicoumarin, 9-acetyl-3'4'-dimethoxy dehydroconiferyl-3-alcohol | Triterpenoid<br><br>Flavone<br><br>Phenolic compound | Kamurthy & Dontha, 2014 |
| 8       | <i>Euphorbia peplus</i>      | Whole plant  | Euphopepluanone L                                                                                                                                                                              | Diterpenoid                                          | Min et al., 2021        |
| 9       | <i>E. geniculata</i>         | Aerial parts | Genicunolide A, B, C                                                                                                                                                                           | Triterpenoid                                         | Farozi et al., 2015     |
| 10      | <i>Euphorbia rapulum</i>     | Roots        | 8,12-Dihydroxy1 $\beta$ H,2 $\alpha$ H-casba-3E,7E,11E-trien-5-one, helioscopinolide A, 19-hydroxyjolkinolide                                                                                  | Casbane diterpenoid                                  | Lui et al., 2018        |

|    |                                   |                     |                                                                                                                                                                                               |                        |                             |
|----|-----------------------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|-----------------------------|
|    |                                   |                     | E,<br>jolkinoide E,<br>euphopilolide                                                                                                                                                          |                        |                             |
| 11 | <i>Euphorbia helioscopia</i>      | Whole plant         | Euphornin N                                                                                                                                                                                   | Jatrophone diterpenoid | Geng et al., 2010           |
| 12 | <i>Euphorbia fischeriana</i>      | Roots               | Cycloart-22-ene-3 $\beta$ , 25-diol, ebracteolatin A, $\alpha$ -linolenic acid, 24-methylenecycloartanol, cycloartenol, $\beta$ -sitosterol, 2, 4-dihydroxy-6-methoxy-3-methyl-1-acetophenone | Diterpenoid            | Xiao-Yang et al., 2012      |
| 13 | <i>Euphorbia phosphorea</i>       | Aerial parts        | Ellagic acid, corilagin                                                                                                                                                                       | Phenolic compound      | De albuquerque et al., 2016 |
| 14 | <i>Euphorbia hyberna</i>          | Aerial parts, roots | 3 $\beta$ -O-Octadecanoillupeol, glut-5-en-3 $\beta$ -ol, 24-metilenocicloartan-3 $\beta$ -ol, ellagic acid, $\beta$ -sitosterol                                                              | Triterpenoid           | Ferreira et al., 2013       |
| 15 | <i>E. hirta</i>                   | Whole plant         | Taraxerol, campesterol, astragalin, hymenoxin, luteolin-7-O- $\beta$ -D-glucopyranoside, quercetin 3-O- $\alpha$ -L arabinofuranoside                                                         |                        | Hue et al., 2020            |
| 16 | <i>E. hirta</i>                   | Aerial parts        | Scopoletin, scoparone, isoscapoletin, quercetin, isorhamnetin, pinocembrin, kaempferol, luteolin, gallic acid                                                                                 | Phenols and flavonoid  | Wu et al., 2012             |
| 17 | <i>E. pulcherrima</i>             | Leaves              | Rutin                                                                                                                                                                                         | Flavonoid              | Shlini et al., 2020         |
| 18 | <i>Euphorbia soongarica</i>       | Roots               | Ellagic acid, 3, 3'-di-O-methylellagic acid, brevifolin, ethyl brevifolin carboxylate                                                                                                         | Polyphenol             | Shi et al., 2006            |
| 19 | <i>Euphorbia formosana Hayata</i> | Aerial parts        | Gallic acid, ellagic acid                                                                                                                                                                     | Polyphenol             | Lan et al., 2020            |
| 20 | <i>Euphorbia helioscopia</i>      | Whole plant         | Euphornin N                                                                                                                                                                                   | Diterpenoid            | Qin, 2018                   |

|    |                             |             |                                                                           |                            |                      |
|----|-----------------------------|-------------|---------------------------------------------------------------------------|----------------------------|----------------------|
| 21 | <i>Euphorbia thymifolia</i> | Whole plant | Catechin, epicatechin                                                     | Flavan-3-ol isomers        | Kainsa & Singh, 2016 |
| 22 | <i>Euphorbia kansui</i>     | Roots       | Euphol, $\alpha$ -amyirin, $\beta$ -amyirin, oleanolic acid, ursolic acid | Diterpenoid & triterpenoid | Peng et al., 2012    |

**Table 3: Different phytochemicals found in *Euphorbia* species and their corresponding biological action**

| Sl. No | Species                      | Plant part   | Chemical compound                                                                                                                            | Biological activities                           | References                |
|--------|------------------------------|--------------|----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|---------------------------|
| 1      | <i>Euphorbia retusa</i>      | Aerial parts | Esculetin, quercetin -3-O-b-D-glucuronide, kaempferol -3-O-b-D-glucuronide, kaempfer-3-O-b-D-glucoside                                       | Antioxidant                                     | Elgamal et al., 2021      |
| 2      | <i>Euphorbia cactus</i>      | Aerial parts | Glutininol, catechin, kaempferol-3-O- $\alpha$ -L-rhamnopyranoside, quercetin-3-O- $\alpha$ -L-rhamnopyranoside                              | Antioxidant/antiproliferative                   | Al-Hamoud et al., 2022    |
| 3      | <i>Euphorbia nematocypha</i> | Aerial parts | $\beta$ -amyirin, $\beta$ -amyirin acetate, betulinic acid, ellagic acid, oleanolic acid, $\beta$ -sitosterol, kaempferol, quercetin, lupeol | Cytotoxic                                       | Xu et al., 2016           |
| 4      | <i>Euphorbia polyacantha</i> | Stem         | 1, 8-dihydroxy -3-(hydroxyl methyl) -5-methoxy anthracene- 9                                                                                 | Anti-inflammatory                               | Elhassan et al., 2015     |
| 5      | <i>Euphorbia ammak</i>       | Leaves       | Euphol $\alpha$ -glutininol stigmasterol                                                                                                     | Cytotoxic                                       | Abdel-Sattar et al., 2015 |
| 6      | <i>Euphorbia tibetica</i>    | Aerial parts | Methyl 4-epi-shikimate-3-O-gallate                                                                                                           | Anti-angiogenesis, anticancer                   | Da-Song et al., 2014      |
| 7      | <i>E. tirucalli</i>          | Whole plant  | Tirucadalenone, euphorol L, euphorol M, euphorol N                                                                                           | Antimicrobial                                   | Le et al., 2018           |
| 8      | <i>Euphorbia macrostegia</i> | Aerial parts | 24-methylenecycloartan-3 $\beta$ -ol, butyrospermol, cycloartenol, 1,2-di-O- $\alpha$ -linolenoyl- <i>sn</i> -glycerol, 1-                   | Analgesic, anticancer, bactericidal, antifungal | Zare et al., 2015         |

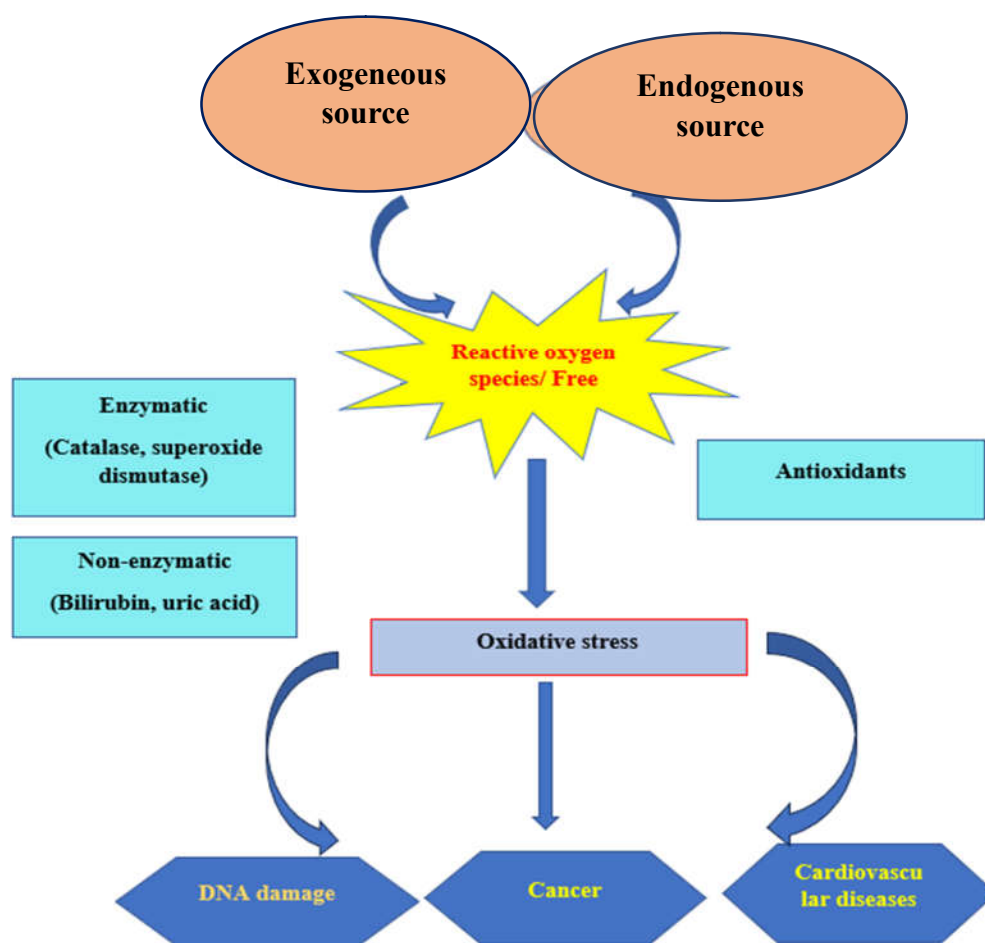
|    |                             |                     |                                                                                                                                                                                                                                                                    |                                                   |                           |
|----|-----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|---------------------------|
|    |                             |                     | <i>O</i> -linoleoyl-3- <i>O</i> -palmitoyl- <i>sn</i> -glycerol, 1- <i>O</i> - $\alpha$ -linolenoyl-2- <i>O</i> -palmitoyl- <i>sn</i> -glycerol                                                                                                                    |                                                   |                           |
| 9  | <i>Euphorbia wallichii</i>  | Roots               | Wallichanol A, wallichanol B, quercetin, kaemferol, myrecetin                                                                                                                                                                                                      | Cytotoxic, antifungal, antibacterial, antioxidant | Hassan et al., 2016       |
| 10 | <i>E. trigona</i>           | Latex               | 3 $\beta$ -friedelinol, taraxerol, stearic acid                                                                                                                                                                                                                    | Anticancer                                        | Anju & Ramesh kumar, 2022 |
| 11 | <i>E. milii</i>             | Aerial parts, Roots | Fraxetin, ellagic acid, kaempferol 3-(6''-acetylglucoside)-7-glucoside, herbacetin 8-acetate                                                                                                                                                                       | Antioxidant                                       | Saleem et al., 2019       |
| 12 | <i>Euphorbia tirucalli</i>  | Roots               | Myricetin, 3,3'-dimethoxy-4- <i>O</i> - $\alpha$ -rhamnopyranoside-ellagic acid, 4- <i>O</i> -methyl-gallic acid, ampelopsin                                                                                                                                       | Antimicrobial                                     | de Lima et al., 2021      |
| 13 | <i>Euphorbia geniculata</i> | Aerial parts        | Gallic acid, ellagic acid, quercetin acid, quercetin-3- <i>O</i> -rutinosid, quercetin-3- <i>O</i> -glucopyranoside-7- <i>O</i> -rhamnoside, quercetin-3- <i>O</i> -rhamnoside, quercetin-3- <i>O</i> -b-D-glucopyranoside, quercetin-3- <i>O</i> -b-D-arabinoside | Nephroprotective/antioxidant                      | Al-Yousef et al., 2020    |
| 14 | <i>E. pulcherrima</i>       | Bark                | 3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4H-chromen-4-one                                                                                                                                                                                          | Multidrug resistance reversal                     | Rauf et al., 2022         |
| 15 | <i>Euphorpia schimperi</i>  |                     | Miquelianin, kaempferol 3- <i>O</i> -glucuronide, quercitrin                                                                                                                                                                                                       | Wound healing                                     | Ahmed et al., 2017        |

Reactive oxygen species are free radicals or molecules with high reactivity derived from molecular oxygen. Hydrogen peroxide, superoxide radical anion, hydroxyl radical, and peroxy radical are a few examples of reactive oxygen species. These molecules are produced either endogenously or exogenously. Endogenous sources include cell organelles with high oxygen consumption, such as peroxisomes,

endoplasmic reticulum, and mitochondria, whereas exogenous sources include harmful chemicals found in the environment, electromagnetic radiation, cosmic radiation,  $\beta$  oxidation of fat, UV light, ozone, cigarette smoking, the low wavelength electromagnetic radiation (Krupanidhi et al., 2022). These molecules are essential for immune defense, cell maturation, pathogen defense, cell signaling, and homeostasis. ROS, on the other hand, can cause cell damage by attacking cell macromolecules such as plasma membrane lipids and nuclear DNA (Parcheta et al., 2021).

Fortunately, under physiological conditions, there is a positive correlation between reactive molecules and antioxidants, and the overproduction of these molecules is controlled and maintained by both enzymatic antioxidant systems (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic mechanisms (bilirubin and albumin) present within the systems (Mans et al., 2020). However, there are times when the production of free radicals in a human system exceeds the capacity of the body's defense mechanisms, which can result in oxidative stress. Oxidative stress is the root cause of serious chronic diseases like cancer, diabetes, cardiovascular disease, Alzheimer's disease, aging, Parkinson's disease and liver disease (Suksungworn & Duangsrirai 2021). A brief outline of the detrimental effects of reactive oxygen species and their inhibition by antioxidants is represented in **Fig. 2**.

Plants are extremely rich in compounds with antioxidant activity (Dumanović et al., 2021) and plant-derived phytoconstituents such as phenols and flavonoids are gaining popularity for their ability to resist and protect the body from the harmful effects of reactive molecules as well as diseases associated with oxidative stress. It has the ability to scavenge reactive species and reduce cellular damage (Joshi & Juyal 2021). As a result, natural antioxidants have received significant attention, increasing the demand for folk medicinal herbs in the modern era. Hence, an appropriate investigation of the phytochemicals responsible for the antioxidant properties of a plant is crucial for eliminating the issues generated by free radicals.



**Fig. 2: Schematic representation of causes and effects of reactive oxygen species**

Members of the genus *Euphorbia* are exploited very well in terms of their scavenging properties. Studies have shown that the members of this genus are a potential source of natural antioxidants with well-known scavenging mechanisms mainly due to the presence of phenolic compounds. Additionally, members of the genus *Euphorbia* are abundant in a wide range of diverse compounds with antioxidant status, which may play a crucial role in the elimination of free radicals (Fais et al., 2021). As a result of their lack of antioxidant activity, it is crucial to investigate the antioxidant potential of the chosen species.

The ability of the well-known plant *E. hirta* to scavenge free radicals has led to its exploitation by numerous workers. Hydroxyl radical scavenging assay,

superoxide anion scavenging assay and DPPH assay were adopted to check the antioxidant potential of *E. hirta*. Among the three various extracts considered, ethanol exhibited a higher percentage of inhibition with low IC<sub>50</sub> values in all conducted assays (Asha et al., 2016). The scavenging potential of the leaf extract of *E. hirta* was also reported by Mekam et al. (2019). They carried out the test by using two different solvents such as water and ethanol and recognized high inhibition against superoxide anion and ABTS free radicals in extracts of ethanol than in water. Phenolic compounds like coumaric acid, gallic acid, epicatechin, caffeic acid, etc. contributed to the scavenging potential of the plant. By measuring DPPH radical scavenging (Ismail et al., 2019), it was established that a beneficial association is observed between the phytochemicals and antioxidant capability of *E. hirta*. As a result of these findings, the plant *E. hirta* can be employed as a natural antioxidant to prevent oxidative stresses and diseases that develop during that exposure period. In addition, Mahomoodally et al. (2020) investigated the antioxidant efficacy of three *Euphorbia* species extracted with solvents such as ethyl acetate, methanol, and water. They discovered that *E. hirta* has a stronger quenching ability toward both DPPH and ABTS radicals than *E. heterophylla* and *E. convolvuloides*. The total flavonoid and phenolic content of *E. hirta* was also high, indicating a positive correlation between phytochemicals and antioxidant properties. Sinan et al. (2021) used the same plant species to analyse the chemical composition of these plants' essential oils using *in vitro* methods. In *E. convolvuloides*, *E. heterophylla*, and *E. hirta*, 33, 42, and 29 essential oil constituents, respectively, were found. In the ABTS assay, every plant species demonstrated radical scavenging capacity. Because essential oils are a natural source of antioxidants, this study highlights the significance of their promising pharmacological profiles.

In order to assess the antioxidant molecules and their antioxidant activity using *in vitro* and *in silico* methods, the phytochemicals derived from the ethanolic extract of *E. hirta* were examined. In the assay, it was found that the extract had a dose-dependent quenching activity against DPPH at increasing concentrations between 10 and 500 µg/ml. The maximum percentage of inhibition was 80.76% at the highest concentration of 500 µg/ml. The extract has a significant antioxidant

capacity with an  $IC_{50}$  value of  $95.1 \pm 10 \mu\text{g/ml}$ , which when compared to that of the standard's ( $68 \pm 0.235 \mu\text{g/ml}$ ), was comparable (Jacob & Sathiyajith, 2022). From the methanolic extracts of the aerial parts of *E. hirta*, twenty chebulic acid and brevifolin carboxylic acid derivatives were isolated, and they showed notable antiradical activity against DPPH (Yang et al., 2020). The importance of the plant as a vast source of natural antioxidants was thus established.

The fresh plant material of *E. royleana* was extracted with methanol, water and hexane in order to investigate the antioxidant properties. The DPPH and ferric ion reduction methods are used to test the scavenging ability. Because of the high distribution of phenolic compounds and flavonoids in hexane extract, it demonstrated effective DPPH scavenging potential and reducing power in comparison to the other extracts (Ashraf et al., 2015). Various parts such as leaf, stem, bark and latex of *E. neriifolia* was screened to evaluate its ability to quench free radicals by using different methods like metal chelation assay, nitric oxide assay (NO), superoxide (SO) scavenging assay, ferric ion reducing antioxidant power assay (FRAP) and  $H_2O_2$  scavenging assay (Chaudhary & Janmeda, 2022). Similarly, cycloartenol, an important compound isolated from the leaves of *E. neriifolia*, demonstrated significant scavenging activity. In a dose-dependent manner, cycloartenol inhibited both DPPH radicals and ferric chloride-induced lipid peroxidation. Furthermore, they concluded that cycloartenol is a natural antioxidant source for the prevention of various diseases caused by free radicals (Sawale et al., 2019).

Kamalanathan et al. (2015) investigated the antioxidant capacity of *E. fusiformis* leaf extracts. The experiments were carried out using solvents such as ethyl acetate, methanol, chloroform, and acetone. Their research included assays for nitric oxide, hydrogen peroxide, superoxide, DPPH radical scavenging activity, and ferrous ion chelating potential. All *E. fusiformis* extracts demonstrated promising radical quenching in the various methods used. However, the remarkable antioxidant potential in DPPH radical inhibitory activity was found in acetone extract with an  $IC_{50}$  value of  $15 \mu\text{g/ml}$ , followed by hydrogen peroxide scavenging activity. They



also reported that the methanolic extract inhibited superoxide and nitric oxide radicals the best. The antioxidant efficacy of acetone extract of *E. golondrina* was reported by Ndam et al., 2016. They stated that significant antioxidant activity was discovered during ABTS and nitric oxide scavenging assays. In a concentration-dependent manner, acetone extract demonstrated lower DPPH scavenging properties than standard vitamin C and rutin. In the ABTS radical scavenging assay, the acetone extract outperformed the standard gallic acid. These findings suggested that *E. golondrina* may be used to avoid the negative effects of pathological conditions such as diabetes and cancer, which were elevated significantly during the production of free radicals.

The free radical scavenging assays DPPH, ABTS, and FRAP were commonly used to quantify the antioxidant potential of the extracts. In their study, Bautista-Hernández et al. (2021) used this method to assess the free radical quenching ability of polyphenolic fractions isolated from two different residues, citric acid residue (CAR) and sulfuric acid residue (SAR) isolated from *E. antisiphilitica*. Their findings revealed significant differences in antioxidant activity between the phenolic fractions tested. When comparing two residues, citric acid residue demonstrated remarkable radical inhibition and can serve as a natural antioxidant. Miquelianin, an important phenolic compound, demonstrated significant antioxidant and free radical scavenging activity (Abdullah et al. 2017). This compound was isolated by Ahmed et al. (2019) from the aerial parts of *E. schimperi* to check the antioxidant potential and demonstrated greater radical scavenging activity in both the DPPH and ABTS assays than the standard, BHA. In the DPPH assay, miquelianin and BHA both displayed IC<sub>50</sub> values of 47.20 ± 4.90 mg/mL and 34.50 ± 6.20 mg/mL, respectively.

To investigate the relationship between total phenolic content and antioxidant activity, dried stem parts of *E. trigona* were extracted with solvents such as methanol, water, and acetone. The extracts were tested for free radical inhibition using a reducing power assay, a DPPH assay, and a Fe<sup>2+</sup> chelating assay. The antioxidant potential of the extracts and the phenolic compounds found in them

showed a positive correlation. Among the three extracts tested, the methanolic extract had the highest total phenolic content as well as the highest quenching ability, with maximum DPPH inhibition (83.2%), Fe<sup>2+</sup> chelating activity (88.59%), and significant reducing power 0.623 at 300 µg/ml. The findings suggested that *E. trigona* extract could be a good source of natural polyphenolic compounds as well as antioxidants (Salar et al., 2015).

Zhang et al. (2017) investigated the antioxidant properties of various parts such as the seed, testa, root, and stem of *E. lathyrus*. The highest DPPH free radical scavenging activity and phenolic content were found in the testa extract. While seed extract demonstrated significant FRAP antioxidant activity. It was also discovered that the DPPH scavenging property of plant extracts is dependent on total phenolic content; the higher the concentration of phenolics, the higher the activity. Based on their research, they came to the conclusion that phytochemicals have various antioxidant qualities, and different parts of a plant have distinct distribution of phytometabolites. In a similar way, Munro et al. (2015) used the correlation between *E. tirucalli*'s total phenolic content (TPC) and scavenging potential, a succulent Mediterranean shrub. For this, aqueous and methanolic extracts were used. In a dose-dependent manner, the aqueous and methanolic extracts both demonstrated scavenging potential against free radicals like DPPH and ABTS. This study unequivocally shows that TPC and antioxidant potential are positively correlated. Here, the methanolic extract had a TPC of 77.33 mg GAE/g, which was twice as high as the aqueous extract's TPC of 34.01 mg GAE/g. The same proportionality was observed in antioxidant activity, where the methanolic extract displayed a doubled scavenging ability compared to the aqueous. The scavenging capacity of three *Euphorbia*, including *E. biumbellata*, *E. terracina*, and *E. dendroides*, was assessed using DPPH radical and ABTS radical scavenging assays. *Euphorbia biumbellata*, one of the three plant species, has high antioxidant activity (Zeghad et al., 2016). With the aid of a Soxhlet apparatus, an ethanolic extract made from aerial plant parts, the antioxidant availability in the plant *E. eriophora* was investigated. Using the DPPH method, the plant extract's capacity to scavenge free radicals was

evaluated. At a concentration of 2 mg/ml, it was discovered that the plant extract's ability to inhibit DPPH activity was  $68.72 \pm 11.694\%$  (Akgül et al., 2022).

Using solvents like methane, dichloromethane, and n-hexane, Ashfaq et al. (2022) investigated the scavenging capability of *E. milii*. To ascertain the amount of phenol in the extracts, the Folin-Ciocalteu reagent method was used. Methanolic extract was found to be the most potential source of antioxidants than the other two solvent extracts, according to the analysis of the three solvents taken into account. They noted the high TPC ( $276.41 \pm 64$  mg GAE/g dry extract wt) in methanolic extract during the total phenolic content assessment by using gallic acid as the reference. Likewise, by using the DPPH assay, the antioxidant activities of three extracts of the *Euphorbia* species (*E. schimperiana*, *E. peplus*, and *E. cuneata*) as well as fractions and isolated compounds of *E. schimperiana* were assessed. Three phenolic compounds—Chrysin, Quercetin-7-O-D-glucuronide and 3-Methyl-Quercetin-7-O-D-glucuronide—and one triterpene—3-Cycloartenol were among the isolated compounds. When compared to *E. peplus* and *E. cuneata*, *E. schimperiana* and its fractions had the highest antioxidant activities. In addition, the antioxidant activities of the pure compound quercetin-7-O-D-glucuronide showed a high antioxidant activity that could be helpful in various *in vivo* studies (Shaker et al., 2015).

The antioxidant capacity exhibited by plants is significantly influenced by polyphenolic compounds. Adam et al. (2020) tested the *E. aegyptiaca* methanolic extract's ability to scavenge the radical DPPH. With a very low IC<sub>50</sub> value ( $0.0449 \pm 0.01 \mu\text{g/ml}$ ), it demonstrated the highest scavenging ability of 89%. The IC<sub>50</sub> value was discovered to be very similar to the value of the reference antioxidant, propyl gallate ( $0.077 \pm 0.01 \mu\text{g/ml}$ ) which was used in the study. Additionally, they inferred the scavenging capacity of n-hexane and chloroform extract derived from the same plant against DPPH and noted the very low activity in hexane (7%) followed by chloroform (23%) extracts. A similar attempt was made using aerial parts of the *E. splendida* total methanolic extract and various subfractions, such as ethyl acetate, petroleum ether, chloroform, and aqueous, to check their concentration to inhibit

50% of radical activity. Aqueous and ethyl acetate subfractions demonstrated good activity in scavenging DPPH in comparison to standard BHT. All of the selected subfractions also exhibited a dose-dependent metal chelating activity (Kefayati et al., 2017).

The DPPH assay was used to test the *in vitro* radical quenching capacity of the methanolic and ethanolic extracts of *E. helioscopia*'s flower, stem, and leaves. With an IC<sub>50</sub> value of  $26.66 \pm 0.000$  µg/ml, the methanolic flower extract had the strongest scavenging effects. The leaf and stem methanolic extract was found to have somewhat inferior antioxidant properties. While taking into account the solvent, the extract with methanol demonstrated good activity (Maoulainine et al., 2012). The oldest drug used in the Moroccan peoples' medicinal tradition is *E. resinifera*. For the purpose of determining antioxidant status, ethanolic and aqueous extracts were chosen. The methods used as tests were DPPH, H<sub>2</sub>O<sub>2</sub>, ABTS, and xanthine oxidase. By comparing the IC<sub>50</sub> values obtained from each assay, it can be seen that ethanol extract demonstrated greater activity than aqueous (Benrahou et al., 2022).

Metin & Bürün (2023) made an effort to determine the potential of *E. rigida* in terms of its antioxidant capacity by using assays including, DPPH radical scavenging activity, metal chelating activity, cupric reducing power method, and β-carotene bleaching method. They deduced that, among the different extracts looked at, acetone, ethanol, and petroleum ether extracts demonstrated high quenching ability, particularly in β carotene bleaching method and in DPPH systems. Thus, *E. rigida* may aid in preventing lipid peroxidation and free radical damage in humans. Through GC-MS analysis, recent studies have found that *E. larica* contains a number of essential oils, with camphene and limonene being the most prevalent. DPPH and ABTS assays revealed that the oil had strong antioxidant activity with IC<sub>50</sub> values of  $133.53 \pm 0.19$  µg/ml and  $154.93 \pm 0.17$  µg/ml, respectively (Shah et al., 2023).

Using different extracts from *E. supina*, including n-hexane, methyl alcohol, and water, Choi & Lim (2014) examined the plant's effects on the generation of

cellular ROS in order to determine its antioxidant capacity. Using the dichlorofluorescein-diacetate (DCFH-DA) assay, the H<sub>2</sub>O<sub>2</sub>-induced ROS generation was quantified. In a concentration-dependent manner, all tested fractions reduced the production of ROS. Therefore, analysis of *E. supina* revealed the antioxidant effects of this plant, indicating its potential to control oxidative stress.

Four different *in-vitro* methods were used to test the ability of the methanolic extracts of the plant *E. granulata* under investigation to scavenge the free radicals in the reaction medium. It was observed that the plant extract had ABTS, DPPH, and NO radical scavenging activities that were higher than 80% and slightly lower than those of the tested controls. From its IC<sub>50</sub> value ( $82.77 \pm 0.30\%$ ), which was lower than ascorbic acid ( $87.76 \pm 1.10\%$ ) and nearly equal to that of BHT ( $83.14 \pm 0.28\%$ ) as controls, it was evident that the extract had a tremendous ability to remove nitric oxide (NO) (Al-Robai et al., 2023). Using ethyl acetate, methanol, and water as solvents, the antioxidant activity of leaf extract from *E. hypericifolia* and *E. prostrata* against DPPH radical was compared. The study took into account various solvent concentrations ranging from 3.12 to 200 µg/ml. The methanolic extract showed the highest rate of scavenging activity, while the ethyl acetate extract and the aqueous extract of both species showed the lowest rates. Comparing the two species, the leaf extract of *E. prostrata* had higher scavenging activity than that of *E. hypericifolia*. Its IC<sub>50</sub> value (23.45 µg/ml), which is lower than that of typical ascorbic acid (31.97 µg/ml), revealed its enormous antioxidant potential (Abbas et al., 2023). Previous studies also noted the antioxidant capacity of the ethanolic leaf extract of *E. prostrata* (Sundaraprabha & Rayan, 2018).

Elagdi et al. (2023) looked into the antiradical effects of methanolic extracts from *E. echinus* and *E. resinifera*. The antioxidant activity of the two species was found to be greater than that of ascorbic acid. The methanolic extract of *E. resinifera* had more antioxidant activity (0.01 mg/ml) than *E. echinus* (0.02 mg/ml). Latex from various *Euphorbia* species showed significant antioxidant properties. For instance, the latex extract of *E. tirucalli* demonstrated quenching ability against DPPH and ABTS with an IC<sub>50</sub> value of 6.0 and 2.0 GAE µg/ml, respectively. With

an EC<sub>50</sub> value of 6.5 GAE µg/ml, the phosphomolybdate assay also demonstrated the latex's strong reduction capability (Benjamaa et al., 2022).

The origin of the word inflammation can be traced back to the Latin word *inflammare*, which means "burned." Inflammation is an immune system's defensive response to any type of tissue damage developed as a result of physical injuries, harmful chemicals, or toxic compounds. It is essential in restoring the body's homeostasis and acts to start the healing process as well as the repair, remodelling, and renewal of various tissues. It is regarded as the first line of defense that shields the host from infections brought on by a variety of pathogens, including bacteria, fungi, and viruses. Thus, it is a crucial defense mechanism that is important for maintaining health (Chen et al., 2018). Acute and chronic inflammation are the two main types. The molecular and cellular events, along with their interactions, typically reduce the severity of infections and injuries during an acute inflammatory response. The recovery of tissue homeostasis is aided by this mitigation process. Uncontrolled or excessive acute inflammation can lead to chronic inflammation, which itself may be a contributing factor to some of these long-lasting illnesses (Zhou et al., 2016). Although the inflammatory process is the body's defense mechanism, it can be easily triggered due to the complex events and mediators involved (Ghauri et al., 2021). In some cases, free radicals (reactive oxygen and nitric oxide) are activated and released from a variety of immune system cells as a result of some types of inflammatory responses, which results in localised tissue damage and lipid peroxidation (Chirumamilla & Taduri, 2023).

The WHO estimates that about 500 million people worldwide suffer from autoimmune syndromes like arthritis and inflammation, which have serious health consequences and substantial economic costs. In traditional Ayurvedic medicine, isolated compounds from therapeutic plants are used to treat a variety of inflammatory conditions. There have been many reports on the ability of COX-2/LOX inhibitors derived from medicinal plants to have the same effects as NSAIDs while having fewer side effects (Shaikh et al., 2016). Some reports available on the anti-inflammatory potential of *Euphorbia* species are mentioned herewith.

Protein denaturation is a well-known contributor to tissue inflammatory conditions that result in symptoms like pain, redness, heat, and swelling. During inflammation, there is an increase in protein denaturation (Gunathilake et al., 2018). It is clear that a substance's ability to prevent protein denaturation is a sign of its anti-inflammatory properties in situations where tissue denaturation—which results from the denaturation of cell protein components—occurs. Consequently, by inhibiting protein denaturation, inflammatory effects can be diminished (Osman et al., 2016).

The anti-inflammatory potential of *E. hirta* was evaluated by Das et al. (2022). Plant parts, including the leaf and root, which were extracted with ethanol, and aspirin, one of the conventional anti-inflammatory agents was used to measure the anti-inflammatory activity *in vitro* by using albumin denaturation, anti-proteinase, and antilipoxygenase assays. Leaf extract significantly outperformed root extract in terms of anti-inflammatory activity at the highest concentration (100 µg/ml) in every assay conducted. Additionally, different extracts of *E. hirta*, including ethanol, methanol, acetone, and water, were tested to see if they had any inhibitory effects on protein denaturation. The most potent anti-inflammatory properties were found in water extract when compared to the other four solvents (dev Sharma et al., 2023). This is supported by research conducted by Rahman et al. (2019) that revealed at the anti-inflammatory potential of *E. hirta* methanolic extract on xylene-induced ear edema in mice model. The plant extract's anti-inflammatory potential was demonstrated by its capacity to inhibit phospholipase A2, the enzyme that initiates all inflammatory activity. At the highest concentration of 800 mg/kg, a significant decrease in ear edema was observed with an inhibition percentage of  $51.06 \pm 6.86\%$ . They also evaluated the anti-inflammatory potential of plant extract using a model of granuloma caused by cotton pellets. Of all the plant extract concentrations, 800 mg/kg had the highest percentage of granuloma formation inhibition ( $56.80 \pm 3.85\%$ ), which was quite close to the average ( $58.69 \pm 3.32\%$ ).

*E. heterophylla* leaf methanolic extract was used by Muhammad et al. (2017) to assess the plant's anti-inflammatory potential. They concluded from their research that methanolic extract significantly reduced the rate of protein denaturation, with a

maximum inhibition of 81.7% at a concentration of 500 µg/ml. They also noted its ability to prevent proteinase and lipoxygenase activity and stabilise red blood cell membranes from hemolysis in heat and hypotonic conditions, which are related to inflammatory processes. This supported the use of *E. heterophylla* in the development of effective anti-inflammatory drugs for the treatment of inflammation.

Through both *in vitro* and *in vivo* studies, tirucallol, a tetracyclic triterpene isolated from *E. lactea* latex, demonstrated its tremendous anti-inflammatory potential. *In vitro* tests showed that the compound significantly reduced nitric oxide levels by inhibiting inducible nitric oxide synthetase (iNOS) expression in lipopolysaccharide stimulated macrophages. Additionally, it reduced ear edema in the mouse model that was brought on by 12-O-tetradecanoylphorbol-acetate (TPA) under *in vivo* conditions by inhibiting neutrophil migration (Fernandez-Arche et al., 2010).

Palit et al. (2016) designed a study to evaluate the anti-inflammatory potential of the fraction enriched in total steroid and terpenoid derived from *E. neriifolia* hydroalcoholic stem extract. Protein denaturation and proteinase activity were found to have significant inhibitory activity by the action of the fraction. Furthermore, the fraction inhibited nitric oxide production by reducing the expression of iNOS, which was expressed as a pathological concentration during the time of inflammation and whose expression was determined by western blot analysis. Furthermore, anti-inflammatory activity was determined *in vivo* using a carrageenan-induced rat hind paw edema model. *In vivo*, studies supported the *in vitro* findings by showing 98.97% protection against carrageenan-induced pain, with a "more relevant" result than the standard indomethacin.

In an experiment using raw 264.7 cells *in vitro*, *E. helioscopia*'s potential anti-inflammatory effects were investigated. Both ethanolic and aqueous extracts were chosen for this. Both extracts demonstrated a dose-dependent reduction in proinflammatory mediators such as nitric oxide, TNF-  $\alpha$ , and IL-6 (Jan et al., 2022). One of the dominant metabolites, diterpenes found in *Euphorbia* members has been reported for its incredible ability to reduce inflammation by inhibiting the formation of inflammatory cytokines such as TNF-  $\alpha$  and IL-6. They also have the ability to inhibit Cox-2 and iNOS, two major inflammatory markers (Wang et al., 2021). For



instance, the LPS-stimulated RAW 264.7 macrophages were used to test the anti-inflammatory activity of the diterpenoid 17-hydroxy-jolkinolide B that was isolated from the dried roots of *E. fischeriana*. It demonstrated strong inhibition of several inflammatory mediators, including nitric oxide (NO) and prostaglandin E2 (PGE<sub>2</sub>), as well as pro-inflammatory cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6). This inhibition was accomplished by reducing the expression of the genes for COX-2, iNOS, IL-6, and TNF-  $\alpha$  by inhibiting MAPK phosphorylation and NF- $\kappa$ B activation (Uto et al., 2012). It was thus proposed that the compound is a promising anti-inflammatory drug capable of suppressing the expression of inflammatory mediators and cytokines via the MAPK and NF- $\kappa$ B pathways.

Lathyrane diterpenoid isolated from *E. lathyrus* seeds inhibited LPS-induced nitric oxide production in raw 264.7 macrophage cells. It has also been noted that it can act as an anti-inflammatory agent by lowering cytokines like IL-6 and iNOS protein expression (Zhang et al., 2019). Similarly, jatrophone polyesters derived from *E. peplus* demonstrated anti-inflammatory activity and significantly reduced NO production (Li et al., 2022). Similar activity was observed in triterpenoids isolated from *E. humifusa*, which inhibited nitric oxide production (Huang et al., 2023). Additionally, by activating NF- $\kappa$ B, the potent bioactive compound known as ebractenoid F, which was found in the roots of *E. ebracteolata* Hayata, significantly decreased the production of nitric oxide (Chun et al., 2023).

The effect of a dichloromethane extract of *E. grantii* on cyclooxygenase 1 and 2 activity as well as the lipoxygenase activity was also investigated. Significant inhibitory activity was found in Cox-1 (IC<sub>50</sub> -  $6.9 \pm 0.2$   $\mu$ g/ml), Cox-2 (IC<sub>50</sub> -  $0.29 \pm 0.01$   $\mu$ g/ml), and Lox activity (IC<sub>50</sub> -  $24 \pm 2.5$   $\mu$ g/ml) (Radi et al., 2023). Chemical profiling of the stem of *E. antiquorum* led to the isolation of 12 compounds as well as five new diterpenoids. Anti-inflammatory studies using isolated diterpenoids demonstrated strong inhibition of nitric oxide with lower IC<sub>50</sub> values (An et al., 2019). In addition, in a recent report, Choodej et al. (2020) detailed the potent effects of diterpenoids on LPS-stimulated nitric oxide production. These diterpenoids were isolated from ethyl acetate extracts of *E. antiquorum* aerial parts.

Cycloarta-23-ene-3-beta,25-diol, a pentacyclic steroid isolated from *E. spinidens*, was studied for its anti-inflammatory properties by Hajhashemi et al. (2022). HPLC purification methods are used to isolate the compound. *In vitro* tests with the substance showed pronounced inhibition of both Cox-1 and Cox-2. Studies conducted in the past on the anti-inflammatory potential of different species of *Euphorbia* have found that these species' anti-inflammatory potential is really remarkable. Therefore, it is essential to ascertain the capacity of selected *Euphorbia* species to decrease inflammation.

The *Allium cepa* test is a significant and highly effective *in vivo* model assay, wherein the root develops in close proximity to the medicinal mixture under examination, thereby permitting the prediction of potential damage to eukaryotic DNA. This *Allium cepa* method was widely used by researchers to determine the potential genotoxicity of medicinal plants (Owolarafe et al., 2020). Kuruppuarachchi et al. (2023) claim that this approach is one of the less expensive plant-based *in vivo* experiments. It is one of the more sensitive tests for analysing chromosomal abnormalities brought on by various chemical poisons. Because the onion (*Allium cepa* L.) has a low chromosomal number ( $2n=16$ ), it is easier to manage and comprehend the chromosomal variation that emerged after the plants were treated with toxic chemicals. This aids in the investigation of the harmful effects of toxins on regular cell division. Information on how harmful substances impact chromosomes and consequently cause chromosomal aberrations is made possible by this test (Sabeen et al., 2020). Other plants such as maize, *Drimia indica*, and *Vicia faba* have also been used for cytotoxicity screening. Nonetheless, *A. cepa* was found to be more useful than them because of its readily visible large chromosomes and ability to show any effects that may have resulted from a low level of interaction between the testing substance and the genetic material (Bonciu et al., 2018).

The Mitotic Index (MI), a biomarker for cell proliferation, was used to determine the proportions of cells in the M-phase of the cell cycle. It is a valid technique for determining the cytotoxicity of any living thing. For example, if the MI value was less than the negative control, it would suggest that there has been an alteration in the development of *A. cepa*. In addition, uncontrolled cell division and the growth of tumour tissues are indicated if the MI value is greater than the negative control (Leme & Marin-Morales, 2009).

Chromosome aberrations are the alterations in a chromosome's normal structure brought on by breaks or excisions in the chromosomal material (Atoyebi et al., 2015). Stickiness of chromosomes is induced as a result of cross-linkage of chromosomal protein leading to improper folding of the chromosome fibres. The cause of chromosome bridges and fragmentation may be related to the stickiness of the chromosome. Likewise, cytokine inhibition is the cause of the bi-nucleated aberrations. On the other hand, laggard chromosomes and multipolar anaphase are produced as a result of changes in spindle formation. When chromosomal proteins cross-link, sticky chromosomes with incorrectly folded chromosome fibres are produced (Ibrahim et al., 2014).

Ping et al. (2012) investigated the cytotoxic properties of *E. hirta* methanolic extracts. In this experiment, different concentrations of 125, 250, 500, and 1000 µg/ml were used. They noticed cytotoxic effects at every concentration they selected. The most common chromosomal abnormalities found are C-mitosis, stickiness, bridges, and vagrant chromosomes.

An engaging online interface, “The Global Cancer Incidence, Mortality and Prevalence” (GLOBOCAN) estimates the total number of cancer sites in 185 countries, as well as the incidence of, and death resulting from, the 36 different types of cancer. Based on data gathered in 2020, it was found that one in five individuals worldwide will develop cancer at some point in their lives, and that one in eight men and one in eleven women will die from the illness. These estimated numbers may have increased due to the aging population and socioeconomic risk factors (Sung et al., 2021). About 36 different types of cancer exist. Males are most affected by lung, prostate, liver, colorectal, prostate, and stomach cancer, whereas females are affected by breast, colorectal, lung, cervix, and thyroid cancer (Khan et al., 2019). Chemotherapy, radiation therapy, and surgery are examples of traditional and cutting-edge cancer treatments. Long-term exposure to any of them, however, has negative health consequences. However, as the problem persists, new approaches to disease control are required, particularly given the failure of conventional chemotherapeutic approaches. As a result, new cancer prevention and treatment strategies are required to control the disease's death rate (Karpuz et al., 2018).

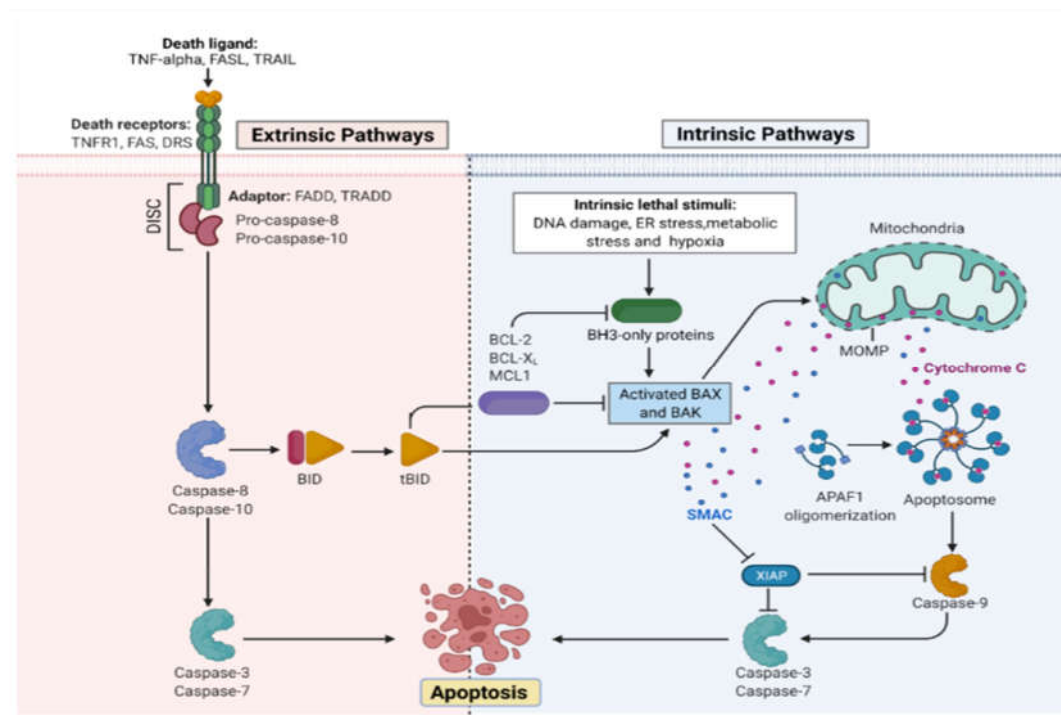
Herbal medicines are important for both cancer prevention and treatment. Some herbs protect the body from cancer by enhancing detoxification functions. Furthermore, some herbs reduce the toxicity of chemotherapy and radiotherapy. Moreover, by regulating the activity of particular hormones and enzymes, herbs can slow the growth of cancer (Bijauliya et al., 2017). Since herbs contain a variety of chemical components, they are thought to counteract the effects of diseases on the body. Vinca alkaloids, podophyllotoxins, and taxanes are just a few of the potent cytotoxic drugs that have been brought to modern medicine through screening of medicinal plants for bioactive constituents with efficacious anticancer properties (Mazumder et al., 2022). Hence, one of the most important aspects of the hunt for anticancer treatments is now the development and discovery of drugs from secondary metabolites in medicinal plants.

The anticancer properties of *Euphorbia* species make them a valuable therapeutic resource in traditional Chinese medicine. There are numerous reports on the effectiveness of secondary metabolites from different species of *Euphorbia* in inhibiting the growth of different types of cancer. Among them, diterpenoids were discovered to be prevalent. Diterpenoids derived from *E. esula*, for example, demonstrate anticancer properties against both non-resistant and multidrug-resistant human gastric cancer cells. Similarly, euphol, a tetracyclic triterpene present in the sap and latex of numerous *Euphorbia* species, has anti-tumour properties. Furthermore, diterpenoids from the ethanolic extract of *E. helioscopia* were shown to be effective against renal cancer cell lines (Mesas et al., 2021).

Since apoptosis is essential for embryonic development, immune system regulation, and the body's reaction to DNA damage, it is a necessary physiological process of cell death that happens without the release of intracellular contents or the subsequent induction of an inflammatory response. Disturbances in apoptosis, however, have noteworthy implications for the development of cancer. A malignant tumour is thought to be characterised by an imbalance between cell death and proliferation. For normal physiological processes to occur, cellular homeostasis between cell proliferation and death must be maintained (Chaudhry et al., 2022).

The regulation of the apoptotic process is greatly aided by several proteins, including pro- and anti-apoptotic ones that are involved in the apoptotic pathway. Thus, these proteins are the focus of cutting-edge cancer therapies and treatments (Chaudhry et al., 2022).

Apoptosis is primarily caused by two signalling pathways: intrinsic and extrinsic. A family of cysteine proteases known as caspases are activated in both pathways by signalling, and these proteases work in a proteolytic cascade to break apart and remove the dying cell. Outside the cell, the extrinsic pathway initiates when conditions in the extracellular environment require a cell to die. While the Intrinsic apoptosis pathway is activated upon cellular damage. Intrinsic stresses such as starvation, oxidative stress, direct DNA damage, and oncogenes can all trigger the intrinsic apoptotic pathway (Wani et al., 2023). **Fig. 3** depicts how programmed cell death is activated both intrinsically and externally by molecules.

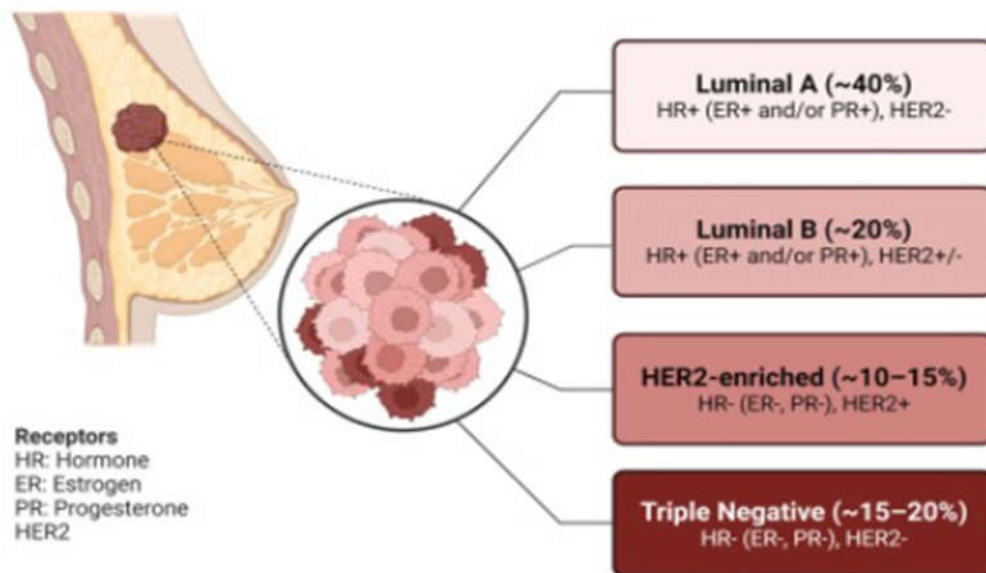


**Fig. 3: Representation of intrinsic and extrinsic path ways of apoptosis** (Wani et al., 2023).

## Breast Cancer (BC)

Carcinogenesis is a multifactorial process mainly triggered by genetic predispositions and environmental factors. Alarming, the death rate from cancer-related causes rises daily. It serves as the primary cause of declining life quality in addition to causing death (Łukasiewicz et al., 2021). Breast cancer (BC) is the most common type of cancer that affects women and is the primary cause of cancer-related death worldwide, with a high incidence rate among those between the ages of 45 and 65. About 30% of cancer cases that affect women each year are related to this.

There are four distinct molecular BC subtypes within it, each with unique morphologies and treatment strategies. It mainly includes luminal-A, luminal-B, human epidermal growth factor receptor 2 (HER2)-enriched and triple-negative breast cancer (TNBC). **Fig. 4** depicts the four subtypes of BC. A patient's chance of survival with breast cancer is primarily determined by the size of the tumor, the hormone receptor profile, and the tumor's growth at the time of diagnosis (Grimm, 2023; Ensenyat-Mendez et al., 2021).



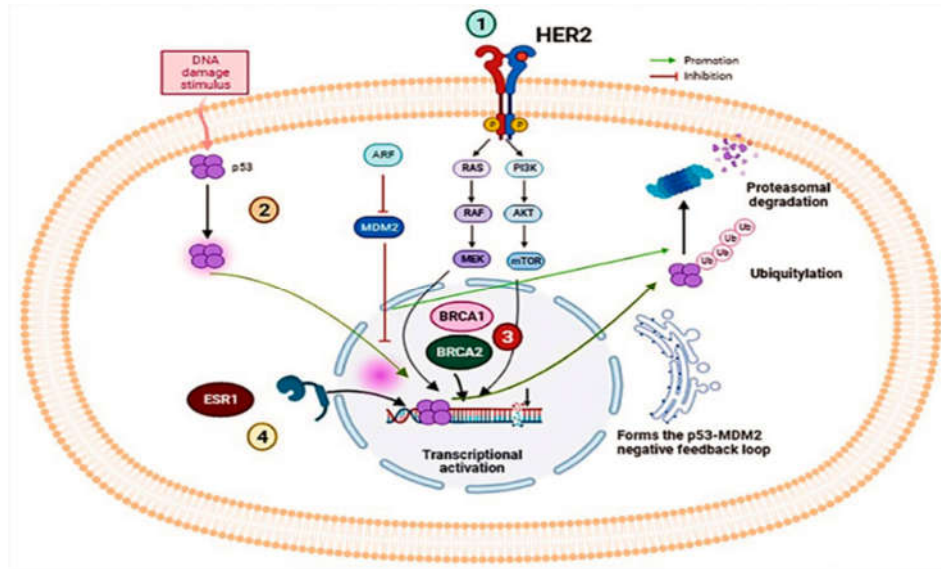
**Fig. 4: Representation of molecular subtypes of cancer** (Pankotai-Bodó et al., 2023)

Breast Cancer can be either sporadic or hereditary. The prominent mutations associated with cancer that are hereditary in nature include those that directly affect genes like BRCA1, BRCA2 (Breast Cancer Associated genes 1 and 2), and TP53 genes as well as DNA damage repair (DDR) genes. Sporadic forms of BC account for about 85% of cases and are caused by exposure to carcinogens such as electromagnetic radiation and air pollution, as well as factors such as aging, family history, and hormonal factors like early or late menopause, as well as lifestyle choices and alcohol consumption (Ortega et al., 2020).

BRCA1 and BRCA2 are two important genes that raise the risk of breast cancer. Any mutation in these genes has an indirect impact on genomic integrity by altering the homologous recombination-mediated DNA repair pathway. BRCA1 and BRCA2 mutation-related breast tumors typically show diverse histopathologies. Breast cancers with BRCA1 mutations are primarily responsible for the development of basal-like breast cancers; in contrast, breast cancers in carriers of BRCA2 mutations are significantly more diverse (Bhat-Nakshatri et al., 2024).

Various signalling pathways are involved in the development of breast cancer. Cell signal transduction is the fundamental mechanism for every cancer's development and survival. Many distinct features, such as apoptosis invasion, genomic instability, and dysregulated progression, are specific to cancer cells. A cancer cell's ability to proliferate, progress, and eventually survive is dependent on modifications and variations in various signalling pathways. The primary causes of the changes in signalling pathways are due to the overexpression of oncogenes, mutated proteins with uncontrolled activity, and the inactivation of tumour suppressor genes (Ortega et al., 2020). For example, excessive expression of oncogenes causes genes linked to cell survival and proliferation to be highly expressed. When EGFR1 and HER2 are overexpressed, breast cancer develops. Moreover, the development and progression of breast tissue in these cases are caused by the malfunctioning of tumour suppression genes such as BRCA1 and BRCA2. Proteins associated with the repair process of DNA double-strand breaks are primarily encoded by these two genes. Thus, these genes' mutations have an impact on the mechanisms that repair DNA, which eventually promotes the growth

of breast cancer (Clusan et al., 2023). **Fig. 5** illustrates the development of breast cancer through the involvement of BRCA1 and BRCA2 tumour suppressor genes.



**Fig. 5: Illustration of various signalling pathways implicated in the oncogenesis of breast cancer (El Hejjoui et al., 2023)**

Cell lines have become increasingly popular, especially when used as models for human biology. It has aided in many areas, such as the development of vaccines and toxicology analysis. The quality of a cell line is crucial, and each cell line has a different quality. It is not feasible to use every cancer cell line as a model for research on human cancer. A common model for human breast cancer is the Michigan Cancer Foundation-7 cell line (MCF-7) (Tran et al., 2021). All across the world in breast cancer research, the MCF-7 cell line is a frequently used xenograft model. Its capacity to generate more data of useful knowledge for patient care makes it more significant than any other breast cancer cell line. Furthermore, the MCF-7 cell line's unique molecular profile and the presence of several key receptors, among other unique characteristics, give it significance in the field of breast cancer research (Comşa et al., 2015).

Various species of the genus *Euphorbia* showed notable resistance against MCF-7 breast cancer cell lines, along with other types of cell lines. Nevertheless, the current study's chosen plant species have been used against MCF-7 for the first time.



One of the important herbs used by traditional medicine practitioners is *E. hirta*. The research was done on the impact of *E. hirta* extract on the HepG2 cell line, a type of liver cancer. The extract demonstrated significant suppression of HepG2 cell proliferation by inducing apoptosis (Kalaivani et al., 2023). Likewise, *E. hirta* extract was tested for its inhibitory potential against a range of cell lines, including the MCF-7, HT 29, Hela, and Vero cell lines. Using the MTT colorimetric cytotoxic assay, the extract's potential was examined. MCF-7 cell survival was significantly inhibited by the extract. It modulated the caspase 3-independent pathway, which in turn caused apoptosis. Additionally, the cell cycle arrest happened during the G2/M and S phases. As a result, the study showed that the plant extract had strong antiproliferative properties and that it might be employed to treat breast cancer (Kwan et al., 2016).

Ethanollic extract of mature *E. lathyris* seeds was tested against colon cancer cell lines, including T84 and HCT-15, to determine its antitumor potential. In both cell lines, the extract caused cell death by overexpressing caspase 3, 8, and 9 and by turning on autophagy. Furthermore, the plant extract demonstrated decreased angiogenic potential and decreased migration of cancer cells in the T84 cell line. These two effects are linked to tumour recurrence. Notably, the results demonstrated significant antitumor effects against colon cancer (Mesas et al., 2021).

*E. fischeriana* is an additional species with potential applications as an anti-tumour drug. This species of plant was used in Chinese traditional medicine to treat liver and lung cancer. Furthermore, one of the well-known traditional Chinese medicines, Langdu, is primarily derived from two sources: *E. fischeriana* and *E. ebracteolata*. It is used to treat conditions like tumors and chronic fatigue. Strong anticancer effects were shown by compounds isolated from *E. fischeriana* roots. Triterpenoid and diterpenoid compounds are among those that were found to be responsible for exerting strong anti-tumor effects (Shi et al., 2017). A different study report revealed that diterpenoids derived from *E. fischeriana* exhibited cytotoxic effects on MCF-7 cells by preventing the growth of the mammosphere in MCF-7 human breast cancer cells (Kuang et al., 2016). The MCF-7 cell line's proliferation

can be inhibited by diterpenoids derived from *E. fisheriana* with a promising IC<sub>50</sub> value. The diterpenoids have a major role in controlling apoptosis, arresting the cell cycle at different checkpoints, and obstructing different signalling pathways to prevent tumor cells from metastasizing (Jain et al., 2018).

The lactone structures found in *Euphorbia*'s diterpenoids are known to confer strong antiproliferative effects. There have also been reports on the diterpenoid of this genus having antineoplastic effects on a variety of cell lines (Ernst et al., 2015). Triterpenoids have also attracted attention lately due to reports of their anti-inflammatory, antiviral, antibacterial, sedative, immunoregulatory, blood pressure-lowering, and antitumor properties. Additionally, it was mentioned that triterpenes could prevent the growth of tumors via a number of different mechanisms (Radi et al., 2023). A recent study states that from the 36 species in the genus *Euphorbia*, approximately 264 compounds with anticancer potential were isolated (Rojas-Jiménez et al., 2024).

The four triterpenoids that were extracted from the *Euphorbia lactea*, taraxerol, friedelan-3 $\alpha$ -ol, friedelin, and friedelan-3 $\beta$ -ol, showed cytotoxic effects on the cell lines HN22, HepG2, HCT116, and HeLa. Of the four identified bioactive metabolites, friedelan-3 $\beta$ -ol demonstrated noteworthy inhibition on cancer cell lines by inducing an S-phase cell cycle arrest (Wongprayoon et al., 2022).

*E. hierosolymitana* is a less well-researched species that is only found in eastern Mediterranean nations. The effectiveness of n-butanol and ethyl acetate fractions derived from the aerial parts of *E. hierosolymitana* has demonstrated their potential as agents against cancer. For this, cell lines like A549, PC-3, MCF-7, and Caco-2 were used and the positive control was doxorubicin. The highest IC<sub>50</sub> value was found at the highest concentration out of various concentrations considered such as 31.25, 62.5, 125, 250, 500, and 1000  $\mu$ g/ml. Additionally, cytotoxic effects were found in a dose-dependent manner. Against the normal cell line Wi38, neither of the two extracts showed any cytotoxic effects. Among the extracts, n-butanol extract showed strong effects on the Caco-2 colon cancer cell line (Al-Saraireh et al., 2021).

Another species that is significant to traditional medicine across various nations is *E. granulata*. The MCF-7 cell line was utilised to evaluate the antitumor potential. The cytotoxic nature of the plant extract was demonstrated by the MTT assay. Through the induction of apoptosis, the plant extract reduced the viability of the cells (Mahdavi et al., 2022).

*Euphorbia tirucalli* has a long history of use in Brazilian folk medicine as a cancer treatment. Using five cancer cell lines—HepG2, HL-60, A549, MCF-7, and HCT116—the anticancer potential of the phenolic extract of *E. tirucalli* was investigated. The extract very effectively inhibited the proliferation of both the MCF-7 and A549 cell lines, with  $IC_{50}$  values of  $31.65 \pm 3.67$   $\mu\text{g/ml}$  and  $35.36 \pm 3.82$   $\mu\text{g/ml}$ , respectively. The extract demonstrated a significant inhibitory effect on the HL-60 cell line, with a promising  $IC_{50}$  value of  $22.76 \pm 2.85$   $\mu\text{g/ml}$ , which was observed to be extremely near to the value demonstrated by doxorubicin, a widely used anticancer medication (Abdel-Aty et al., 2019). Furthermore, in a different investigation, the growth of gastric adenocarcinoma cancer cell lines was inhibited by the extract of *E. tirucalli*. The compounds euphol and tirucallol found in the plant extract are primarily responsible for this effect (de Souza et al., 2019).

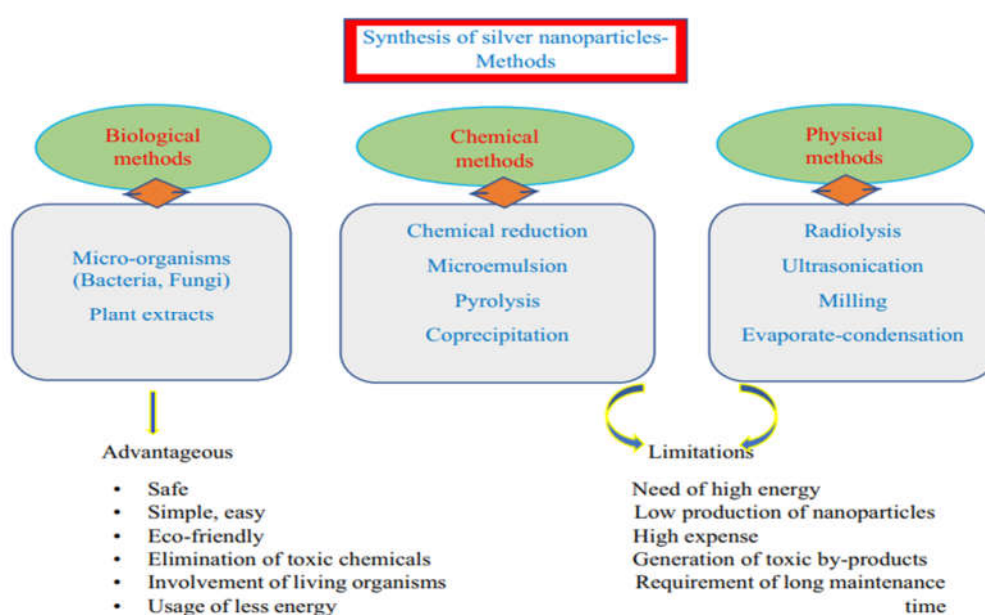
*E. greenwayi* is one of the important species whose applications have been studied in a variety of bioactivities, including cancer research. The anticancer effects of utilising *E. greenwayi* methanol extract and its different fractions (n-hexane, chloroform, and ethyl acetate fraction) against cancer cell lines, namely HepG-2 hepatocellular carcinoma, SW620 colorectal adenocarcinoma, and MCF-7 breast adenocarcinoma, were determined by using two different concentrations (10 and 100  $\mu\text{g/ml}$ ) of each sample. The three selected cell lines were significantly affected by the cytotoxic effects of both the n-hexane and chloroform fractions. Additionally, MCF-7 was discovered to be the most susceptible cell line among the others. Phytochemicals with anticancer properties, including  $\beta$ -sitosterol, lupeol, and cycloartenol, were identified as the possible cause of the cytotoxic effects (Zaghlol et al., 2024).

With wide applications across numerous industries, nanotechnology is a rapidly and promisingly developing technology in today's world. Numerous industries, including food, medicine, water treatment, and cosmetics, use nanotechnology. Small materials, ranging in size from 1 to 100 nm, are known as nanoparticles and have distinct properties. The emergence of metal nanoparticles, including silver, platinum, copper and gold, has garnered significant interest due to their diverse range of applications across various industries (Ruddaraju et al., 2020). Due to their remarkable antimicrobial and localised surface plasmon resonance properties, silver nanoparticles (Ag NPs) are significant among the many metals. These properties give them unique qualities like a broad spectrum of antimicrobial properties, surface-enhanced Raman spectroscopy (SERS), chemical/biological sensors, biomedicine materials, and biomarker applications (Kumar et al., 2022). In addition, the medical industry is particularly interested in silver nanoparticles due to their broad range of bactericidal and therapeutical potential, unique thermal conductivity, chemical stability, unique morphology, size, shape, high surface area, and widespread application in optical, photo-electro-chemicals, magnetism, photonics, and pharmaceuticals. Their important applications as antimicrobial, anti-inflammatory, and anticancer agents in drug delivery and diagnostics are also noteworthy (Zahoor et al., 2021). Ag NPs are a great option and have been utilised in medical devices such as breathing tubes, wound care products, and antimicrobial dressings. Because of Ag NPs' superior biocompatibility and viability, they have antimicrobial, anticancer, cytotoxicity, and anti-inflammatory properties (Dhaka et al., 2023).

### **Types of silver nanoparticle synthesis**

The two most popular methods for generating silver nanoparticles are physical and chemical ones. It is costly to produce nanoparticles using both chemical and physical methods. In addition to being expensive, these methods produce toxic byproducts that are harmful to both the environment and human health because they involve the use of toxic chemicals. High energy consumption and low synthesis rates are two more disadvantages of these technologies (Iravani et al., 2014). Different approaches used to synthesize nanoparticles are depicted in **Fig. 6**

Green strategies are now recognised as practical choices. Among the materials used in the biosynthesis of silver nanoparticles are plant extracts, actinomycetes, algae, fungi, bacteria, viruses, and diatoms (**Fig. 7**). Despite the microorganisms' ability to efficiently produce nanoparticles, they still face challenges like contamination and culture maintenance. Because of its ease of use and quick formation in a single step, researchers are increasingly turning to plant extracts for the production of nanoparticles. Moreover, the phytometabolites found within the plant extract act as natural reducing and stabilising agents, which drive the development of nanoparticles (Sameena & Thoppil, 2022).

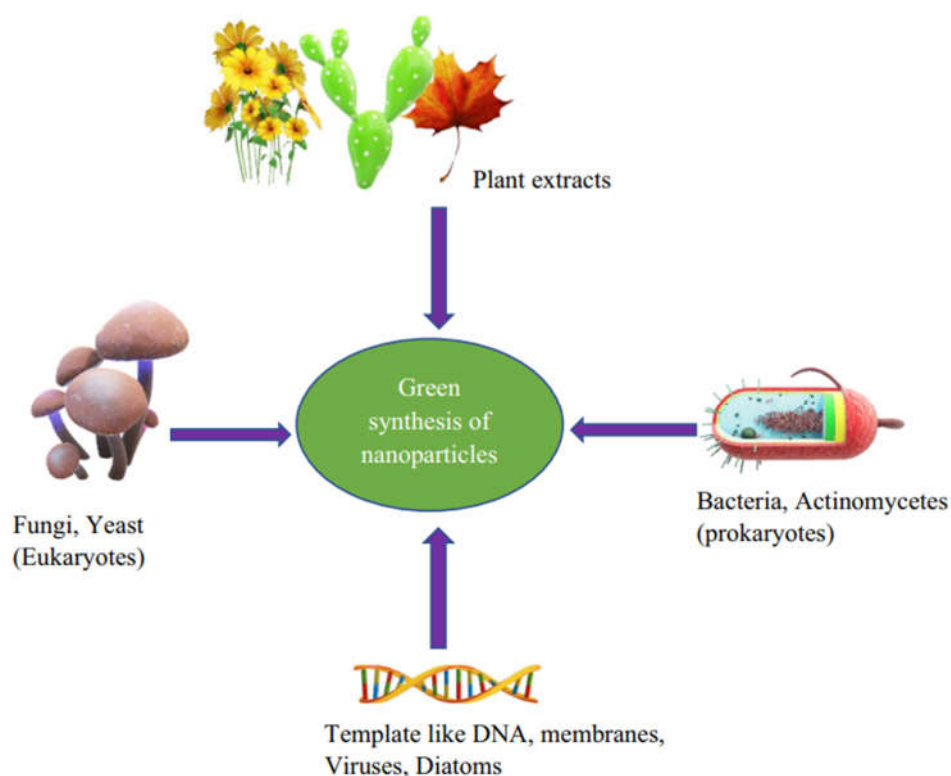


**Fig. 6: Different methods for nanoparticle synthesis** (Sameena & Thoppil, 2022)

### Green synthesis of nanoparticles using plant extracts

Plant extracts contain phytoconstituents that function as both capping and reducing agents during the process of nanoparticle formation. The selection of plants for the synthesis of nanoparticles has several advantages, chief among them being the abundance and diversity of secondary metabolites that are easily accessible. Large quantities of pollution-free, environment-benign nanoparticles with minimal

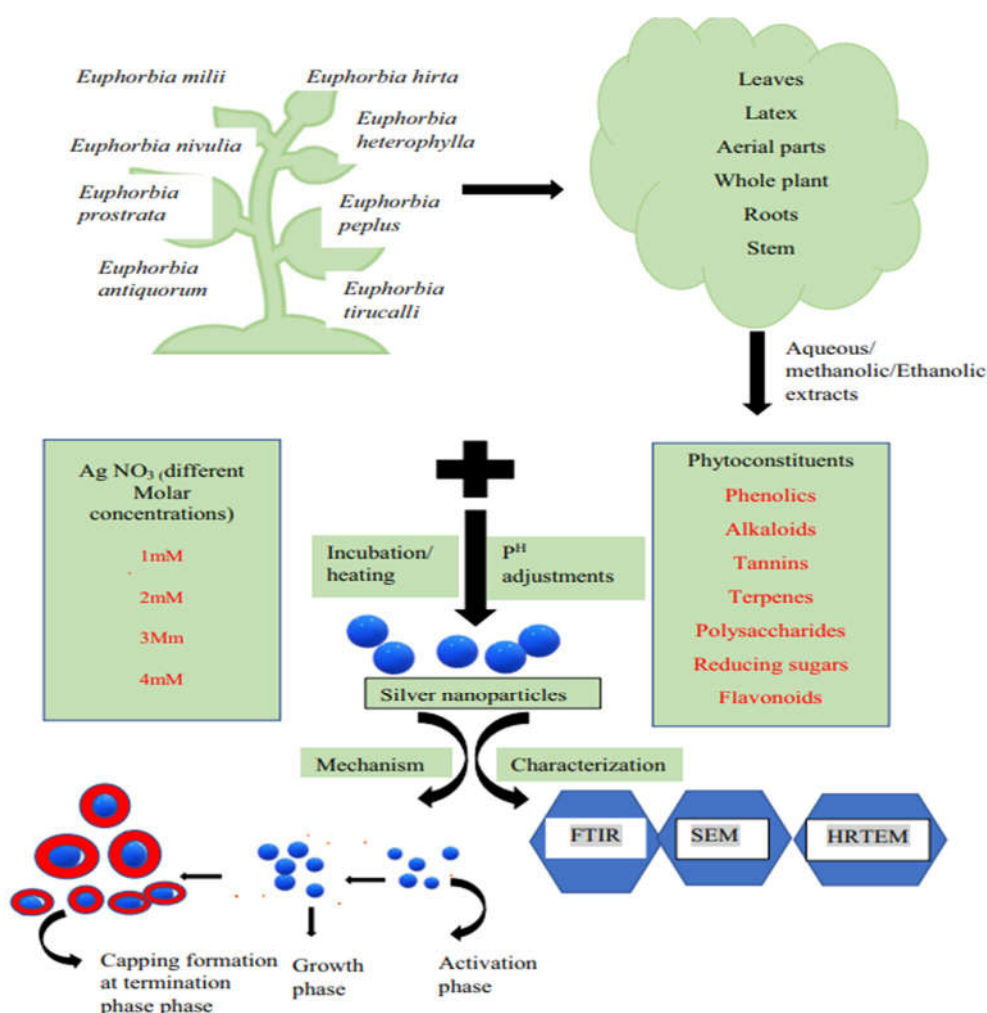
negative effects on the environment are produced by the process of biogenic synthesis. This approach is widely used due to its simplicity, inexpensive, easy-to-use, eco-friendliness and biocompatibility (Arif & Uddin, 2021). Recently, a wide range of plant species and plant parts, including stems, roots, leaves, latex, bark, flowers, and fruits, have been applied for the formulation of nanoparticles for a range of applications (Mustapha et al., 2022).



**Fig. 7: Various sources of green synthesis of nanoparticles** (Sameena & Thoppil, 2022)

Different plant species extracts contain a wide range of primary and secondary phytoconstituents including phenolic acids, flavonoids, alkaloids, quinones, terpenoids, polysaccharides, antioxidants, and organic acids (ascorbic, oxalic, malic, tartaric, and protocatechuic acid). Metal ions undergo reduction to form metallic nanoparticles by all of these compounds. Plant extracts have been used in several studies to synthesise silver nanoparticles (Mustapha et al., 2022). The majority of the plant extract-based nanoparticle production mechanism consists of three phases (**Fig. 8**).

- i) The activation phase, in which phytoconstituents with reducing potential reduce metal ions and further nucleation of reduced metal atoms takes place.
- ii) The growth stage, in which small metal nanoparticles combine to form larger molecules in a variety of shapes, including spheres, triangular shape and rods.
- iii) The third phase, known as the termination phase, during which the nanoparticles assume their final form. The characterization of Ag NPs can be used to understand the structural morphology and stability of synthesised nanomaterial. There are numerous characterization techniques available, such as microscopic and spectral analyses (Makarov et al., 2014).



**Fig. 8: Mechanism of silver nanoparticle formation in genus *Euphorbia*** (Sameena & Thoppil, 2022)

The bioactive constituents found in *Euphorbia* species are widely recognised and exhibit a range of biological impacts. The various functional groups and secondary metabolites, including tannins, flavonoids, phenolic compounds, terpenoids, and saponins, that are present in the genus *Euphorbia* play a significant and potentially beneficial role in the production of Ag NPs by acting as a natural capping and stabilising agent. Because of its many uses, the genus is successfully used by many researchers to synthesise silver nanoparticles. Many research works have documented the importance of silver nanoparticles produced from various *Euphorbia* species, especially when it comes to biological properties like antimicrobial, antioxidant, and anticancer properties. **Table. 4** presents the green synthesis of silver nanoparticles using different species of *Euphorbia*.

Khattak et al. (2019) treated the ethanolic extract with a 1 mM silver nitrate solution in a magnetic stirrer with continuous stirring and sporadic heating (45-50°C) to create spherical-shaped Ag NPs with a size range of 14 to 45.6 nm from *Euphorbia dracunculoides*. Additionally, the examination found the presence of different types of phytochemicals that contributed significantly to the reduction of silver ions and capping of fabricated Ag NPs.

A lot of white, milky latex is produced by Euphorbias. Numerous studies have confirmed the effectiveness of latex in developing nanoparticles. To produce silver nanoparticles, Rajkuberan et al. (2017) for instance, mixed 1 mM Ag NO<sub>3</sub> with 3% aqueous latex extract obtained from *Euphorbia antiquorum* and left the reaction mixture in the dark for a full day. In addition to showing strong anticancer potential against human cervical carcinoma cells, synthesised silver nanoparticles were also found to be effective against blood-sucking parasites that spread disease, such as *Aedes aegypti* and *Culex quinquefasciatus*. These nanoparticles also exhibited larvicidal activity against human pathogens. In addition, Bharathi et al. (2022) produced silver nanoparticles from the same plant's aqueous extracts by treating them with a 0.01 M Ag NO<sub>3</sub> solution and then letting the reaction mixture sit in the dark for 24 hours at room temperature. They observed that the silver



nanoparticles had a remarkable cytotoxic effect on the breast cancer MCF-7 cell line.

The ability of latex-bearing Euphorbiaceae plants, such as *E. milii*, *E. hirta*, *E. franckiana*, *E. ingens*, *P. tithymaloides*, and *E. lacteal*, to produce silver nanoparticles in an environmentally safe manner was also investigated by Patil et al. (2012) using 1 mM Ag NO<sub>3</sub> solution. The production of silver nanoparticles was verified using a variety of characterization techniques, including TEM and zeta potential analysis.

Recent research by Aljubiri et al. (2023) produced nearly spherical-shaped silver nanoparticles with an average particle size of 20 - 34 nm from *E. balsamifera*. They observed that it guarantees water purification and has an effective ability to eliminate dangerous water-based dyes like bromophenol blue (BPB) and bromocresol green (BCG) from water bodies. The morphological parameters of the developed nanoparticles were determined using a variety of characterization techniques, such as Fourier transform infrared spectroscopy (FT-IR), X-ray diffractometry (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). In order to create silver nanoparticles, *E. milii* leaves were extracted using a variety of solvents, including methanol, ethyl acetate, distilled water, acetone, chloroform, and hexane, depending on their polarity (Ramli et al., 2023). For this purpose, each extract was mixed with a 1 mM AgNO<sub>3</sub> solution. They obtained spherical-shaped silver nanoparticles for each extract that was chosen, and SEM images were used to examine them. The presence of nanoparticles was also confirmed using UV-vis spectrophotometer and Fourier-transform infrared spectroscopy (FTIR).

In terms of the synthesis of silver nanoparticles, the plant *E. hirta* was extensively used by the researchers. Various parts of the plant like leaf, root and stem were employed for this purpose. Recently, Ramachandran et al. (2022) created Ag NPs from *E. hirta* and found their immense potential as an anticancer agent against human lung adenocarcinoma A549 cells. Similarly, Kumar et al. (2014) synthesised globe-shaped nanocrystals using *E. hirta* leaf extract. For a thorough

understanding of nanoparticles, a variety of characterization techniques were used, including particle size analysis, field emission scanning electron microscopy (FESEM), UV-visible spectroscopy, and Fourier transform infrared spectroscopy (FTIR). Researchers discovered that derived nano molecules have a remarkable ability to inhibit the growth of several bacteria, including *Salmonella typhi*, *Vibrio cholera*, and *Bacillus subtilis*. In addition, Selvam et al. (2019) explained the cytotoxic potential of silver nanoparticles derived from the ethanolic extract of the aerial parts of *E. hirta*.

### **Antimicrobial activity of silver nanoparticles**

One of the main concerns of the pharmaceutical industry for the past 30 years has been the development of antibiotics that have the ability to significantly inhibit bacterial DNA replication and cell wall synthesis. Even so, high mortality rates continue to occur and rise daily as a result of antibiotic resistance (Crisan et al., 2021). The emergence of disease-causing microbes that are drug-resistant to traditional antibiotics is a problem for public health that is getting worse. A significant amount of research has been done to develop new, effective antimicrobial agents. Due to the sharp increase in antibiotic resistance at this time, there is a lot of interest in studying the therapeutic potential of metallic nanoparticles like silver nanoparticles (Ag NPs). The bactericidal effect of silver (Ag) on microorganisms is particularly notable because it is known to have a toxic effect on a variety of small organisms (Tang & Zheng, 2018).

One of the most popular bioactivities of silver nanoparticles is their antimicrobial activity. The antimicrobial properties of silver are superior to those of all other metals in strength, power, and significance. Fungi, bacteria, viruses, and other eukaryotic micro creatures are among the microbes that it kills. In terms of fungi and bacteria, Ag NPs have demonstrated notable antimicrobial activities. Consequently, silver-based mixtures are frequently utilised in antimicrobial applications. Silver is also used to control bacterial growth in a wide range of biomedical applications, including catheters, the treatment of some dental problems, and burn injuries. Before the invention of silver nanoparticles, silver's salts were

used as a powerful antimicrobial agent. However, the overuse of silver agents has reduced their effectiveness as antimicrobial agents. The growth of nanotechnology in recent years has rekindled interest in the use of silver nanoparticles as antibacterial agents, and there is now more interest in using nanoparticles to treat skin microbial infections. Numerous studies have looked at Ag NPs' antibacterial abilities against multidrug-resistant bacteria and a plethora of reports have shown how effective Ag NPs' antimicrobial properties are.

#### **i) Antibacterial activity**

The quasi-spherical Ag NPs synthesised from an aqueous extract of *E. graminea* showed broad-spectrum antimicrobial activity against bacteria and fungi (Thomas et al., 2023). Muchanyereyi et al. (2017) found that the silver nanoparticles made from the stem extract of *E. confinalis* showed remarkable inhibition of the growth of gram-positive and gram-negative bacterial strains, including *S. aureus* and *E. coli*. The antibacterial activity was assessed using the agar disc diffusion method. In a different study, Arif et al. (2022) created Ag NPs using leaf extract from *E. wallichii*. The study tested the activity of Ag NPs against *Xanthomonas axonopodis* at concentrations ranging from 15.62 µg/ml to 1000 µg/ml for determining the effect of different concentrations on bacterial growth. They noticed that the highest concentration selected exhibited the strongest antibacterial activity.

The biogenic development of silver nanoparticles from *E. serpens* was reported by Ahmed et al. (2022). 1 mM AgNO<sub>3</sub> and 25% aqueous extract were combined and agitated for three hours. The obtained silver nanoparticles have an average particle size of 30 to 80 nm and are spherical in shape. The antibacterial and antifungal properties of the created Ag NPs were also examined. The examined bacteria, including *P. aeruginosa*, *S. typhi*, *S. aureus*, and *E. coli*, showed good inhibition of growth by the Ag NPs. It demonstrated the strongest antibacterial activity against *E. coli* at the maximum concentration of 0.1 M Ag NPs, which also created a noticeable 20 ± 06 mm zone of inhibition. In addition, the Ag NPs demonstrated strong antibacterial properties even at the lowest concentration of 0.0001 M. To find out how well Ag NPs worked against fungi, an agar well diffusion

method was used to measure its antifungal activity. *Candida albicans*, *Fusarium gramineum*, and *Alternaria alternata* are among the fungi that were selected. They observed that the plant extract *E. serpens*-mediated Ag NPs had weak or only moderate antifungal activity.

The development of Ag NPs from *E. maculate* leaf extract through green synthesis was reported by George et al. (2024). A distinct absorbance peak at 420 nm was found by UV-visible spectral analysis of Ag NPs colloidal solution. Furthermore, TEM analysis revealed the spherical nature of Ag NPs. They also noted the antibacterial potential of biofabricated nanoparticles. It was found that, compared to *S. aureus*, Ag NPs showed significantly more antibacterial activity against *E. coli*.

Ag NPs made from the aqueous extract of *E. hirta* were screened for antibacterial efficacy by Bennet Rohan et al. (2020). They produced spherically shaped nanoparticles that ranged in size from 50 to 80 nm and found that the synthesised Ag NPs exhibited good potential for inhibiting the growth of bacteria, including *B. subtilis* and *P. aeruginosa*. Ag NPs with a spherical form and a size range of 50 - 75 nm was created using an aqueous leaf extract of *E. rigida*. Using DLS and SEM analysis, the morphology and surface details of the nanoparticles were evaluated. The evaluation of Ag NPs' antimicrobial activity revealed that they had strong antimicrobial properties against common human pathogens, including *E. coli*, *S. aureus*, *S. faecium*, *S. epidermidis*, *S. enteritidis*, *S. typhimurium*, *L. monocytogenes*, and *C. albicans* (Kocazorbaz et al., 2021).

Ag NPs made from *E. umbellatum* leaf extract demonstrated strong antimicrobial activity. *S. aureus* and *K. pneumoniae* were found to be effectively inhibited by the nanoparticle due to their greatest microbiological properties (Rosa et al., 2022). In a similar way, Nirmala et al. (2023) observed that Ag NPs exhibited a more potent effect on *B. subtilis*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *E. coli* growth inhibition than did the plant extract of *E. helioscopia*. The presence of elemental silver was shown by a sharp peak in the UV-visible spectrum at 442 nm. With the use of an FTIR spectrum, several functional groups from the plant extract

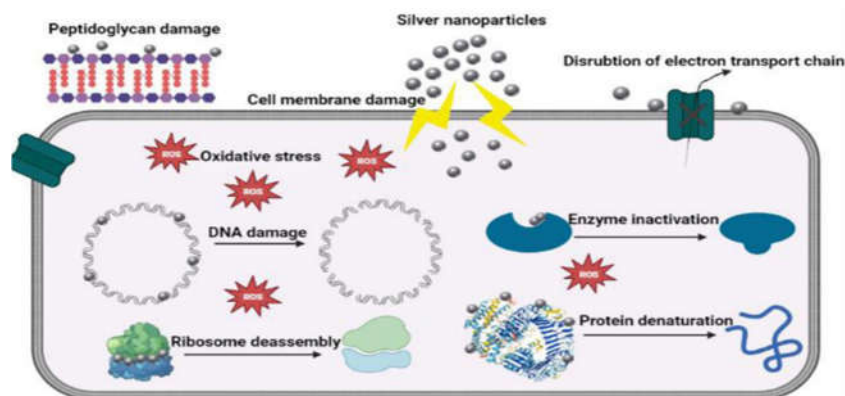
that were significantly involved in the creation and activation of the Ag NPs were evaluated.

## ii) Antifungal activity

The fungicidal potential of *Euphorbia* sp. has been documented in fewer reports. The production of silver nanoparticles (Ag NPs) from the extract of the medicinal plant *E. prostata* has been documented by Dzoyem et al. (2022). A distinct peak from the spectral analysis that fell between 402 and 493 nm in wavelength indicated the formation of nanoparticles. After testing synthesised Ag NPs against two strains of *Candida albicans* and *Candida parapsilosis*, they discovered a noteworthy anticandidal property. Ag NPs were produced using the stem extract of *E. poissonii*, a medicinal plant, with an average particle size of 10.25 nm. These Ag NPs showed evidence of antifungal activity against *Candida albicans*, with a discernible zone of inhibition (Owoseni et al., 2023). According to Elumalai et al. (2010) Ag NPs derived from *E. hirta* showed a strong antifungal activity. Nanoparticles inhibited the growth of *C. albicans* with a zone of inhibition of  $16.25 \pm 0.03$ , followed by *C. Kefyr* ( $15.00 \pm 0.02$ ) and *C. tropicalis* ( $12.01 \pm 0.21$ ) respectively.

Ag NPs' explicit antimicrobial activity has not yet been fully explained, but numerous studies have suggested that this is because of their large surface area, which allows them to release sufficient amounts of Ag<sup>+</sup> ions more quickly and make good contact with microorganisms by adhering to their cell membranes and penetrating them. It disrupts the bacterial or fungal envelope once it has entered the bacterial cells. Additionally, these Ag<sup>+</sup> ions bind to DNA and protein inside of a cell, increasing the production of ROS. This denaturing of the organelles and cell membrane ultimately leads to cell lysis (Mukherjee et al., 2023; Dakal et al., 2016; Yin et al., 2020). According to some scientists, the nanoparticles enter bacteria or fungi and interact with their DNA and proteins, harming them. Reactive oxygen species (ROS) are created as a result, which increases oxidative stress. ROS buildup in bacterial mitochondria results in dysfunctional mitochondria, which in turn

prevents bacterial growth (Al-Otibi et al., 2020). The possible antibacterial action exhibited by the Ag NPs against various bacterial species was illustrated in **Fig. 9**.



**Fig. 9:** Antibacterial mechanism of AgNPs against bacterial cells (Aljeldah et al., 2023)

**Table 4.** Reports on the synthesis of silver nanoparticles by utilizing various species of *Euphorbia* (Sameena & Thoppil, 2022)

| Species                    | Plant part      | Size (nm)     | Shape             | Application/ Activity        |
|----------------------------|-----------------|---------------|-------------------|------------------------------|
| <i>Euphorbia wallichii</i> | Rhizome         | 20 - 58       | -                 | Antileishmanial<br>Analgesic |
| <i>E. thymifolia</i>       | Whole plant     | -             | -                 | Toxicological                |
| <i>E. prostrata</i>        | Leaves          | 12.82 - 2.50  | Spherical         | Antileishmanial              |
| <i>E. geniculata</i>       | Aerial parts    | 13 - 45       | Spherical         | Antimicrobial                |
| <i>E. hirta</i>            | Leaves          | 40 - 50       | Spherical         | Antifungal                   |
| <i>E. hirta</i>            | Aqueous extract | 50 - 80       | -                 | Antibacterial                |
| <i>E. hirta</i>            | Leaves          | 40-50         | Polydisperse      | Antibacterial                |
| <i>E. milii</i>            | Latex           | 10 - 50       | -                 | -                            |
| <i>E. tirucalli</i>        | Latex           | 20 - 30       | Spherical & cubic | Nematicidal                  |
| <i>E. milii</i>            | Stem            | 6 - 50        | Spherical         | Antimicrobial                |
| <i>E. helioscopia</i>      | Leaves          | 2 - 14        | Spherical         | Optical & catalytic          |
| <i>E. peplus</i>           | Dried leaves    | 5 - 10        | Spherical         | Catalytic                    |
| <i>E. antiquorum</i>       | Latex           | 10 - 50       | Spherical         | Anticancer                   |
| <i>E. confinalis</i>       | Stem            | 12 - 18       | Spherical         | Antibacterial                |
| <i>E. pseudocactus</i>     | Fresh leaves    | 5.48 - 60     | Spherical         | Antibacterial                |
| <i>E. rothiana</i>         | Leaves          | 18.16 - 27.78 | Spherical         | Antimicrobial                |
| <i>E. milii</i>            | Latex           | -             | Round             | Antimicrobial                |
| <i>E. lactea</i>           | Latex           | -             | Round             | Antimicrobial                |

|                          |              |             |                   |                                           |
|--------------------------|--------------|-------------|-------------------|-------------------------------------------|
| <i>E. ingens</i>         | Latex        | -           | Round             | Antimicrobial                             |
| <i>E. hirta</i>          | Latex        | -           | Round             | Antimicrobial                             |
| <i>E. franckiana</i>     | Latex        | -           | Round             | Antimicrobial                             |
| <i>E. heterophylla</i>   | Dried leaves | -           | Spherical         | Catalytic                                 |
| <i>E. nivulia</i>        | Stem latex   | 10 - 20     | Spherical         | Antimicrobial                             |
| <i>E. amygdaloides</i>   | Whole plant  | 7 - 20      | Spherical         | Antibacterial                             |
| <i>E. hirta</i>          | Fresh leaves | 30-60       | Spherical         | -                                         |
| <i>E. milii</i>          | Leaves       | 20 - 50     | Spherical         | Antibacterial                             |
| <i>E. prostrata</i>      | Fresh leaves | 25 - 80     | Rod               | Antiparasitic                             |
| <i>E. cyathophora</i>    | Leaves       | 30 - 50     | Spherical         | Anticancer                                |
| <i>E. hirta</i>          | Fresh leaves | 30 - 60     | Spherical & Cubic | Antiplasmodial                            |
| <i>E. hirta</i>          | Fresh leaves | 2.9 - 206.3 | Spherical         | Anticancer                                |
| <i>E. dracunculoides</i> | Whole plant  | 14.0 - 45.6 | Spherical         | Phytotoxic<br>Analgesic<br>Cytotoxic      |
| <i>E. wallichii</i>      | Roots        | -           | -                 | Antioxidant<br>Cytotoxic<br>Antimicrobial |
| <i>E. fusiformis</i>     | Leaves       | -           | Spherical         | Antibacterial                             |
| <i>E. hirta</i>          | Leaves       | 30 - 60     | Spherical & cubic | Larvicidal                                |
| <i>E. nivulia</i>        | Stem bark    | 20-90       | Spherical         | Antibacterial                             |
| <i>E. umbellata</i>      | Leaves       | -           | Spherical         | Antibacterial                             |
| <i>E. hirta</i>          | Leaves       | 13-61       | Spherical         | Antibacterial                             |

## MATERIALS AND METHODS

The current study was finished in three parts. The first part discusses the phytometabolites that were revealed in the methanolic extracts of the aerial parts of *Euphorbia rothiana*, *Euphorbia deccanensis*, and *Euphorbia thymifolia*, respectively. The bioactivities of plant extracts, such as their capacity to scavenge free radicals, reduce inflammation, cytotoxicity assay using *Allium cepa*, and anticancer properties, are covered in the second part. The part three primarily examined the green synthesis of silver nanoparticles using methanolic plant extracts and antimicrobial activity exerted by the developed nanoparticles.

### PLANT SPECIMENS

*Euphorbia deccanensis* V. S. Raju, *Euphorbia rothiana* Spreng., and *Euphorbia thymifolia* L. are the plant species chosen for this investigation (**Plate 1**). Among these, *E. deccanensis* is native to South India (Kerala), while *E. rothiana* is found in the regions, including Bangladesh, Sri Lanka, Europe, Africa, and Pakistan. On the other hand, *E. thymifolia* is found throughout tropical Asia. *E. thymifolia* was gathered from Calicut University Botanical Garden, *E. deccanensis* from Madayippara in Kannur district, and *E. rothiana* from Kundhakod manth, Nanjanad in Ooty, Tamil Nadu. Dr. A. K. Pradeep and Dr.V. Pramod, Assistant Professors in the Department of Botany, University of Calicut identified the plants. The voucher specimens were placed in the herbarium at Calicut University (CALI).

- **Botanical Name:** *Euphorbia deccanensis* V. S. Raju (CALI N0. 7129)
- **Common name:** Mulachurathi
- **Synonym:** *Euphorbia katrajensis* var. *kasaragodensis*, *Euphorbia liniarifolia* Roth.
- **Habit:** Herb
- **Habitat:** Lateritic rocky areas



- **Flowering & fruiting:** July-December
- **Key identification features:** An annual herb with a pinkish cyathium
- **Endemism:** Deccan Peninsula
- **Plant description:** It is a small, glabrous, prostrate or decumbent herb. The leaves are glabrous, simple, opposite, linear, 1 - 2 x 0.3 - 0.5 cm, with entire margins, an oblique base, an acuminate apex, and a petiole that is 1- 2 cm long. The cyathium is axillary, with a green, cupular involucre tube with pinkish white lobes. Style short, 3-lobed; ovary 3-celled. Fruits 3 mm across and pyrenes 3. It grows most commonly in the seasonally dry tropical biome.

**2. Botanical Name:** *Euphorbia rothiana* Spreng. (CALI N0. 7118)

**Common name:** Merasupalchedi

**Synonym:** *Euphorbia laeta* Heyne ex Roth

**Habit:** Undershrub

**Habitat:** Margins of green forests and grasslands

**Flowering & fruiting:** September – April

**Key features:** Milky latex, inflorescence unique (Cyathium). Umbrella-like whorls of branches.

**Native:** India and Sri Lanka

**Plant description:**

It is a perennial branching shrub that can reach a height of one metre and is frequently found as a hill spurge. Its smooth, leafy stem is frequently tinged with red. An umbrella-like whorl of thin branches emerges from the top of the stem. Sessile and 5–10 cm long, the leaves are arranged alternately in a linear–oblong, typically lance-like pattern. The flower cyathium is  $2.5 \times 3$  cm across, with male



**Plate 1:** Habit of the plants. **a.** *Euphorbia deccanensis* V. S. Raju; **b.** *Euphorbia rothiana* Spreng.; **c.** *Euphorbia thymifolia* L.

florets encircling the female floret. The flowers are green. White milky latex is present throughout the plant.

**3. Botanical Name:** *Euphorbia thymifolia* L. (CALI N0. 7357)

**Common name:** Duddhi, Dudhiya, Choti-dudhi

**Synonym:** *Chamaesyce thymifolia* (L.) Millsp.

**Habit:** Herb

**Habitat:** Scrub forest and plains

**Flowering & fruiting:** September - November

**Key features:** An annual, stoloniferous, delicate herb. Inflorescences are clustered in leaf axils.

**World distribution:** Tropical Asia

**Plant description:**

It is a small prostrate annual or perennial herb that typically grows along the ground. Its stem is slender, smooth, thin, cylindrical, hairy, and mat-forming. Typically, the stem's colour is pink when it's fresh and turns greyish-green or dark purple when it dries. The stem can grow up to 10–20 cm in length and 1-3 mm in diameter. The leaves are elliptic or oblong, 4 mm long, 2-5 mm wide, with a rounded and oblique base at the apex and a simple, opposite phyllotaxy arrangement. Stipules are linear-subulate, whole or slightly parted, and distinct or slightly connate at the base with a length of 0.9–1.2 mm. Petiole 0.5-1 mm, sparsely strigose-tomentulose; blade broadly elliptic to narrowly oblong ovate-lanceolate, 3-10 × 1.8-5 mm, base asymmetric, one side typically angled or round, with the other truncate and expanded into a small, rounded auricle; margins serrate (larger leaves) to serrulate (smaller leaves); apex blunt to acute; abaxial surface sparsely tomentulose to glabrate, adaxial surface glabrate, palmately veined at the base. Cyathia are typically found on crowded, axillary branches in small, cymose clusters; the peduncle is 0.1 to 0.3 mm. The involucre is broadly obconic, but the base of partially included

capsules causes it to swell and distort. Flower styles range from conic to truncate-ovoid, and the ovary of pistillate and staminate flowers is densely strigose, with styles of 0.4 to 0.5 mm. Seeds are narrowly ovoid to white, with a sharp 4-angle cross- section.

## **METHODOLOGY**

### **PART I- PHYTOCHEMICAL PROFILING**

Aerial parts of the plants were collected, shade-dried and powdered into fine particles by using a grinder. The preliminary analysis was carried out in each plant by using three different solvents, such as distilled water, methanol, and n-hexane. For this purpose, 3 g of powdered plant samples were soaked overnight the complete dissolution in the corresponding solvents of 30 ml. The solutions were filtered using a Whatman filter paper No.1, and the obtained filtrates were used for further preliminary qualitative analysis.

#### **a) Qualitative phytochemical analysis**

Three different extracts of each plant were subjected to preliminary qualitative analysis using conventional chemical tests to determine the presence and absence of various phytometabolites (Harborne, 1998; Edeoga et al., 2005; Shanmugam et al., 2019).

#### **i) Test for phenolic compounds (Harborne, 1998; Kumar et al., 2007)**

- **Ferric chloride Test**

1 ml of the extract was mixed with 5% ferric chloride. It is checked for the presence of a deep blue colour.

- **Spot Test**

On a piece of clean filter paper, 1 ml of extract was spotted. A single drop of the phosphomolybdic acid reagent was then added to the spot, and it was then

exposed to ammonia vapors. The spot's blue colour formation indicates the presence of phenols.

ii) **Test for flavonoids (Harbourne, 1998)**

- **Alkaline reagent Test**

1 ml of the extract was mixed with a few drops of the 20% sodium hydroxide solution. When a few drops of diluted hydrochloric acid were added, the intensified yellow colour began to fade, which is a sign that flavonoids are present.

- **Lead acetate Test**

A scanty amount of 10% lead acetate solution was added to 1 ml of the extract. The development of a yellow-coloured precipitate indicates the occurrence of flavonoids.

iii) **Tests for alkaloids (Shanmugam et al., 2019)**

The three different extracts of each plant of the *Euphorbia* species were dissolved in dilute HCl, and the obtained filtrate was used to identify the presence of alkaloids.

- **Wagner's Test**

The addition of a few drops of Wagner's reagent (**Appendix 1**) to the test tube containing 1 ml of filtrate results in the formation of a reddish-brown precipitate, indicating the presence of alkaloids.

- **Hager's Test**

To 1 ml of the extract, a few drops of Hager's reagent (Saturated Picric Acid Solution) were added. The presence of alkaloids is indicated by the appearance of orange-yellow-coloured precipitates.

**iv) Tests for tannins (Evans & Evans, 2002)**

- **Breymer's test**

2 ml of the extract was treated with 10% alcoholic ferric chloride solution. The formation of blue or greenish colour reveals the presence of tannins.

- **Lead acetate Test**

A few drops of the 1% lead acetate solution were added to 5 ml of the test solution. The presence of tannins is indicated by precipitation that is yellow in colour.

**v) Test for coumarins**

- **Ferric chloride test**

1 ml of each extract was treated with 1 ml of ferric chloride solution. The production of a dark yellow colour indicates the existence of coumarins.

**vi) Test for anthraquinones (Kumar et al., 2007)**

- **Bontrager's Test**

1 ml of a 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid were added to 2 ml of the extract, which was then heated. After that, the test solution was cooled and filtered. A similar amount of diethyl ether was then added to the filtrate. This solution was then extorted with strong ammonia. The presence of anthraquinones is indicated by the development of pink or deep red colouration in the solution.

**vii) Test for terpenoids (Shanmugam et al., 2019)**

- **Salkowski Test**

About 2 ml of extract was subjected to treatment with 2 ml of chloroform and filtered. The obtained filtrate was shaken with a few drops of concentrated sulphuric acid to keep it undisturbed. A yellow colour with green fluorescence indicates the presence of terpenoids.

**viii) Test for saponins**

- **Foam Test (Adegoke et al., 2010)**

In a test tube, approximately 2 ml of extract is shaken vigorously with 2 ml of distilled water. The emergence of a long-lasting foam indicates the presence of saponins.

**ix) Test for phlobatannins (Edeoga et al., 2005)**

- **Precipitation test**

The mixture containing 2 ml of extract and 2 ml of hydrochloric acid (1%) was boiled. The formation of a red coloured precipitate is taken as a shred of evidence for the occurrence of phlobatannins.

**x) Test for glycosides**

- **Legal's test**

Pyridine is blended with 2 ml of the sample solution. A few drops of sodium nitroprusside and 10% sodium hydroxide were added to this mixture. The presence of glycosides is indicated by the deep red colour.

**xi) Test for phytosterols**

- **Liebermann-Burchard test (Siddiqui & Ali, 1997)**

After being treated with 2 ml of chloroform, the extract was filtered. Acetic anhydride (2 ml) and concentrated sulfuric acid (2 ml) were added to 2 ml of filtrate. A shift from violet to blue in colour indicates the presence of phytosterols.

- **Sulphuric acid test**

A few drops of concentrated sulphuric acid were added to the mixture by the test tube sides after 2 ml of the extract and 2 ml of chloroform were combined. An indication of phytosterols is a red upper layer with a yellow-green fluorescence in the sulphuric acid layer.

## **xii) Test for triterpenoids**

### **• Chloroform test**

The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. Concentrated sulphuric acid (1 ml) was added to the solution. The formation of reddish-violet colour indicates the presence of triterpenoids.

## **b) Quantitative estimation of phytochemicals**

### **❖ Extraction**

The qualitative preliminary assays revealed the distribution of a greater number of phytoconstituents in the methanolic extract. Therefore, the methanolic extract of selected plant species was used for Soxhlet extraction procedures and further analysis. Hence, about 20 g of the powder was extracted with 200 ml methanol using the Soxhlet apparatus. Extraction was done for 6-7 h of 7 extraction cycles. The crude methanolic extract was allowed to cool and then filtered by using Whatman No.1 filter paper. The crude extract was concentrated by evaporating the complete solvent by placing it in a water bath. It was then stored in an amber-coloured bottle and kept at 4°C for further analysis.

Quantification of major phytochemicals like terpenoids, phenolics, and flavonoids, which were analysed in the preliminary qualitative studies, was performed as per the standard protocols.

### **i) Total terpenoid estimation**

The total terpenoid content of selected *Euphorbia* species was examined by the protocol of Ghorai et al. (2012) with slight modifications. Here, the linalool was the standard for estimation. About 0.2 ml of the sample is derived from a 1 mg/ml stock solution. Next, 1.5 ml of chloroform and 1 ml of concentrated sulphuric acid were added to this sample, as well as various standard concentrations. The chloroform layer was visible as a distinct layer and was a yellow-green coloured solution. 2 ml of methanol was added to the mixture after the layer of chloroform



had been removed. There was no longer any trace of the yellow-green solution and the absorbance was calculated at 538 nm against blank i.e., 95% (v/v) methanol. The calibration curve of linalool was plotted as 20, 40, 60, 80, and 100 µg/ml and a regression equation were obtained. The total terpenoid content of the extract was expressed as milligram linalool equivalent per gram dry weight (mg LE/g DW). Samples were scrutinized in triplicate.

## **ii) Total flavonoid estimation**

The total flavonoid content of the samples was screened by using the colorimetric method suggested by Oueslati et al. (2012) with slight variations. Quercetin was used as the standard for the procedures of estimation. About 0.5 ml of extracts (1mg/ml) and standard solutions of quercetin having different concentrations, such as 20, 40, 60, 80, and 100 µg/ml, were added to a mixture containing 0.5 ml of distilled water and 0.3 ml of sodium nitrate (7%) solution. Incubate the solution for 5 minutes at 25<sup>0</sup> C, then add 0.3 ml of (10%) AlCl<sub>3</sub> solution (w/v) immediately. Into this, add 2 ml of 1M NaOH solution after shaking the reaction mixture properly. The absorbance was read at 510 nm. The calibration curve of quercetin was plotted, calculated the total flavonoid content of the extract, and expressed as milligram quercetin equivalent per gram of dry weight (mg QE/g DW) by using a regression equation. Each sample was estimated in triplicate.

## **iii) Total phenolic estimation**

The total phenolic content was determined by using the Folin-Ciocalteu assay (Ouesleti et al., 2012). The standard curve was drawn by taking the different concentrations of standard gallic acid such as 20, 40, 60, 80, and 100 µg/ml. From the stock solution, 0.1 ml of the sample was taken. Then add 0.5 ml of distilled water followed by 125 µl of Folin-Ciocalteu reagent (1N). The mixture is shaken thoroughly and kept undisturbed for about 5-6 minutes. After that, add 1.25 ml of sodium carbonate. The final volume was adjusted to 3 ml with the addition of distilled water. Incubate the mixture at room temperature in the dark for 90 minutes after proper mixing. The absorbance was noted at 760 nm against a blank using a spectrophotometer (Elico SL 218, India). A regression equation was created by

plotting the calibration curve of gallic acid. Using the equation, the total phenolic content was determined and expressed as milligrams of gallic acid equivalent per gram of dry weight. All the samples were analyzed in triplicate.

#### **c) High Resolution Liquid Chromatography - Mass Spectrometry (HR LC-MS)**

Non-volatile components are identified and separated using Orbitrap; Biopharma Q-Exactive Plus-High Resolution LCMS was used for this purpose. There was a direct infusion mass with both positive and negative mode ionisation (APCI) and ESI. It could reach up to 280,000 resolutions. 12 Hz was the highest scan rate. An ion source known as an RF-lens was used to increase sensitivity. Intelligent ion beam management using advanced active beam guides was used for high-flux ion sources. It had a mass range of 50-8000 amu (m/z) and a resolution of 1,40,000 @ m/z 200.

#### **d) Gas Chromatography-Mass Spectrometry (GC-MS) analysis**

With an Agilent Model 8890 GC system fitted with a single quadrupole mass spectrometer (5977B MSD) analyser, the extract's volatile components were identified and separated. The temperature of the GC oven was approximately 450°C. Retention time repeatability was less than 0.008 %, and area reproducibility was less than half of the chromatographic performance. The inlet split ratio was 7500:1. Accurate mass measurement: 100 pg/μl OFN injected in 1 μl; monoisotope will be obtained at m/z 271 using standard scanning beginning at 50.350 u.

### **PART II – BIOACTIVITY SCREENING**

#### **A) Free radical scavenging activity of selected species of *Euphorbia***

Oxidative stress is brought on by an imbalance between oxidants and antioxidants. Five separate *in-vitro* experiments were carried out to evaluate the effectiveness of plant extracts towards scavenging free radicals. The experiments are all carried out in triplicate.

### **Extract stock preparation**

The antioxidant potential of the methanolic extracts of selected species was evaluated. For all assays except the DPPH radical scavenging assay, different concentrations of 125, 250, 500, 1000, and 2000 µg/ml were prepared from a stock solution of 10 mg/ml. The concentrations used in the DPPH method were 12.5, 25, 50, 100, and 200 µg/ml.

#### **i) DPPH radical scavenging activity**

The DPPH scavenging potential of plant extracts was determined on the method described by Chang et al (2001). DPPH (1,1-diphenyl-2-picryl hydrazyl) is a pink-colored stable free radical. It becomes yellow when scavenged by antioxidants. The antioxidants present in the plant extract react with the DPPH radical and reduce it into DPPH-H by donating hydrogen. The degree of discolouration of DPPH indicates the scavenging potential of antioxidants, and it can be read spectrophotometrically.

4 mg of DPPH was dissolved in 100 ml of methanol to make a 0.1 mM DPPH solution. Ascorbic acid (10 mg/ml DMSO) served as a control. Various concentrations of samples (12.5, 25, 50, 100, and 200 µg/ml) and standards were made. As a control, distilled water without the test compound was used. They were made up to 20 µl by adding 1.48 ml of DPPH (0.1 mM) solution and DMSO. After 20 minutes of incubation in the dark at room temperature, the absorbance at 517 nm was measured using 3ml of DPPH as a blank.

The percentage of inhibition was calculated by using the equation given below:

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

Triplicate of each extract were tested, and the mean value was calculated.

#### **ii) Hydroxyl radical scavenging activity**

This test evaluated the plant extract's ability to scavenge the hydroxyl radical produced by the  $\text{Fe}^{3+}$ - ascorbate – EDTA -  $\text{H}_2\text{O}_2$  system (the Fenton reaction)

(Elizabeth & Rao, 1990). Different concentrations of plant extract and standard (125, 250, 500, 1000 and 2000 µg/ml) were added to the reaction mixture, which is made up of 2 deoxy-2-ribose (2.8 mM), Fe Cl<sub>3</sub> (100 mM), EDTA (100 mM), H<sub>2</sub>O<sub>2</sub> (1.0 mM), and ascorbic acid (100 mM), in a buffer solution of KH<sub>2</sub>PO<sub>4</sub>-KOH (20 mM pH 7.4). The mixture is then put into a final volume of 1 ml. Gallic acid was taken as the reference standard, along with distilled water without extract as a control. The entire reaction mixture was incubated at 37°C for 1 hour before adding 1 ml of 2.8% trichloro acetic acid (TCA) and 1 ml of 1% aqueous thiobarbituric acid (TBA) and incubating at 90°C for 15 minutes. After cooling, the absorbance at 532 nm was measured against a suitable blank solution. The potential of plant extracts to inhibit hydroxyl radical was expressed as inhibition percentage, and using triplicate of each test sample, the mean values were calculated by using the following formula given below.

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

### **iii) Superoxide-free radical scavenging activity**

Injurious physiological circumstances are caused by an increase in the production of the physiologically significant superoxide radical, which can be scavenged by utilising plant extracts. The reduction of nitroblue tetrazolium (NBT) during the production of superoxide radicals in the riboflavin NADH system provides the basis for this test. Ascorbic acid was used as a reference. The reaction mixture consists of different concentrations of extract and standard such as 125, 250, 500, 1000 and 2000 µg/ml and 0.2 ml of EDTA solution [0.1M], 0.05 ml of Riboflavin solution (0.12 mM) and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5mM] diluted up to 2.64 ml with phosphate buffer [0.067M]. A control with all reagents except extract but with an equal amount of distilled water was also maintained [Valentao et al, 2002]. At 560 nm, the absorbance was measured following 5 and 30 minutes of illumination with fluorescent light. The OD difference was calculated. The ability to scavenge harmful superoxide free radical scavenging potential of selected plant species was expressed in inhibition percentage and can be determined using the following equation,

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

#### **iv) Nitric oxide radical scavenging activity**

The method is based on the idea that sodium nitroprusside in aqueous solution at physiological pH spontaneously produces nitric oxide, which reacts with oxygen to form nitrite ions that can be measured with the Griess reagent (**Appendix 2**). The ability of the compounds to compete with oxygen in order to decrease the amount of nitrite ions is the basis for their ability to quench the nitric oxide radical. Nitric oxide levels above a certain point cause significant tissue damage.

A slightly modified version of Kumaran & Karunakaran's method (2006) was used to test the plant extract's ability to scavenge nitric oxide. Sodium nitroprusside (5 mmol/l) in phosphate buffer (PBS) (**Appendix 3**) was combined with various concentrations of the sample (125 – 2000 µg/ml) and incubated at 25°C for 30 min. 30 min of incubation resulted in the removal of 1.5 ml of the solution, which was then diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). At 546 nm, absorbance was measured against a blank. Here, gallic acid was used as positive control. The ability to scavenge nitric oxide radicals was calculated as a percentage of inhibition using the equation below.

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

#### **V) 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity**

To evaluate the antioxidant potential of the plant extract, the ABTS cation decolorization assay was conducted using standard techniques. Various concentrations of the sample were prepared by mixing 0.2 ml of different sample concentrations, ranging from 125 to 2000 µg/ml, obtained from a stock concentration of 10 mg/ml. The resulting solution was then adjusted to a final

volume of 1.36 ml by adding 0.16 ml of ABTS solution. After an incubation period of 20 min, the absorbance of the reaction mixture was measured at a wavelength of 734 nm. Here, ascorbic acid served as a positive control. An equal volume of distilled water was used as the control instead of the test compound. This measurement resulted in the determination of the antioxidant activity of the plant extract based on its capacity to decolorize the ABTS cation. The potential of plant extracts to quench the ABTS radicals was expressed as a percentage of inhibition, which was derived from using the following equation.

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

## **B) Anti-inflammatory activity**

The ability of selected species to reduce inflammation was primarily evaluated using various biochemical techniques. The most effective one from there was selected to see how it affected the inflammatory mediators in LPS-stimulated RAW 264.7 macrophage cell lines. The indirect ELISA was then carried out in order to determine the precise quantity of the major inflammatory mediator that was involved in the inflammation process.

### **i) Evaluation of anti-inflammatory potential using biochemical assays**

Initially, *in vitro* biochemical assays were used to assess the anti-inflammatory potential of the methanolic plant extracts of three chosen plant species. After determining the inhibition percentage, the most potent plant extract was selected for additional research.

#### **a) Inhibition of protein denaturation**

Mizushima and Kobayashi's (1968) methodology was used to analyse the plant extracts' capacity to prevent protein denaturation. For the study, a stock solution containing 10 mg/ml was used to create samples with varying concentrations, ranging from 62.5 µg/ml to 500 µg/ml. The test control is made up of 0.05 ml of distilled water and bovine serum albumin. The test solution is made up

of 0.45 ml of bovine serum albumin and various concentrations of the sample. The product control contains 0.45 ml of distilled water and various concentrations of the sample. The standard solution consisted of diclofenac sodium. 1N HCl was used to bring all of the aforementioned solutions up to pH 6.3. After 20 minutes of incubation at 37°C, the samples were heated to 57°C for three minutes. 2.5 ml of phosphate buffer was added to the solutions once they had cooled. The UV-Visible Spectrophotometer was used to measure the absorbance at 416 nm. The percentage inhibition of protein denaturation can be calculated as

$$\% \text{ of inhibition} = 100 - \frac{(\text{OD of test solution} - \text{OD of product control})}{\text{OD of test control}} \times 100$$

**b) Inhibition of proteinase activity**

A modified version of Oyedepo and Femurewa's (1995) methodology was used to assess which species' methanolic extracts had the greatest potential to inhibit proteinase activity. For this, different concentrations of plant extracts, including 62.5, 125, 250, and 500 µg/ml, were derived from a stock solution of 10 mg/ml DMSO. The standard was diclofenac sodium. Using the phosphate buffer, 1 ml of different concentrations of plant samples was prepared. After that, add 1 ml of Tris-HCl buffer (20 Mm, pH 7.4) (**Appendix 4**) containing 0.06 mg trypsin to each sample and incubate for 5 minutes. It was then followed by the addition of 1 ml (0.8%) casein, after which the reaction mixture was incubated for another 20 minutes. A 2 ml dose of perchloric acid is added to each test tube to end the reaction. After getting a cloudy solution, the mixture was centrifuged at 3000 rpm for 10 minutes. A UV-Visible spectrophotometer was used to measure the absorbance at 200 nm against a blank sample of buffer. The following formula was used to calculate the percentage of proteinase inhibitory activity.

$$\% \text{ of inhibition} = 100 - \frac{(\text{OD of test solution} - \text{OD of product control})}{\text{OD of test control}} \times 100$$

**ii) Anti-inflammatory activity on the RAW 264.7 macrophage cell line**

The powerful extract's anti-inflammatory properties were further examined using the RAW 264.7 macrophage cell line. It was also determined how the

methanolic extract of *E. deccanensis* affected the levels of cellular nitrite (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX), and lipoxygenase (LOX).

- **Cell culture**

RAW 264.7 macrophage cells initially procured from the National Centre for Cell Sciences (NCCS), Pune, India, were used to assess the anti-inflammatory activity of the plant extract. To cultivate the cells, Dulbecco's modified Eagle's medium (DMEM) (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate, and antibiotics (penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (2.5 µg/ml) was employed. The cells were incubated at 37°C with 5% CO<sub>2</sub> humidity (NBS Eppendorf, Germany).

Using a cyclomixer, dissolve 1 mg of the plant extract in 1 ml of DMEM to create a stock of the extract. Further experiments were conducted using the filtered solution. The cells were treated with lipopolysaccharide (LPS: 1µg/ml) if they had reached 60% confluency. Then, the LPS-treated RAW cells were treated with different concentrations of the plant extract, followed by a 24-hour incubation period. Following incubation, cell lysates were used to conduct anti-inflammatory assays. The positive control consisted of various concentrations of diclofenac sodium, a common anti-inflammatory medication, which corresponds to the concentration of the sample solution.

**a) Cyclooxygenase (Cox) activity**

Walker and Gierse's (2010) methodology was used to measure the impact of the plant extract on COX activity. To initiate the reaction, 100 µl of cell lysate was combined with Tris-HCl buffer (pH 8), glutathione (5 mM/l), and haemoglobin (5 mM/l) at a temperature of 25°C, followed by incubation for one minute. The reaction was initiated by adding arachidonic acid (200 mM/l). After 20 min, the reaction was stopped by adding 200 µl of a solution containing 10% trichloroacetic acid in 1 N hydrochloric acid. The tubes were then centrifuged, and the resulting



supernatant was mixed with 200 µl of 1% thiobarbituric acid solution. The tubes were subsequently boiled for 20 min, and the absorbance was measured at 632 nm to analyse the reaction. In comparison to the positive control diclofenac sodium, the plant extract's cyclooxygenase activity was measured. The percentage inhibition of COX was calculated using the formula,

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

here, Ac shows the absorbance of the control, and As shows the absorbance of the sample. After analysing the sample in triplicate, the mean value was calculated.

#### **b) Lipoxygenase (Lox) activity**

With the aid of the positive control diclofenac sodium, the method outlined by Axelrod et al. (1981) was used to measure Lipoxygenase (LOX) activity. The reaction mixture, in summary, contained 200 µl of sodium linoleate, 50 µl of cell lysate, and Tris-HCl buffer (pH 7.4). A rise in absorbance at 234 nm, a sign of the formation of 5-hydroxyeicosatetraenoic acid, was used to measure the LOX activity. The percentage inhibition of the enzyme was calculated using the formula,

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

here, Ac shows the absorbance of the control, and As shows the absorbance of the sample. After taking into account the sample's values in triplicate, the mean values were calculated.

#### **c) Inducible nitric oxide synthase activity**

The method described by Salter et al. (1996) was employed to measure nitric oxide synthase (iNOS) activity. To start, the cell lysate was mixed thoroughly with 2 ml of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Appendix 5). The reaction mixture consisted of L-arginine (0.1 ml, 2 µmol/l), manganese chloride (0.1 ml, 4 µmol/l), tetrahydropterin (0.1 ml, 4 µmol/l), oxygenated haemoglobin, and 0.1 ml of cell lysate. Using diclofenac sodium as a

positive control, the absorbance was read at 401 nm to determine the iNOS activity. The percentage inhibition of the enzyme was calculated using the formula,

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

here, Ac shows the absorbance of the control, and As shows the absorbance of the sample. After analysing the sample in triplicate, the mean value was calculated.

#### **d) Estimation of cellular nitrite level**

The method outlined by Lepoivre et al. (1990) was used to calculate the nitrite level. The cell lysate (0.5 ml) was combined with 0.1 ml of 3% sulphosalicylic acid, and the mixture was vortexed vigorously for 30 min. The obtained supernatant was combined with 200 µl of the supernatant and 30 ml of 10 % NaOH after centrifugation at 5000 rpm for 15 min. 300 µl of Tris–HCl buffer was added after the mixture had been thoroughly blended. After that, Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthyl ethylene diamine dihydrochloride) was added, and the resulting mixture was incubated for 10–15 min in the dark. In order to calculate the nitrite level, the absorbance at 540 nm was measured. The standard used was sodium nitrite solution. It was estimated how much nitrite was in the sample based on the standard curves that were produced. A triplicate test of the sample was conducted.

#### **iii) Cytotoxic evaluation using MTT assay on RAW 264.7 cells**

The MTT assay was performed on RAW 264.7 cells in order to ascertain the appropriate concentration of the plant extract for additional analysis, as well as to prevent the plant extract's predicted cytotoxicity and its impact on the plant's anti-inflammatory activity. The study used a RAW 264.7 cell line that was kept in DMEM medium with supplements.

- **Cells seeding in 96-well plate**

Confluent monolayer cells that were two days old were trypsinised and suspended in 10% growth medium. A 100 µl cell suspension was then seeded into a

96-well tissue culture plate and incubated at 37°C in an incubator with 5% CO<sub>2</sub> humidification.

- **Preparation of compound stock**

Using a cyclomixer, 1 mg of the sample was weighed and dissolved in 1 ml of 0.1% DMSO. To guarantee sterility, the sample solution was passed through a 0.22 µm Millipore syringe filter.

- **Cytotoxicity evaluation**

The growth medium was taken out after 24 hours. Each compound that had been freshly prepared in DMEM was serially diluted five times using a two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, and 6.25 µg in 500 µl of DMEM). Each concentration was then added in triplicate to the corresponding wells, and the wells were then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Control cells that were not treated were also kept.

- **Cytotoxicity assay by direct microscopic observation**

After a full day of treatment, 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 observations were captured. Cytotoxicity was determined by looking for any discernible changes in the morphology of the cells, such as rounding or shrinking, granulation, or vacuolization in the cytoplasm.

- **Cytotoxicity assay by the MTT method**

In 3 ml of PBS, 15 mg of MTT was reconstituted until it was fully dissolved, and it was then sterilised using a filter. Following the incubation period of 24 hours, the sample content in the wells was removed, and 30 µl of reconstituted MTT solution was added to each test and cell control well. The plate was then gently shaken thoroughly, and it was incubated for 4 hours at 37°C in a humidified 5% CO<sub>2</sub> incubator. The supernatant was discarded after the incubation period, and 100 µl of MTT Solubilisation Solution was added. The wells were then gently pipetted up and down to dissolve the formazan crystals. Using a microplate reader set at 540 nm, the

absorbance values were determined (Talarico et al., 2004). The lethal concentration 50% (LC<sub>50</sub>) and the percentage of cell viability were computed using the formula below.

**iv) Estimation of inflammatory mediator using enzyme-linked immunosorbent assay (ELISA)**

To measure the levels of COX-2, an inflammatory mediator, ELISA was utilized. Following sufficient confluency, LPS-stimulated cells were treated with the sample's LC<sub>50</sub> concentration and incubated for 24 hours at 37°C in an incubator with 5% CO<sub>2</sub> that had been humidified. Treatment-free LPS-stimulated cells were also kept as a control. Following incubation, the 96-well plate was filled with 100 µl of the supernatant and left to incubate overnight at 37° C. The wells were then drained and given two PBS washes. One hour of room temperature incubation was followed by the addition of 200 µl of freshly made blocking buffer (**Appendix 6**). The blocking buffer was eliminated after incubation, and PBS Tween was used twice to wash the wells. After that, 100 µl of primary antibody (COX-2) was added, and the mixture was left at room temperature for two hours. Following incubation, PBS Tween was used twice to wash the wells in order to remove the primary antibody. After adding 100 µl of secondary antibody (anti-human HRP conjugated secondary antibody), the mixture was allowed to sit at room temperature for an hour. After that, it was cleaned twice using PBS Tween. O-dianizdine hydrochloride (200 µl) was then added, and the mixture was allowed to sit at room temperature for 30 minutes in the dark. The reaction was then halted by adding 50 µl of HCl (5 N). 415 nm was the OD reading in an ELISA reader, and the antibody's activity were computed using the following formula.

$$\text{Activity of antibody} = \frac{\text{OD value}}{\text{Protein concentration}}$$

**C) Screening for cytotoxicity with *Allium cepa* L.**

Several assays were used to assess the antiproliferative efficacy of the methanolic extract of the three selected *Euphorbia* species. On *Allium cepa*, a preliminary examination of the plant extracts' cytotoxic potential was conducted.

**Cytotoxicity studies using the *Allium cepa* bioassay**

To determine the plant extract's potential for cytotoxicity, the *Allium cepa* root tip assay was employed. To do this, fresh, healthy onion bulbs were chosen, their outer dry scales were removed without harming the root primordia, and cultivated on moist, sterile sand. Following germination, onion bulbs with 1-2 cm roots were harvested between 9 and 10 am, when mitotic activity was at its highest. They were then rinsed with distilled water. After a thorough wash, the bulbs were suspended in varying concentrations of the plant extract (12.5, 25, 50, and 100 µg/ml) for a 24-hour treatment. It was ensured that only the bulb roots are submerged in extracts. The positive control (PC) was hydrogen peroxide (0.1%), and the negative control (NC) was distilled water. Roots were removed from the bulb's after treatment, cleaned in distilled water, and then fixed for one hour in modified Carnoy's fluid (Appendix 7). With a few minor modifications, mitotic squash was prepared using the methodology of Sharma and Sharma (1990). After being cleaned with distilled water, the fixed root tips were hydrolysed for five minutes in 1 N HCl. The roots were subsequently cleaned and stained for three hours with acetocarmine (**Appendix 8**). Following that, use 45% acetic acid to destain it. The roots were then placed on clean glass slides in 45 % acetic acid and squashed. Cells were viewed under a 40X objective of a light microscope (Leica DM 2000 LED, Germany). The objects mounted on sterile slides were examined, and photomicrographs were captured. To ascertain the total number of cells, aberrated cells, and mitotic cells, six distinct fields were selected at random. The following formulas were used to calculate the mitotic index (%) and abnormality percentage (%), and the results were presented as mean ± SE.

$$\text{Mitotic index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Abnormality percentage (\%)} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

**D) Antiproliferative activity****i) *In vitro* cytotoxicity evaluation by MTT assay (L929)**

The L929 (fibroblast) cell line was first acquired from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's modified Eagle's medium (Appendix 9), or DMEM (Sigma Aldrich, USA). In a 25 cm<sup>2</sup> tissue culture flask, the cell line was cultured using DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany), and an antibiotic solution that contained Amphotericin B (2.5 µg/ml), Streptomycin (100 µg/ml), and Penicillin (100 U/ml). The cultured cell lines were maintained in a humidified incubator with 5% CO<sub>2</sub> at 37°C (NBS Eppendorf, Germany). Cell viability was determined through direct observation of cells using an inverted phase contrast microscope, followed by the MTT assay method.

**Cells seeding in 96-well plate:**

After two days, confluent monolayer cells were trypsinised and suspended in 10% growth medium. A 96-well tissue culture plate was then seeded with a 100 µl cell suspension ( $5 \times 10^3$  cells/well), and the cells were cultivated at 37°C in an incubator with humidified 5% CO<sub>2</sub>.

**Preparation of compound stock:**

A cyclomixer was used to weigh 1 mg of the sample and dissolve it in 1 ml of 0.1% DMSO. To ensure sterility, the sample solution was filtered through a 0.22 µm Millipore syringe filter.

**Cytotoxicity evaluation:**

Following the removal of the growth medium after 24 hours, each of the freshly prepared compounds in DMEM was serially diluted five times using a two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, and 6.25 µg in 500 µl of DMEM). Additionally, 100 µl of each concentration was added in triplicate to the corresponding wells, and the wells were then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Also, untreated control cells were kept.

**Cytotoxicity assay by direct microscopic observation:**

After a full day of treatment, the entire plate was examined under an Olympus CKX41 inverted phase contrast tissue culture microscope equipped with an Optika Pro5 CCD camera. Microscopic observations were captured as images. Any discernible alterations in the cells' morphology, such as rounding or shrinking, granulation, or vacuolization in the cytoplasm, were regarded as markers of cytotoxicity.

**Cytotoxicity assay by MTT method:**

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml of PBS until completely dissolved, and then filter sterilised.

Following the incubation period of 24 hours, the sample content in the wells was removed, and 30 µl of reconstituted MTT solution was added to each test and cell control well. The plate was then gently shaken thoroughly, and it was incubated for 4 hours at 37°C in a humidified 5% CO<sub>2</sub> incubator. After incubation, remove the supernatant and add 100 µl of MTT solubilisation solution (DMSO, Sigma Aldrich, USA) to the wells. Gently pipette up and down to dissolve the formazan crystals. A microplate reader was used to measure the absorbance values at a wavelength of 540 nm (Riss et al., 2013).

The following formula was used to determine the percentage of growth inhibition:

$$\% \text{ of viability} = \frac{\text{Mean OD of Samples}}{\text{Mean OD of control group}} \times 100$$

**ii) *In Vitro* anticancer effect determination by MTT assay (MCF-7)**

Dulbecco's modified Eagle's medium, or DMEM (Sigma Aldrich, USA), was used to maintain the MCF-7 (human breast cancer) cell line, which was originally purchased from the National Centre for Cell Sciences (NCCS), Pune, India. In a 25 cm<sup>2</sup> tissue culture flask, antibiotics were added to DMEM supplemented with 10% FBS, L-glutamine, and sodium bicarbonate (Merck,

Germany). These antibiotics included penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (2.5 µg/ml). In a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany), cultured cell lines were maintained at 37°C. To evaluate the viability of the cells, the MTT assay method was applied soon after direct cell observation under an inverted phase contrast microscope (Riss et al., 2013).

#### **Cells seeding in 96-well plate:**

Trypsinization was performed on a confluent monolayer of cells that were two days old. The cells were then suspended in 10% growth medium, seeded in 96-well tissue culture plates with 100 µl of the cell suspension ( $5 \times 10^3$  cells/well), and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

#### **Preparation of compound stock:**

Using a cyclomixer, 1 mg of the sample was weighed and dissolved in 1 ml of 0.1% DMSO. To guarantee sterility, the sample solution was passed through a 0.22 µm Millipore syringe filter.

#### **Anticancer evaluation:**

The growth medium was taken out after 24 hours. Each compound that had been freshly prepared in DMEM was serially diluted five times using a two-fold dilution (100 µg, 50 µg, 25µg, 12.5 µg, and 6.25 µg in 500 µl of DMEM). Additionally, 100 µl of each concentration was added in triplicate to the corresponding wells, and the wells were then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Also, untreated control cells were kept.

#### **Anticancer assay by direct microscopic observation:**

Following a 24-hour treatment period, the entire plate was examined under an Olympus CKX41 inverted phase contrast tissue culture microscope equipped with an Optika Pro5 CCD camera. Microscopic observations were captured as images. Any discernible alterations in the cells' shape, such as rounding or shrinking, granulation, or vacuolization in the cytoplasm, were regarded as markers of cytotoxicity. Cytotoxicity was determined by looking for any discernible changes in



the morphology of the cells, such as rounding or shrinking, granulation, or vacuolization in the cytoplasm.

**Anticancer assay by MTT method:**

15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml of PBS until it was fully dissolved, and then it was filtered to ensure it was sterile. The sample content 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 thoroughly, and it was incubated for four hours at 37°C in a humidified 5% CO<sub>2</sub> incubator. The supernatant was discarded after the incubation period, and 100 µl of MTT Solubilisation Solution (Dimethyl Sulfoxide, DMSO, Sigma Aldrich, USA) was added. The wells were then gently pipetted up and down to dissolve the formazan crystals. A microplate reader set to 540 nm was used to measure the absorbance values.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of samples}}{\text{Mean OD of control group}} \times 100$$

**iii) Determination of apoptosis by acridine orange (AO) and ethidium bromide (EtBr) double staining**

The morphological identification of necrotic and apoptotic cells was done using DNA-binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) (Sigma, USA) (Gherghi et al, 2003). Both living and dead cells absorb AO, which, when intercalated into double-stranded DNA, produces green fluorescence. Only non-viable cells can absorb EtBr, which gives off red fluorescence when it intercalates into DNA. After 24 hours of exposure to the material at the LC<sub>50</sub> concentration, cells were washed with cold PBS and stained with a mixture of AO (100 µg/ml) and EtBr (100 µg/ml) at room temperature for 10 minutes. Following two rounds of washing with 1X PBS, the stained cells were examined using a fluorescence microscope in blue filter (Olympus CKX41 with Optika Pro5 camera). The cells were separated into four groups: necrotic cells (uniformly orange-stained cell nuclei), early apoptotic cells (bright green nucleus with condensed or

fragmented chromatin), late apoptotic cells (orange-stained nuclei with chromatin condensation or fragmentation), and living cells (normal green nucleus).

**iv) Analysis of DNA content and cell cycle distribution using the cell cycle kit by flow cytometry**

In order to evaluate a cell's health during toxicity studies, it is essential to track its capacity for proliferation. The most precise way to do this is to measure DNA synthesis directly. The fundamental idea behind the MUSE cell cycle kit is that standard ethanol fixation and detergent permeabilization are adequate to access DNA while the cell cycle is active. To enhance the specificity of DNA staining in each phase (G0/G1, S, and G2/M), the kit employs a premixed reagent containing propidium iodide (PI), a nuclear DNA intercalating stain that separates cells at various cell cycle stages according to the differential DNA content in the presence of RNAase.

The MCF-7 cell line was grown using the previously described standard protocols, treated with a compound at its LC<sub>50</sub> concentration (ED: 93.21809 µg/ml), and then incubated for a full day. A 50 ml conical flask or a 12 x 75 mm polystyrene tube was used to hold the cell sample. When fixing cells in a tube, a minimum of 1×10<sup>6</sup> cells is advised. The samples were then centrifuged for five minutes at 3000 rpm. The pellet was not agitated during the removal of the supernatant. Following centrifugation, the cell pellet either forms a white film on the tube's bottom or a visible pellet.

Each tube was filled with the proper amount of PBS (1 ml per 1×10<sup>6</sup> cells), and the contents were combined by gently vortexing or pipetting multiple times. For five minutes, the cells were centrifuged at 3000 rpm. Without affecting the cell pellet, the supernatant was disposed of, leaving about 50 µl of PBS per 1×10<sup>6</sup> cells. Repeatedly pipette the pellet or gently vortex it in the remaining PBS to resuspend it. The reconstituted cells were vortexed at a medium speed and added drop by drop to the tube containing one millilitre of ice-cold 70% ethanol. Store the tube at -20°C after closing it.

### **Staining of the cell cycle**

Following an overnight incubation period, the samples were centrifuged for five minutes at room temperature at 3000 rpm. After removing the supernatant, 250 µl of PBS was added to the pellet. The centrifugation was then repeated at the same time and rpm. The supernatant was discarded, and 250 µl of cell cycle reagent was added to the pellet. Because it is light-sensitive, this was incubated for 30 minutes in the dark. After that, a Flow Cytometer was used to analyse it. Following an analysis of the samples, gating was carried out in relation to untreated control cells.

### **v) Gene expression studies**

In accordance with the manufacturer's instructions, total RNA was isolated using the total RNA isolation kit (Invitrogen, product code 1029610). Cell disruption and RNA release occur when TRIzol solution is added. After centrifugation, proteins are in the interphase and organic phase, while chloroform is only extracted in the aqueous phase. When RNA is mixed with isopropanol, it precipitates as a white pellet on the tube's side and bottom.

After being cultivated using the previously described standard procedures, the MCF-7 cell line was treated with a compound containing its LC<sub>50</sub> concentration (ED: 93.21809 µg/ml) and incubated for a full day. Wells that were not treated were also kept up. One millilitre of TRIzol reagent was added to a culture well plate and incubated for five minutes after the DMEM media was aseptically removed. A brand-new, sterile Eppendorf tube was then used to hold the contents. Centrifugation at 14000 rpm for 15 minutes at 4°C was performed after 200 µl of chloroform was added, shaken vigorously for 15 seconds, and incubated for 2–3 minutes at room temperature. Following the collection of the aqueous layer, 500 µl of 100% isopropanol was added.

After 10 minutes at room temperature, it was centrifuged for 15 minutes at 4°C at 14,000 rpm. After discarding the supernatant, 200 µl of 75% ethanol (Merck) was used to wash the pellet that was produced. After that, it was centrifuged in a cooling centrifuge (Remi CM12) at 14,000 rpm for five minutes at 4°C. TE buffer was used to suspend the dried RNA pellet.

### cDNA Synthesis

Trizol was used to extract total RNA (Invitrogen, USA). The concentration and purity of total RNA were ascertained. The cDNA preparation kit (G BIOSCIENCES, Product code: 786-5019s, 786-5020, master premix for first-strand cDNA synthesis) was used to create the template complementary DNA. An RNase-free tube was filled with roughly 5 µl of RT Easy mix, 0.5 µl of oligodT, and 2 µl of RNA template (0.5 µg of total RNA). Sterile distilled water was then added to bring the total reaction volume up to 10 µl. A pipette was used to gently mix the solution up and down. It was programmed to perform cDNA synthesis using the Eppendorf Master Cycler. The cycling conditions used were 20 minutes at 42°C and 5 minutes at 85°C.

| Step           | Temperature°C | 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 for Real-Time qRT-PCR analysis on a Light Cycler 96 (Roche). The  $\Delta\Delta C_t$  method was used to analyse the data, and all reactions were carried out in triplicate (using Light Cycler 96 SW 1.1 software). The Table lists all of the steps that are involved, along with the temperature and time required for each step.

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

The forward and reverse primer used:

| 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<br>(20) | 57.3 | TGTGTTTGTGCCCAGAATGT<br>(20) | 55.3 |

### Agarose gel electrophoresis

One technique for separating and displaying DNA fragments is agarose gel electrophoresis. Upon exposure to an electric field, the fragments separate according to size and charge and flow through the agarose gel matrix. By applying potential across an electrolyte solution (buffer), an electric field is created. Agar dissolves when boiled in an aqueous buffer and solidifies into a gel when cooled. 1x TE buffer was used to prepare a 1.5% agarose gel, which was then melted in a hot water bath at 90°C. The agarose that had melted was then allowed to cool to 45°C. The gel comb was used to pour 6 µl of 10 mg/ml ethidium bromide into a gel casting apparatus. The comb was removed from the gel once it had set. The platform containing the gel was submerged in the electrophoresis buffer after it had been poured into the gel tank. After loading the samples onto the gel, it was run for 30 minutes at 50 V. A gel documentation system (E gel imager, Invitrogen) was used to visualise the stained gel.

## Part III

### i) Green synthesis of silver nanoparticles

10 mg of the methanolic extract is mixed with 20 ml of distilled water. The ingredients are combined using a vortex mixer before being filtered to produce a clear solution. Using a measuring cylinder, 20 ml of the prepared methanolic extract of the three plant species chosen for the study was transferred to a 250-ml volumetric flask, to which 80 ml of 2 mM AgNO<sub>3</sub> was added, heated at 60°C for

about 10 minutes, and kept in the dark for 20 minutes. After 20 minutes of incubation, the solution colour changes into reddish brown, indicating the formation of silver nanoparticles. The solution is then centrifuged for 15 minutes at 13,000 rpm, after which the supernatant is discarded and washed with distilled water. The centrifugation and washing steps were repeated to ensure that the silver nanoparticles were completely separated. The pellets were stored at room temperature for characterisation and further analysis.

## **ii) Characterization techniques**

### **a) UV-visible spectroscopy**

The Agilent Technologies Cary 5000 UV-Vis-NIR spectrophotometer was used to accomplish this. The decrease in Ag<sup>+</sup> ions and the formation of a peak at a wavelength of 200-800 nm were studied using approximately 1 ml of the synthesised solution.

### **b) Field emission scanning electron microscopy (FE-SEM)**

The dried silver nanoparticle pellets were mounted on an aluminium stub and examined with a field emission scanning electron microscope (ZEISS Gemini SEM 300 with magnification of 12 X - 2,000,000 X, acceleration voltage was 0.02 – 30 kV). 100 µg of the samples were assessed with this apparatus in order to ascertain the average size measurement and to further illustrate the formation of nanoparticles.

### **c) Fourier transform infrared spectroscopy (FTIR)**

The functional groups of prepared nanomaterials were measured using an Agilent Cary 620 FTIR imaging system and an Agilent Cary 660 Fourier Transform Infrared Spectroscopy in the 4000-400 cm<sup>-1</sup> range (FTIR). The cleaned pellet was used for FTIR analysis, for which the silver nanoparticle solution was centrifuged at 13,000 rpm and allowed to air dry at room temperature.

## **iii) Antimicrobial activity**

### **a) Antibacterial activity**

33.8 g of the Muller Hinton Agar Medium (MHI Agar Media), which is sold commercially, was dissolved in 1000 millilitres of distilled water to create the

medium. For 15 minutes, the dissolved medium was autoclaved at 121°C and at 15 pounds of pressure. Once thoroughly combined, the autoclaved medium was poured, still molten, onto 100 mm Petri plates (25–30 ml/plate). In order to make one litre of nutrient broth, 13 g of commercially available nutrient medium (HI Media) were dissolved in 1000 millilitres of distilled water and then brought to a boil to completely dissolve the medium. Following the desired dispensing, the medium was autoclaved for 15 minutes at 121°C and at 15 lbs of pressure to sterilise it. *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus mutans* (ATCC 25175), and *E. coli* (ATCC 25922) bacterial cultures were seeded into Petri plates with 20 ml of Muller Hinton Agar Medium (growth of culture adjusted according to McFarlands Standard, 0.5%). Sterile discs containing the appropriate test samples were positioned on plates. For the next 24 hours, the plates were incubated at 37°C. In order to measure the antibacterial activity, the diameter of the inhibition zone that developed around the discs was measured (Hudzicki, 2009).

#### **b) Antifungal activity**

To make the medium, 39 g of the commercially available Potato Dextrose Agar Medium (Hi Media) was dissolved in 1000 millilitres of distilled water. For 15 minutes, the dissolved medium was autoclaved at 121°C and at 15 pounds of pressure. After thoroughly mixing, the autoclaved medium was poured onto 100 mm Petri plates (25–30 ml/plate). After preparing potato dextrose agar plates, *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231), two fungal species that grow overnight, were swabbed. The corresponding test samples were placed on plates with sterile paper discs. After being incubated for the entire night at room temperature, the zone of inhibition was measured and compared with that of a typical antifungal drug clotrimazole.





## RESULTS

### PART I – PHYTOCHEMICAL SCREENING

The presence and absence of various secondary metabolites found in selected *Euphorbia* species were assessed using qualitative phytochemical screening. Estimation techniques were adopted to quantify the specified amount of secondary phytometabolites. Additionally, potential volatile and non-volatile components present in selected species were evaluated using analytical techniques such as GC-MS and HR LC-MS analysis.

#### a) Qualitative screening of phytometabolites

By observing the colour change and precipitate formation in various reactions, it was possible to determine the distribution of different secondary metabolites belonging to various classes. It was carried out using standard protocols. Three different solvents, including n-hexane, methanol, and distilled water, were taken into consideration for this. All the species that were chosen displayed distinct types of metabolites, including terpenoids, triterpenoids, phenolic compounds, flavonoids, etc. The results of a preliminary qualitative screening of metabolites were compiled in **Table. 5**.

Flavonoids, phenolic compounds, terpenoids, saponins, tannins, anthraquinones and triterpenoids were found in both methanolic and aqueous extracts of *E. deccanensis*, but alkaloids were only found in the methanolic extract. In the methanolic and aqueous extracts of *E. rothiana*, flavonoids, phenols, tannins, and coumarins were found, while glycosides and triterpenoids were only present in the methanolic extract. Likewise, *E. thymifolia* methanol and aqueous extracts showed alkaloids, flavonoids, terpenoids, tannins, and phenolics, while saponins, phytosterols and glycosides were only detected in the methanolic extract. Thus, the present research discovered that while phlobatannins were lacking in all three tested plant extracts, phenolics, tannins, and flavonoids were present in significant amounts. Further investigation into methanolic extract was prompted by the preliminary screening, which showed that it was the most appropriate solvent with a higher number of phytocompounds.

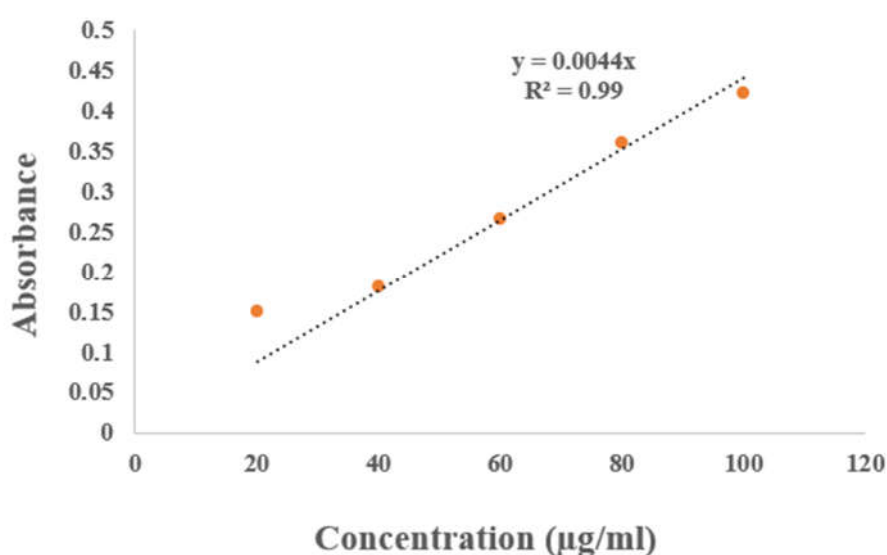
**Table 5: Preliminary phytochemical analysis using three different solvent extracts of *E. deccanensis*, *E. rothiana* and *E. thymifolia***

| Sl. No. | chemical class | Tests                    | ED  |      |     | ER  |      |     | ET  |      |     |
|---------|----------------|--------------------------|-----|------|-----|-----|------|-----|-----|------|-----|
|         |                |                          | Hex | MeOH | Aqs | Hex | MeOH | Aqs | Hex | MeOH | Aqs |
| 1       | Phenols        | Ferric chloride test     | -   | +    | -   | -   | +    | +   | -   | -    | -   |
|         |                | Spot test                | -   | -    | +   | -   | -    | -   | -   | +    | +   |
| 2       | Flavonoids     | Alkaline reagent test    | +   | +    | +   | +   | +    | +   | -   | +    | +   |
|         |                | Lead acetate test        | -   | +    | +   | -   | -    | -   | -   | -    | -   |
| 3       | Alkaloids      | Hager's test             | -   | +    | -   | -   | +    | -   | +   | +    | +   |
|         |                | Wagner's test            | -   | +    | -   | -   | +    | +   | +   | +    | -   |
| 4       | Tannins        | Braymer's test           | -   | +    | +   | -   | +    | +   | -   | +    | +   |
|         |                | Lead acetate test        | -   | +    | +   | -   | +    | +   | -   | -    | -   |
| 5       | Coumarins      | Ferric chloride test     | -   | -    | -   | -   | +    | +   | -   | -    | -   |
| 6       | Anthraquinones | Borntrager's test        | -   | +    | +   | -   | +    | +   | -   | +    | -   |
| 7       | Terpenoids     | Salkowski test           | +   | +    | +   | +   | +    | +   | -   | +    | +   |
| 8       | Saponins       | Foam test                | -   | +    | +   | -   | +    | +   | -   | +    | -   |
| 9       | Phlobatannins  | Precipitation test       | -   | -    | -   | -   | -    | -   | -   | -    | -   |
| 10      | Glycosides     | Legal's test             | -   | +    | +   | -   | +    | -   | -   | +    | -   |
| 11      | Phytosterols   | Liebermann-Burchard test | -   | +    | -   | -   | +    | -   | -   | -    | -   |
|         |                | Sulphuric acid test      | -   | -    | -   | -   | -    | -   | -   | +    | -   |
| 12      | Triterpenoids  | Chloroform test          | -   | +    | +   | -   | +    | -   | -   | +    | -   |

**Aqs: Aqueous extract, MeOH: Methanolic extract, Hex: Hexane extract, ED: *E. deccanensis*; ER: *E. rothiana*; ET: *E. thymifolia***

### b) Quantitative estimation of major phytometabolites

The quantitative estimation of major metabolites revealed the exact amount of secondary metabolites within the extract. The quantitative estimation of total phenolic content, total flavonoid content and total terpenoid content were determined. The total terpenoid content was tested against linalool (**Fig. 10**). It was found that both the *E. deccanensis* ( $135.51 \pm 2.67$  mg LE/g DW) and *E. rothiana* ( $134.4 \pm 3.34$  mg LE/g DW) methanolic extracts exhibited almost equal amounts of terpenoid content. While in the case of *E. thymifolia*, the terpenoid content was found to be very less. The total phenolic content of the methanolic extracts of the selected species was derived from the calibration curve of gallic acid. The highest phenolic content was observed in *E. rothiana* ( $229 \pm 3.11$  mg GAE/g DW) followed by *E. deccanensis* ( $139 \pm 2.25$  mg GAE/g DW) and *E. thymifolia* ( $132 \pm 0.78$  mg GAE/g DW) respectively. Similar to the overall terpenoid content, the flavonoid content of the *E. deccanensis* ( $59.3 \pm 3.3$  mg QE/g DW) and *E. rothiana* ( $57.52 \pm 0.89$  mg QE/g DW) extracts were nearly equal, with *E. thymifolia* ( $38.96 \pm 0.31$  mg QE/g DW) following closely behind. Total flavonoid content was determined against quercetin. The distribution of metabolites like terpenoids, phenols, and flavonoids in sufficient amounts was discovered in the current study. These phytochemicals may have had a significant impact on the bioactive potential of particular plant species.



**Fig. 10: Standard calibration curve of linalool for the determination of total terpenoid content**

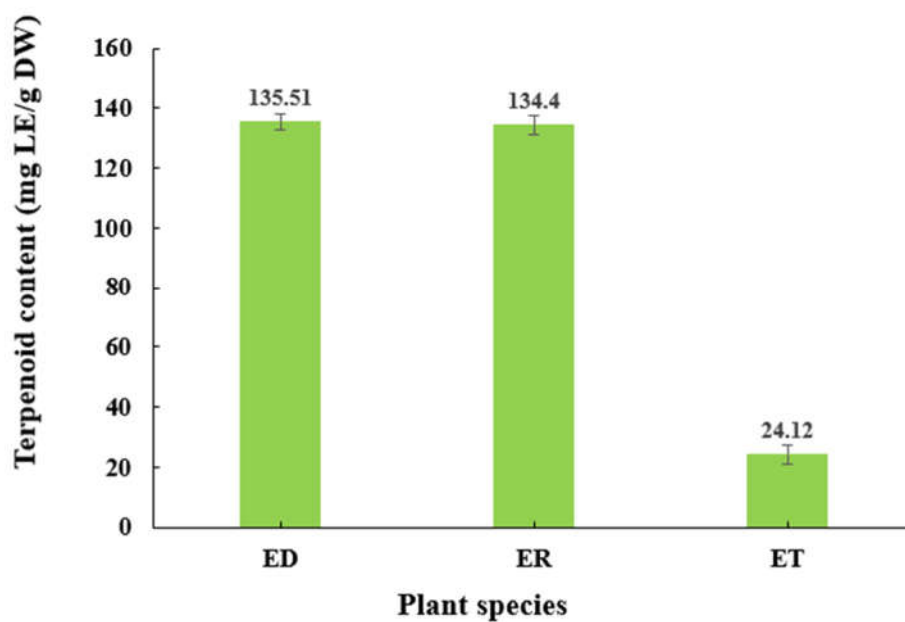


Fig. 10: Quantitative estimation of total terpenoid content in the selected species of *Euphorbia*. ED - *Euphorbia deccanensis*; ER - *Euphorbia rothiana*; ET- *Euphorbia thymifolia*

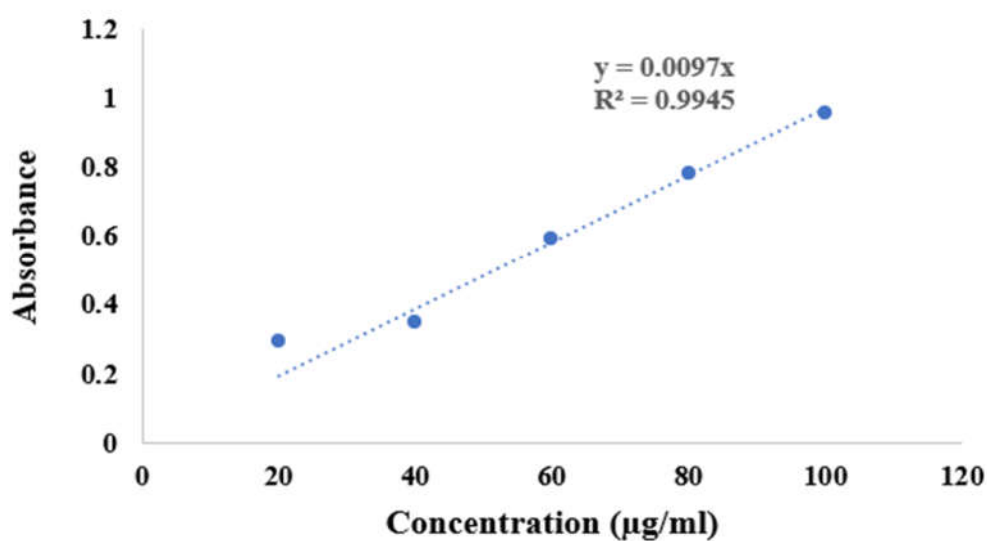
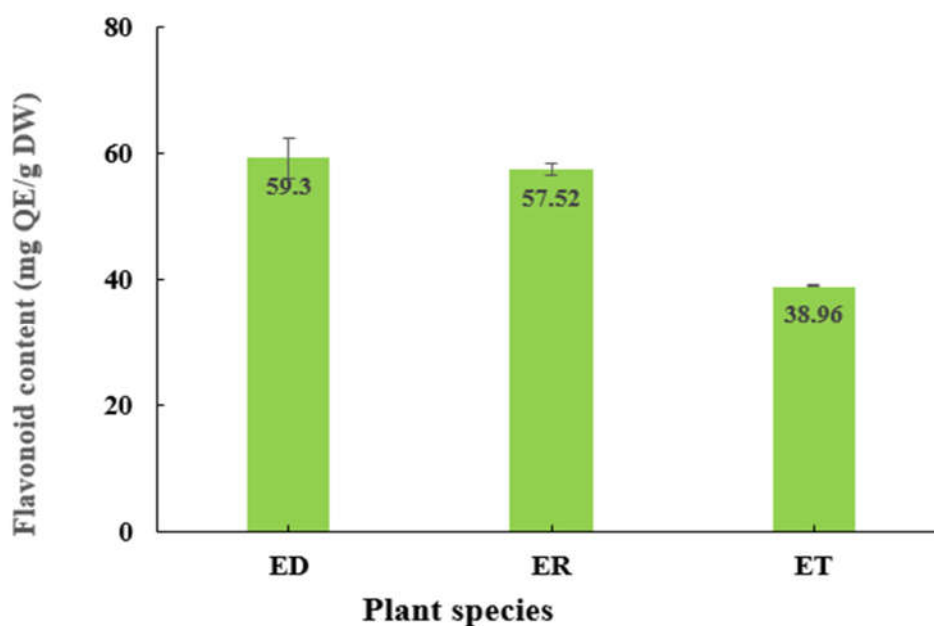
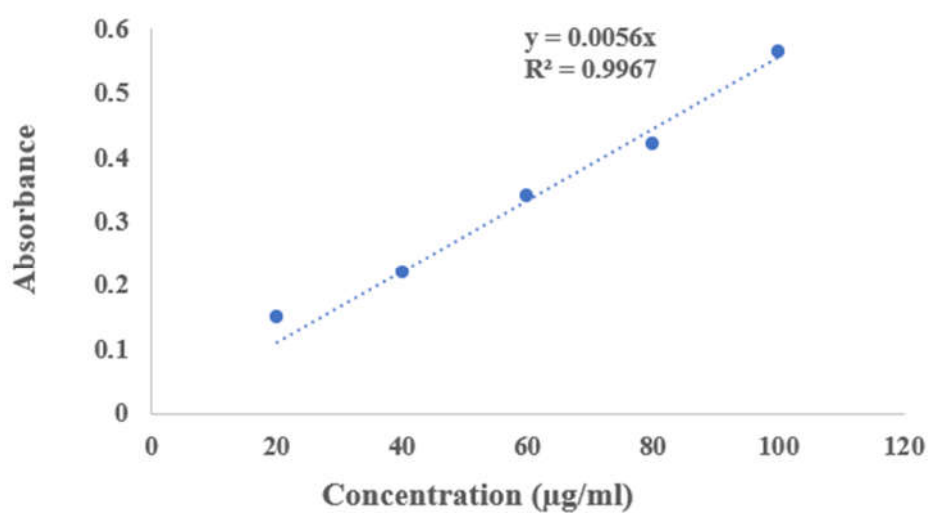


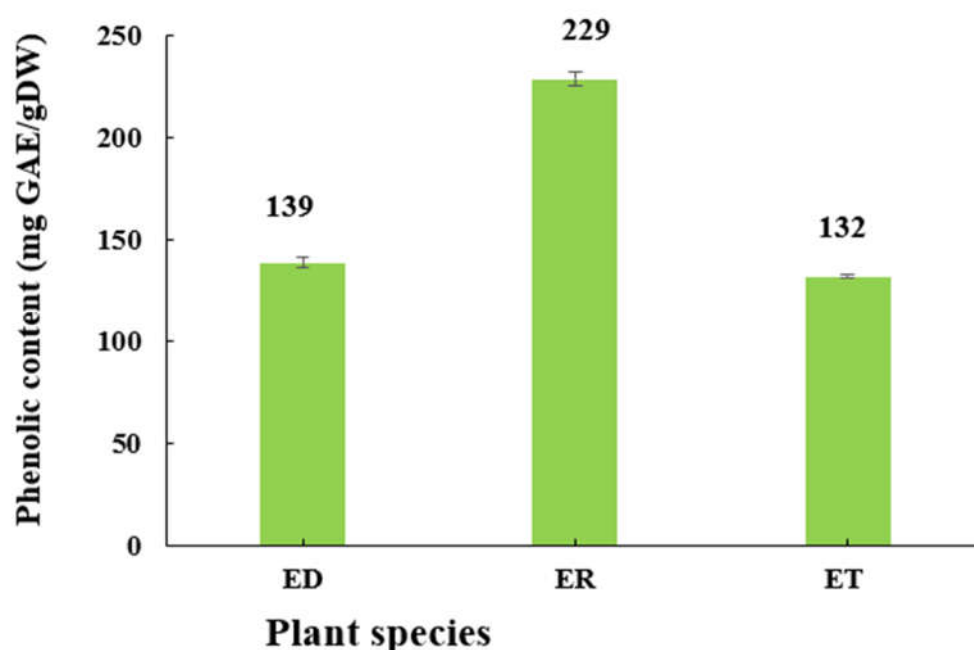
Fig. 11: Standard calibration curve of quercetin for the determination of total flavonoid content



**Fig. 11: Quantitative estimation of total flavonoid content in the selected species of *Euphorbia*.** ED - *Euphorbia deccanensis*; ER - *Euphorbia rothiana*; ET- *Euphorbia thymifolia*



**Fig. 12: Standard calibration curve of gallic for the determination of total phenolic content**



**Fig. 12: Quantitative estimation of total phenolic content in the selected species of *Euphorbia*.** ED - *Euphorbia deccanensis*; ER - *Euphorbia rothiana*; ET- *Euphorbia thymifolia*

**c) High Resolution Liquid Chromatography-Mass Spectrometry (HR LC-MS) analysis**

By using HR LC-MS analysis, the non-volatile elements that were found in the methanolic extract of the chosen species of *Euphorbia* were identified. About 83 phytochemicals from various classes were found in the results, with retention times ranging from 1.083 to 20.472. Quinic acid was the first compound eluted at the lowest retention time and 25-Methylgramisterol was eluted at the highest retention time. The majority of the metabolites that were eluted are flavonoids, followed by terpenoids, phenolics, and tannins. The outcome of the HR LC-MS analyses were summarised in **Table. 6**

In the case of *E. deccanensis*, 43 non-volatile components were identified, including potent phytochemicals with known bioactivities such as flavonoids (hibiscitrin, myricetin 3 - galactoside, kaempferol, myricetin) terpenoids (camellidionol) tannin (beta-Boswellic acid acetate, chesnatin) Fatty alcohol

(momordol) and coumarin (fraxetin). Most compounds were eluted from *E. deccanensis* out of the three species that were examined. **Fig. 13 (i)** depicts the liquid chromatogram of the *E. deccanensis* methanolic extract. Seven compounds with reported biological properties were detected out of the 40 compounds examined in the case of the *E. rothiana* methanolic extract, including cryptochlorogenic acid, caffeic acid, rutin, melitric acid A, quercetin 3,7- dirhamnoside and 1-O-caffeoylquinic acid. Quercetin-3-O-glucuronide, also known as miquelianin, is a phenolic compound with well-known antioxidant properties that was also found in this plant extract. The liquid chromatogram of *E. rothiana* methanolic extract is shown in **Fig. 13 (ii)**. The lowest number of metabolites, 29, were eluted from the *E. thymifolia* methanolic extract, with the most significant bioactive compounds being peltatoside, rustoside, guajavarin and fukinolic acid. **Fig. 13 (iii)** shows the *E. thymifolia* methanolic extract's liquid chromatogram. The mass spectra of each compound from each of the three plant species that were chosen and evaluated by HR LC-MS analysis are given in **Figs. 14(i) - 14 (xvii)**.

Among the so many phytometabolites eluted, gallic acid, corchorifatty acid F, O-methylsomniferine, isodiospyrin, and rhamnazin 3-sophoroside are common in all three plant extracts. Additionally, both *E. deccanensis* and *E. rothiana* eluted euphornin, a well-known diterpenoid found in several species of the genus *Euphorbia*.

**Table. 6** Phytocompounds revealed by HR LC-MS analysis of the selected species of *Euphorbia*

| SI NO. | RT    | Compound        | Molecular mass | Class                       | ED | ER | ET |
|--------|-------|-----------------|----------------|-----------------------------|----|----|----|
| 1      | 1.083 | Quinic acid     | 192.0622       | Cyclohexane carboxylic acid |    | +  | +  |
| 2      | 1.127 | Retronecine     | 155.0952       | Alkaloid                    |    | +  | +  |
| 3      | 1.387 | Gentisic acid   | 154.0258       | Phenolic acid               |    | +  | +  |
| 4      | 1.443 | Prolyl-Arginine | 271.1645       | Dipeptide                   |    |    | +  |
| 5      | 1.499 | Justicidin A    | 394.1018       | Organic compound            |    |    | +  |
| 6      | 1.589 | Gallic acid     | 170.0201       | Phenolic compound           | +  | +  | +  |

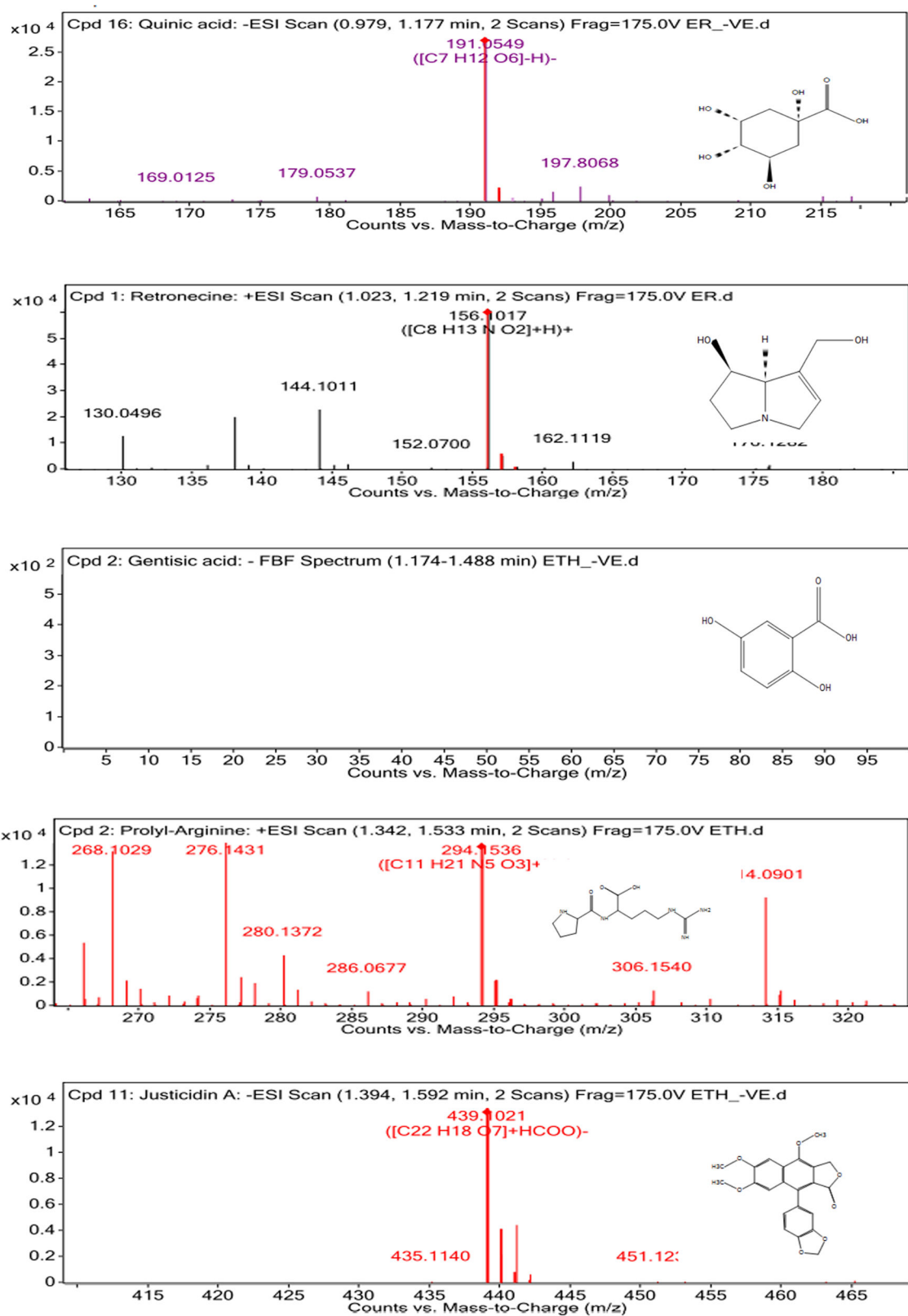
|    |       |                                                                      |          |                          |   |   |   |
|----|-------|----------------------------------------------------------------------|----------|--------------------------|---|---|---|
| 7  | 1.838 | N-(1-Deoxy-1- fructosyl) phenylalanine                               | 327.1305 | Carboxylic acid          |   | + |   |
| 8  | 2.128 | 2-O-Caffeoyl hydroxycitric acid                                      | 370.053  | Carboxylic acid          | + |   |   |
| 9  | 2.309 | 1-O-Galloylglycerol                                                  | 244.0566 | Phenolic compound        | + |   |   |
| 10 | 3.045 | Punicacortein B                                                      | 634.0783 | Polyphenol               | + | + |   |
| 11 | 3.066 | Crypto chlorogenic acid                                              | 354.0948 | Phenolic acid            |   | + |   |
| 12 | 3.211 | Indole acrylic acid                                                  | 187.06   | Indole                   |   | + |   |
| 13 | 3.223 | Caffeic acid                                                         | 180.0409 | Phenol                   |   | + |   |
| 14 | 3.287 | (3'x,5'a,9'x,10'b)-O-(3-Hydroxy-6-oxo-7-drimen-11- yl) umbelliferone | 396.1846 | Coumarin                 |   | + |   |
| 15 | 3.722 | Hibiscitrin                                                          | 496.0866 | Flavonoid                | + |   |   |
| 16 | 3.907 | 4-Hydroxycinnamic acid                                               | 164.0462 | Coumaric acid            | + |   |   |
| 17 | 4.093 | Chesnatin                                                            | 638.1144 | Tannins                  | + |   |   |
| 18 | 4.162 | 1-O-Caffeoylquinic acid                                              | 354.094  | Quinic acid              |   | + |   |
| 19 | 4.174 | Vanillic acid                                                        | 168.0416 | Monohydroxy benzoic acid | + |   |   |
| 20 | 4.189 | 5-O-Feruloylquinic acid                                              | 368.1108 | Quinic acid              |   | + |   |
| 21 | 4.194 | Chlorogenic acid                                                     | 354.0944 | Polyphenol               | + |   |   |
| 22 | 4.198 | Vescalin                                                             | 632.0682 | Tannins                  | + |   |   |
| 23 | 4.366 | Fraxetin                                                             | 208.0377 | Coumarin                 | + |   |   |
| 24 | 4.468 | Amlaic acid                                                          | 652.0957 | Allyl sulfur compound    | + |   |   |
| 25 | 4.6   | Rutin                                                                | 610.1526 | Flavonoid                |   | + |   |
| 26 | 4.717 | Sanguiin H11                                                         | 952.0824 | Tannins                  | + | + |   |
| 27 | 4.981 | Quercetin 3,7-dirhamnoside                                           | 594.1585 | Flavonoid                |   | + |   |
| 28 | 5.034 | Osmanthuside A                                                       | 446.1536 | Coumaric acid ester      | + | + |   |
| 29 | 5.237 | Myricetin 3- galactoside                                             | 480.0884 | Flavonoid                | + |   |   |
| 30 | 5.311 | Punicafolin                                                          | 938.1062 | Tannins                  |   |   | + |
| 31 | 5.416 | Quercetin                                                            | 302.0414 | Flavonoid                | + |   | + |
| 32 | 5.418 | Peltatoside                                                          | 596.1371 | Organic compound         |   |   | + |
| 33 | 5.609 | Citrusin A                                                           | 538.207  | Glycoside                |   |   | + |
| 34 | 5.649 | Myricetin                                                            | 318.0385 | Flavonoid                | + |   |   |
| 35 | 5.665 | Ellagic acid                                                         | 302.0074 | Phenolic compound        |   | + | + |



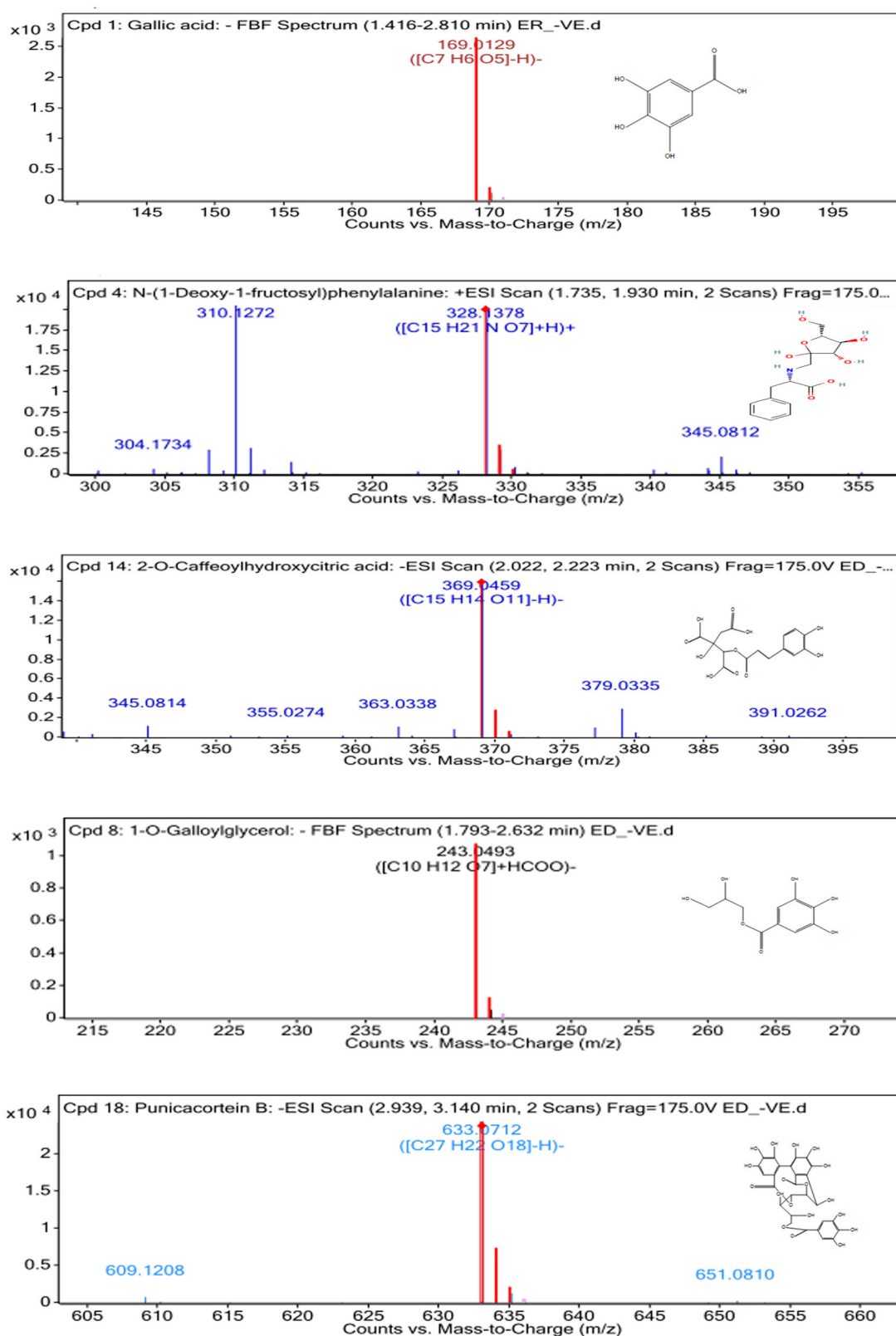
|    |        |                                                              |          |                       |   |   |   |
|----|--------|--------------------------------------------------------------|----------|-----------------------|---|---|---|
| 36 | 5.757  | Myricetin 7- rhamnoside                                      | 464.0916 | Flavonoid             | + |   |   |
| 37 | 5.775  | Myricitrin                                                   | 464.0973 | Flavonoid             | + |   | + |
| 38 | 5.833  | Rustoside                                                    | 580.1457 | Flavonoid             |   |   | + |
| 39 | 5.837  | Herbacetin                                                   | 302.0443 | Flavonoid             | + | + |   |
| 40 | 5.847  | N-Malonyl tryptophan                                         | 290.0916 | Carboxylic acid       |   | + |   |
| 41 | 5.868  | Quercetin 3-O-glucuronide (Miquelianin)                      | 478.0721 | Phenol                |   | + |   |
| 42 | 5.894  | Tyrosyl-Serine                                               | 268.1059 | Dipeptide             |   |   | + |
| 43 | 6.158  | Isodiospyrin                                                 | 374.0855 | Naphthoquinone        | + | + | + |
| 44 | 6.186  | Fukinolic acid                                               | 434.0811 | Hydroxy cinnamic acid |   |   | + |
| 45 | 6.206  | Guajavarin                                                   | 434.0856 | Flavonoid             |   |   | + |
| 46 | 6.27   | Luteolin 4'-O-glucoside                                      | 448.1034 | Flavonoid             | + |   | + |
| 47 | 6.332  | 3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D-glucopyranoside) | 440.0954 | Phenolic glycoside    |   | + |   |
| 48 | 6.829  | Apigenin 7-glucoside                                         | 432.1029 | Flavonoid             |   | + |   |
| 49 | 6.983  | Diosmetin 7-O-beta-D-glucurono pyranoside                    | 476.0922 | Flavonoid             |   | + |   |
| 50 | 7.283  | Kaempferol                                                   | 286.048  | Flavonoid             | + |   |   |
| 51 | 7.9    | Melitric acid A                                              | 538.1135 | Organic compound      |   | + |   |
| 52 | 8.679  | 6- De methoxy capillarisin                                   | 286.0472 | Coumarin              |   |   | + |
| 53 | 8.704  | Corchorifatty acid F                                         | 328.2257 | Fatty acyl compound   | + | + | + |
| 54 | 10.034 | Iso syringinose                                              | 534.1856 | Phenolic glycoside    | + |   |   |
| 55 | 10.245 | (9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid    | 290.1873 | Fatty acyl compound   |   |   | + |
| 56 | 10.275 | Gibberellin A75                                              | 380.1473 | Lipid                 |   |   | + |
| 57 | 11.503 | Sphinganine                                                  | 301.297  | 1,2-Aminoalcohol      |   | + | + |
| 58 | 12.052 | 6-Deoxocastasterone                                          | 450.3708 | Steroid               | + |   |   |
| 59 | 12.536 | Rodiasine                                                    | 622.3147 | Alkaloid              | + |   |   |
| 60 | 12.758 | 1,4-beta-D-Glucan                                            | 536.1596 | Oligosaccharide       |   | + | + |
| 61 | 14.049 | 6-Deoxytyphasterol                                           | 434.3758 | Brassino steroid      | + |   |   |
| 62 | 14.164 | 16-Hydroxy hexadecanoic acid                                 | 272.2365 | Fatty acid            |   | + | + |

|    |        |                                                               |          |                      |   |   |   |
|----|--------|---------------------------------------------------------------|----------|----------------------|---|---|---|
| 63 | 14.89  | Rhamnazin 3-sophoroside                                       | 654.1757 | Flavonoid            | + | + | + |
| 64 | 14.908 | Momordol                                                      | 440.3456 | Fatty alcohol        | + |   |   |
| 65 | 15.457 | Camelledionol                                                 | 440.3311 | Triterpenoid         | + |   |   |
| 66 | 15.622 | 23- Acetoxy soladulcidine                                     | 473.3489 | Steroidal alkaloid   | + |   |   |
| 67 | 16.228 | Phaeophorbide b                                               | 606.2479 | Tetrapyrrole         |   | + | + |
| 68 | 16.516 | O-Methyl somniferine                                          | 622.2808 | Alkaloid             | + | + | + |
| 69 | 16.648 | 16-Hydroxy hexadecanoic acid (Juniperic acid)                 | 272.2358 | Fatty acid           | + | + |   |
| 70 | 16.71  | (3 $\alpha$ OH,20S,24S)-3,19:20,24-Diepoxydammarane3,25-diol  | 474.3721 | Triterpenoid saponin | + |   |   |
| 71 | 16.84  | Kanokoside D                                                  | 624.271  | Terpene glycoside    | + |   |   |
| 72 | 16.937 | Longistylin A                                                 | 294.1608 | Stilbene             |   | + |   |
| 73 | 17.387 | 3-O-p-trans-Coumaroyl alphitolic acid                         | 618.3934 | Triterpenoid         | + |   |   |
| 74 | 17.503 | Leucodelphinidin 3-[galactosyl-(1 $\rightarrow$ 4)-glucoside] | 646.183  | Flavonoid            |   | + |   |
| 75 | 17.548 | Pheophorbide a                                                | 592.2673 | Tetrapyrrole         | + | + |   |
| 76 | 18.406 | beta-boswellic acid acetate                                   | 498.3699 | Triterpenoid         | + |   |   |
| 77 | 18.902 | N-Hexadecanoyl pyrrolidine                                    | 309.3019 | Organic compound     |   | + |   |
| 78 | 18.974 | Euphornin                                                     | 584.2998 | Diterpenoid          | + | + |   |
| 79 | 18.977 | 3beta-3-Hydroxy-18-lupen-21-one                               | 440.3654 | Triterpenoid         | + | + |   |
| 80 | 19.272 | 6-Deoxocathasterone                                           | 418.3813 | Brassinosteroid      | + |   |   |
| 81 | 19.723 | 1-Hexanol arabinosyl glucoside                                | 396.1994 | Fatty acyl glycoside |   | + |   |
| 82 | 20.174 | (3beta,24xi)-Cycloartane-3,24,25-triol                        | 460.393  | Steroid              | + |   |   |
| 83 | 20.472 | 25-Methylgramisterol                                          | 426.3864 | Steroid              |   |   | + |

RT – retention time; ED – *E. deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia*

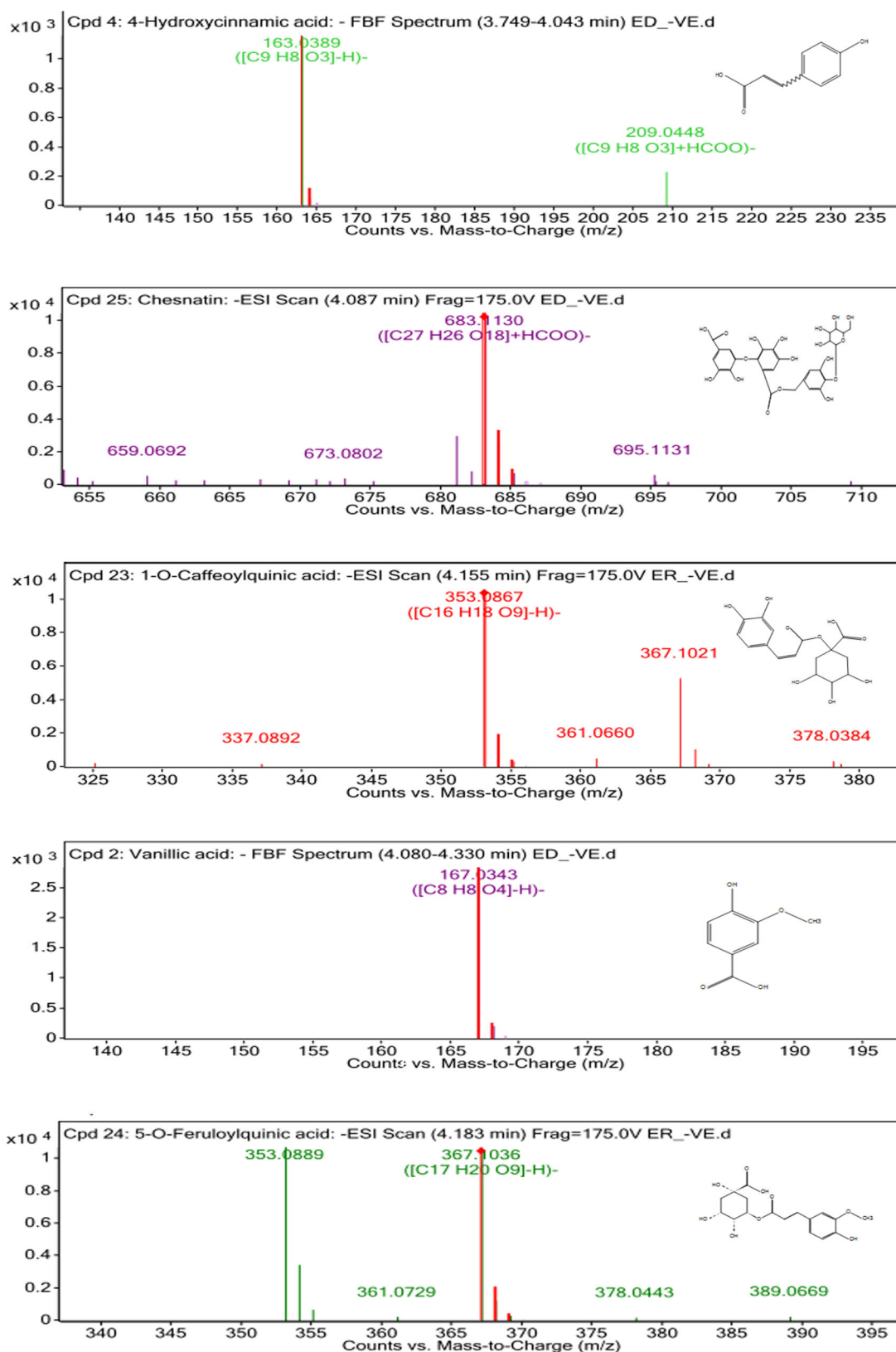


**Fig. 14 (i) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

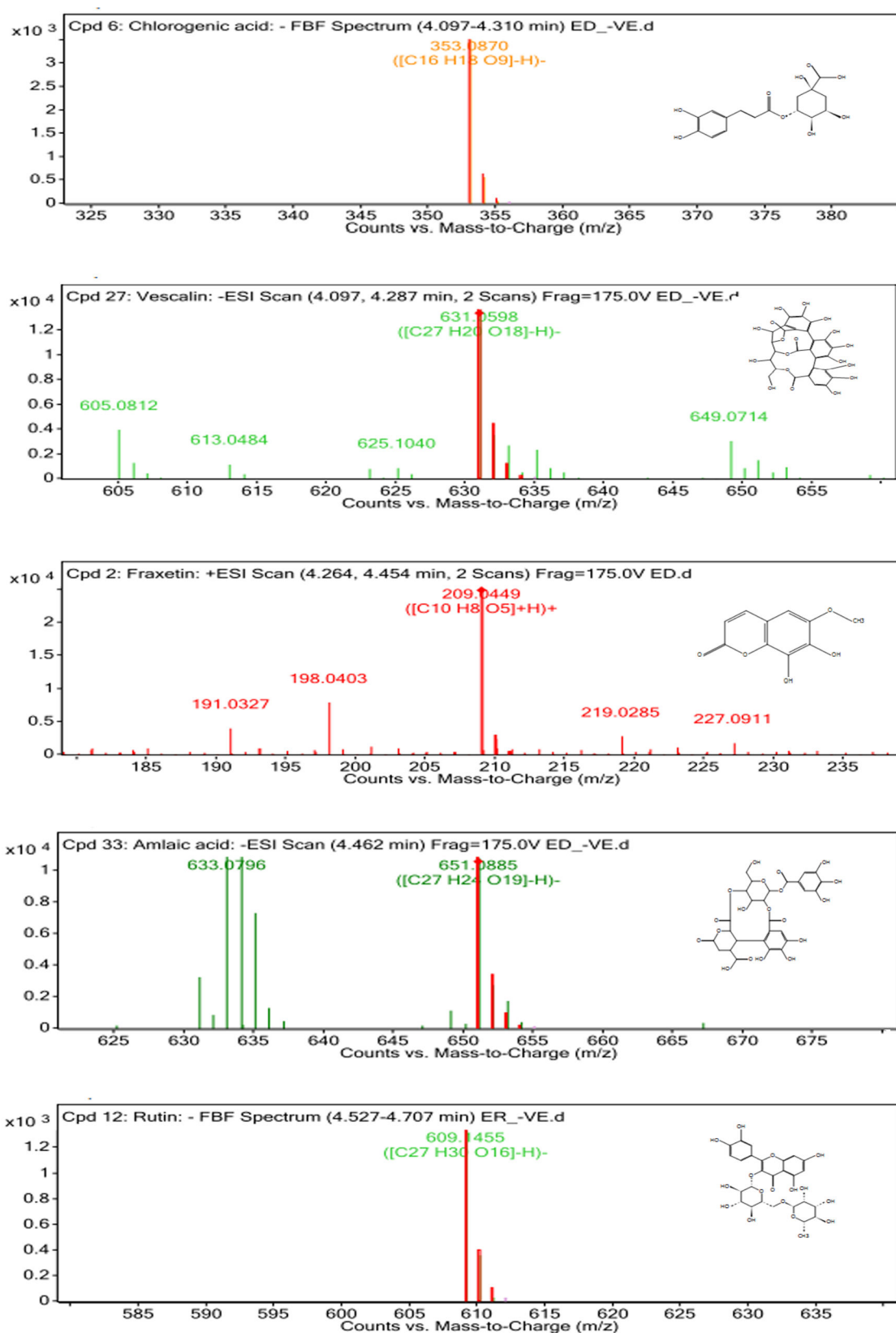


**Fig. 14 (ii) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

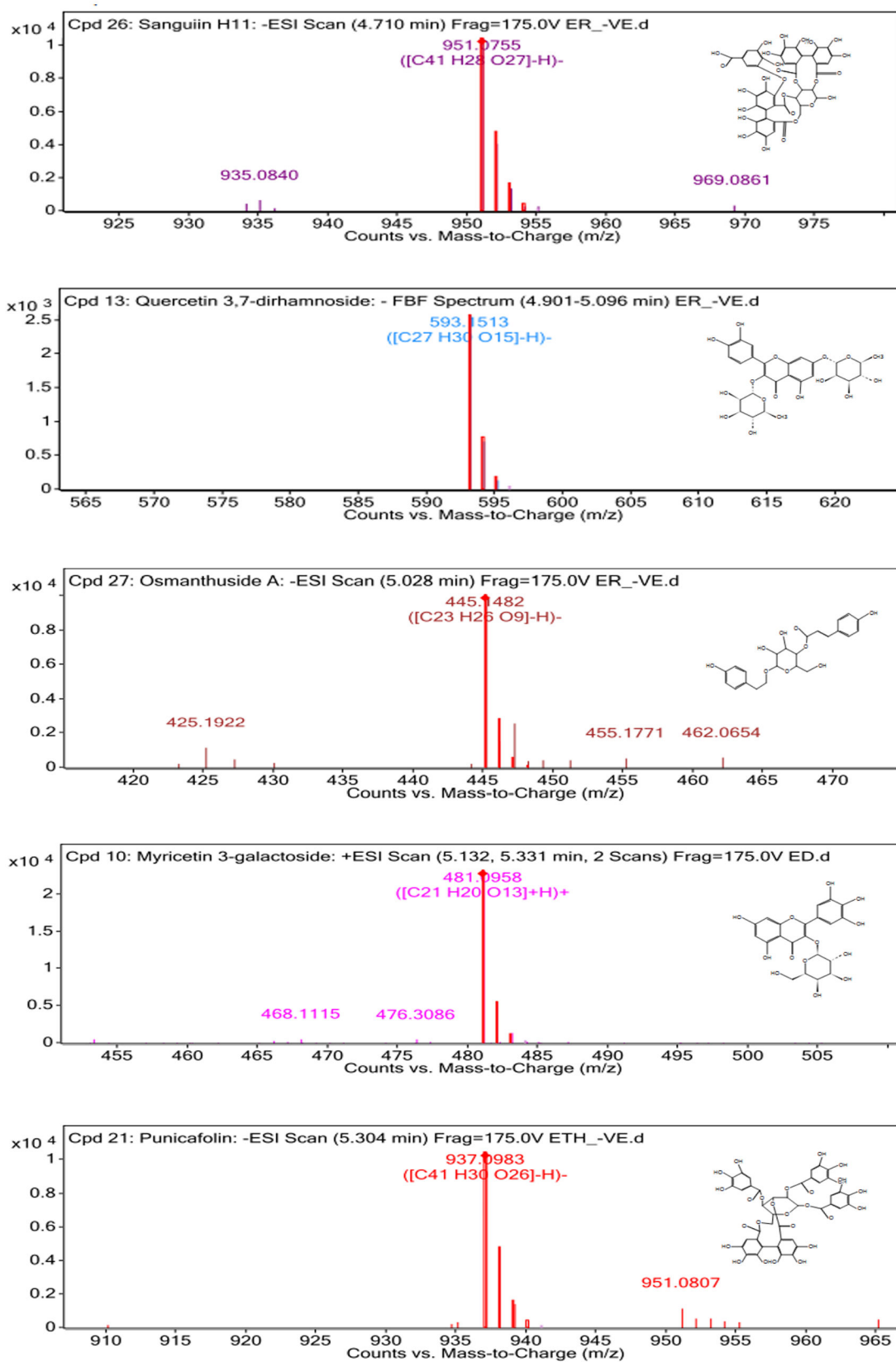




**Fig. 14 (iv) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

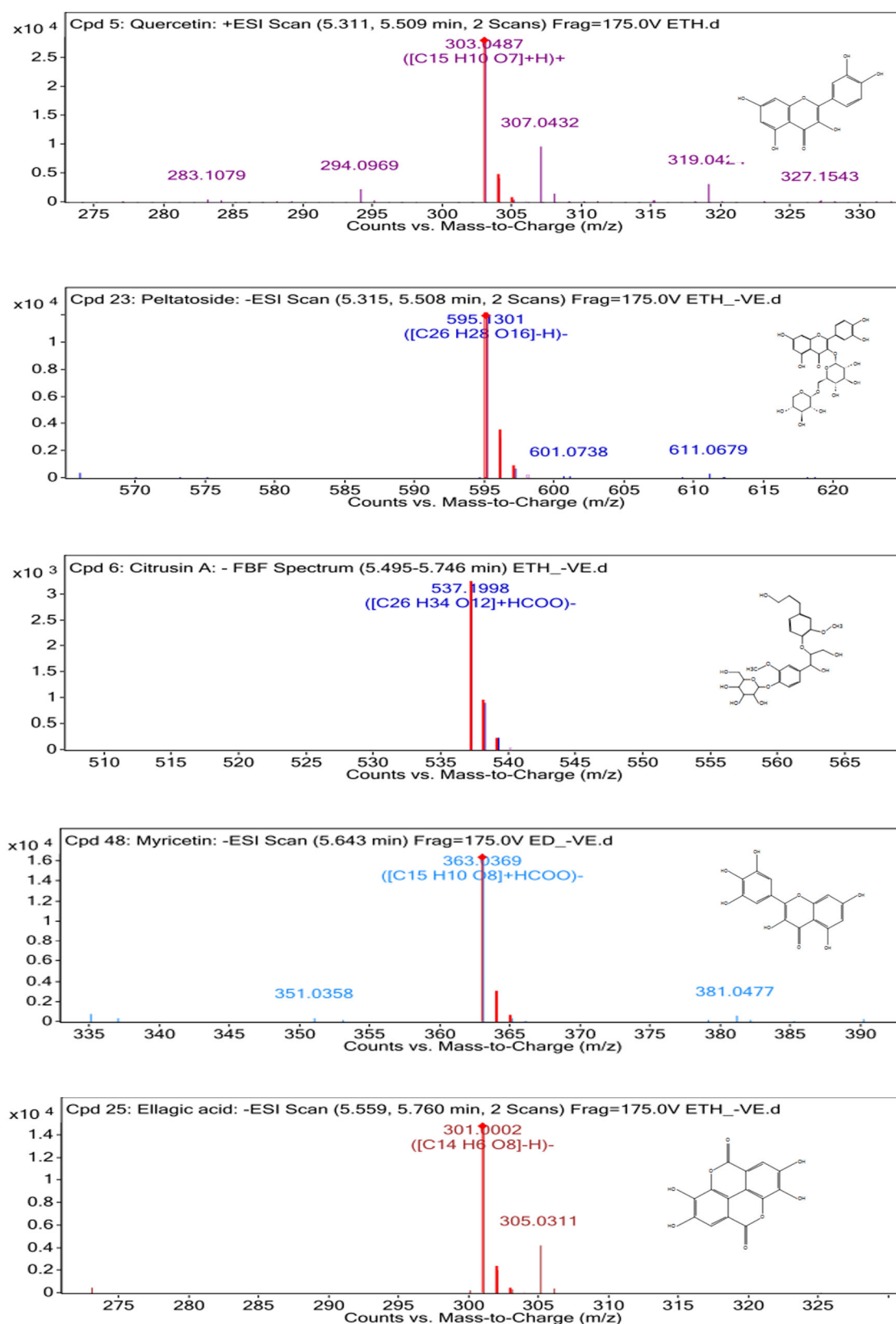


**Fig. 14 (v) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

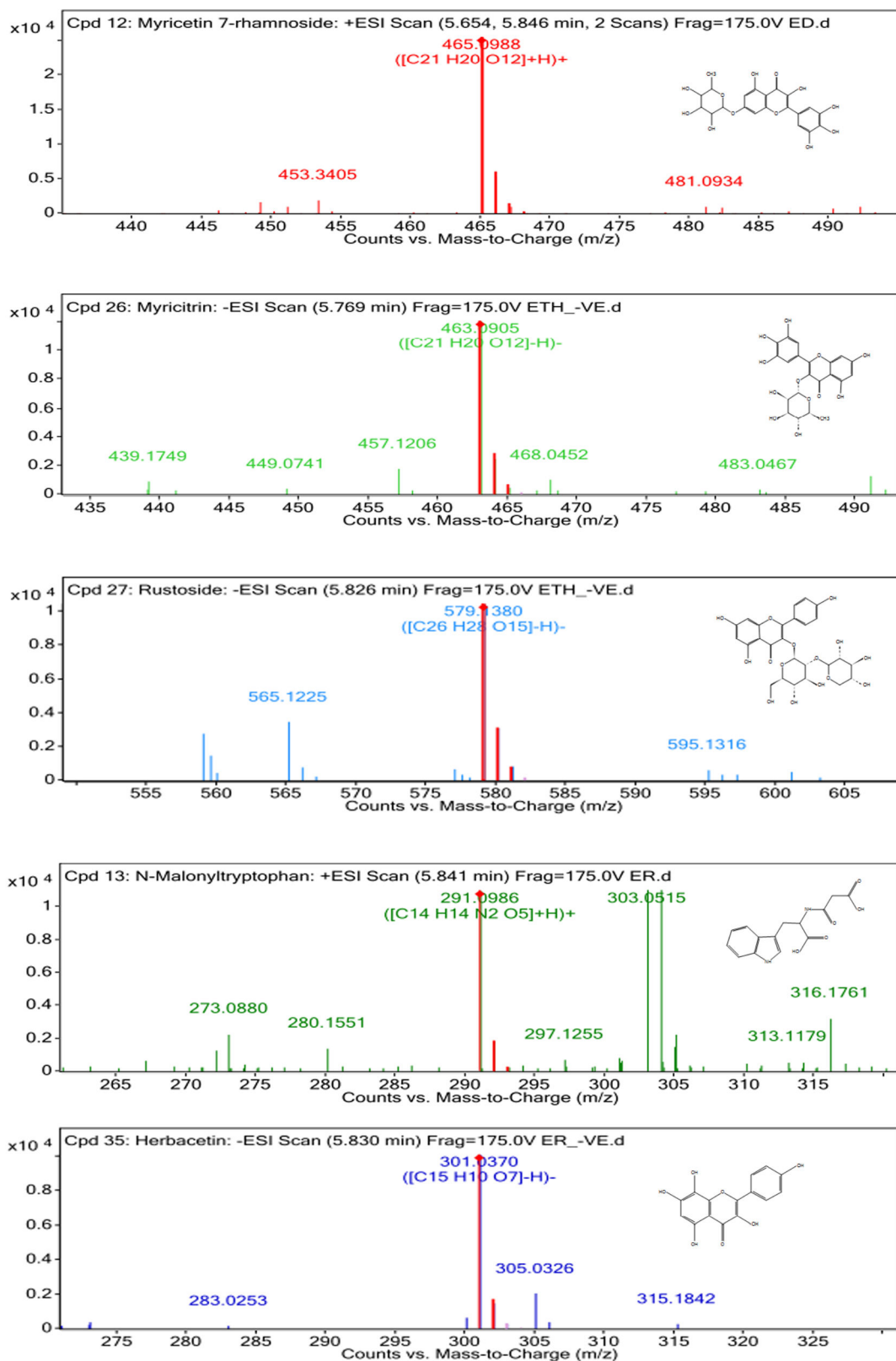


**Fig. 14 (vi)** : Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

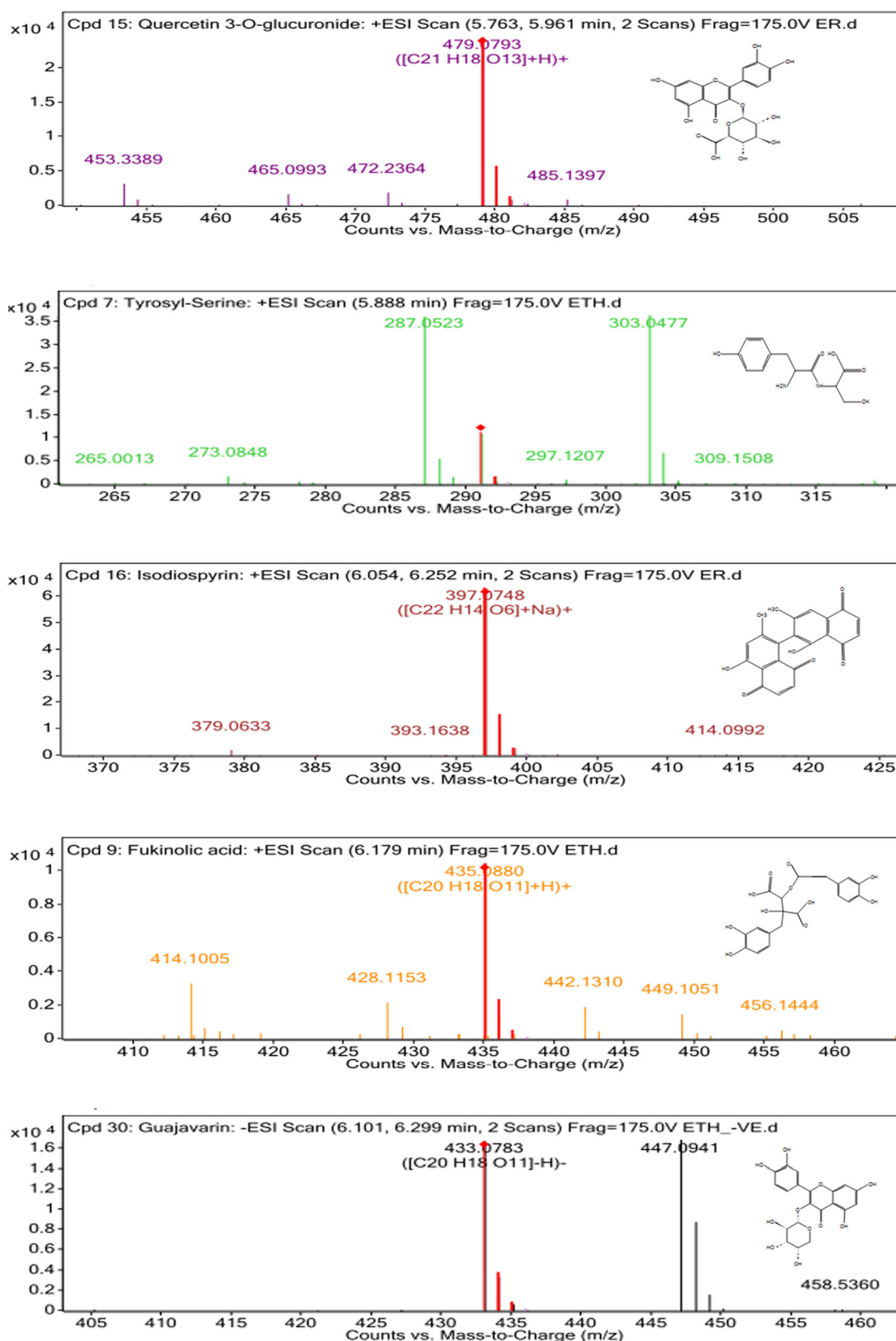




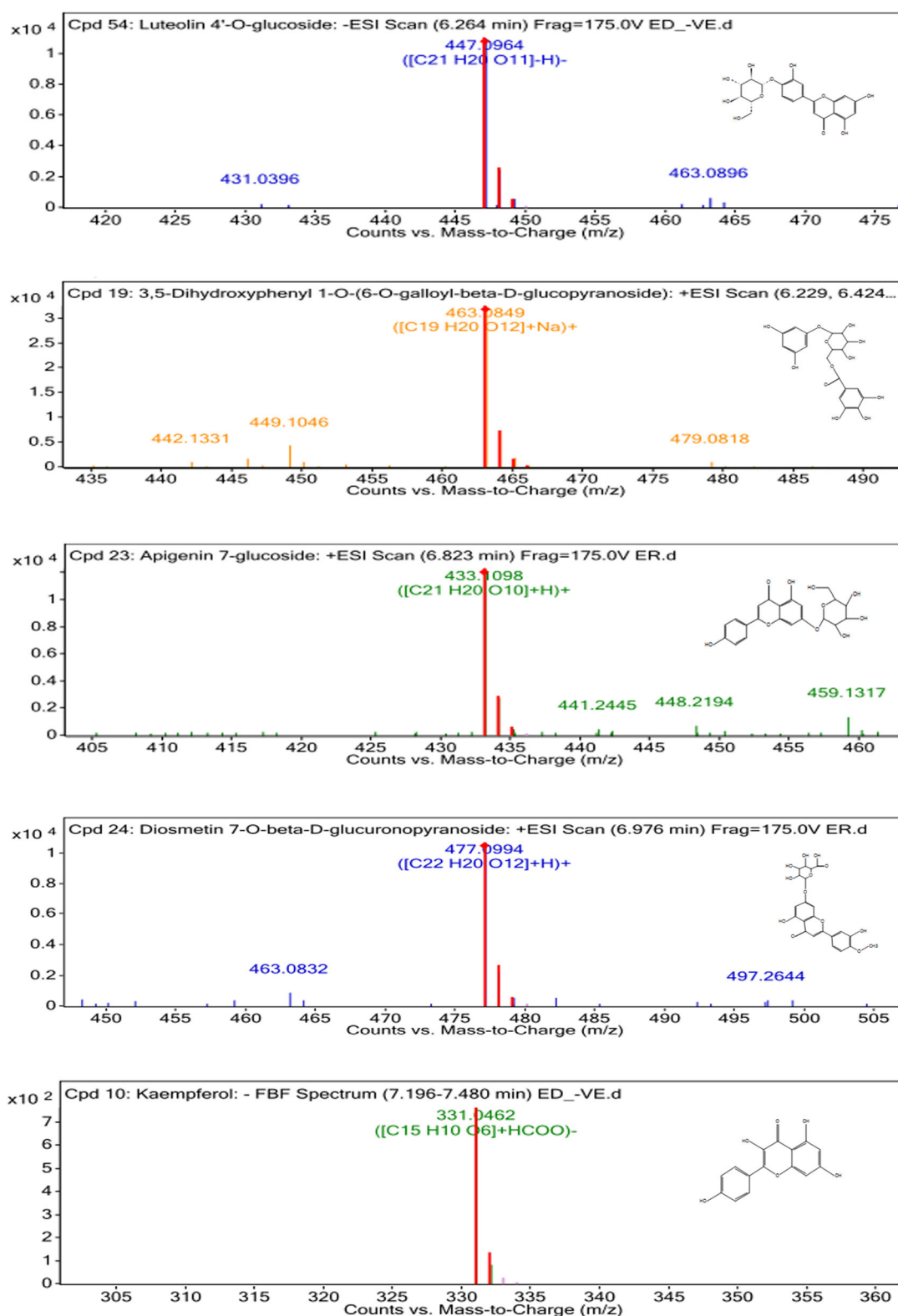
**Fig. 14 (vii) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



**Fig. 14 (viii)** : Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

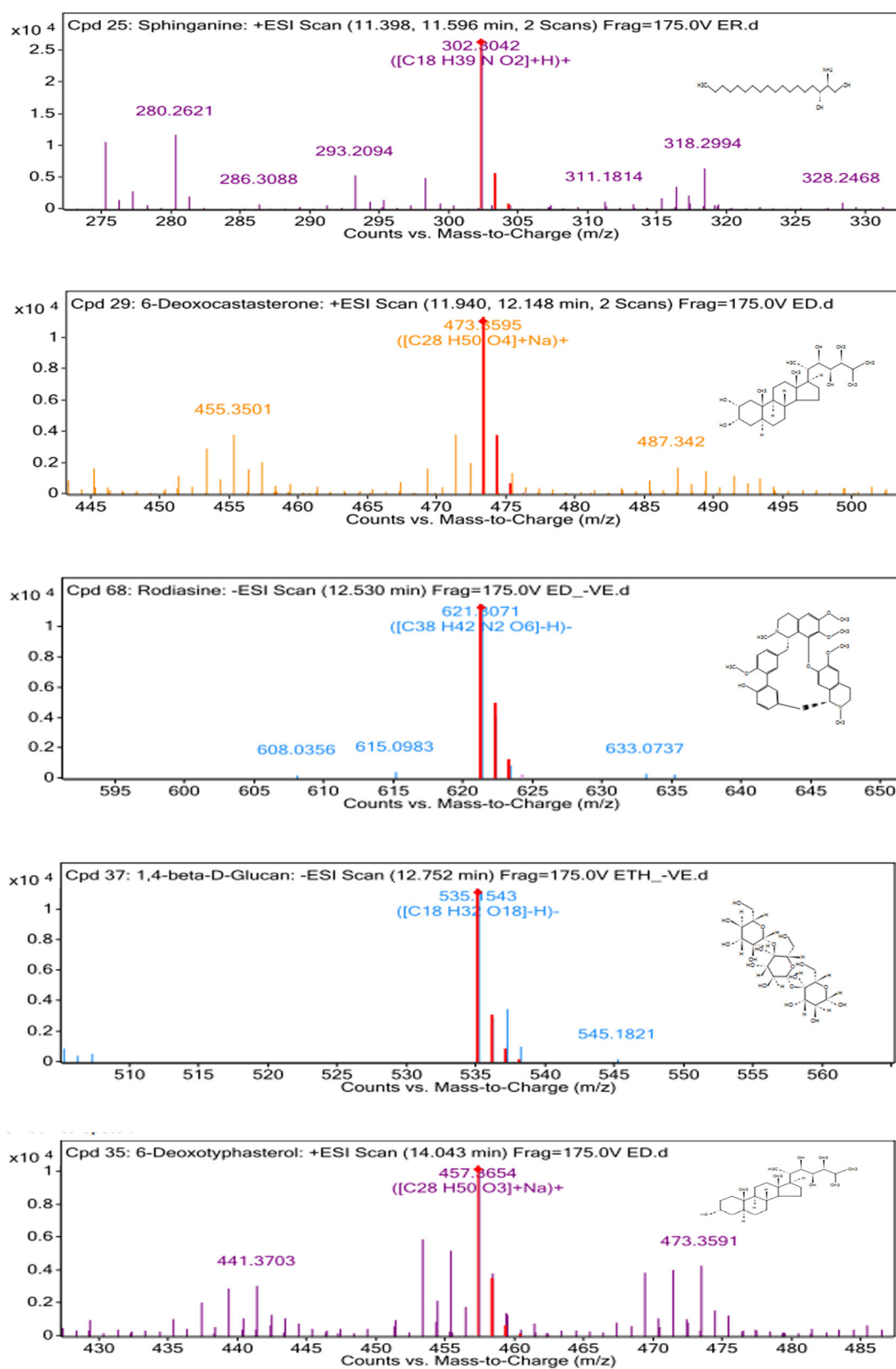


**Fig. 14 (ix) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



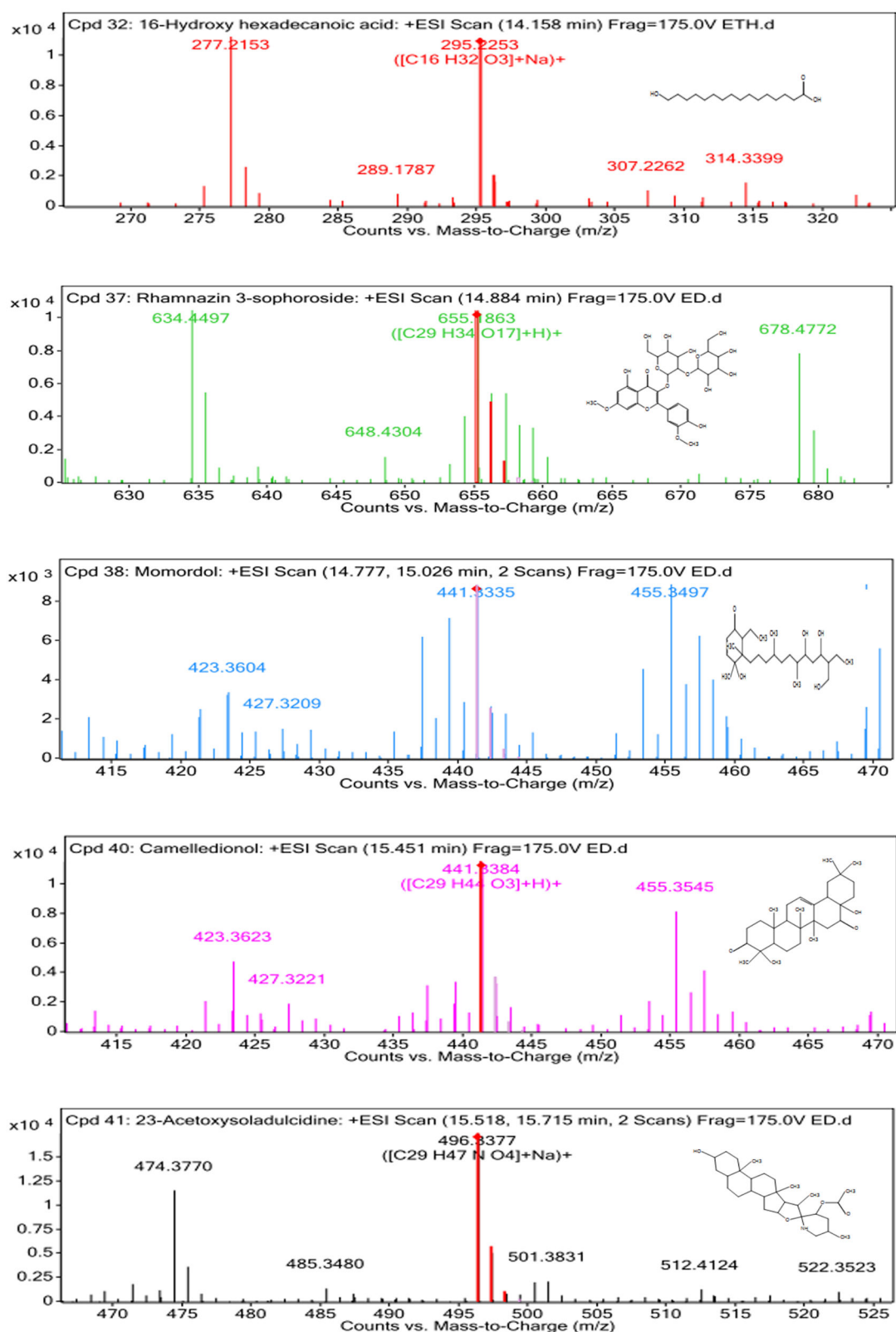
**Fig. 14 (x) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



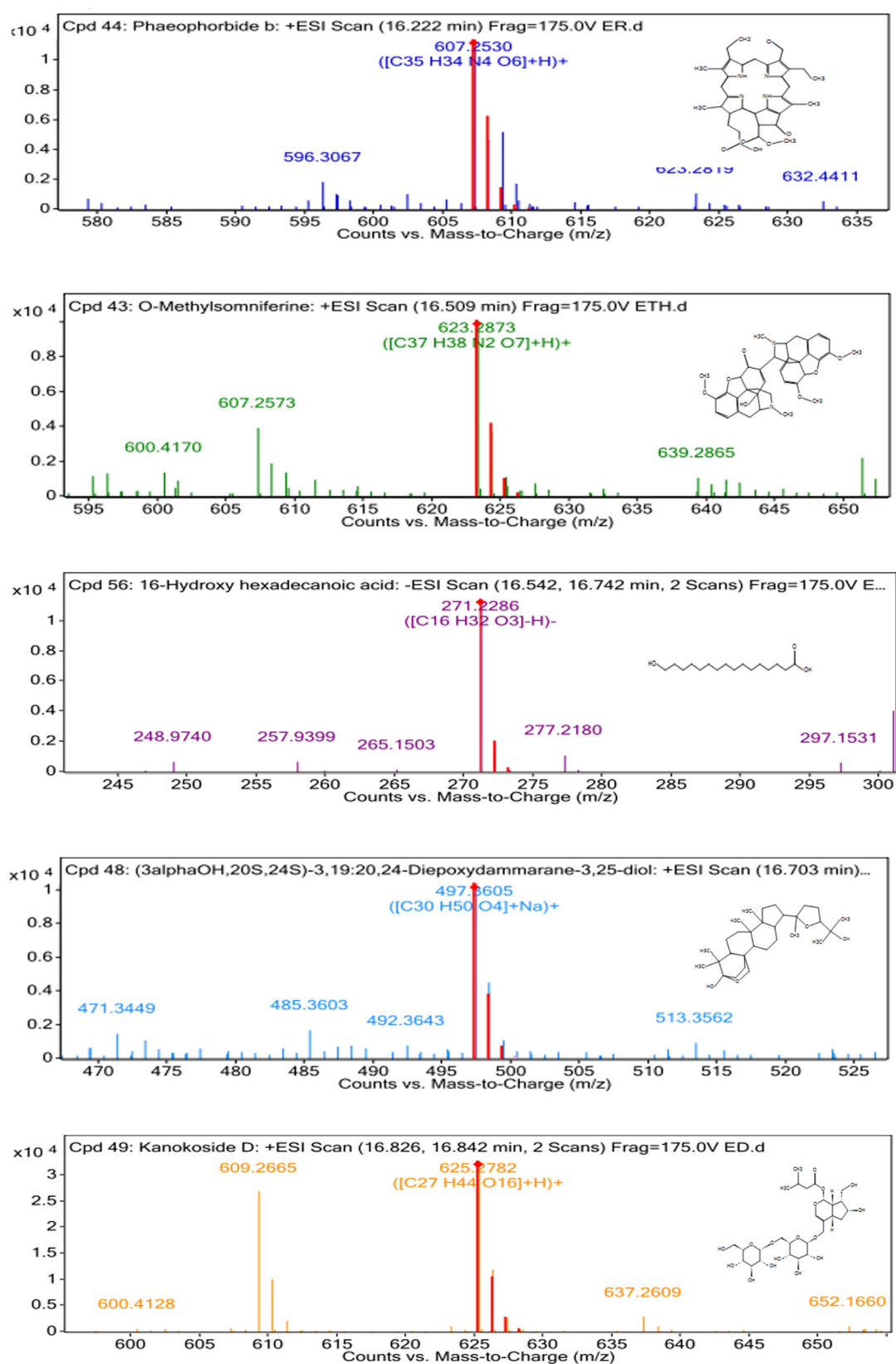


**Fig. 14 (xii) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



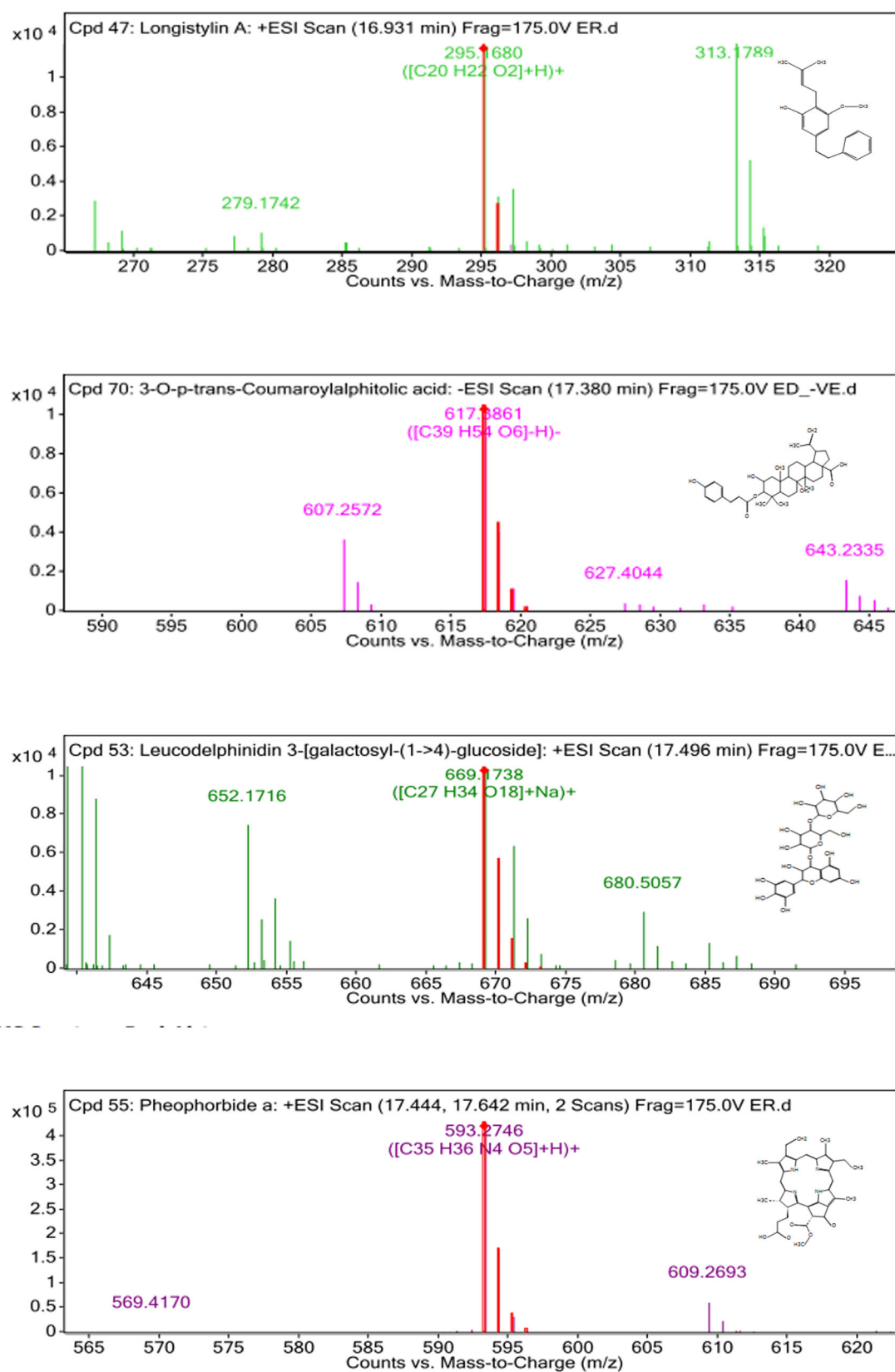


**Fig. 14 (xiii)** : Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

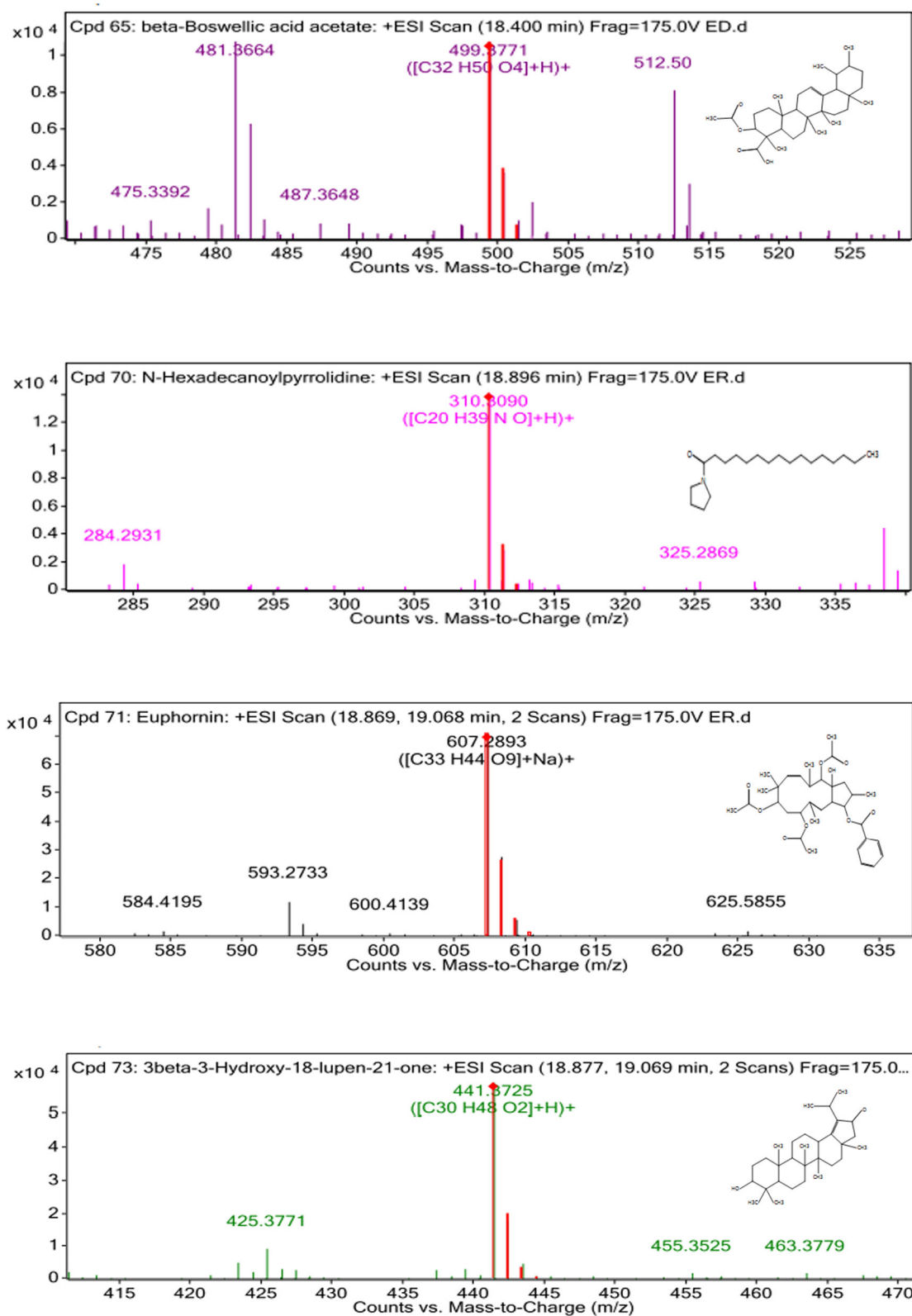


**Fig. 14 (xiv) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*

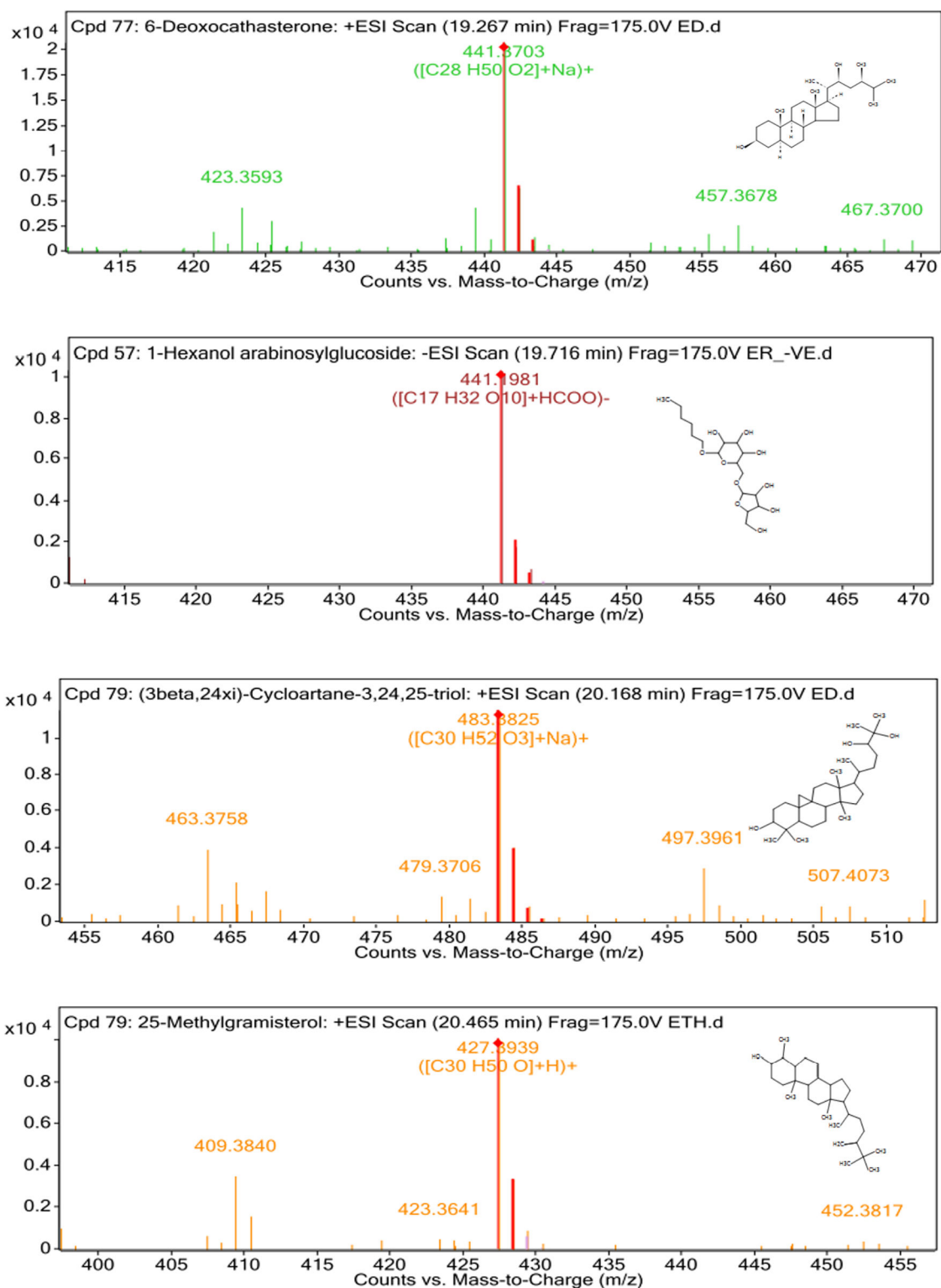




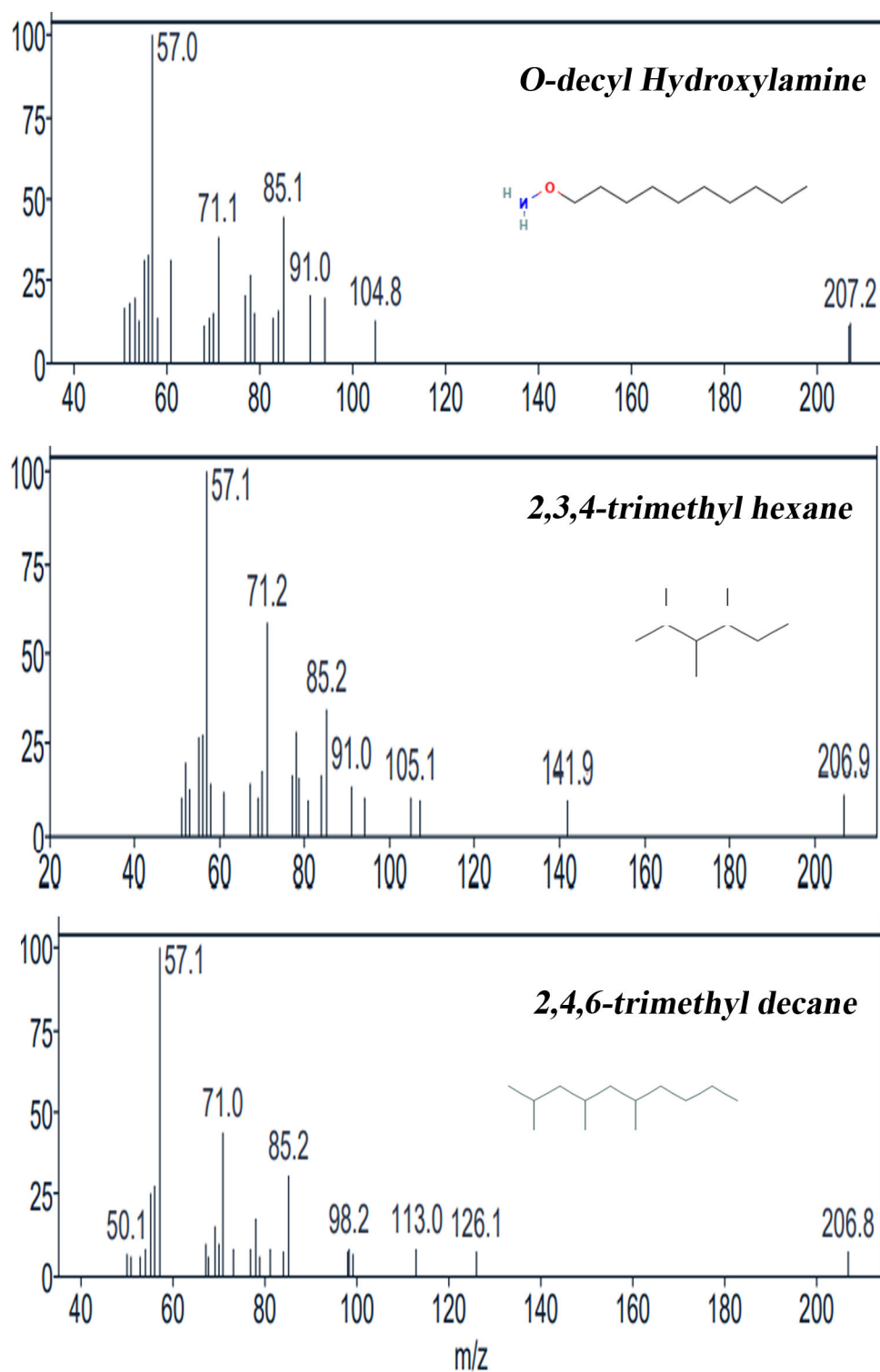
**Fig. 14 (xv) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



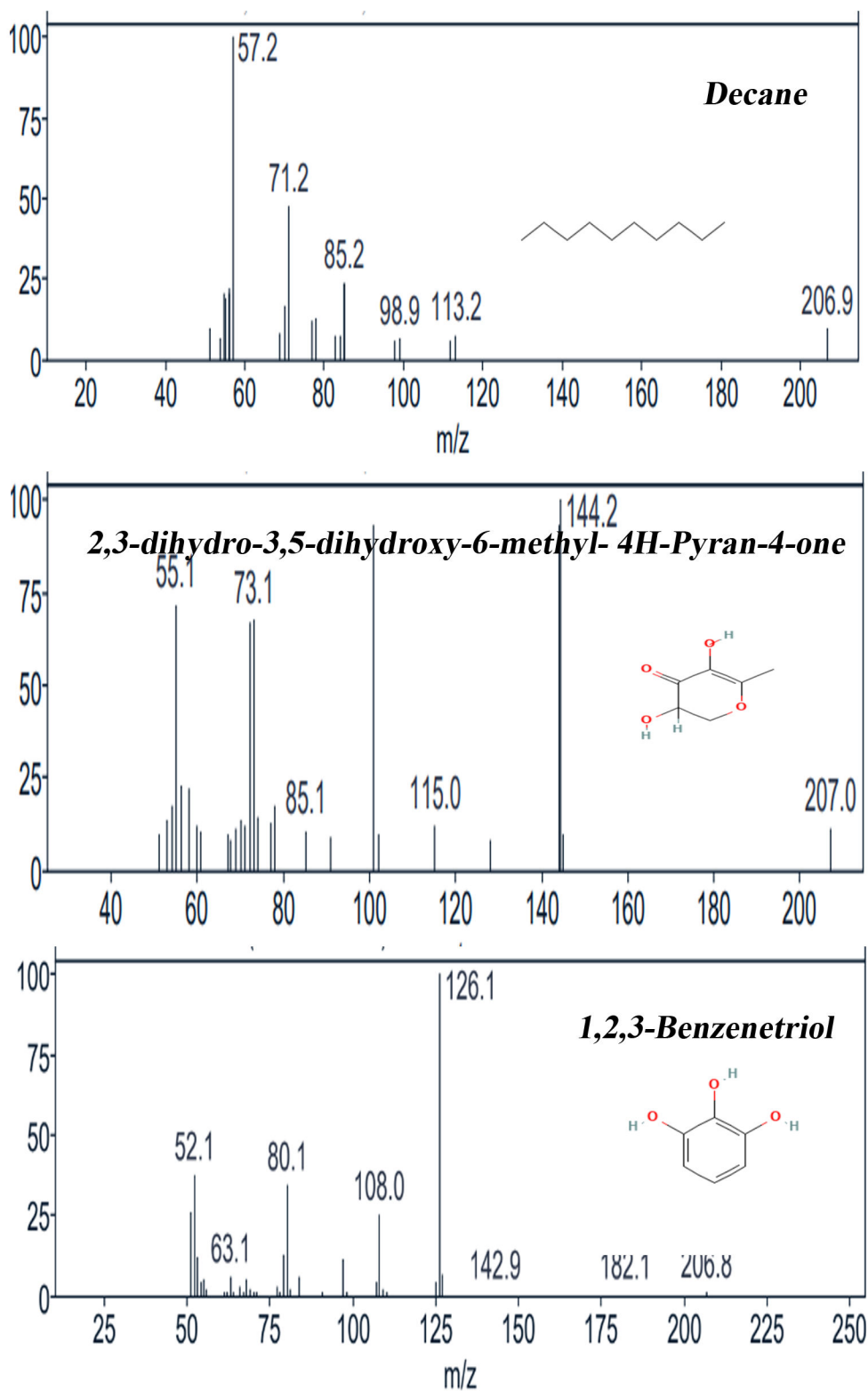
**Fig. 14 (xvi) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



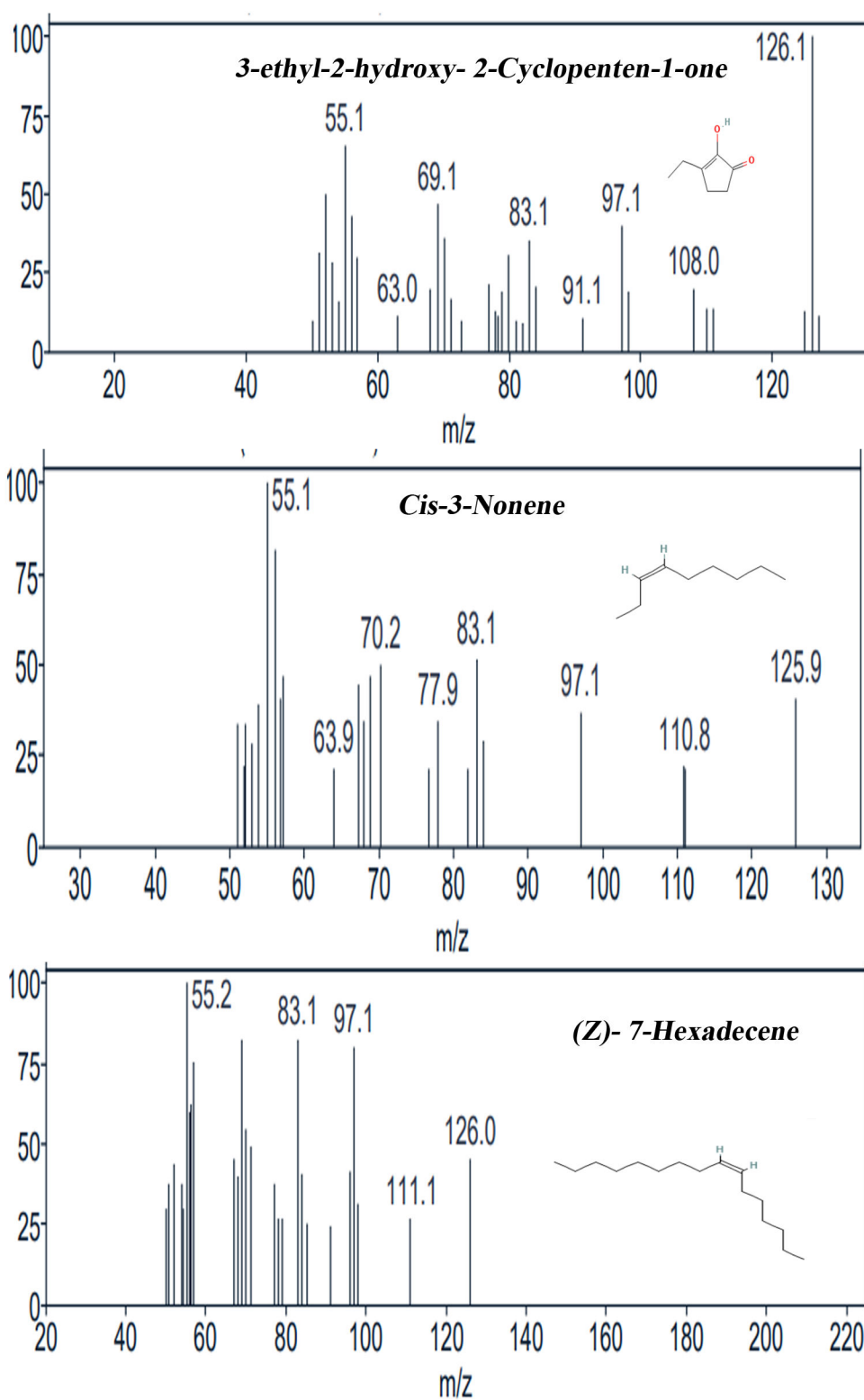
**Fig. 14 (xvii) :** Mass spectra of compounds detected by HR LC-MS analysis in the methanolic extract of selected species of *Euphorbia*



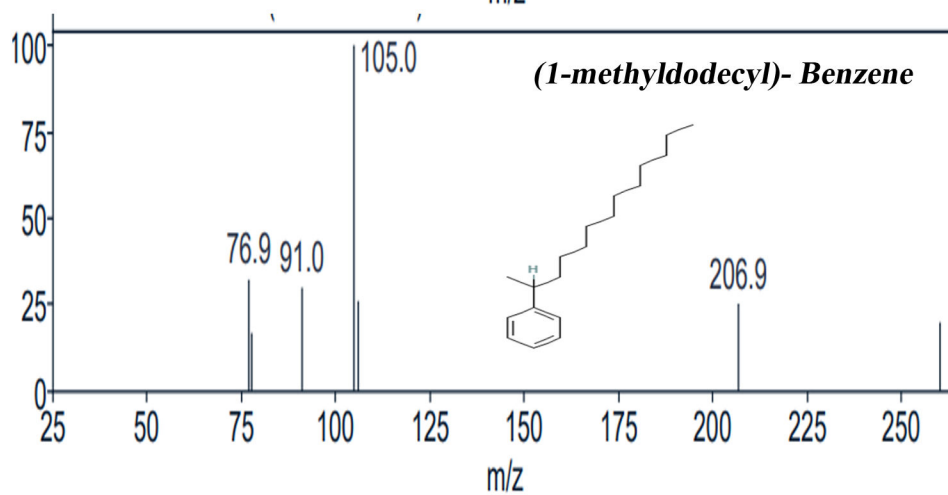
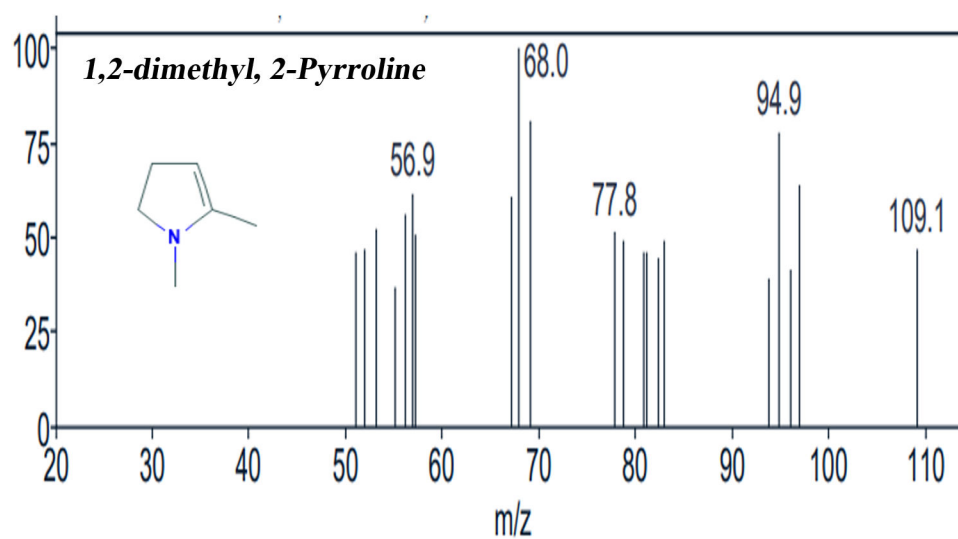
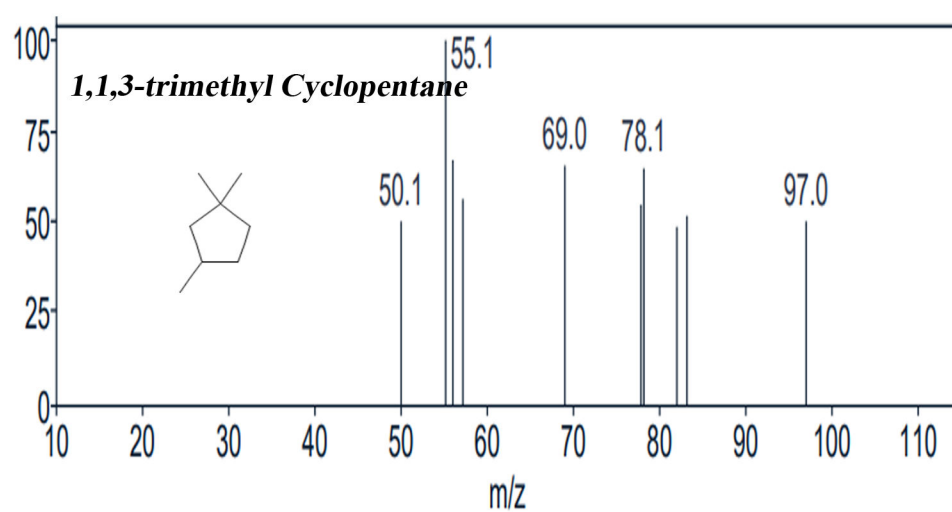
**Fig. 16 (i):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*



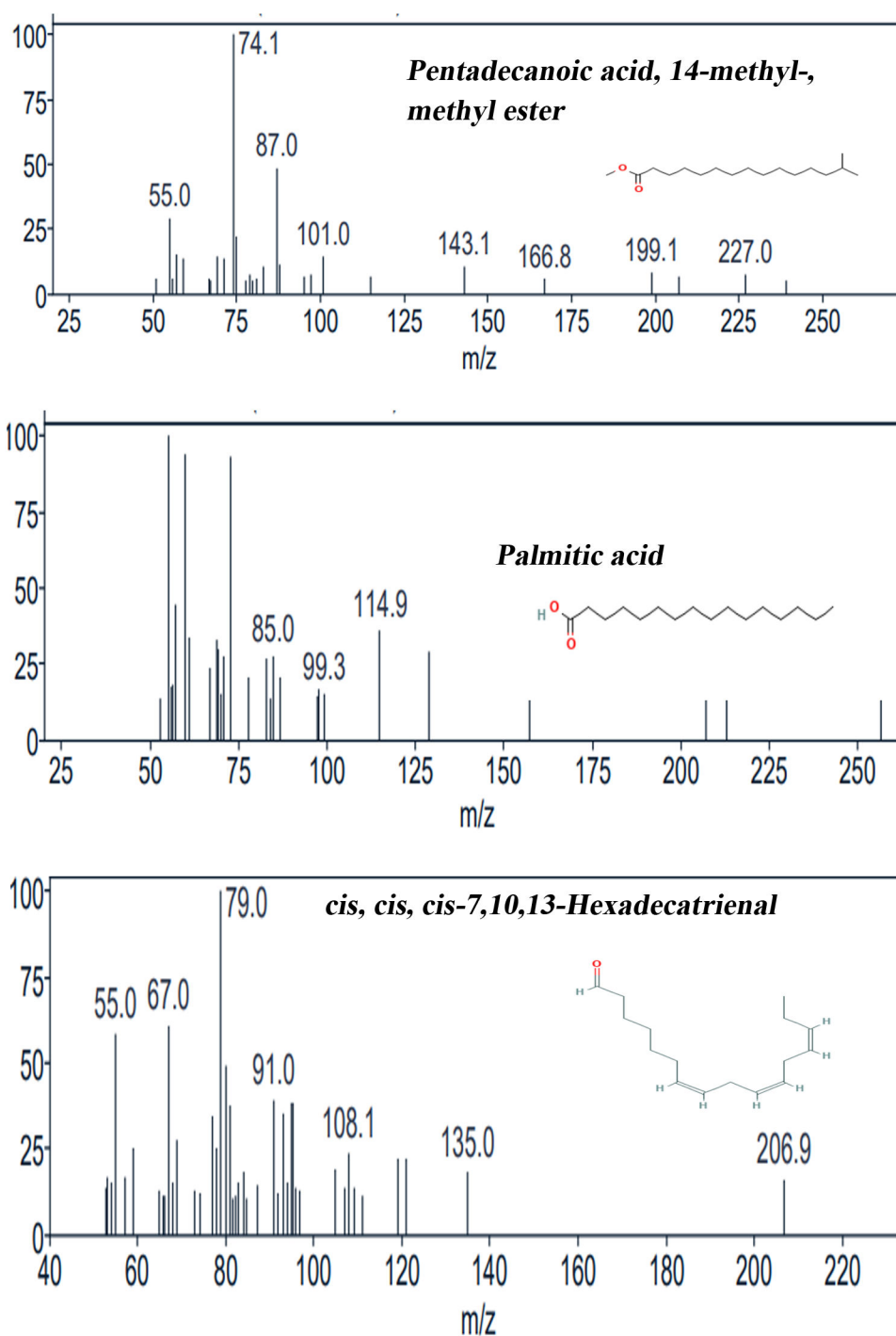
**Fig. 16 (ii):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*



**Fig. 16 (iii):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*

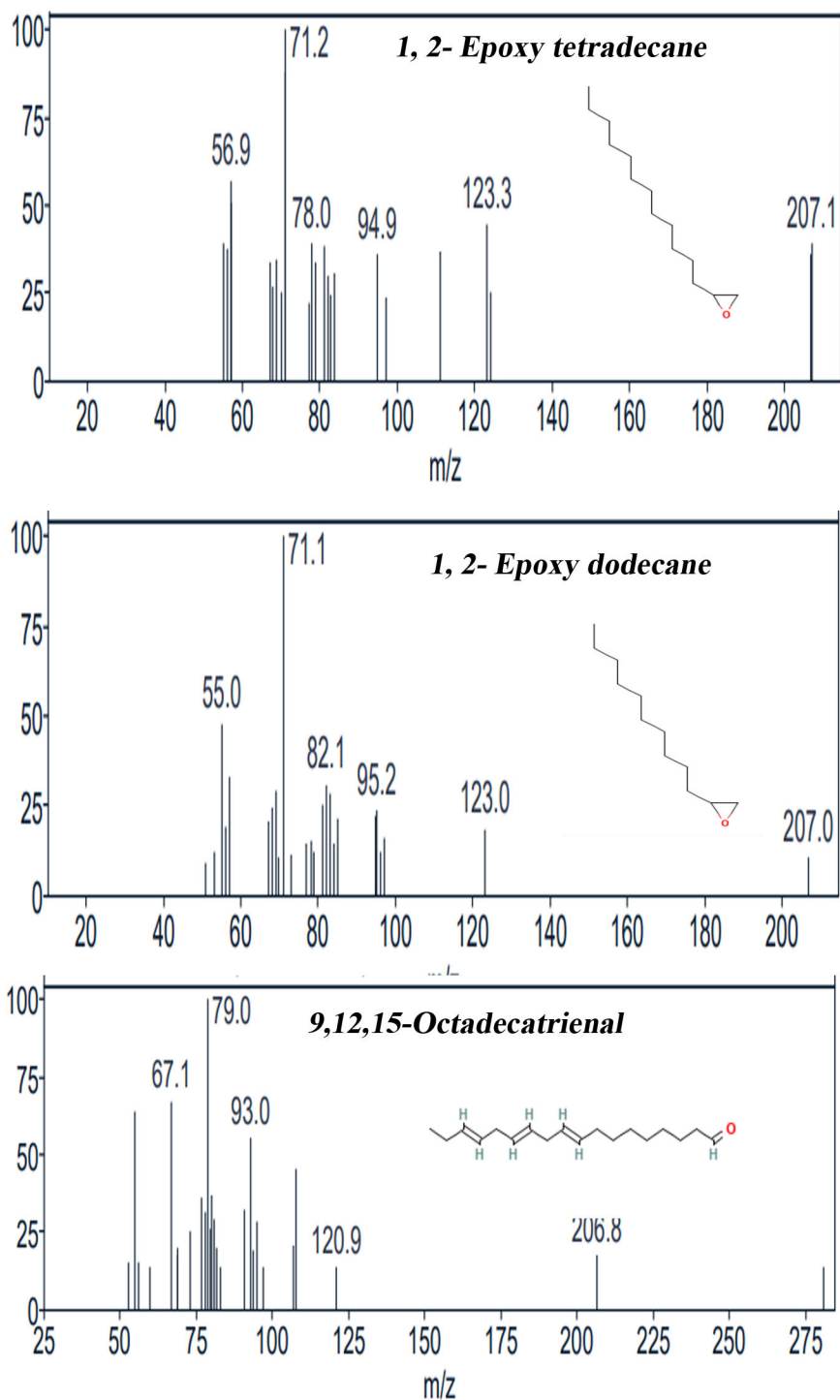


**Fig. 16 (v):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*

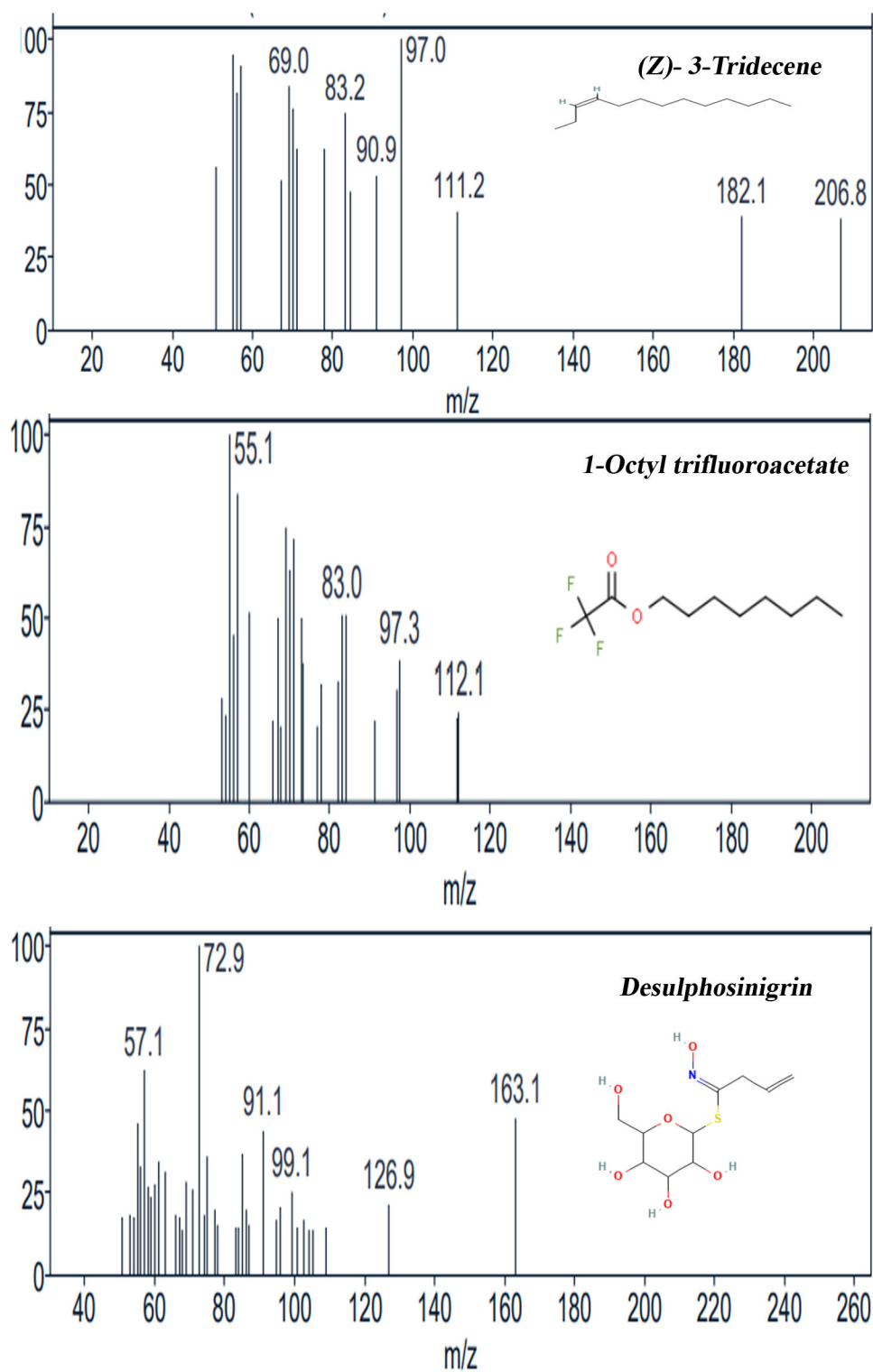


**Fig. 16 (vi):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*

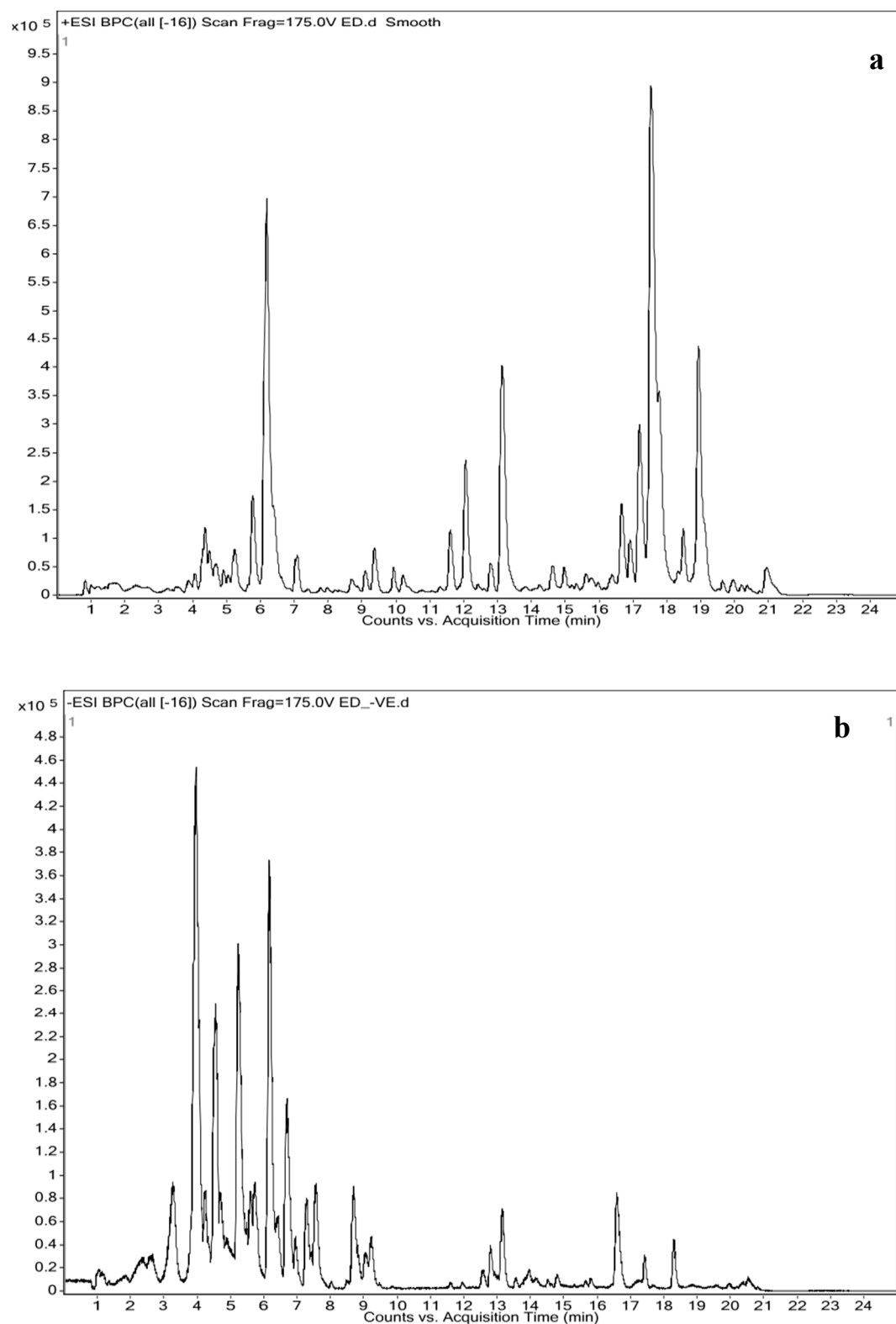




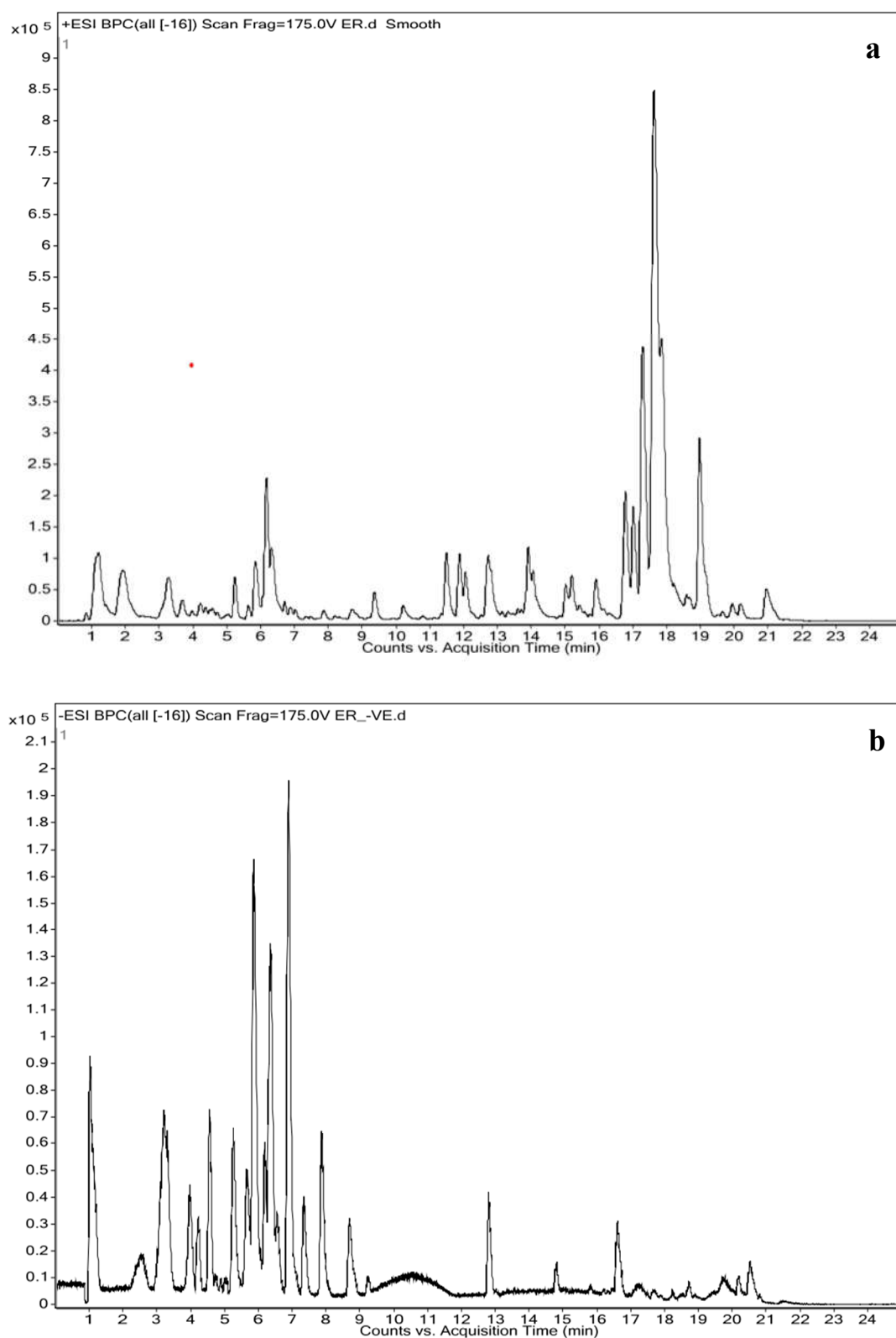
**Fig. 16 (vii):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*



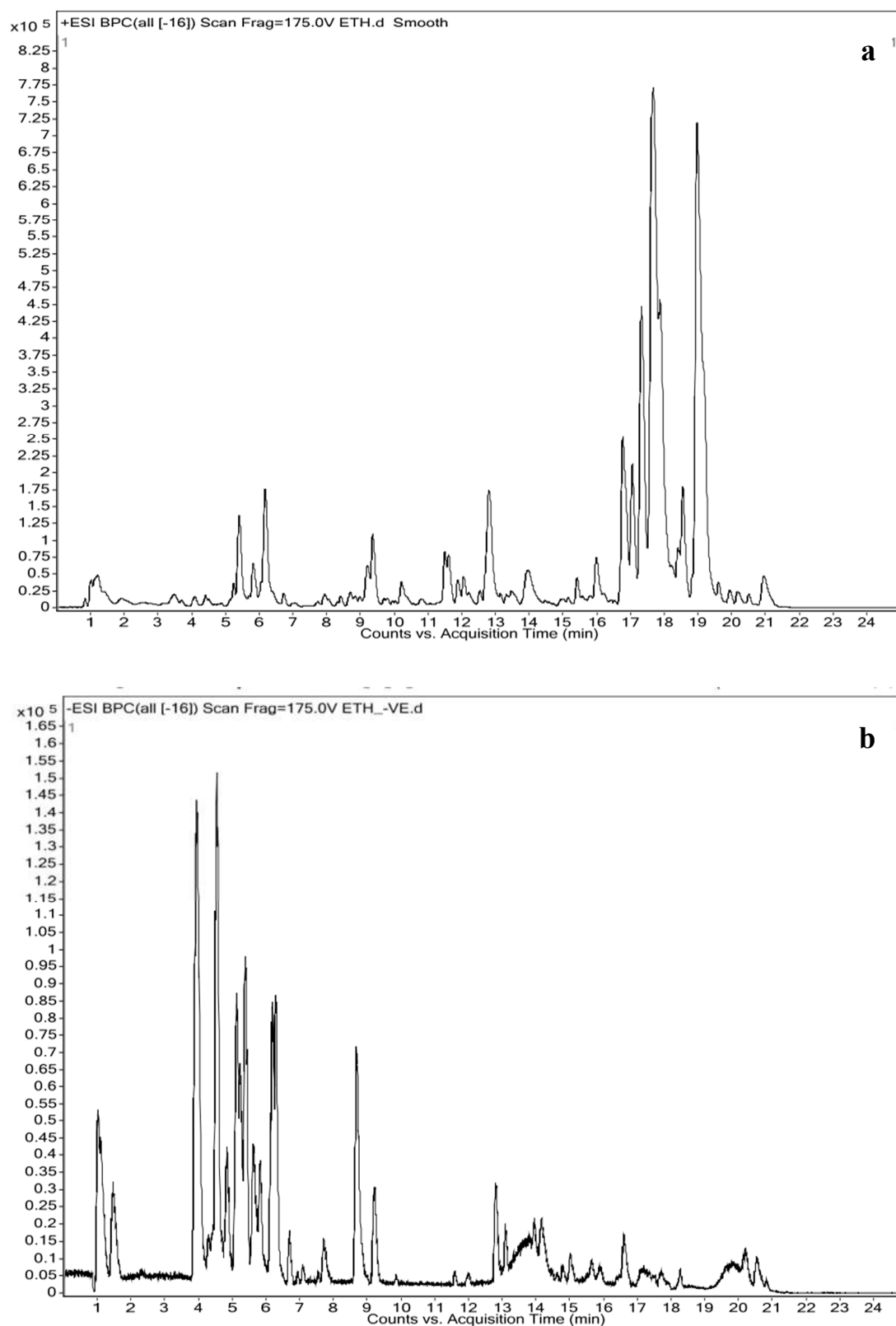
**Fig. 16 (iv):** Mass spectra of compounds detected in the GC-MS analysis of methanolic extract of selected plant species of *Euphorbia*



**Figure. 13 (i):** Liquid chromatogram of methanolic extract of *E. deccanensis* – a) Positive ionisation mode; b) Negative ionization mode



**Figure. 13 (ii):** Liquid chromatogram of methanolic extract of *E. rothiana* – a) Positive ionisation mode; b) Negative ionization mode



**Figure. 13 (iii):** Liquid chromatogram of methanolic extract of *E. thymifolia* – a) Positive ionisation mode; b) Negative ionization mode

#### d) Gas Chromatography-Mass Spectrometry (GC-MS) analysis

To identify the volatile components, present in the chosen species of *Euphorbia*, the GC-MS analysis technique was employed. The phytocompounds were resolved based on the retention time. Peak area, molecular mass, and retention time were used to identify the compounds. Analyses showed that there are about 21 volatile phytoconstituents. The main groups of identified compounds include fatty acids, alkanes, hydrocarbons, and phenols. The eluted compounds, O-decyl hydroxylamine and 9,12,15-octadecatrienal, were the first and last, respectively, within the range of retention times of 4.169 to 30.692. The **Table.7** provides a summary of the phytocompounds investigated through GC-MS analysis.

Six different compounds were altogether eluted in the methanolic extract of *E. deccanensis*. Decane was one of these compounds with the highest content (35.80%). The gas chromatogram for *E. deccanensis* is shown in **Fig. 15(i)**. Similar to this, six compounds were noted in *E. rothiana*. Here, compound 1, 2-epoxytetradecane had the lowest content (8.17%), while O-decyl hydroxylamine had the highest content (24.2%). *E. thymifolia* exhibited the highest number of compounds detected (10), out of the three species taken into consideration. 1,2,3-Benzenetriol, a phenolic compound, was present in significant amounts in this plant species (67.5%).

The only compound found in both the methanolic extracts of *E. deccanensis* and *E. rothiana* is an organooxygen compound called O-decyl hydroxylamine. Its presence was found to be abundant in *E. rothiana* (24.2%), and *E. deccanensis* (15.76%). The three plant species that were picked for the investigation don't have any other common volatile phytocompounds. Consequently, it is evident from the results that there are comparatively lesser volatile phytoconstituents in the methanolic extract of selected *Euphorbia* species.

**Table. 7** Phytocompounds revealed by GC-MS analysis of the selected species of *Euphorbia*

| Sl No. | RT     | Compound                                           | Class                   | Peak area (%) |       |      |
|--------|--------|----------------------------------------------------|-------------------------|---------------|-------|------|
|        |        |                                                    |                         | ED            | ER    | ET   |
| 1      | 4.169  | O-decyl Hydroxylamine                              | Hydrocarbon             | 15.76         | 24.2  |      |
| 2      | 4.181  | 2,3,4-trimethyl hexane                             | Alkane                  |               | 12.47 |      |
| 3      | 6.013  | 2,4,6-trimethyl decane                             | Alkane                  |               |       | 2.8  |
| 4      | 6.025  | Decane                                             | Alkane                  | 35.80         |       |      |
| 5      | 6.975  | 2,3-dihydro-3,5-dihydroxy-6-methyl- 4H-Pyran-4-one | Pyrone                  |               |       | 4    |
| 6      | 12.495 | 1,2,3-Benzenetriol                                 | Phenol                  |               |       | 67.5 |
| 7      | 12.908 | 3-ethyl-2-hydroxy- 2-Cyclopenten-1-one             | Cyclic ketone           |               |       | 2.2  |
| 8      | 12.914 | cis-3-Nonene                                       | Alkane                  | 21.22         |       |      |
| 9      | 12.914 | (Z)- 7-Hexadecene                                  | Hydrocarbon             |               | 17.05 |      |
| 10     | 17.64  | (Z)- 3-Tridecene                                   | Hydrocarbon             | 14.19         |       |      |
| 11     | 17.640 | 1-Octyl trifluoroacetate                           | Organofluorine compound |               | 19.82 |      |
| 12     | 17.859 | Desulphosinigrin                                   | Glucosinolate           |               |       | 2.73 |
| 13     | 22.009 | 1,1,3-trimethyl Cyclopentane                       | Hydrocarbon             | 3.24          |       |      |
| 14     | 23.122 | 1,2-dimethyl, 2-Pyrroline                          |                         |               | 18.24 |      |
| 15     | 25.091 | (1-methyldodecyl)-Benzene                          | Benzene                 | 9.78          |       |      |
| 16     | 25.591 | Pentadecanoic acid, 14-methyl-, methyl ester       | Fatty acid              |               |       | 3.28 |
| 17     | 26.491 | Palmitic acid                                      | Fatty acid              |               |       | 4.64 |
| 18     | 29.917 | cis, cis, cis-7,10,13-Hexadecatrienal              | Aldehyde                |               |       | 5.8  |
| 19     | 30.173 | 1, 2- Epoxytetradecane                             | Epoxide                 |               | 8.17  |      |
| 20     | 30.173 | 1, 2- Epoxydodecane                                | Epoxide                 |               |       | 3.7  |
| 21     | 30.692 | 9,12,15-Octadecatrienal                            | Fatty aldehyde          |               |       | 2.99 |

RT– Retention time; ED – *Euphorbia deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia*

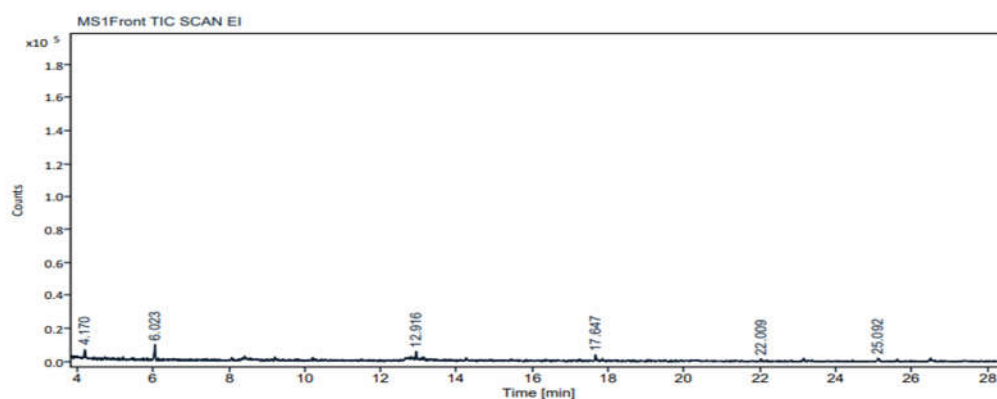


Figure 15 (i): Gas chromatogram of methanolic extract of *E. deccanensis*

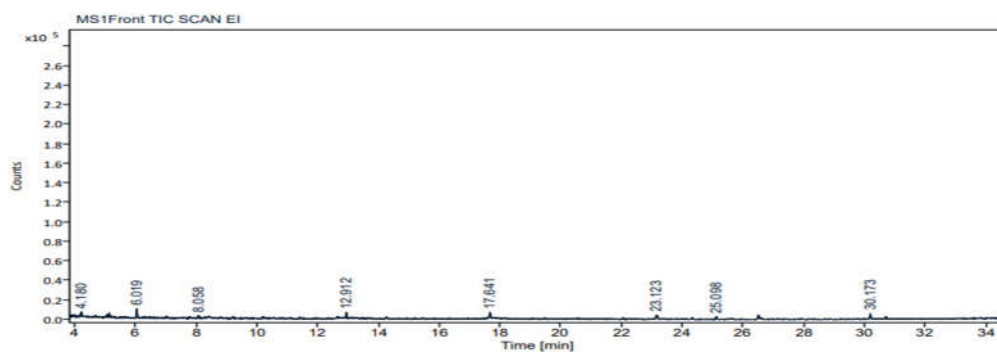


Figure 15 (ii): Gas chromatogram of methanolic extract of *E. rothiana*

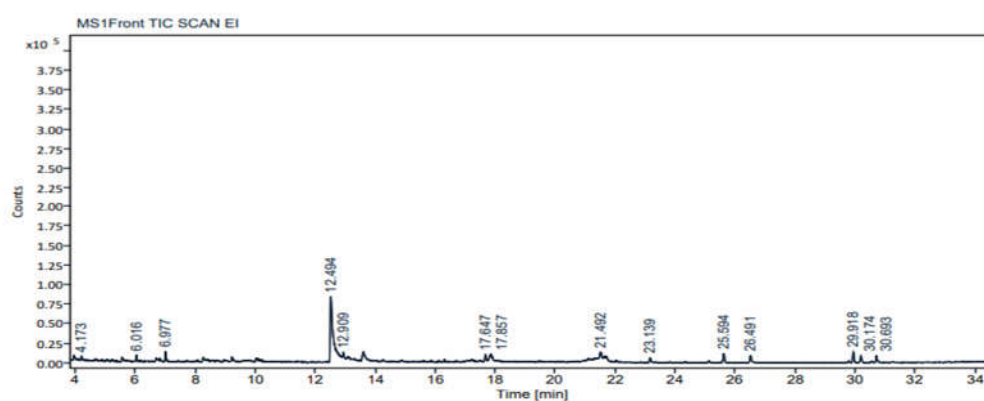


Figure 15 (iii): Gas chromatogram of methanolic extract of *E. thymifolia*



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## PART II- BIOACTIVITY SCREENING

### A. Free radical scavenging activity of selected species of *Euphorbia*

Antioxidants are molecules with the ability to reduce the harm caused by reactive oxygen species and also have the ability to prevent other molecules from oxidising. A wide variety of plants synthesize phytoconstituents naturally, including polyphenolics and flavonoids, which function as antioxidants and also significantly contribute to crucial roles in numerous biological processes. Since free radicals can be inhibited, plants and natural products can serve as a major source of antioxidants. Each antioxidant assay has unique operating procedures and free radical scavenging tenets. The ability of the methanolic extract to inhibit harmful radicals like the DPPH, hydroxyl, superoxide, nitric oxide, and ABTS radicals was evaluated here using the chosen *Euphorbia* species.

#### i) DPPH free radical scavenging activity

To determine whether plant extracts have the ability to scavenge free radicals, one of the widely used and popular free radical scavenging assay is DPPH. In methanol solution, it is violet or purple in colour, but when it is scavenged by an antioxidant, it turns yellow. For this assay, different sample concentrations of 12.5, 25, 50, 100, and 200 µg/ml were prepared. The same concentrations of ascorbic acid, the standard, were also made. The degree of yellow colouration or discolouration indicates that the antioxidants in plant extracts have scavenging potential. The percentage of radical inhibition increased in proportion to the increase in extract concentrations from 12.5 to 200 µg/ml. *E. deccanensis* had the highest inhibition percentage among the chosen species with a range of inhibition percentage of  $21.64 \pm 0.46$  to  $81.17 \pm 0.20$ . At the lowest concentration of 12.5 µg/ml, *E. rothiana* and *E. thymifolia* both displayed the same inhibition percentage of  $20.21 \pm 0.91$  and  $20.21 \pm 0.87$ , respectively. At the highest concentration of 200 µg/ml, they also displayed very similar inhibition percentages of  $72.53 \pm 0.58$  and  $74.77 \pm 0.78$ . The  $IC_{50}$  is a concentration at which 50% of the potential for radical scavenging is reached, and the standard's  $IC_{50}$  value was determined to be  $32.179 \pm 0.86$  µg/ml. The  $IC_{50}$  value for *E. deccanensis* was  $40.739 \pm 0.39$ , which was nearly

very close to the value for the standard. While *E. rothiana*'s IC<sub>50</sub> value was  $45.997 \pm 0.94$  µg/ml, and *E. thymifolia*'s IC<sub>50</sub> value was  $45.0193 \pm 0.71$  µg/ml. These IC<sub>50</sub> values were found to be very close to each other. **Fig. 17** presents the results of the DPPH radical scavenging activity of selected species. The table contains the IC<sub>50</sub> value discovered by the DPPH assay.

When the IC<sub>50</sub> value was taken into account, all of the chosen species' IC<sub>50</sub> values were close to that of the standard, with *E. deccanensis* showing more similar values. Accordingly, all of the chosen plant's methanolic extracts showed a significant ability to scavenge DPPH radicals, with *E. deccanensis* exhibiting particularly strong DPPH quenching activity.

#### ii) Hydroxyl radical scavenging activity

The ability of the methanolic extracts of *E. deccanensis*, *E. rothiana*, and *E. thymifolia* to quench hydroxyl radicals was observed to be in a dose-dependent manner. The inhibition percentage rises in direct proportion to the extract concentration when it goes from 200 µg/ml to 2000 µg/ml. Standard gallic acid showed an inhibition percentage of  $93.07 \pm 0.41$  with an IC<sub>50</sub> value of  $305.93 \pm 2.85$  µg/ml at the highest concentration of 2000 µg/ml. At the highest concentration, all three of the chosen species showed nearly comparable inhibition percentages. When IC<sub>50</sub> values were taken into account, it was discovered that *E. deccanensis*' IC<sub>50</sub> value was relatively close to the value of the standard. Both *E. thymifolia* and *E. rothiana* displayed high IC<sub>50</sub> values. The IC<sub>50</sub> value for *E. thymifolia* was  $619.89 \pm 2.60$  µg/ml, while for *E. rothiana* it was  $405.82 \pm 2.38$  µg/ml. The findings from the hydroxyl radical scavenging activity of each of the chosen species are shown in **Fig. 18**. The analysis's IC<sub>50</sub> result was compiled in **Table 8**. The methanolic extract of *E. deccanensis* was comparable to the standard in its ability to scavenge hydroxyl radicals. Although they were not significantly better than the standard, the other two plant extracts had a moderate level of hydroxyl radical inhibition.

#### iii) Nitric oxide free radical scavenging activity

The ability of plant extract to inhibit nitric oxide radical was determined using the nitric oxide scavenging assay. For the assay, plant extracts at various

concentrations of 125, 250, 500, 1000, and 2000  $\mu\text{g/ml}$ , as well as standard gallic acid at the same concentrations, were taken into consideration. A positive correlation between the concentration of plant extract and the percentage of inhibition in all plant species was noted. The maximum inhibition percentage ( $94.02 \pm 0.93$ ) was found in standard gallic acid, which is significantly higher than the value from the test samples. All three of the test samples were almost equally effective at scavenging nitric oxide radicals at the highest concentration. Similar to the hydroxyl radical assay, inhibition percentages were found to be comparable in three plant species, ranging from  $70.98 \pm 0.45$  to  $79.54 \pm 0.38$ . However, among the test samples, *E. deccanensis* showed the greatest inhibition at a concentration of 2000  $\mu\text{g/ml}$ . All three extracts demonstrated essentially identical  $\text{IC}_{50}$  values. *E. deccanensis*, with a  $410.9 \pm 3.2$   $\mu\text{g/ml}$   $\text{IC}_{50}$  value, however, showed the lowest value among them. The experiment's reference substance, ascorbic acid, had an  $\text{IC}_{50}$  value of  $226.1 \pm 3.1$   $\mu\text{g/ml}$ . Hence, it was found that plant extracts had a significant level of activity based on the inhibition percentage and  $\text{IC}_{50}$  values that were obtained from the analysis. The results of nitric oxide scavenging activity of all the selected species are given in **Fig. 19** and  $\text{IC}_{50}$  values obtained for each plant extract are tabulated in **Table 8**.

#### iv) Superoxide free radical scavenging activity

All the chosen methanolic extracts of chosen *Euphorbia* species demonstrated concentration-dependent antioxidant activity in the superoxide scavenging assay. Inhibition of free radicals was greatly increased from low to higher concentrations. The highest percentage of free radicals were scavenged by *E. deccanensis*, which displayed a percentage of inhibition ranging from  $24.95 \pm 0.33$  to  $80.59 \pm 0.67$ . Nearly equal percentages of inhibition were shown by *E. rothiana* and *E. thymifolia* from  $20.02 \pm 0.84$  to  $73.68 \pm 0.46$  and  $17.26 \pm 0.244$  to  $75.11 \pm 0.45$  respectively.

The results of superoxide radical scavenging activity are shown in **Fig. 20**. The  $\text{IC}_{50}$  value obtained for each test sample along with the standard was tabulated in **Table 8**. The  $\text{IC}_{50}$  value for the reference ascorbic acid was  $344.42 \pm 1.2$   $\mu\text{g/ml}$ .

*E. deccanensis*, one of the chosen species, had a lower IC<sub>50</sub> value ( $409.11 \pm 1.5$  µg/ml) than the other two, demonstrating significant superoxide radical scavenging activity. An IC<sub>50</sub> value of  $780.05 \pm 2.7$  µg/ml was observed for *E. rothiana*, demonstrating its moderate superoxide radical scavenging ability.

#### V) ABTS free radical scavenging activity

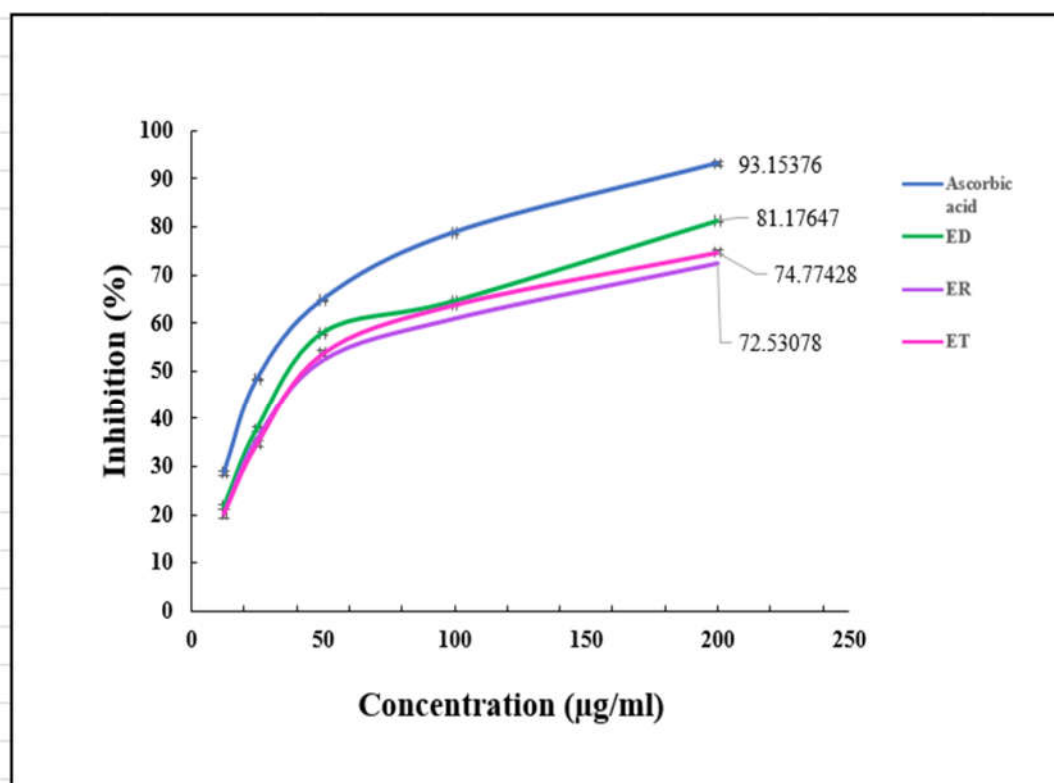
The scavenging of ABTS radicals by the antioxidants present in the test samples was examined in the ABTS free radical scavenging assay. For this, different concentrations of methanolic plant extract (125, 250, 500, 1000, and 2000 µg/ml) as well as standards were used. The IC<sub>50</sub> values for each plant extract were clearly visible and each one demonstrated significant scavenging activity (**Table. 8**). The IC<sub>50</sub> value for the reference ascorbic acid was  $226.83 \pm 0.55$  µg/ml. When compared to other test samples, *E. deccanensis* had the lowest IC<sub>50</sub> value ( $405.99 \pm 0.62$  µg/ml). Additionally, the same plant extract of *E. deccanensis* showed the highest level of inhibition. The other two had nearly equal ranges of inhibition on ABTS radicals as revealed by *E. rothiana* ( $62.22 \pm 0.875$ ) and *E. thymifolia* ( $64.41 \pm 0.38$ ) respectively. The inhibition percentage and IC<sub>50</sub> values of the plant extracts were not higher than the standard results. However, in the case of *E. deccanensis*, it demonstrated significant activity. Hence, the activity against ABTS radical in the other two species appears to be moderate.

**Table 8: Inhibition potential of chosen *Euphorbia* in antioxidant assays**

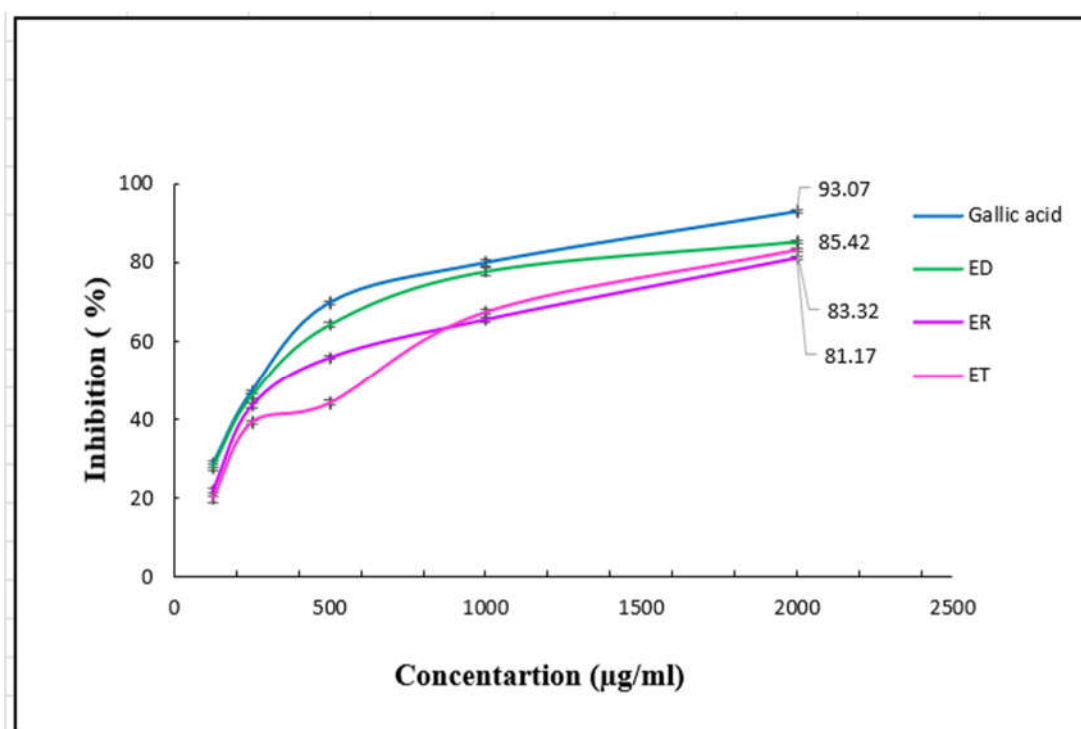
| Plant                 | IC <sub>50</sub> Value (µg/ml)        |                                      |                                        |                                  |                                  |
|-----------------------|---------------------------------------|--------------------------------------|----------------------------------------|----------------------------------|----------------------------------|
|                       | DPPH free radical scavenging activity | Hydroxyl radical scavenging activity | Superoxide radical scavenging activity | Nitric oxide scavenging activity | ABTS radical scavenging activity |
| <i>E. deccanensis</i> | $40.739 \pm 0.39$                     | $334.63 \pm 2.5$                     | $409.11 \pm 1.5$                       | $410.9 \pm 3.2$                  | $405.99 \pm 0.62$                |
| <i>E. rothiana</i>    | $45.997 \pm 0.94$                     | $405.82 \pm 2.38$                    | $780.05 \pm 2.7$                       | $470.32 \pm 1.4$                 | $965.31 \pm 0.69$                |
| <i>E. thymifolia</i>  | $45.0193 \pm 0.71$                    | $619.89 \pm 2.60$                    | $674.53 \pm 2.07$                      | $463.08 \pm 2.8$                 | $885.9 \pm 0.694$                |
| Standard              | $32.179 \pm 0.86$                     | $305.93 \pm 2.85$                    | $344.42 \pm 1.2$                       | $226.1 \pm 3.1$                  | $226.83 \pm 0.55$                |

Thus, the findings revealed the radical scavenging potential of methanolic extracts of selected species. Among the three species tested, *E. deccanensis* had the

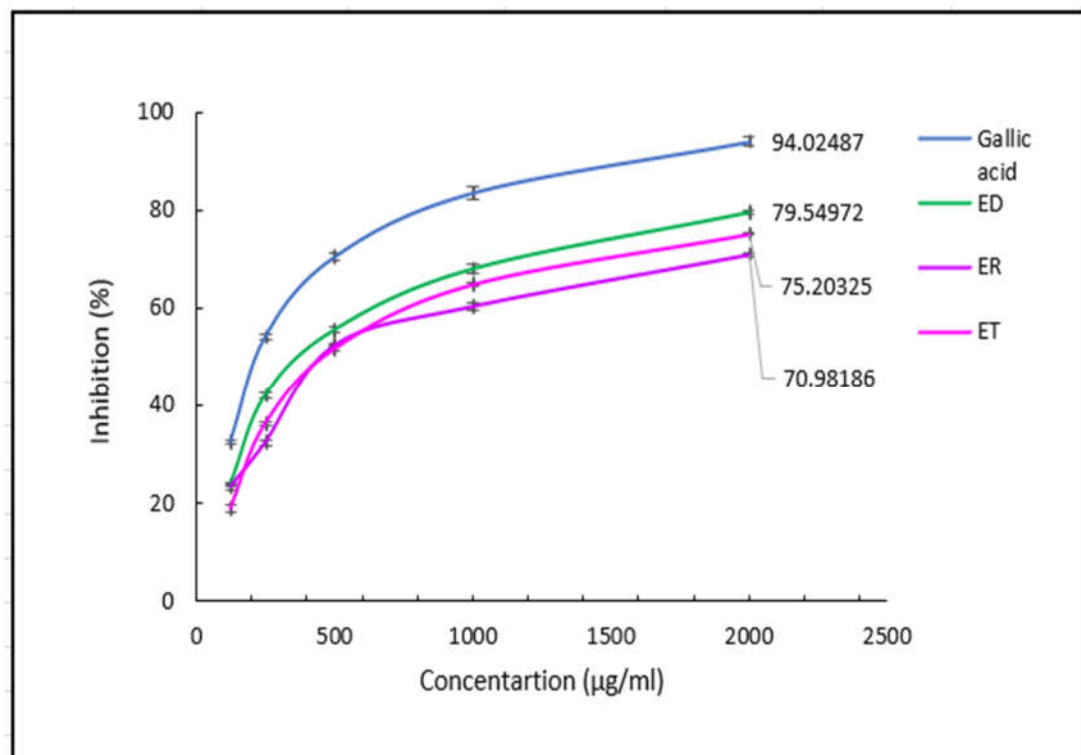
highest scavenging potential against all of the radicals tested, particularly DPPH, superoxide and hydroxyl radicals. In these cases, *E. deccanensis* had nearly the close  $IC_{50}$  value as the standard. Both *E. rothiana* and *E. thymifolia* had moderate scavenging effects in inhibiting free radicals. In the DPPH and hydroxyl radical assays, *E. rothiana* outperformed *E. thymifolia*. In the case of superoxide and ABTS assays, *E. thymifolia* showed better activity than *E. rothiana*. In the nitric oxide scavenging assay, all of the selected species had nearly equal inhibition on nitric oxide. Overall, the selected species demonstrated distinct antioxidant ability in five different assays.



**Fig. 17: DPPH radical scavenging activity of methanolic extracts of selected *Euphorbia* species along with standard**



**Fig. 18: Hydroxyl radical scavenging activity of methanolic extracts of selected *Euphorbia* species along with standard**



**Fig. 19: Nitric oxide free radical scavenging activity of methanolic extracts of selected *Euphorbia* species along with standard**

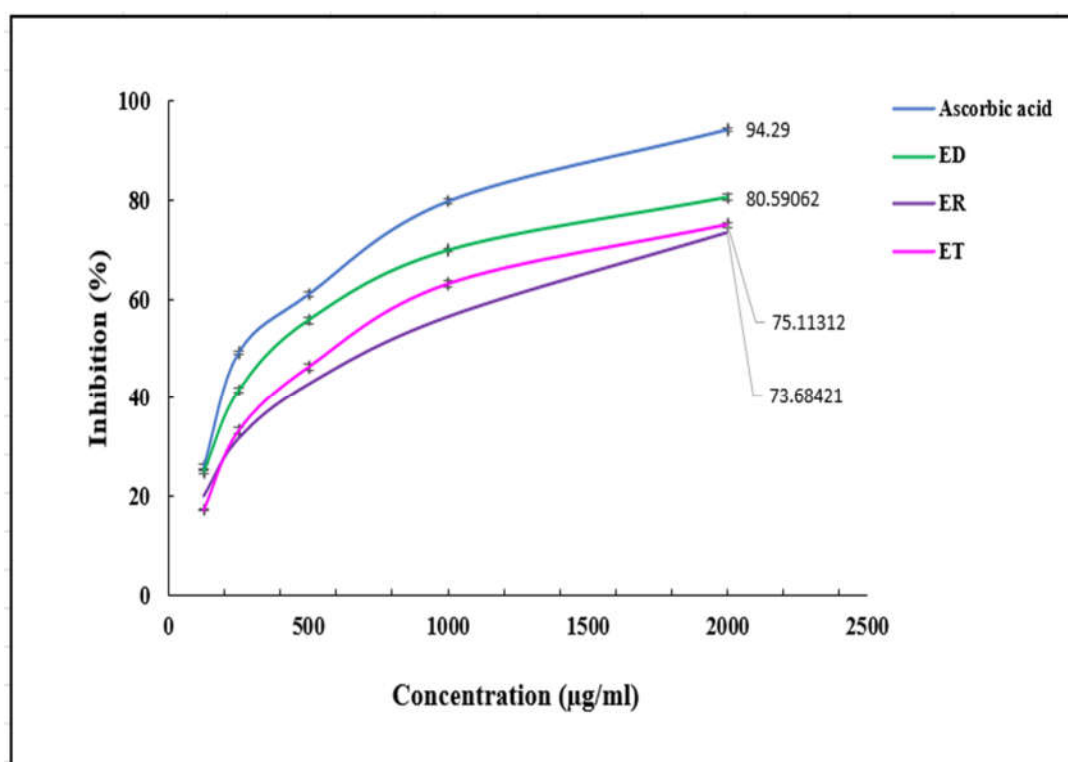


Fig. 20: superoxide free radical scavenging activity of methanolic extracts of selected *Euphorbia* species along with standard

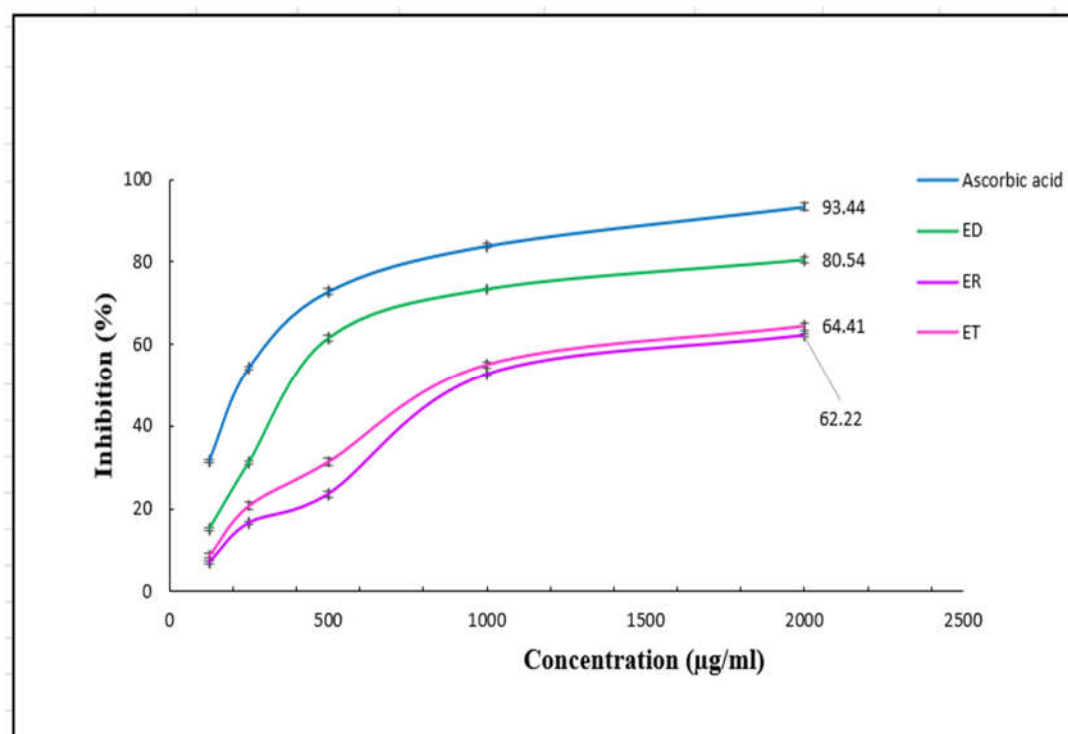


Fig. 21: ABTS free radical scavenging activity of methanolic extracts of selected *Euphorbia* species along with standard

## **B) ANTI-INFLAMMATORY ACTIVITY**

The capacity of methanolic extract of chosen *Euphorbia* species to lessen inflammation and thereby act as an anti-inflammatory agent was tested. It was initially determined which sample had the best anti-inflammatory activity through *in vitro* biochemical assays. Then, using that best sample, its effects on the lipopolysaccharide (LPS)-induced proinflammatory mediators on Raw 264.7 macrophage cell lines were detected. This allowed for the establishment of the best sample's anti-inflammatory activity.

### **i) Determination of anti-inflammatory effects using biochemical assays**

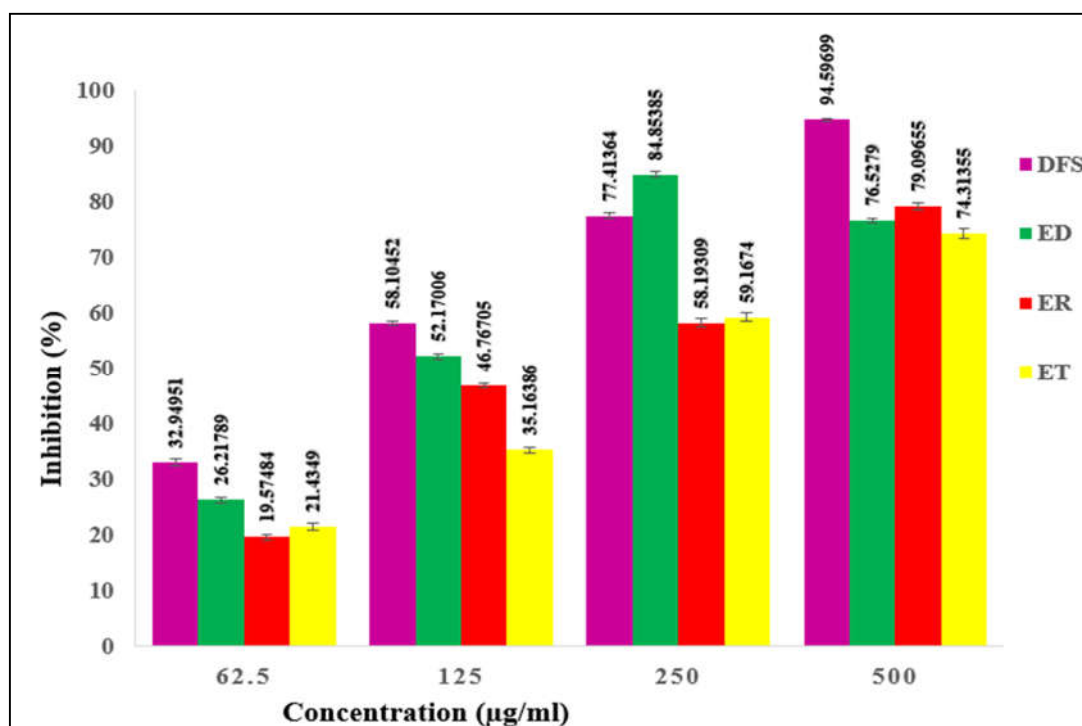
#### **a) Inhibition of protein denaturation**

Protein denaturation is the process by which proteins lose the orientation of their secondary and tertiary structures due to various external factors such as heat, organic solvents, potent acids, and bases. It may lead to a number of diseases connected to inflammation. Therefore, it is possible to inhibit inflammatory activity by preventing protein denaturation. The methanolic extracts of specific *Euphorbia* species in the current study inhibited the denaturation of the protein in a concentration-dependent manner. The concentrations considered for this purpose were 62.5, 125, 250, and 500 µg/ml. When the three plant species were compared, all of the *Euphorbia* species displayed very similar inhibition percentages at the highest concentration. The standard, diclofenac sodium, displayed an inhibition percentage of  $94.597 \pm 0.36$ . Despite the fact that all of the chosen species demonstrated comparative potential in terms of the percentages of inhibition on the inhibition of protein denaturation, better results were observed in *E. deccanensis* with the lowest  $IC_{50}$  value ( $119.77 \pm 0.35$  µg/ml). The  $IC_{50}$  value obtained from both the biochemical assays were included in **Table 9**. The summary of the results is represented in **Fig. 22**.



**Table 9: Inhibition concentration observed in *in vitro* biochemical assays**

| Plant species | Inhibition of protein denaturation (µg/ml) | Proteinase inhibitory activity (µg/ml) |
|---------------|--------------------------------------------|----------------------------------------|
| DFS           | 104.86 ± 0.66                              | 106.35 ± 0.69                          |
| ER            | 202.911 ± 0.66                             | 238.7 ± 0.82                           |
| ED            | 119.77 ± 0.35                              | 198.78 ± 0.46                          |
| ET            | 190.603 ± 0.77                             | 236.6 ± 0.43                           |



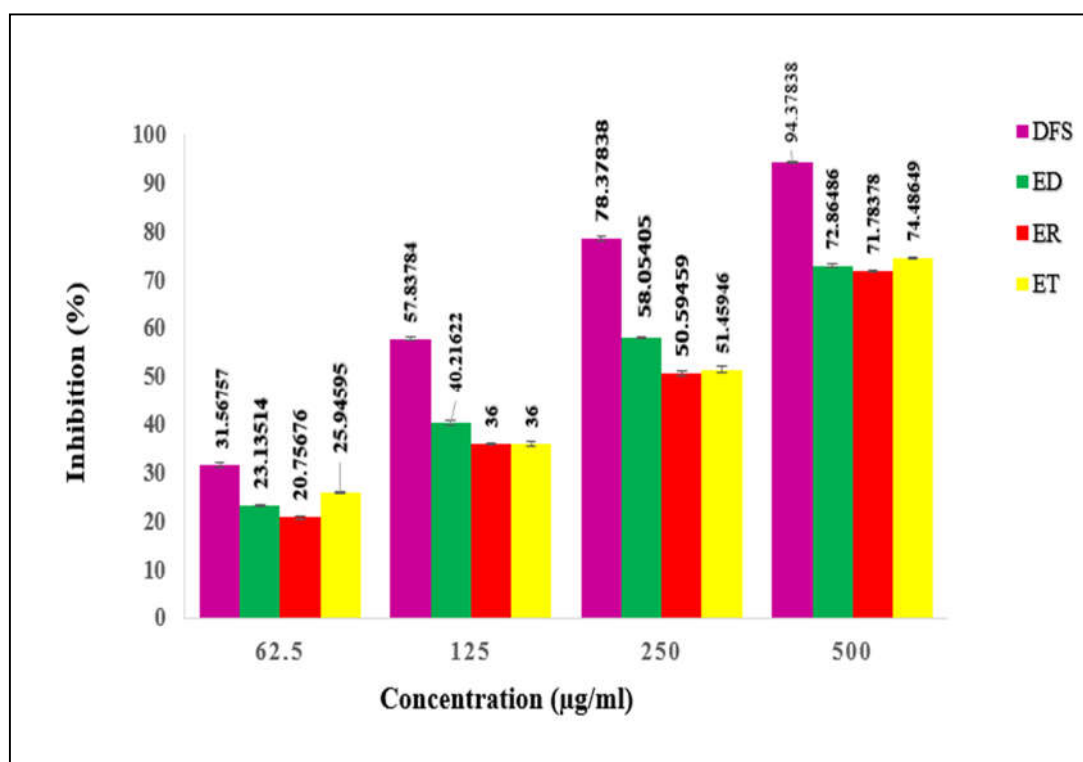
**Fig. 22: Protein denaturation inhibitory activity exhibited by methanolic extracts of selected *Euphorbia* species. DFS - Diclofenac sodium; ED - *E. deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia***

#### **b) Inhibition of proteinase activity**

Proteinase activity inhibition has a significant impact on the reduction of inflammation and any substance with the ability to do so can be thought of as a potential anti-inflammatory agent. Concentration-dependent activity, a trend that had already been noticed in the protein denaturation inhibitory assay, was observed in the proteinase activity assay. When concentration was increased from 62.5 to 500 µg/ml, a corresponding increase in inhibition percentage was found. This pattern

was present in each of the three test samples. The standard showed a  $94.37 \pm 0.16\%$  inhibition at the highest concentration. While at the same concentration, the inhibition percentage exhibited by all of the selected extracts ranged between  $71.78 \pm 0.33$  to  $74.48 \pm 0.25\%$ , indicating their ability to inhibit proteinase activity.

While taking into account  $IC_{50}$  values, it was found that *E. deccanensis*'s  $IC_{50}$  value ( $198.78 \pm 0.46 \mu\text{g/ml}$ ) was much closer to the standard ( $106.35 \pm 0.69 \mu\text{g/ml}$ ) and the  $IC_{50}$  values for the other two extracts were comparable to one another and higher than the standard. The observations found in the analysis are represented in **Figure 23**.



**Fig. 23:** Proteinase inhibitory activity exhibited by methanolic extracts of selected *Euphorbia* species. DFS - Diclofenac sodium; ED - *E. deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia*

According to the results of the *in vitro* assays, *E. deccanensis* was more active than the other two species studied in terms of both inhibition of protein denaturation and proteinase activity. Therefore, this plant extract was chosen for further analysis.

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**ii) Anti-inflammatory potential on LPS-induced RAW 264.7 macrophage cell line**

The methanolic extract of the test sample, *E. deccanensis* was examined for its effects on the activity of several proinflammatory mediators, including lipooxygenase, cyclooxygenase, and inducible nitric oxide synthase. Additionally, the level of cellular nitrite on LPS-induced RAW 264.7 macrophages was also determined.

**a) Cyclooxygenase (COX) activity**

Three different plant extract concentrations, including 6.25, 12.5 and 25 µg/ml, were used to test *E. deccanensis*'s ability to lower the level of COX enzyme. In terms of the percentage of enzyme activity inhibition, the potential of the plant extract was calculated. On the activity of the COX enzyme, a concentration-dependent inhibition was detected. At the lowest possible concentration of 6.25 µg/ml, the *E. deccanensis* extract exhibited an inhibition percentage of  $10.50 \pm 0.43$ . At the same concentration, standard diclofenac sodium inhibited the enzyme activity with an inhibition percentage of  $25.02 \pm 0.32$ . In the highest tested concentration, the plant extract's inhibition activity was found to be  $57.21 \pm 0.74\%$ , and that of the standard was  $72.51 \pm 0.70\%$ . When  $IC_{50}$  values were examined, the standard's  $IC_{50}$  value was discovered to be 15.7813 µg/ml, while the value for plant extract was 21.939 µg/ml. From these, it is obvious how well the plant extract inhibits COX activity. **Figure 24** depicts the COX activity inhibition of both the plant extract and the standard.

**b) Lipooxygenase (LOX) activity**

Three different concentrations of plant extracts were taken into consideration in this case as well to evaluate their potential to reduce the activity of the LOX enzyme. At a concentration of 6.25 µg/ml, the test sample displayed an inhibition percentage of  $14.75 \pm 0.68$ . The standard displayed a percentage of inhibition of  $52.45 \pm 0.87$  at the same concentration. A sudden increase in the ability of *E. deccanensis* extract to inhibit enzyme activity was observed when the

concentration was increased from 6.25 to 12.5 µg/ml. At the highest concentration of 25 µg/ml, the plant extract also significantly affected enzyme activity, which was also comparable to the activity of standard.

In the context of IC<sub>50</sub> values, the *E. deccanensis*'s IC<sub>50</sub> value was 13.677 µg/ml, while the standard's was 4.41640 µg/ml. The **Figure 25** illustrates the results of the assay. Comparing the plant extract to standard, it was clear that it had a much greater ability to inhibit LOX activity.

### c) Inducible nitric oxide synthase (iNOS) activity

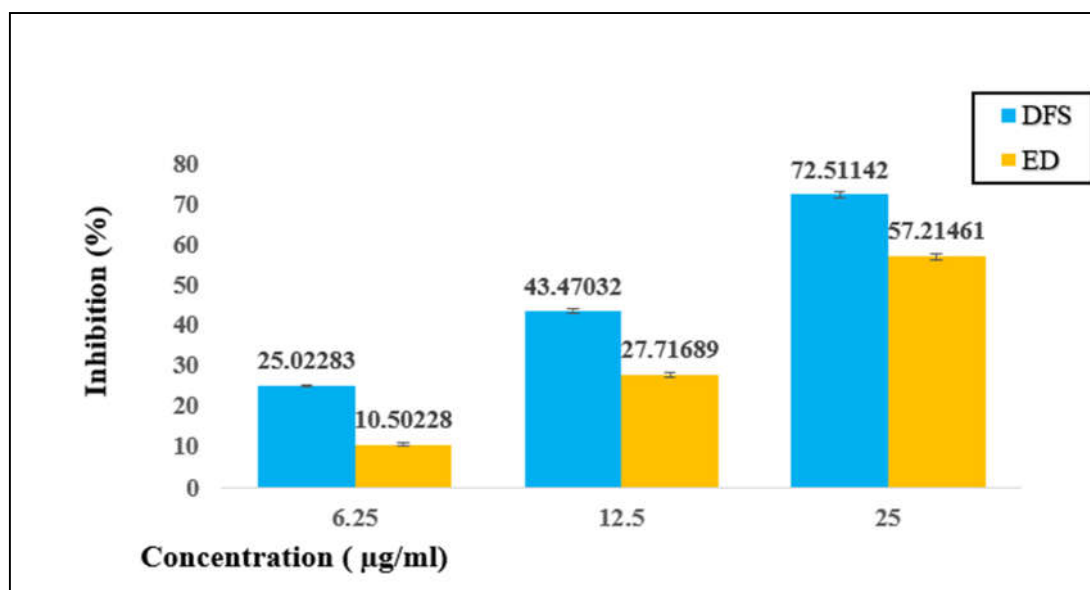
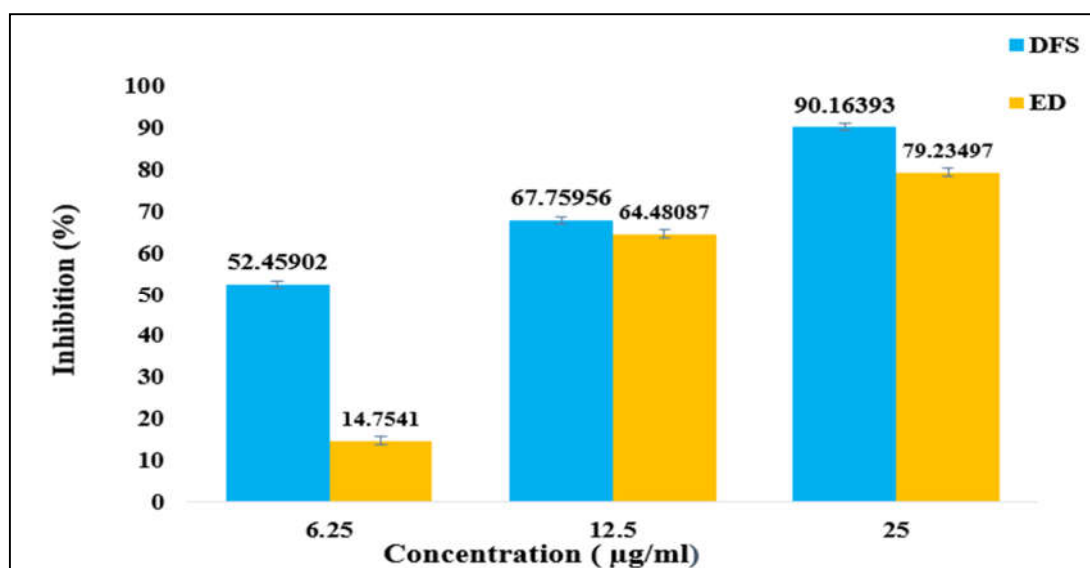
The ability of a plant extract to reduce the level of an important proinflammatory mediator called iNOS was calculated as a percentage of the enzyme's inhibition. The sample showed an inhibition percentage that was in line with standards for all concentrations tested, including 6.25, 12.5 and 25 µg/ml. At concentrations of 6.25, 12.5 and 25 µg/ml, respectively, the plant extract had an inhibition percentage of  $43.73 \pm 0.58$ ,  $51.53 \pm 1.07$ , and  $67.13 \pm 0.51\%$ . Likewise, the standard drug, diclofenac sodium, had an inhibition percentage of  $48.22 \pm 0.76$ ,  $55.08 \pm 0.67$ , and  $73.52 \pm 0.69\%$  in respective concentrations. **Figure 26** displays the assay results. It was determined from the assay that *E. deccanensis* extract has a significant ability to inhibit iNOS activity.

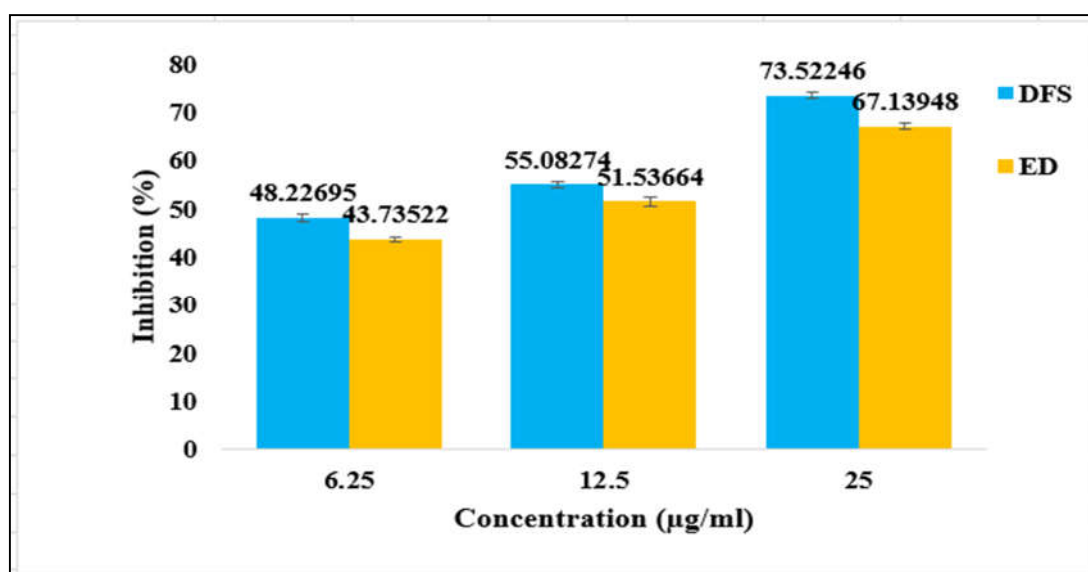
### d) Level of cellular nitrite

In order to assess the extract's potential as an anti-inflammatory agent, the level of cellular nitrite must be determined. High levels of cellular nitrite are a sign of elevated inflammatory responses, which are harmful to health. Using different extract concentrations (6.25, 12.5 and 25 µg/ml), the ability of plant extract to reduce the amount of cellular nitrite was evaluated in this assay. Cellular nitrite levels dropped significantly from  $718.74 \pm 1.25$  µg/ml to  $464.80 \pm 0.827$  µg/ml from lower to higher concentrations. Regarding diclofenac, the drop was from  $737.055 \pm 0.63$  g/ml to  $377.19 \pm 0.59$  µg/ml. In the present study, a significant decrease in the concentration of nitrite ions was observed, suggesting that plant extracts may have the ability to prevent inflammation and avoid the diseases associated with inflammation.

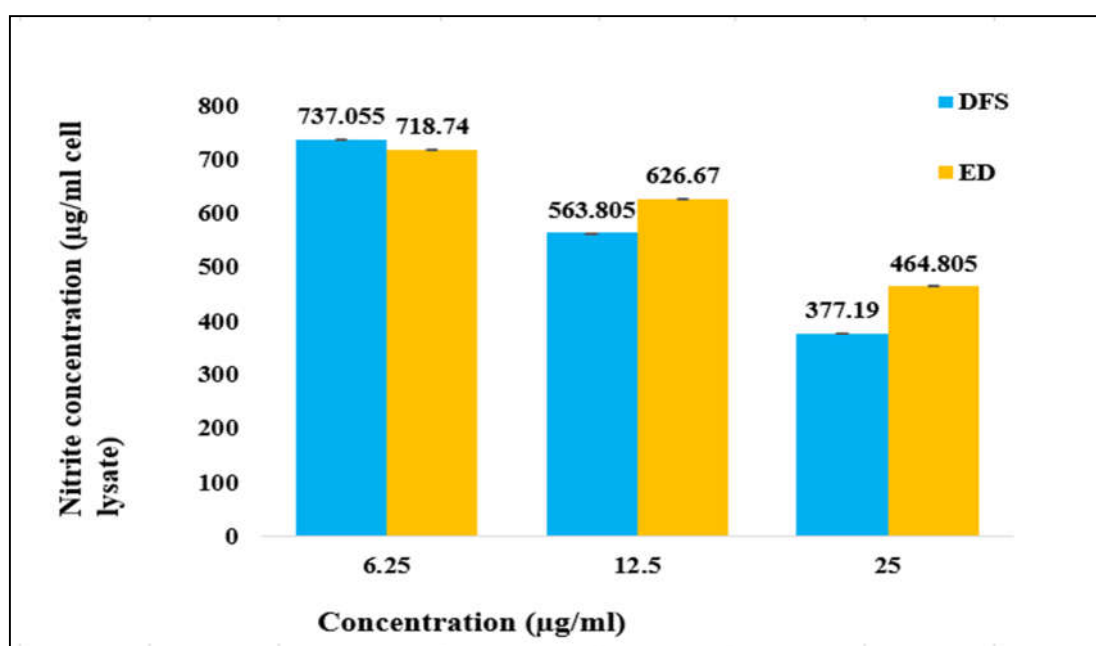
**Table 10: IC<sub>50</sub> values for anti-inflammatory activities**

| Anti-inflammatory assays | IC <sub>50</sub> values (µg/ml) |               |
|--------------------------|---------------------------------|---------------|
|                          | Diclofenac sodium               | Plant extract |
| COX activity             | 15.7813                         | 21.939        |
| LOX activity             | 4.41640                         | 13.677        |

**Fig. 24: Effect of methanolic extract of *E. deccanensis* on cyclooxygenase activity. ED – *E. deccanensis*; DFS – Diclofenac sodium****Fig. 25: Effect of methanolic extract of *E. deccanensis* on lipoxygenase activity. ED – *E. deccanensis*; DFS – Diclofenac sodium**



**Fig. 26:** Effect of methanolic extract of *E. deccanensis* on inducible nitric oxide synthase (iNOS) activity. ED – *E. deccanensis*; DFS – Diclofenac sodium



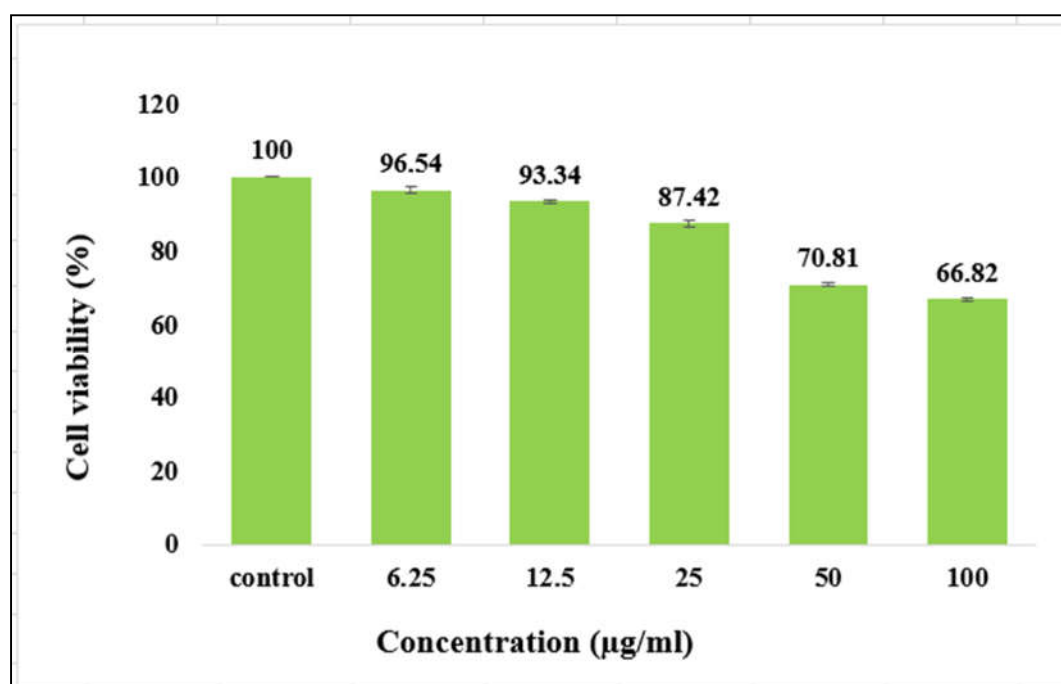
**Fig. 27:** Effect of methanolic extract of *E. deccanensis* on cellular nitrite level. ED – *E. deccanensis*; DFS – Diclofenac sodium

In each of the four different assays that were performed, the methanolic extract of *E. deccanensis*'s anti-inflammatory ability showed notable results. The chosen pro-inflammatory enzymes in LPS-induced macrophage cells were highly susceptible to inhibition by the plant extract. Through the indirect inhibition of the

activity of the inducible nitric oxide synthase enzyme, it also had the capacity to significantly lower the level of cellular nitrite.

### iii) Evaluation of cytotoxicity using MTT assay on RAW 264.7 macrophage cells

The MTT assay was used to determine how the methanolic plant extract of *E. deccanensis* affected the viability of RAW 264.7 macrophage cells after a 24-hour treatment with various concentrations (6.25, 12, 25, 50, and 100  $\mu\text{g/ml}$ ). Cell viability percentages were calculated for each concentration and are displayed in **Fig. 28**. From this assay, the  $\text{LC}_{50}$  value was calculated and found to be  $139.63 \pm 0.68 \mu\text{g/ml}$ . RAW cells had a lower viability than the control, but the difference was not very noticeable. As a result, the plant extract's  $\text{LC}_{50}$  value, which was found to be  $139.63 \pm 0.68 \mu\text{g/ml}$ , was barely toxic to RAW 264.7 cells. This value is therefore taken into account for future analysis. The cytotoxic effects exhibited by the plant extract of *E. deccanensis* on RAW 264.7 cells were shown in the **Plate. 2**

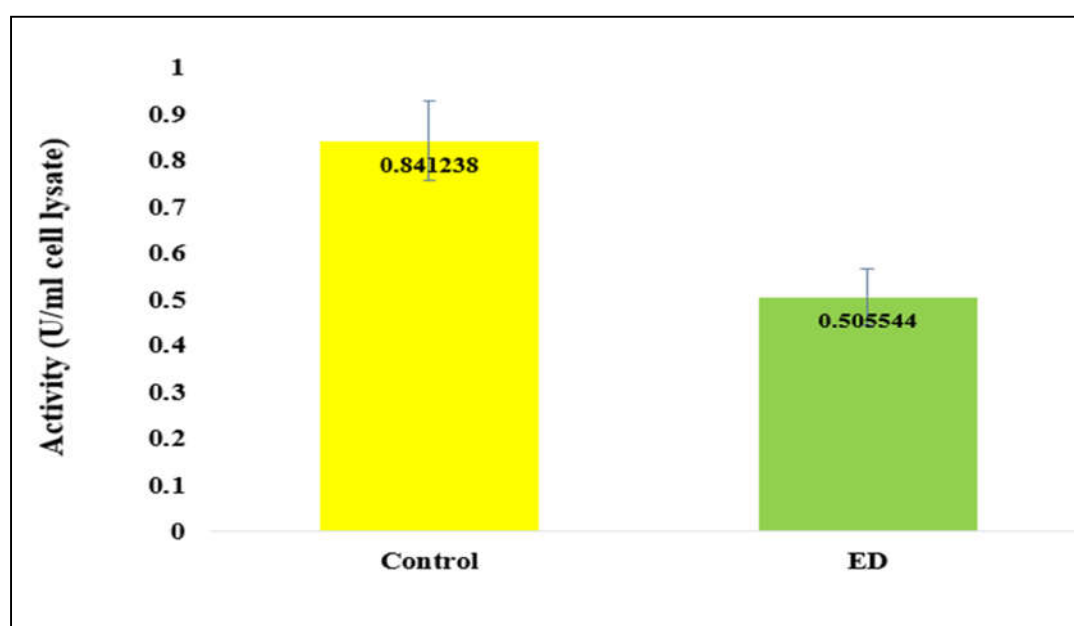


**Fig. 28:** Cytotoxic effect of methanolic extract of *E. deccanensis* on RAW 264.7 cells

#### iv) Estimation of inflammatory mediator COX-2 using ELISA

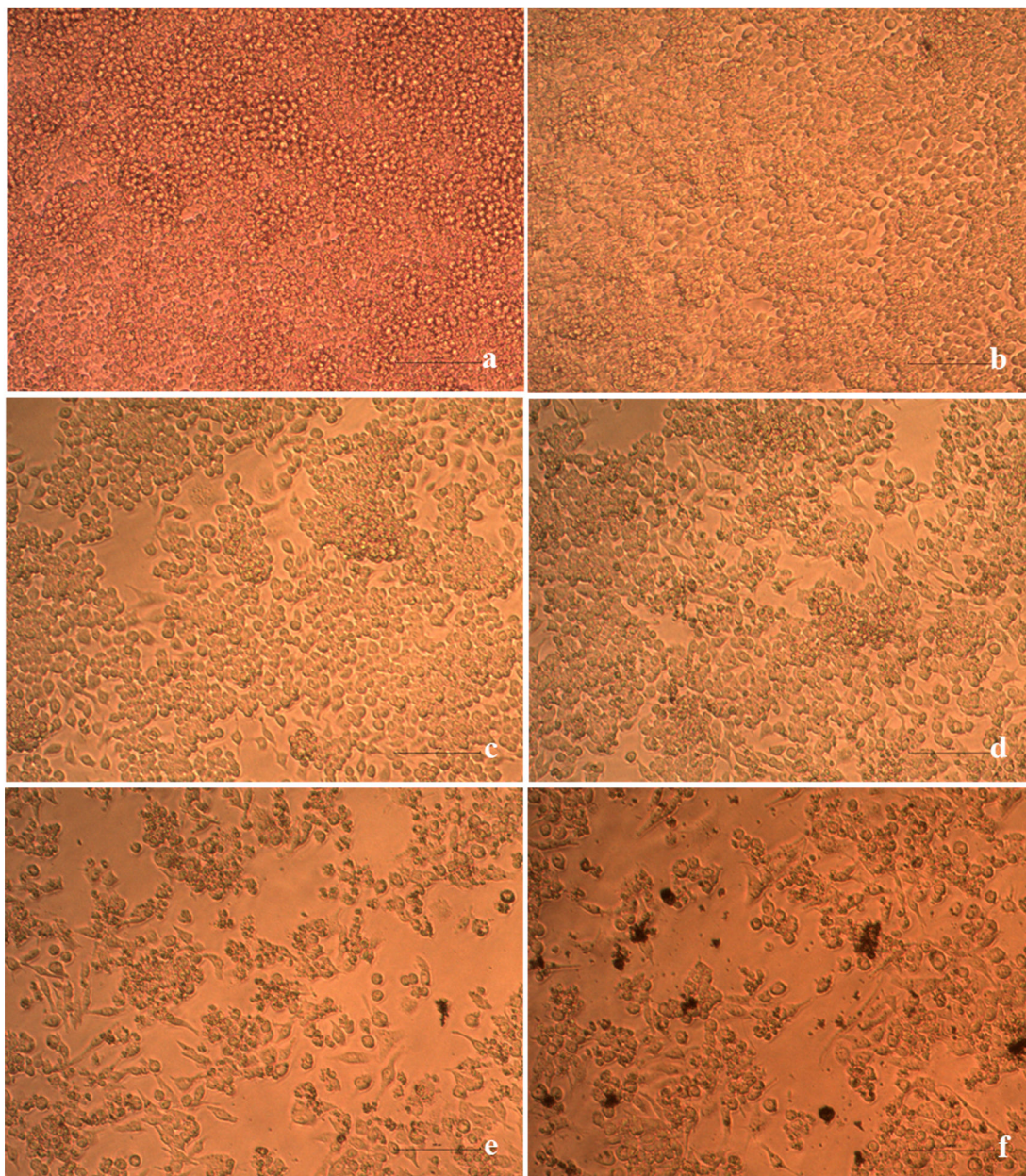
In this study, ELISA was used to measure the effect of methanolic extract of *E. deccanensis* on the amount of COX-2, one of the main inflammatory mediators, in LPS-stimulated RAW cells. As the control, RAW cells that had only been exposed to LPS were kept. In order to measure the level of COX-2, RAW cells were exposed to the plant extract at a concentration of  $139.63 \pm 0.68 \mu\text{g/ml}$ . The level of COX-2 was found to be significantly reduced by the plant extract, dropping from  $0.841 \pm 0.08$  to  $0.505 \pm 0.062$  U/ml of cell lysate. The result of the experiment is shown in Fig. 29.

The potent anti-inflammatory properties of the methanolic extract of *E. deccanensis* were thus demonstrated by the outcome of the experiments and the plant extract's powerful inhibition of a variety of inflammatory enzymes. A significant drop in COX-2 levels, a well-known proinflammatory mediator that is crucial in inflammation, provided additional support for this. Based on the results of all assays, the methanolic extract of *E. deccanensis* appears to be a strong candidate for anti-inflammatory research and might be used to prevent issues related to inflammations.



**Fig. 29:** Effect of methanolic extract of *E. deccanensis* on the production of COX-2





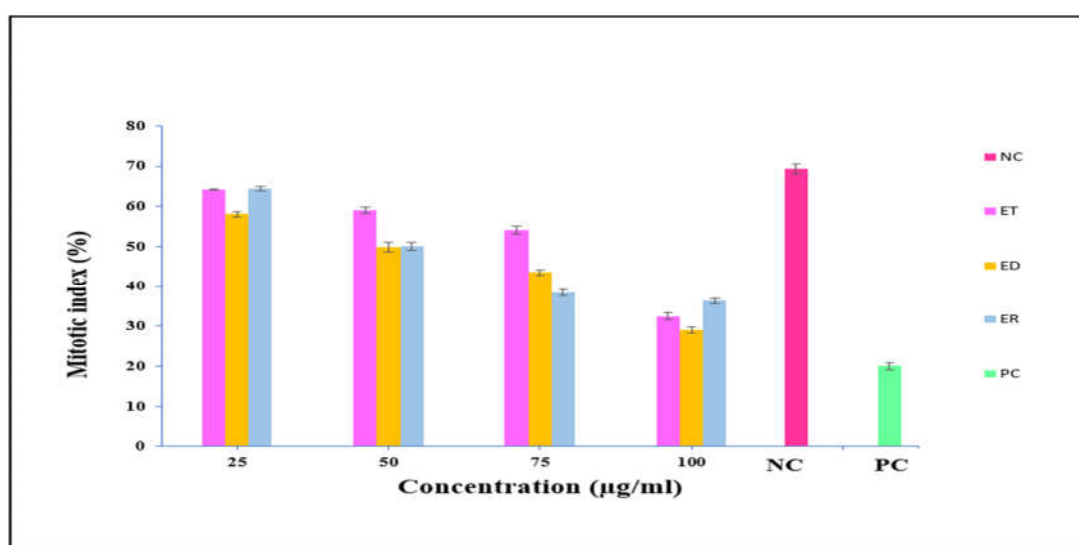
**Plate 2:** Cytotoxic effect of methanolic extract of *E. deccanensis* on RAW 264.7 cells. **a.** Control; **b.** 6.25  $\mu\text{g/ml}$ ; **c.** 12.5 $\mu\text{g/ml}$ ; **d.** 25 $\mu\text{g/ml}$ ; **e.** 50 $\mu\text{g/ml}$ ; **f.** 100 $\mu\text{g/ml}$ . Scale bars = 100  $\mu\text{m}$

### C) Cytotoxicity study using *Allium cepa* bioassay

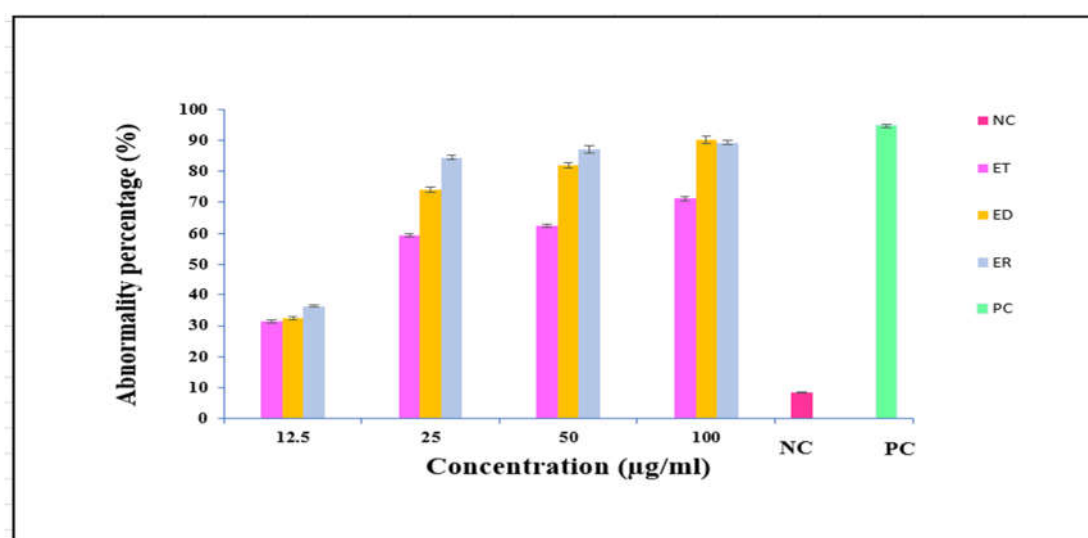
*Allium cepa* assay was designed to assess the cytotoxic nature of methanolic extracts of chosen *Euphorbia* species. To accomplish this, four various concentrations of each plant extract were treated on the root tips of *Allium cepa* for 24 hours. The various plant concentrations include 12.5, 25, 50 and 100 µg/ml and distilled water as negative and hydrogen peroxide as positive control. Mitotic index and aberration percentage are two important parameters associated with cytotoxicity. Thus, both these parameters were calculated to understand the extent of the cytotoxicity exhibited by each plant species. Concerning concentration, the mitotic index was found to decrease prominently in every *Euphorbia* species considered (**Figure 30**). In contrast to this, direct relationship was observed between the aberration percentage and concentration. In every *Euphorbia* species, the aberration percentage increases predominantly with the concentration (**Figure 31**).

In all living organisms, the mitotic index is an important parameter to measure the level and extent of cytotoxicity and the lowering of the mitotic index value will reveal the nature of cytotoxicity. After 24 hours of treatment of root tips with that of methanolic plant extract, a gradual reduction in the mitotic index was noticed in every *Euphorbia* species. Among the three species considered, the highest mitotic index value was found in the *E. rothiana* extract ( $64.35 \pm 0.51\%$ ) at its lowest concentration. At the same time, the lowest mitotic index was observed in the highest concentration of *E. deccanensis* and it was noted to be  $29.11 \pm 0.81\%$ . In *E. thymifolia*, the mitotic index reduction was from  $64.35 \pm 0.51\%$  to  $36.4 \pm 0.66\%$ . When positive and negative controls were taken into account, the mitotic index of negative control was found to be very high ( $69.3 \pm 1.2\%$ ), while that of positive control was found to be very low. The enhancement in the percentage of abnormality is another indicator of the cytotoxicity potential of plant extracts. In every species, dose-dependent increase in the percentage of abnormality was detected. Both *E. rothiana* and *E. deccanensis* methanolic extracts show the highest count of abnormal cells, which ranges from  $36.32 \pm 0.41\%$  to  $89.2 \pm 0.64\%$  and from  $32.32 \pm 0.53\%$  to  $90.3 \pm 1.2\%$  respectively. In the case of *E. thymifolia*, the percentage of abnormal cells at the highest concentration was found to be  $71.2 \pm 0.56\%$ .

Various types of chromosomal aberrations were observed during the microscopic visualization of *Euphorbia* extract-treated root tip cells. It reveals the aberrations that occur at every stage of mitotic division. The normal mitotic cell division stages are represented in **Plate 3**. Both clastogenic and non-clastogenic aberrations were detected in *A. cepa* treated with the extracts of three species of *Euphorbia*.

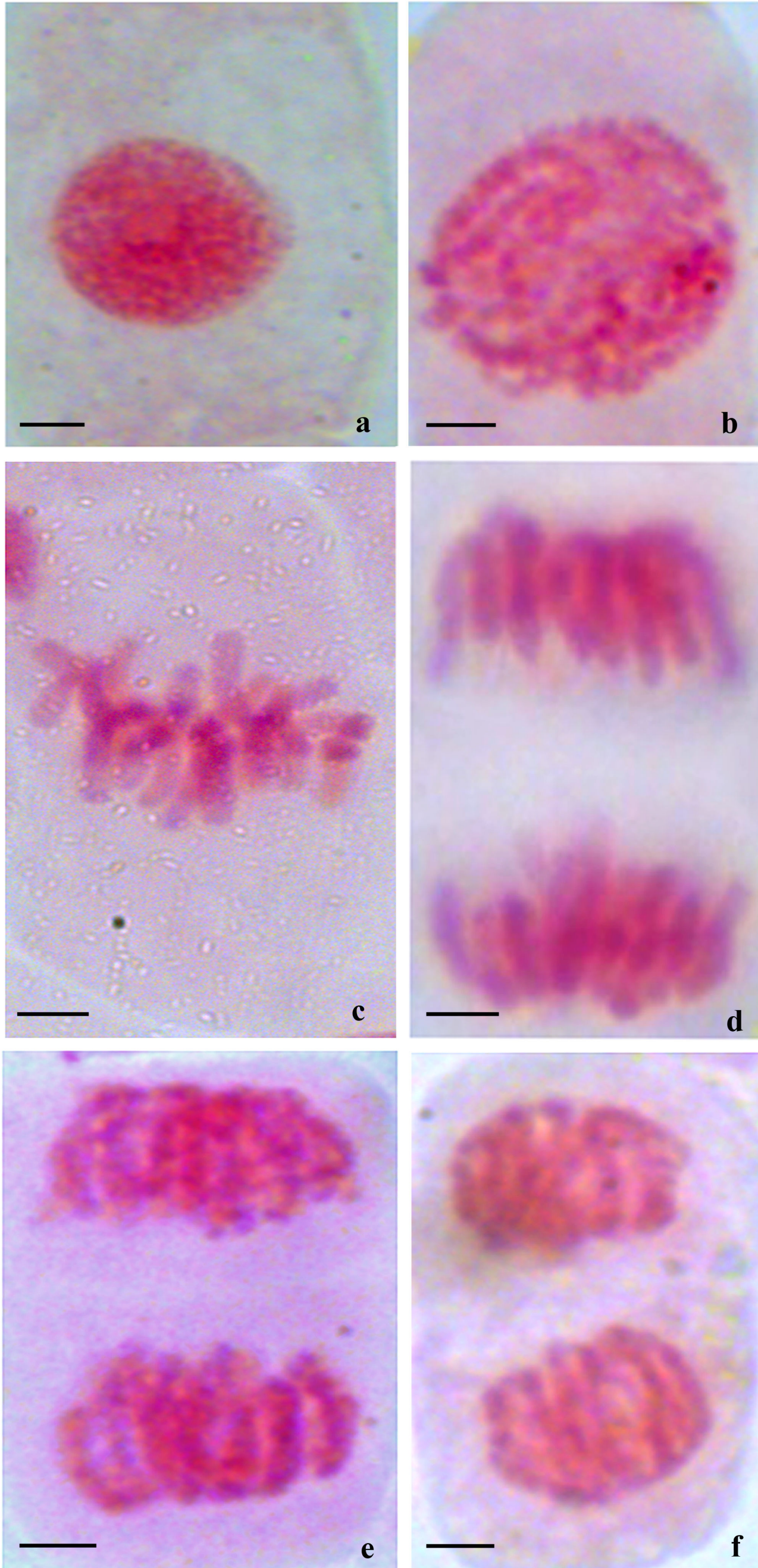


**Fig. 30:** The effects of different concentrations of methanolic extract of selected species of *Euphorbia* on *A. cepa* meristematic root tip cells after treatment for 24 h (Mitotic index)



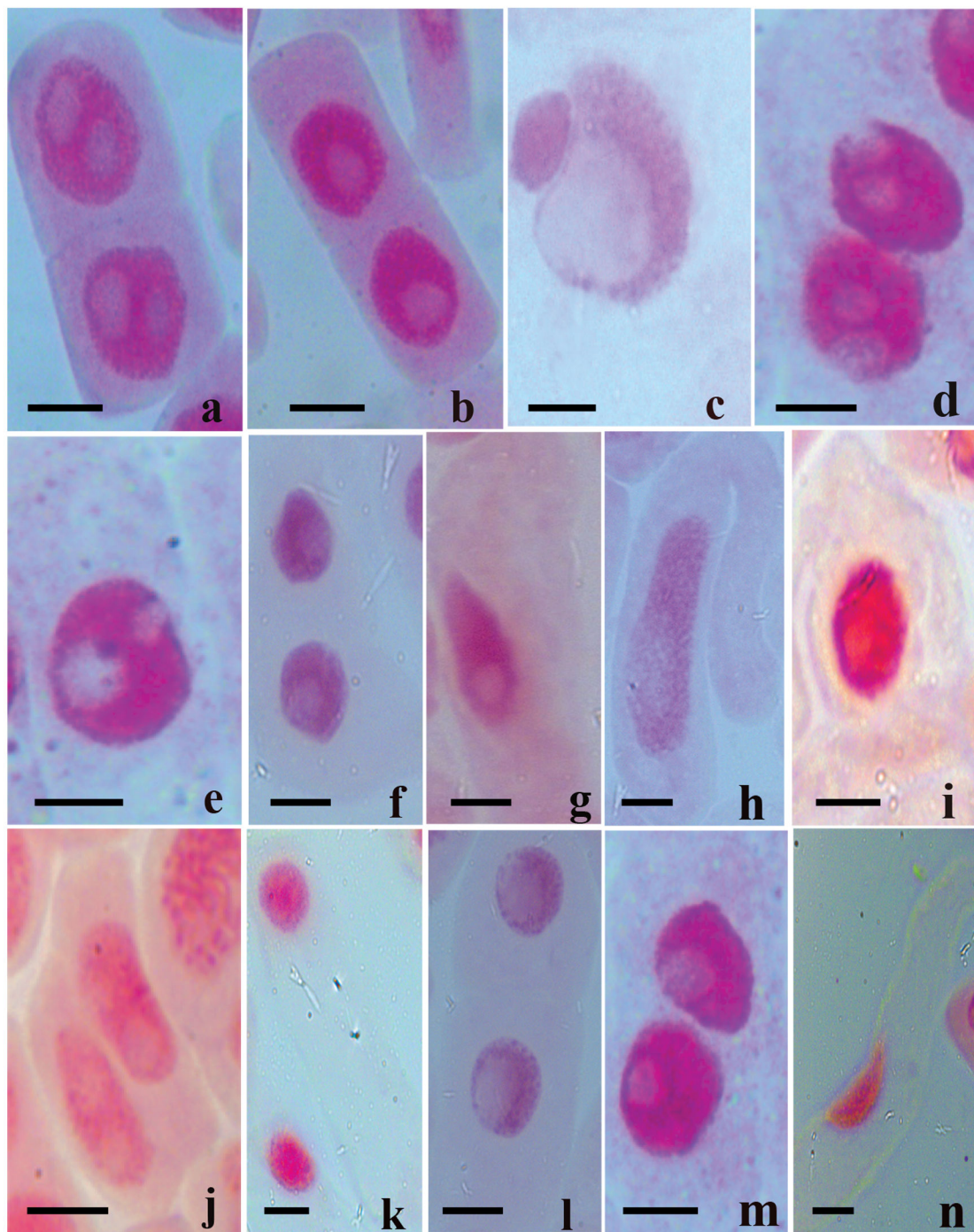
**Fig. 31:** The effects of different concentrations of methanolic extract of selected species of *Euphorbia* on *A. cepa* meristematic root tip cells after treatment for 24 h (Abnormality percentage)





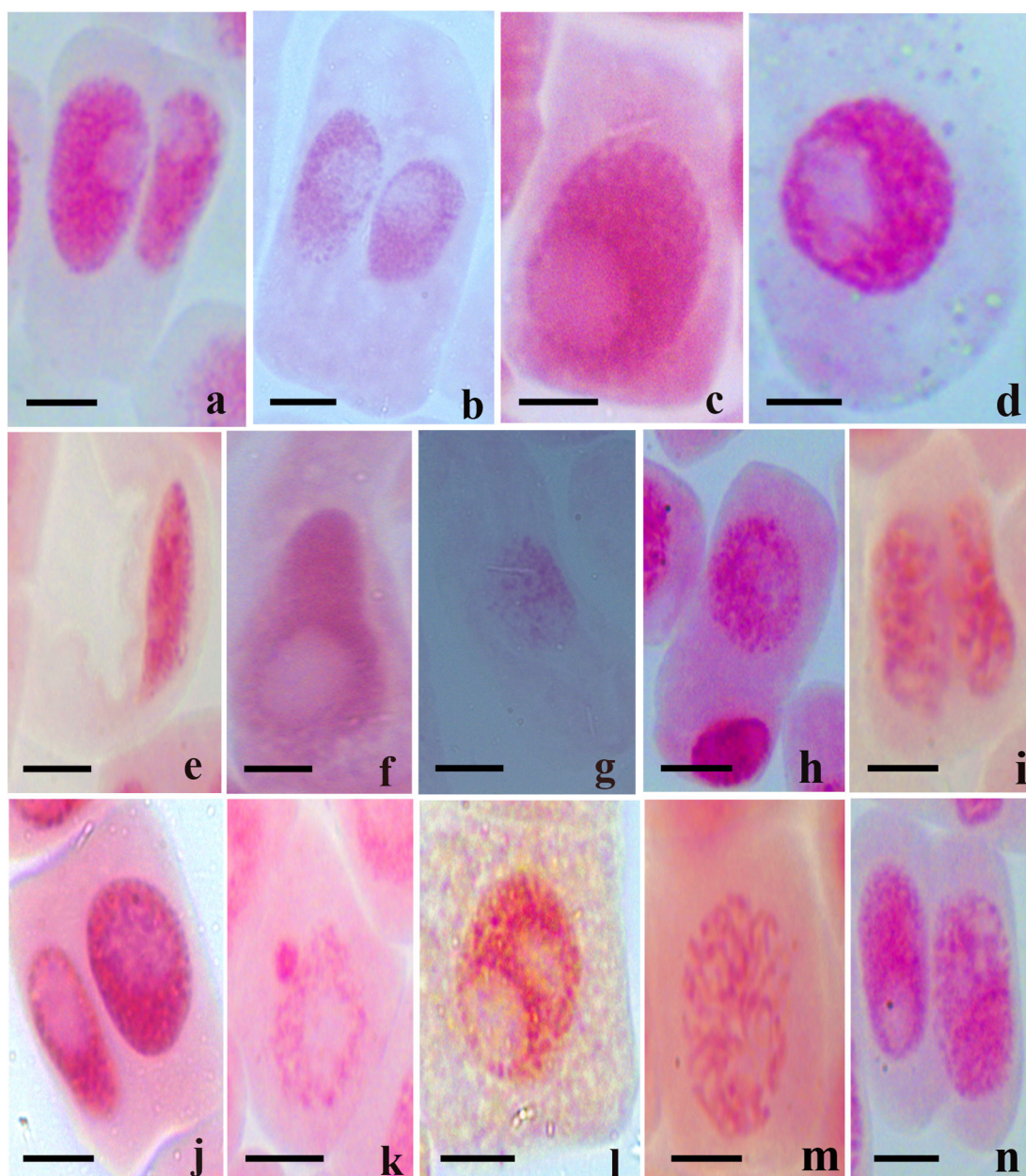
**Plate 3 : Normal stages of mitotic cel division in *Allium cepa* root tip cells. a: Interphase; b: Prophase; c: Metaphase; d: Anaphase; e: Telophase; f: Cytokinesis. Scale bars = 10  $\mu$ m**





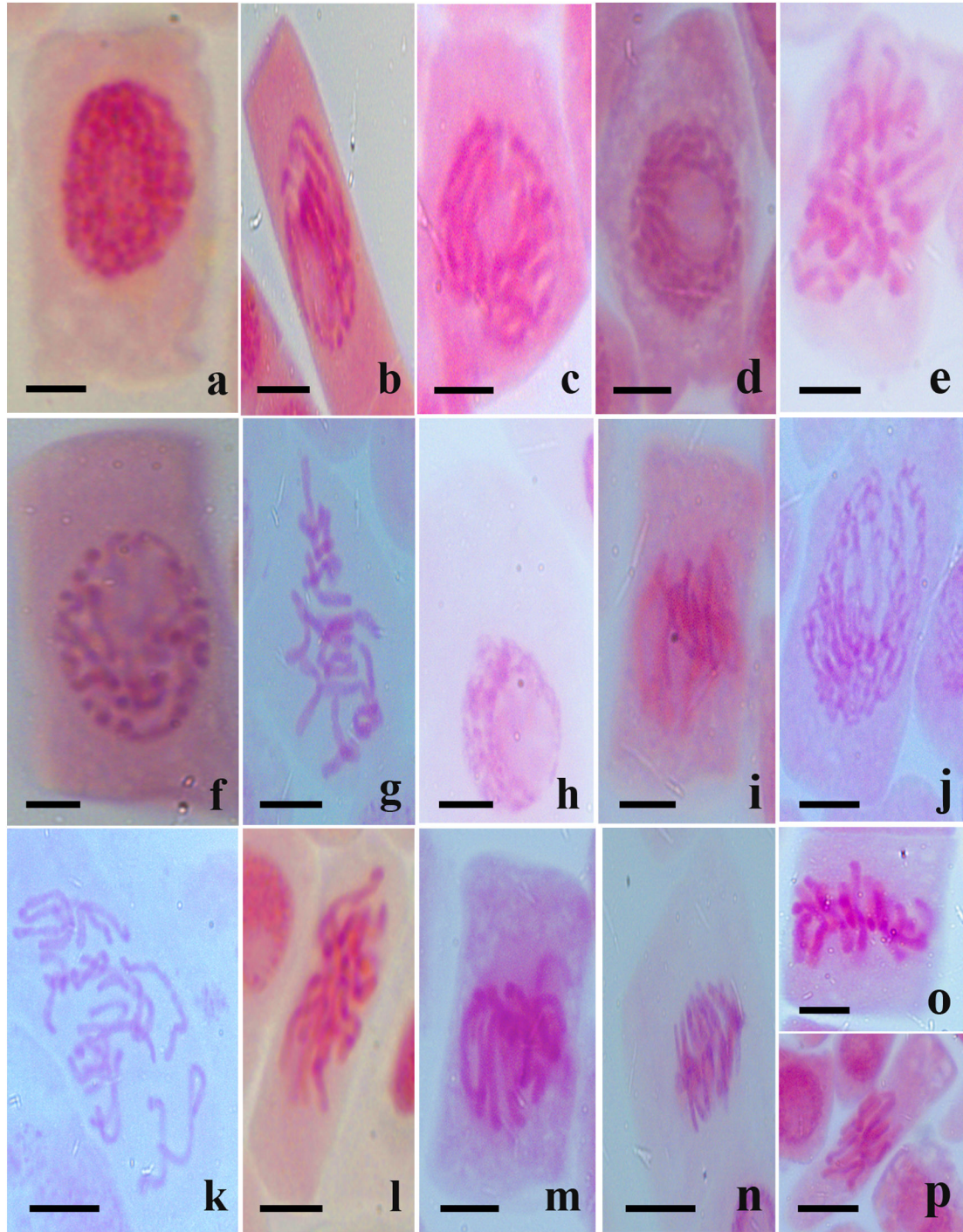
**Plate 4:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at interphase. **a.** Cells showing double nuclear lesions; **b.** Cells showing single nuclear lesions; **c.** Macronucleus with a lesion and a micronucleus; **d.** Single nuclear lesions and erosions in a binucleate cell; **e.** Double nuclear lesion; **f.** Formation of lesions in a binucleate cell; **g.** Nuclear peak, erosion and lesion; **h.** Chromatin erosion in a strap cell; **i.** Nuclear and cytoplasmic breakage; **j.** Strap cells showing chromatin disintegration; **k.** Strap cells; **l.** Cells showing single nuclear lesion; **m.** Single nuclear lesion in a binucleate cell; **n.** Spindle-shaped nucleus in a strap cell Scale bars = 10  $\mu$ m





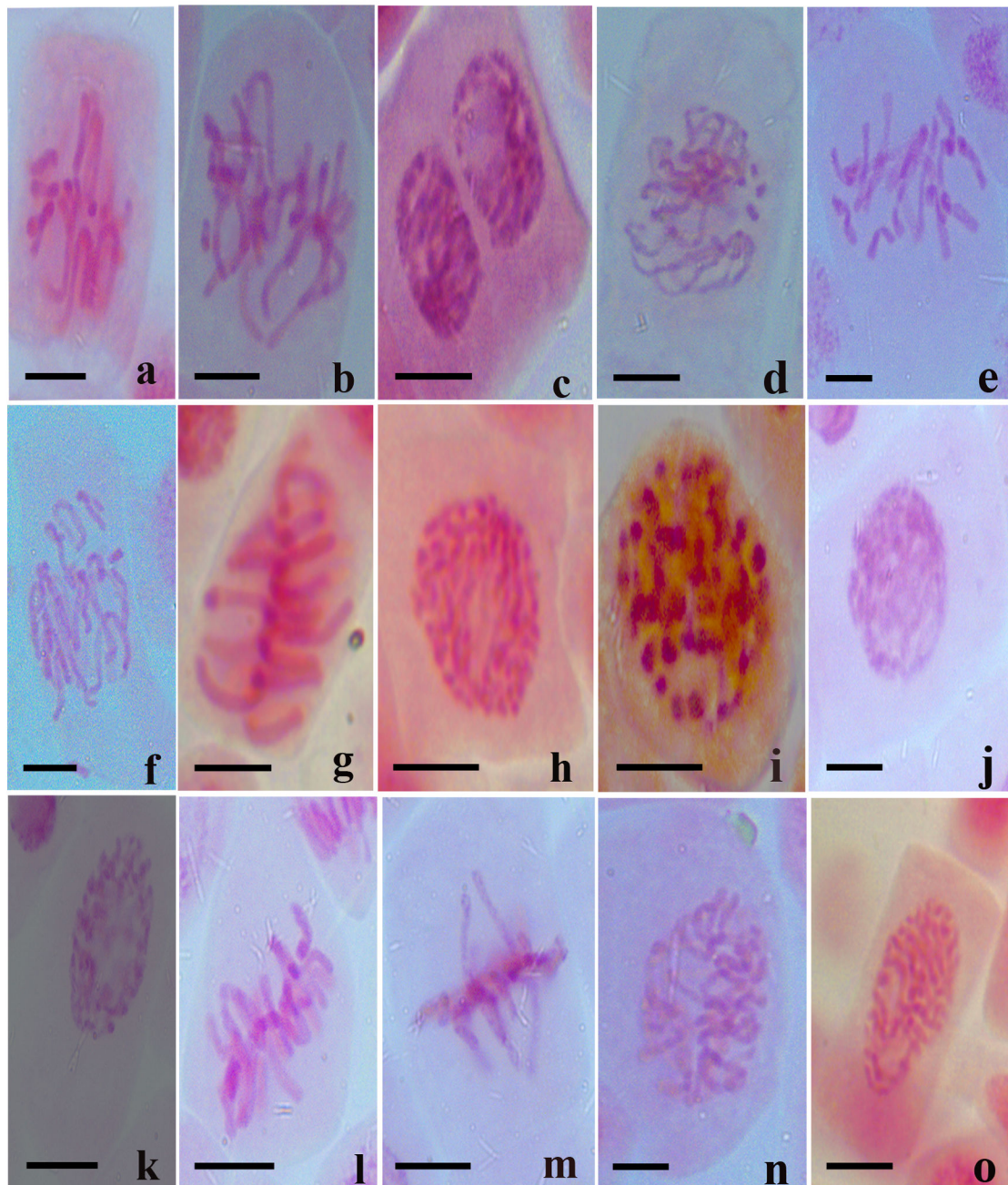
**Plate 5:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at prophase. **a.** Single nuclear lesion at prophase in a binucleate cell; **b.** Single nuclear lesion and erosion at prophase in a binucleate cell; **c.** Single nuclear lesion at early prophase; **d.** Single nuclear lesion at prophase; **e.** Nuclear peak in a strap cell at prophase; **f.** Nuclear peak and lesion at prophase; **g.** Nuclear disintegration; **h.** Non-synchronised interphase and prophase stages in a binucleate cell; **i.** Chromatin erosion at prophase in a binucleate cell; **j.** Formation of lesions in a large and small nuclea in a binucleate cell at prophase; **k.** Nuclear disintegration and micronucleus; **l.** Double nuclear lesion; **m.** Formation of lesion; **n.** Binucleate cell showing nuclear lesion. Scale bars = 10  $\mu$ m





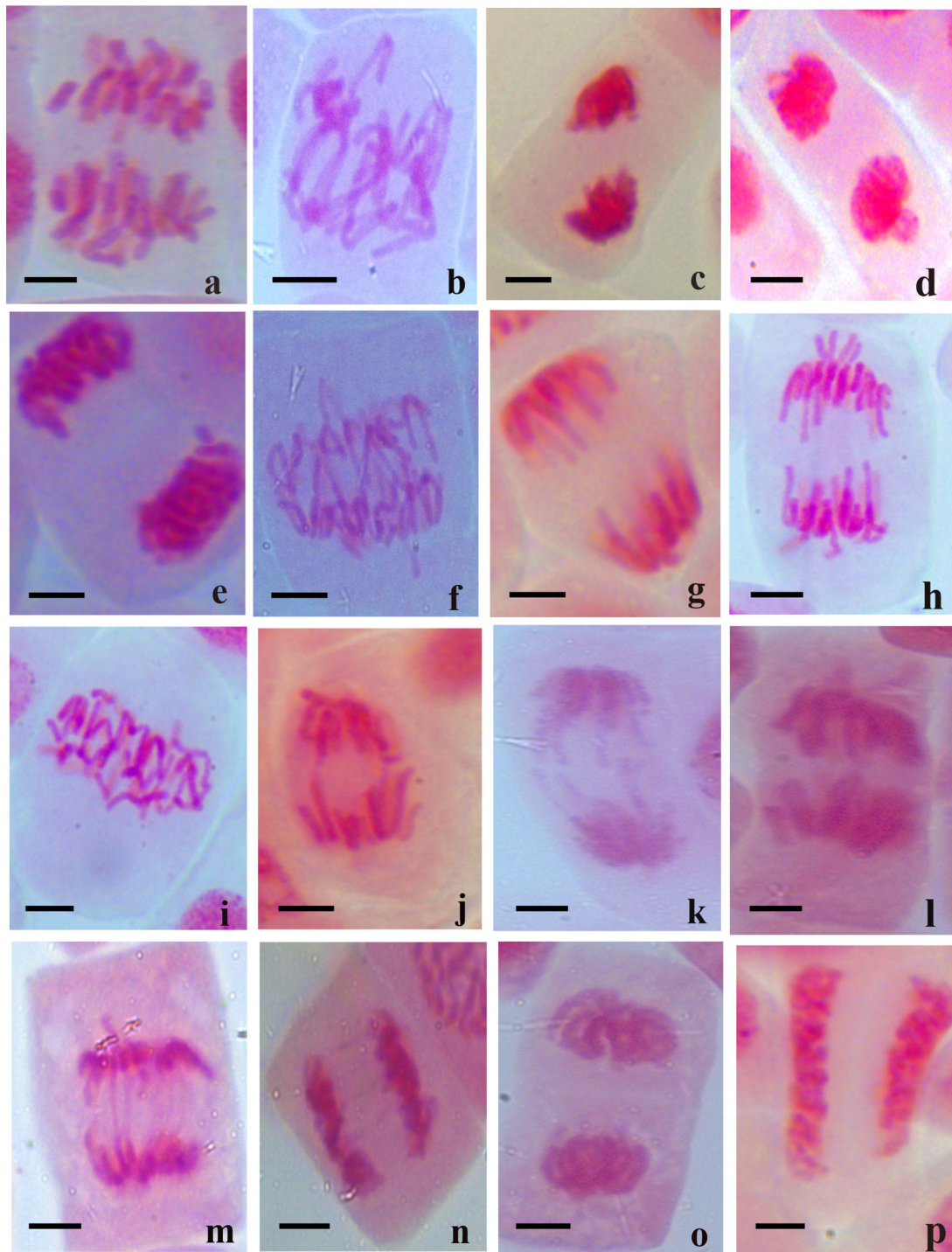
**Plate 6:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at metaphase. **a.** Early ball metaphase; **b.** Formation of lesion in a ball metaphase; **c.** Formation of lesion at ball metaphase; **d.** Lesion in ball metaphase; **e.** Metaphase in a hyperploid cell; **f.** Nuclear lesion; **g.** Partial C-metaphase; **h.** Pulverised ball metaphase; **i.** Partially coagulated metaphase; **j.** Scattered early ball metaphase; **k.** Scattered metaphase; **l.** Sticky disturbed metaphase; **m.** Sticky metaphase in a hypoploid cell; **n.** Sticky metaphase showing chromosome erosion; **o.** Tropokinensis; **p.** Vagrants at metaphase. Scale bars = 10 µm





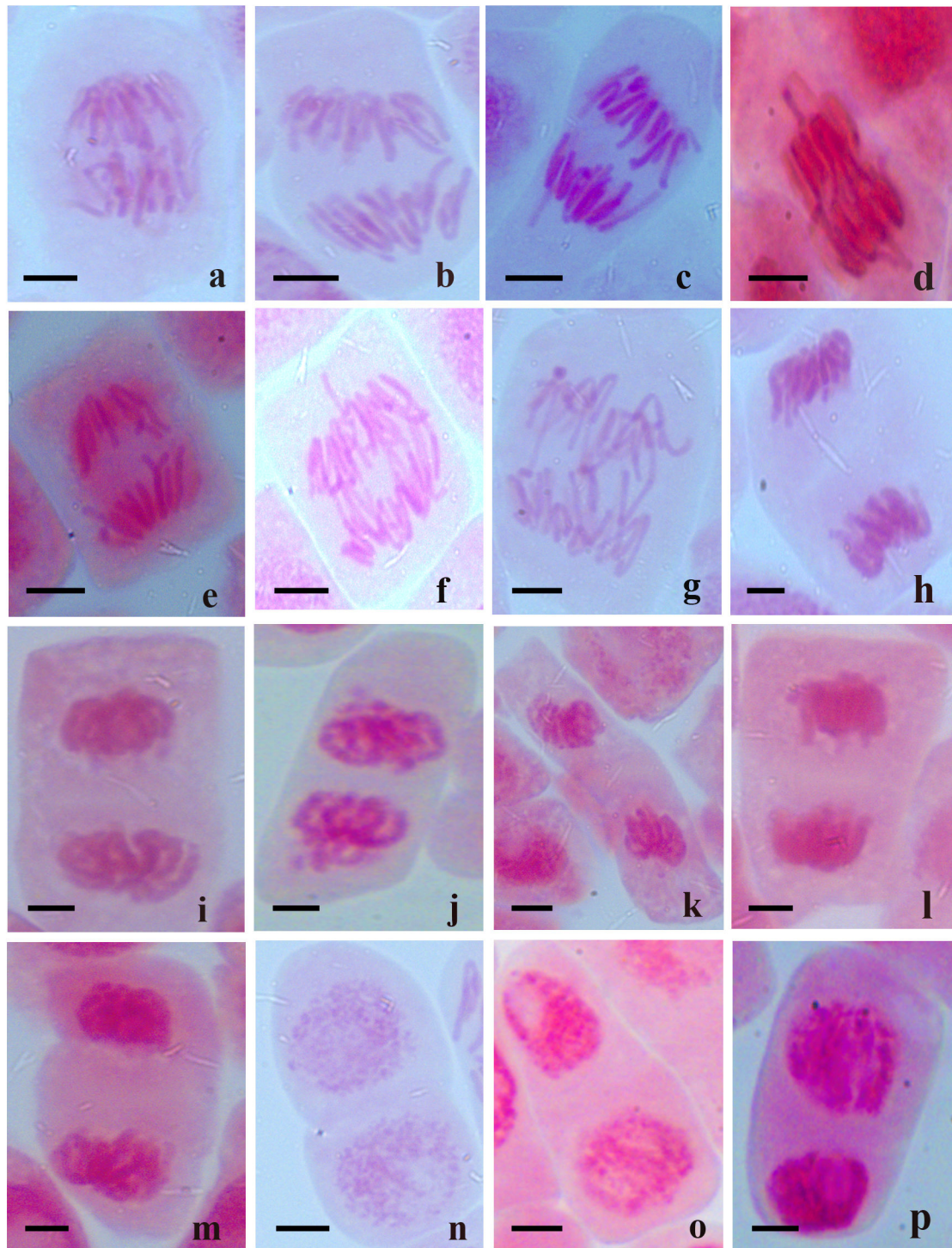
**Plate 7:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at metaphase. **a.** Disturbed metaphase showing vagrants; **b.** Chromosome fragment in a disturbed metaphase; **c.** Chromosome erosion at synchronised metaphase in a binucleate cell; **d.** Chromosome fragments in an early ball metaphase; **e.** C-metaphase; **f.** Chromosome fragments; **g.** Diagonal metaphase; **h.** Ball metaphase in a polyploid cell; **i.** Chromosome erosion in a ball metaphase; **j.** Diagonal partial C-metaphase; **k.** Chromosome erosion in a ball metaphase; **l.** Chromosome erosion; **m.** Chromosome erosion at metaphase; **n.** Disturbed metaphase in a polyploid cell; **o.** Ball metaphase with lesion. Scale bars = 10 μm





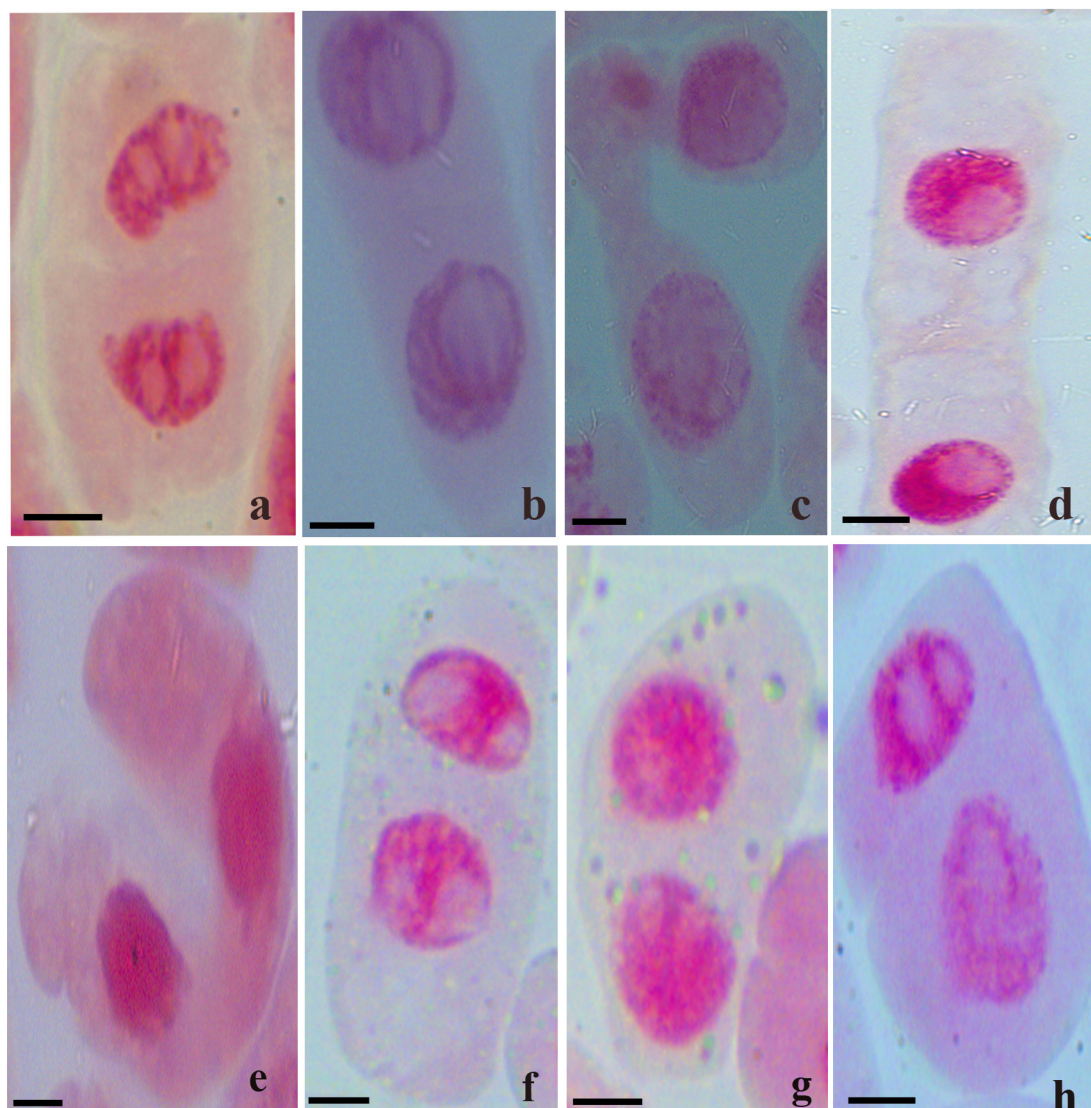
**Plate 8:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at metaphase (**a, b**) and at anaphase (**c -p**). **a.** Binucleate cell showing synchronised partial sticky C-metaphases with a persistent broken chromosome bridge; **b.** Diagonal disturbed metaphase showing unequal condensation of chromosomes; **c.** Diagonal coagulated anaphase; **d.** Coagulated anaphase showing chromosome erosion and early movement; **e.** Chromosome fragment at late anaphase; **f.** Chromosome bridges, laggards, vagrants and early movement at anaphase; **g.** Diagonal sticky anaphase; **h.** Misorientation at anaphase; **i.** Stathmo-anaphase; **j.** Sticky anaphase in a hypoploid cell; **k.** Sticky anaphase showing broken chromosome bridges; **l.** Sticky anaphase showing early movement of chromosomes; **m.** Sticky anaphase showing multiple chromosome bridges; **n.** Sticky anaphase with narrow chromosome bridges after equatorial separation; **o.** Sticky anaphase; **p.** Sticky chromosomes at late anaphase after equatorial separation. Scale bars = 10  $\mu$ m





**Plate 9:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at anaphase(**a-i**), telophase (**j-m**), cytokinesis (**n-p**). **a.** Chromosome bridges and erosion at anaphase; **b.** Chromosome erosion in a disturbed anaphase; **c.** Vagrants at anaphase; **d.** Sticky early anaphase showing early movement of chromosomes; **e.** Sticky anaphase; **f.** Multiple chromosome bridges at anaphase; **g.** Chromosome laggards in a disturbed anaphase; **h.** Chromosome fragment at anaphase; **i.** Chromosome erosion at anaphase; **j.** Chromosome erosion at early telophase; **k.** Telophase in a strap cell; **l.** Chromosome erosion at telophase; **m.** Abnormal cell plate formation leading to micro and macrocell at early telophase; **n.** Nuclear disintegration at cytokinesis in a binucleate cell; **o.** Nuclear lesion and erosion at early cytokinesis; **p.** Large and small groups of chromatin at cytokinesis. Scale bars = 10  $\mu$ m





**Plate 10:** Chromosomal aberration induced by the extracts of selected species of *Euphorbia* in *Allium cepa* at cytokinesis. **a.** Abnormal cytokinesis showing nuclear lesions and erosions; **b.** Nuclear lesion at cytokinesis; **c.** Chromatin disintegration and a micronucleus at the cell plate at cytokinesis; **d.** Nuclear and cytoplasmic lesion at cytokinesis; **e.** Nuclear erosion at cytokinesis in a strap cell; **f.** Formation of nuclear lesions and erosion at cytokinesis; **g.** Abnormal condensation of chromatin at cytokinesis; **h.** Macro and micro cell formation at cytokinesis showing nuclear lesions. Scale bars = 10  $\mu$ m

**D) Antiproliferative activity of *Euphorbia* species**

Both normal cell line (L929) and human breast cancer cell line MCF-7 were used to assess the antiproliferative activity of the methanolic extract of chosen *Euphorbia* species. The cytotoxic effect exerted by various concentrations of plant extracts were assessed using the MTT assay. The LC<sub>50</sub> values obtained from the two cell lines as well as the viability percentage of both cells after the treatment using tested extracts were compared in order to select the most effective plant extract for further research.

**i) *In vitro* anticancer activity using MTT assay in normal L929 cell line**

Using the MTT test, the nature of cytotoxic potential of the methanolic extracts of *E. deccanensis*, *E. rothiana*, and *E. thymifolia* was initially examined on normal L929 cell lines. To achieve this, concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml of each species were treated for 24 hours on the normal cells. The normal cells were significantly cytotoxically affected by all three of the tested plant extracts, and the effect was dose-dependent. The viability of normal cells was reduced due to the cytotoxic effects produced by all plant extracts. From the reduction of percentage of viable cells detected, it is clear that the action of the *E. thymifolia* plant extract significantly reduced the viability of normal cells compared to the other two species. The cytotoxic potential exhibited by each species on the viability of the normal L929 cell line was provided in **Fig. 32**, and the obtained results were displayed in **Plates 11, 12 and 13**. For every species, the LC<sub>50</sub> values were determined and tabulated. When compared to the other two plant species, *E. deccanensis*'s LC<sub>50</sub> value was found to be very high, indicating that it had less cytotoxic effects on normal cell line. The LC<sub>50</sub> value obtained for *E. deccanensis* was (218.04 ± 0.40 µg/ml) followed by *E. rothiana* (172.17 ± 0.48 µg/ml) and *E. thymifolia* (140.67 ± 0.65 µg/ml) respectively. In addition, it was observed that all three extracts had higher LC<sub>50</sub> values than the LC<sub>50</sub> values found in the MCF-7 cell line. Using a system microscope, the cell death characteristics of the treated and control L929 cell lines were noted. The ED50 PLUS V1.0 Software was used to compute the LC<sub>50</sub> values.

**ii) *In vitro* anticancer activity using MTT assay in MCF-7 cell line**

To ascertain the capacity to suppress the progression of breast cancer MCF-7 cell line, the methanolic extract of three *Euphorbia* spp. were analysed. For this,

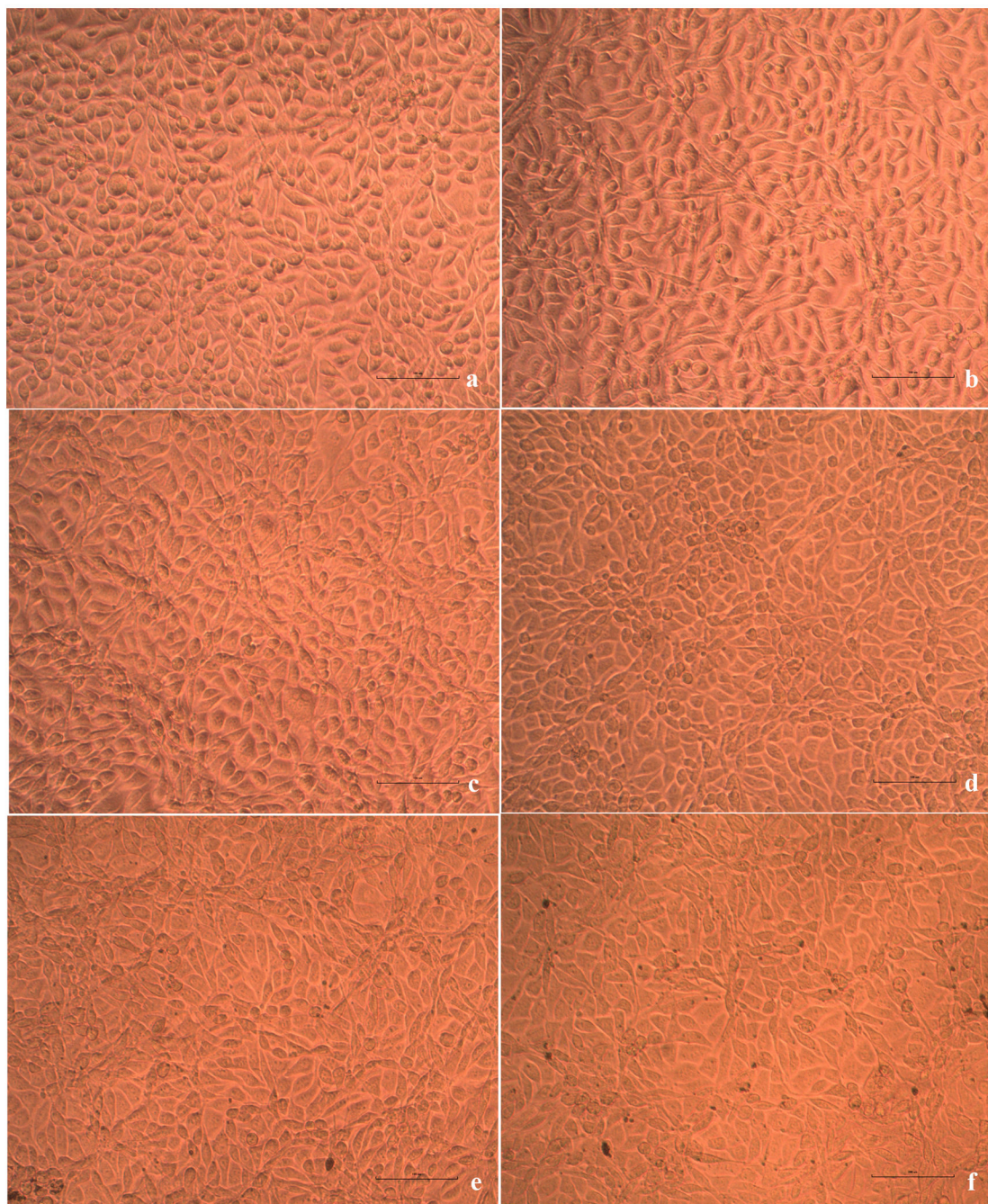
various concentrations of plant extracts, including 6.25, 12.5, 25, 50, and 100 µg/ml, were taken. The system microscope was used to examine the control and treated MCF-7 cell lines' cell death traits. Through microscopic observation, it was discovered that the plant extract treated cell line exhibited a variety of aberrations, including the condensed nucleus, apoptotic bodies, membrane blebbing, budding, and cell shrinkage. It can be concluded from the results that the three selected extracts had an observable cytotoxic effect on the breast cancer cell line that were dependent on concentration. Cell viability progressively decreased from the lower concentration of 6.25 to the higher concentration of 100 µg/ml of the plant extracts. The highest cytotoxic nature was detected in *E. deccanensis* with a great reduction in viability of MCF-7 cell line ranging from  $99.18 \pm 0.61\%$  to  $49.40 \pm 0.99\%$ . In addition, the  $LC_{50}$  values were calculated for the proper assessment of the cytotoxicity potential of the extract and noted almost equal  $LC_{50}$  value in both the case of *E. thymifolia* and *E. rothiana*. Thus, based on its lowest  $LC_{50}$  value ( $93.21 \pm 0.58$  µg/ml) and decreased cell viability percentage, it was concluded that *E. deccanensis* had a significant cytotoxic potential. **Fig. 33** displays the cytotoxic effect of each species on the viability of breast cancer cell lines. The results are presented in Plates **14, 15 and 16**.

On assessing the percentage of cell viability in both the cell lines as well as  $LC_{50}$  values of all the chosen species, it was found that *E. deccanensis* significantly inhibited the proliferation of the MCF-7 cell line than the other two plant extracts, and it also exhibited very less cytotoxicity on the normal L929 cell line. This indicates that the cancer cell-specific toxic effect of the extract of *E. deccanensis*. Thus, methanolic extract of *E. deccanensis* appeared to be a better candidate for further analysis.

**Table 11: Lethal concentrations of selected species of *Euphorbia* species in both MCF-7 and L929 cell lines**

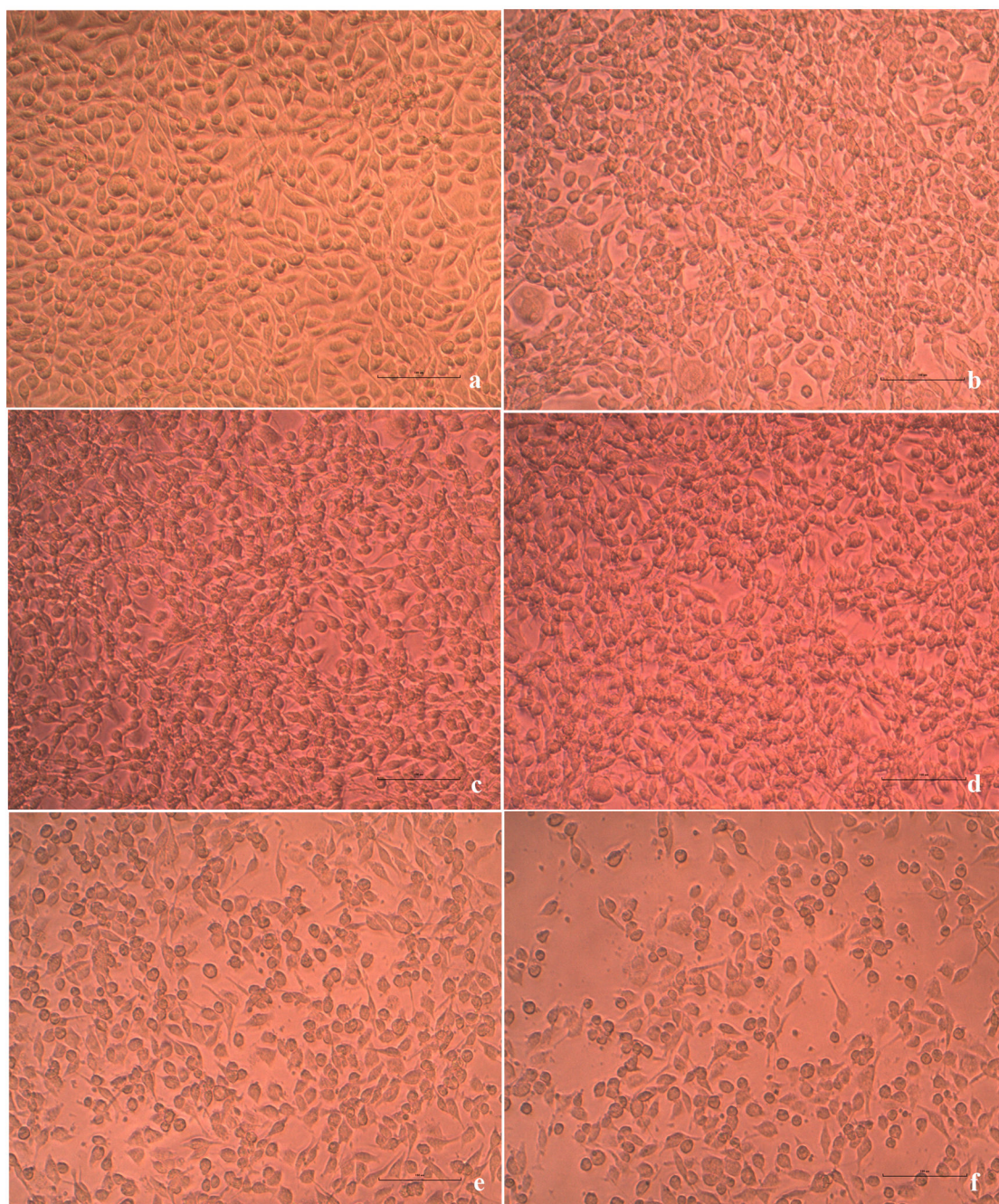
| Selected species of<br><i>Euphorbia</i> | $LC_{50} \pm SE$ (µg/ml) |                   |
|-----------------------------------------|--------------------------|-------------------|
|                                         | MCF-7                    | L929              |
| <i>E. deccanensis</i>                   | $93.21 \pm 0.58$         | $218.04 \pm 0.40$ |
| <i>E. rothiana</i>                      | $161.17 \pm 0.34$        | $172.17 \pm 0.48$ |
| <i>E. thymifolia</i>                    | $137.55 \pm 0.67$        | $140.67 \pm 0.65$ |





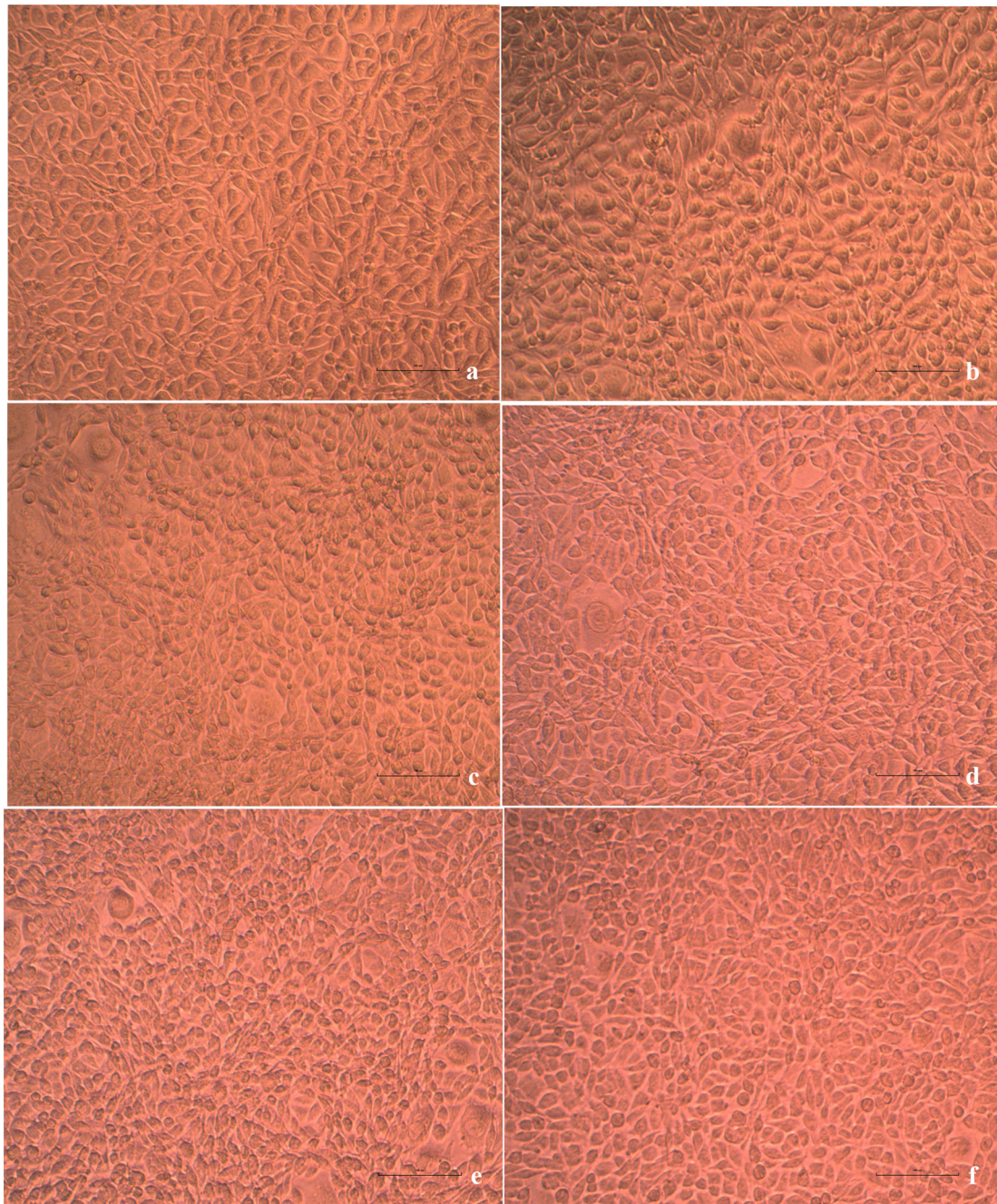
**Plate 11:** Cytotoxic effect of methanolic extract of *E. deccanensis* on L929 cells. **a.** Control; **b.** 6.25 µg/ml; **c.** 12.5 µg/ml; **d.** 25 µg/ml; **e.** 50 µg/ml; **f.** 100 µg/ml. Scale bars = 100 µm





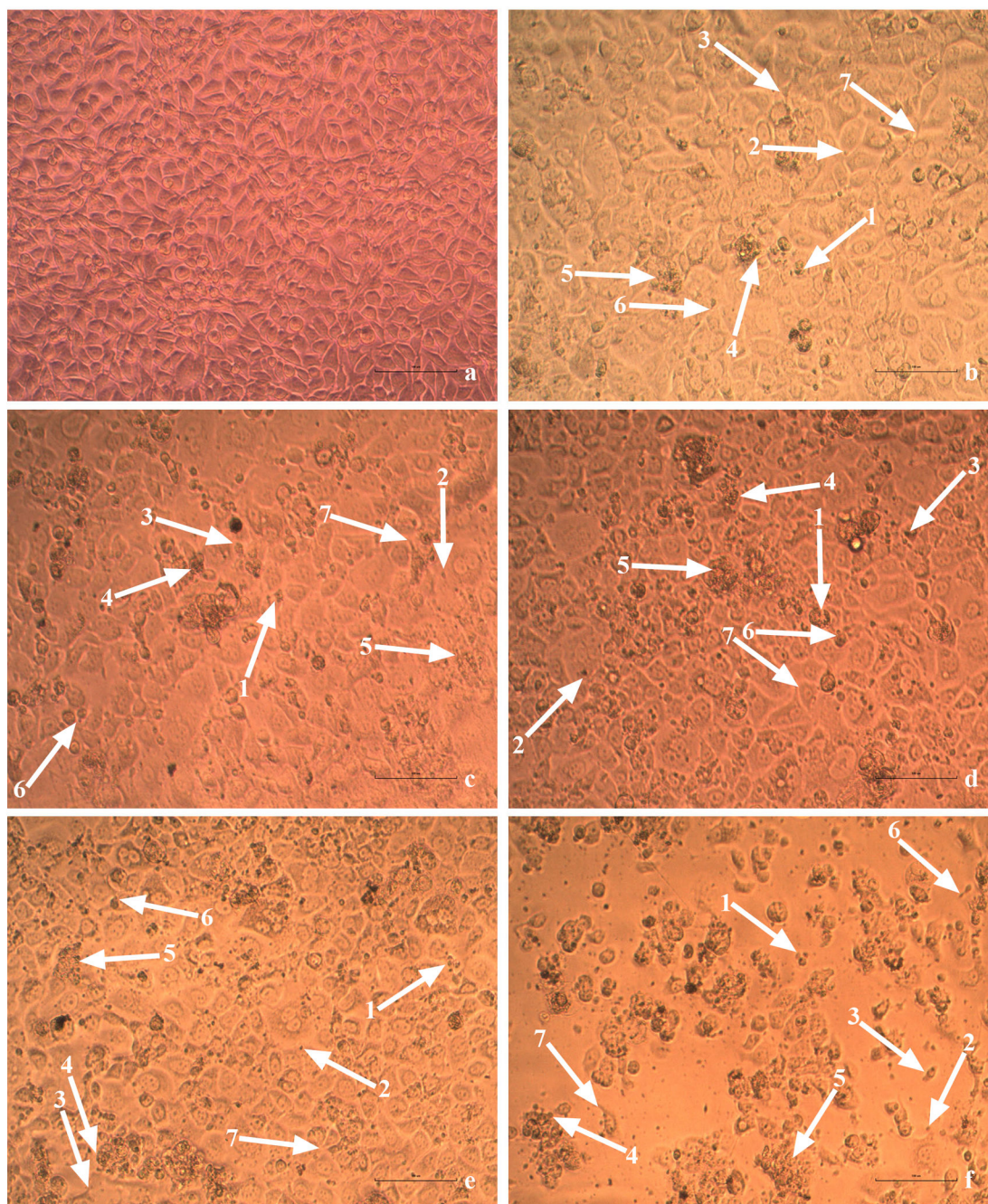
**Plate 12:** Cytotoxic effect of methanolic extract of *E. rothiana* on L929 cells. **a.** Control; **b.** 6.25 µg/ml; **c.** 12.5 µg/ml; **d.** 25 µg/ml; **e.** 50 µg/ml; **f.** 100 µg/ml. Scale bars = 100 µm





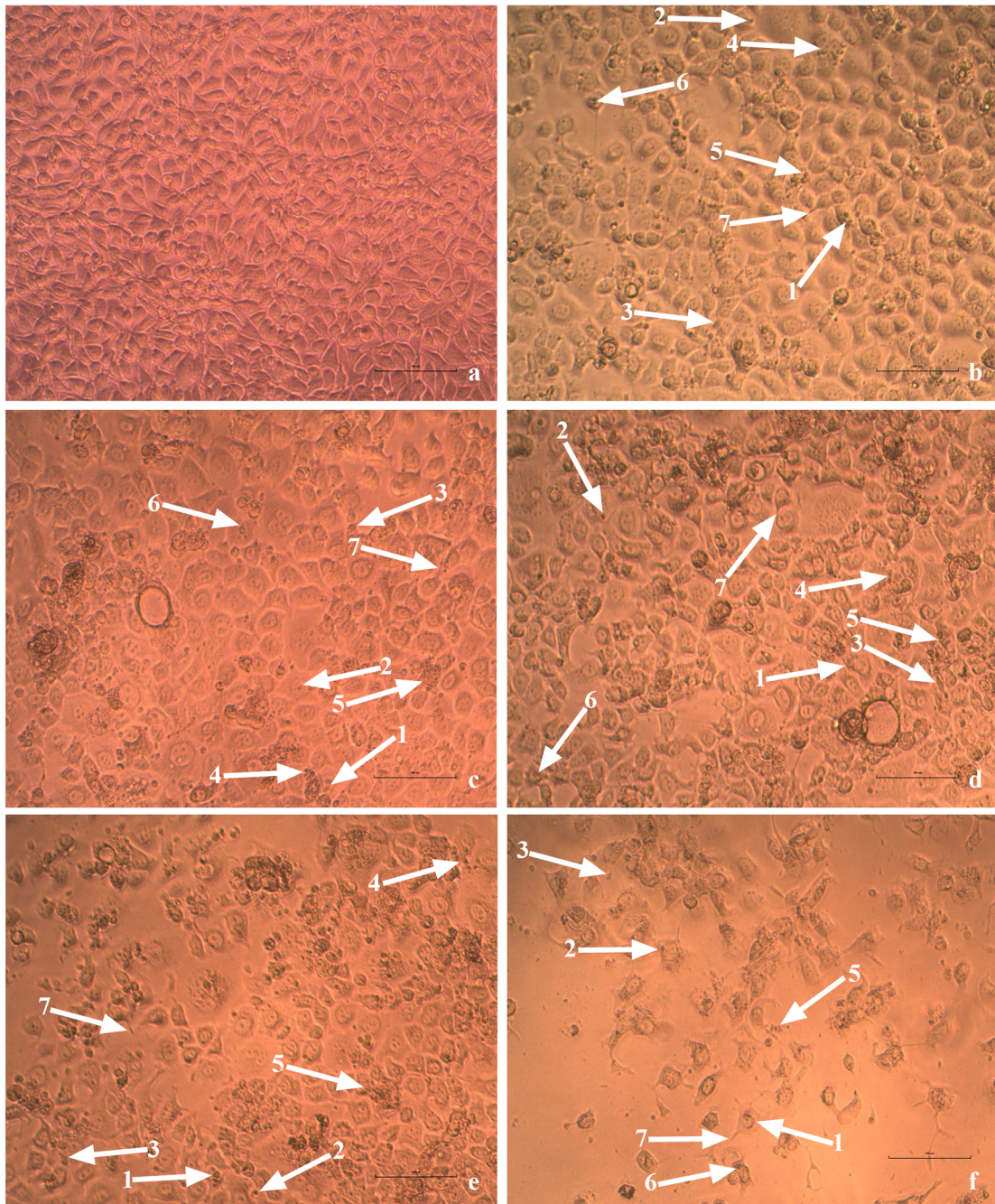
**Plate 13:** Cytotoxic effect of methanolic extract of *E. thymifolia* on L929 cells. **a.** Control; **b.** 6.25 µg/ml; **c.** 12.5 µg/ml; **d.** 25 µg/ml; **e.** 50 µg/ml; **f.** 100 µg/ml. Scale bars = 100 µm





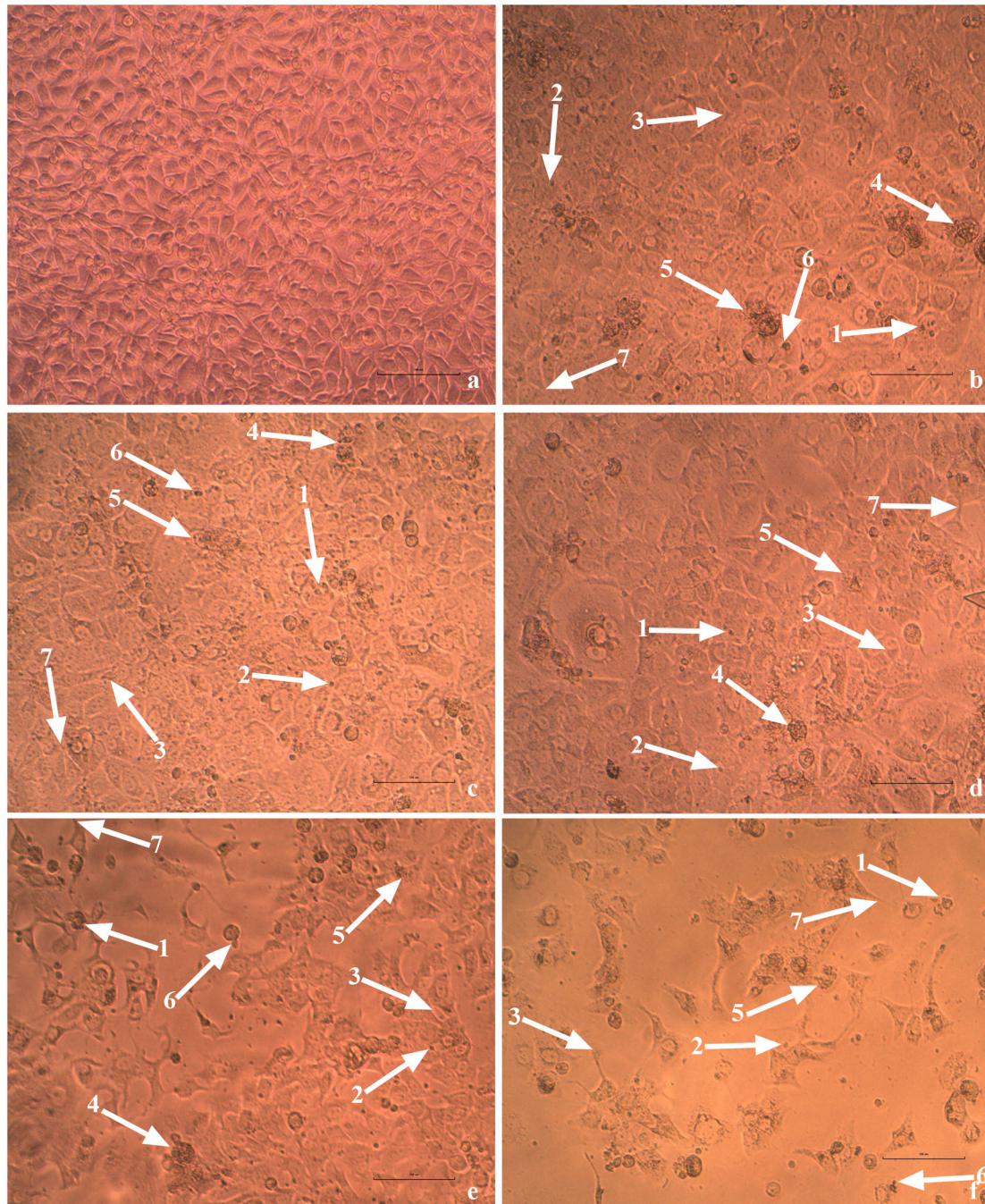
**Plate 14:** Cytotoxic effect of methanolic extract of *E. deccanensis* on MCF-7 cells, **a:** Control; **b:** 6.25 µg/ml; **c:** 12.5 µg/ml; **d:** 25 µg/ml; **e:** 50 µg/ml; **f:** 100 µg/ml. Arrows indicates apoptotic signals 1. Nuclear fragmentation, 2. Condensed nuclei, 3. Cell shrinkage, 4. Membrane blebbing, 5. Apoptotic bodies, 6. Budding, 7. Echinoid spikes. Scale bars = 100 µm





**Plate 15:** Cytotoxic effect of methanolic extract of *E. rothiana* on MCF-7 cells. **a.** Control; **b.** 6.25 µg/ml; **c.** 12.5 µg/ml; **d.** 25 µg/ml; **e.** 50 µg/ml; **f.** 100 µg/ml. Arrows indicate apoptotic signals: 1. Nuclear fragmentation, 2. Condensed nuclei, 3. Cell shrinkage, 4. Membrane blebbing, 5. Apoptotic bodies, 6. Budding, 7. Echinoid spikes. Scale bars = 100 µm





**Plate 16:** Cytotoxic effect of methanolic extract of *E. thymifolia* on MCF-7 cells, **a.** Control; **b.** 6.25 μg/ml; **c.** 12.5 μg/ml; **d.** 25 μg/ml; **e.** 50 μg/ml; **f.** 100 μg/ml. Arrows indicates apoptotic signals **1.** Nuclear fragmentation, **2.** Condensed nuclei, **3.** Cell shrinkage, **4.** Membrane blebbing, **5.** Apoptotic bodies, **6.** Budding, **7.** Echinoid spikes. Scale bars = 100 μm

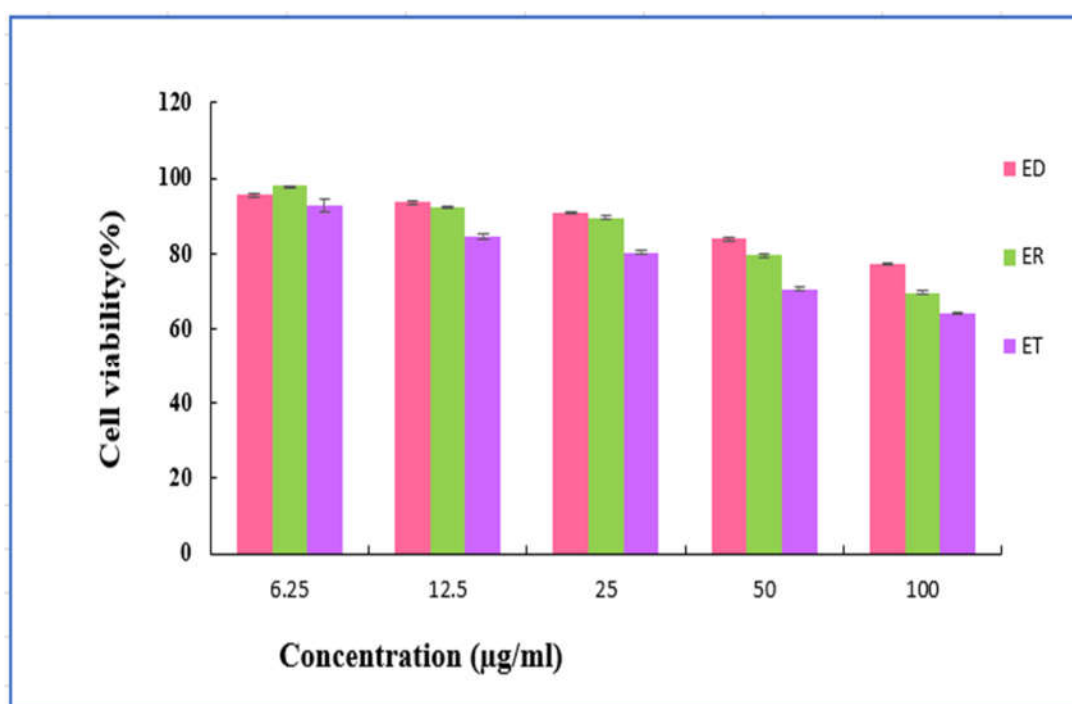


Fig. 32: Cytotoxic effects of selected species of *Euphorbia* on L929 normal cells using MTT assay. ED - *E. deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia*

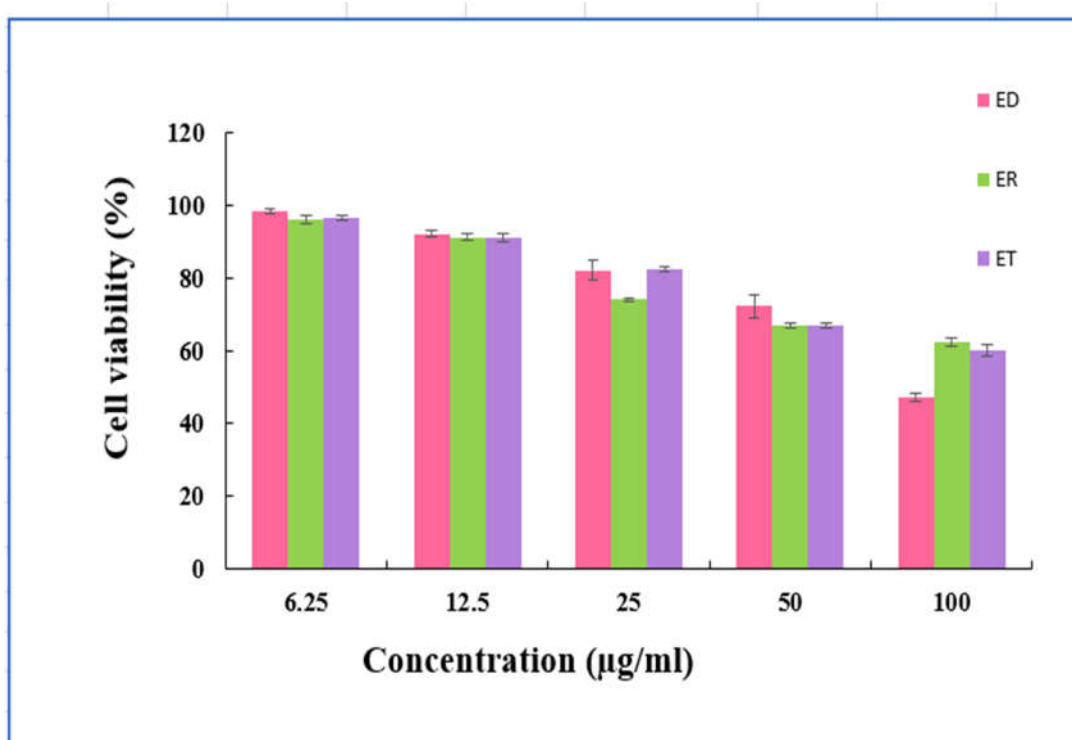


Fig. 33: Cytotoxic effects of selected species of *Euphorbia* on MCF-7 cancer cells using MTT assay. ED - *E. deccanensis*; ER - *E. rothiana*; ET - *E. thymifolia*

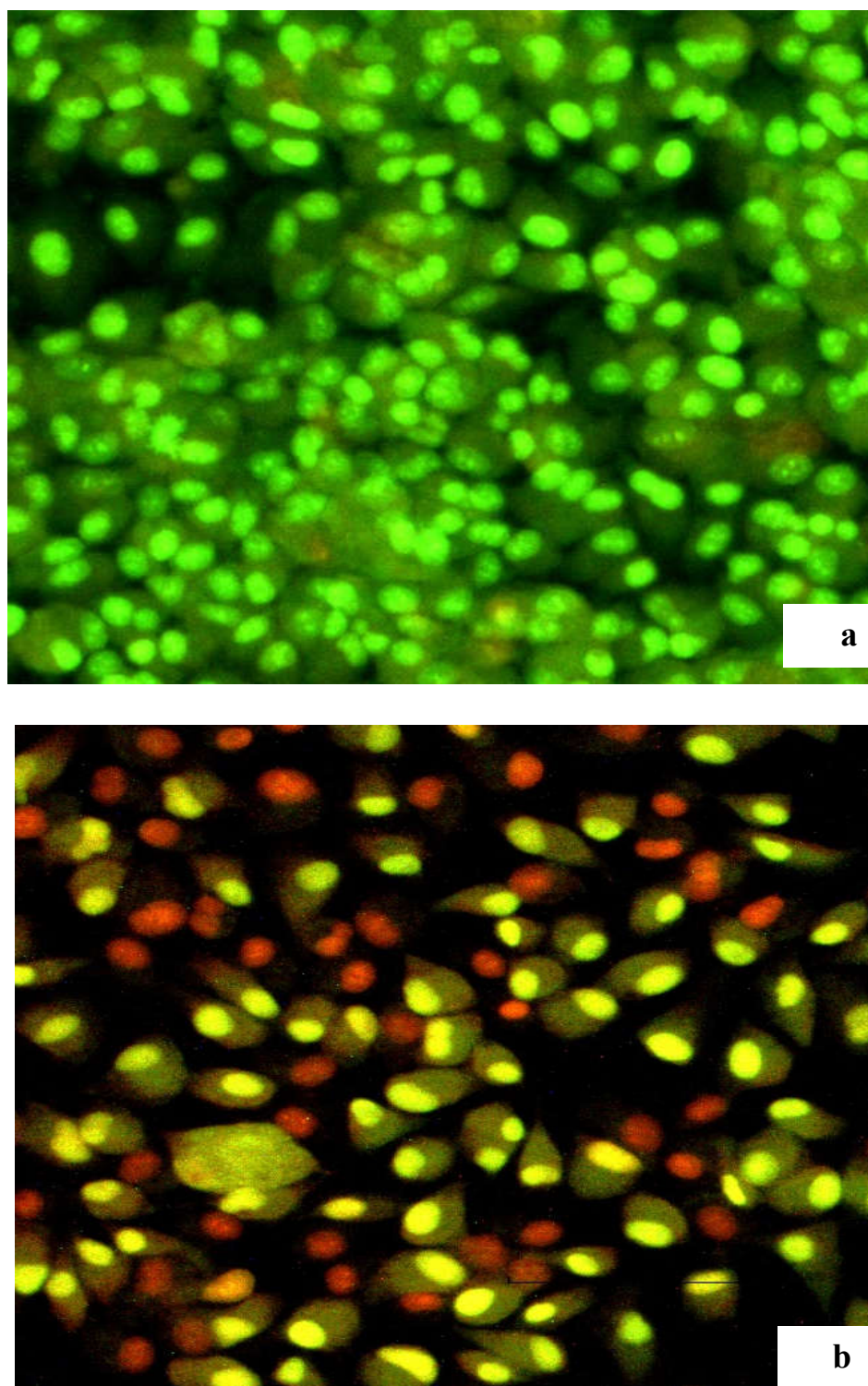
### iii) Determination of apoptosis by acridine orange (AO) and ethidium bromide (EtBr) double staining

The two dyes acridine orange/ethidium bromide (AO/EB), which binds with DNA are used in order to detect alterations in cell membranes and cells associated with apoptosis. Apoptotic cells can have basic morphological changes that can be detected using dual AO/EB fluorescent staining. It also permits the separation of necrotic cells from other cell types, including early and late apoptotic cells. The *in vitro* antiproliferative effectiveness of *E. deccanensis* was demonstrated to have a notable inhibitory effect on the cancer cell line MCF-7. Therefore, in order to perform the double staining, an LC<sub>50</sub> concentration (93.21 µg/ml) of *E. deccanensis* was subjected to treatment using the MCF-7 cell line. This was followed by staining with ethidium bromide and acridine orange. A significant representation of both the live and dead cells was revealed by this technique and also differentiate between necrotic and apoptotic cells. Ethidium bromide (EtBr) only stains cells that have lost membrane integrity to red, but acridine orange (AO), a vital dye, will turn the nuclei of both living and dead cells to green. Four distinct cell types can be distinguished morphologically, which include,

- i) Normal cell with green nucleus, which is clearly visible in the control.
- ii) Early apoptotic cells have yellowish nuclei that are either fragmented or condensed.
- iii) Cells in the late stages of apoptosis had nuclei-stained orange and showed signs of chromatin condensation or fragmentation.
- iv) Necrotic cells, whose cell nuclei are uniformly stained red.

Based on the images in **Fig. 34**, it appears that apoptosis is the primary mechanism by which the methanolic extract of *E. deccanensis* induces cell death.





**Fig. 34:** Detection of apoptosis by AO/ EtBr staining on MCF-7 cells.

**a) Control; b) Cells treated with methanolic extract of *E. deccanensis* ( $93.93.21 \pm 0.58 \mu\text{g/ml}$ )**

**iv) Analysis of cell cycle using flow cytometry**

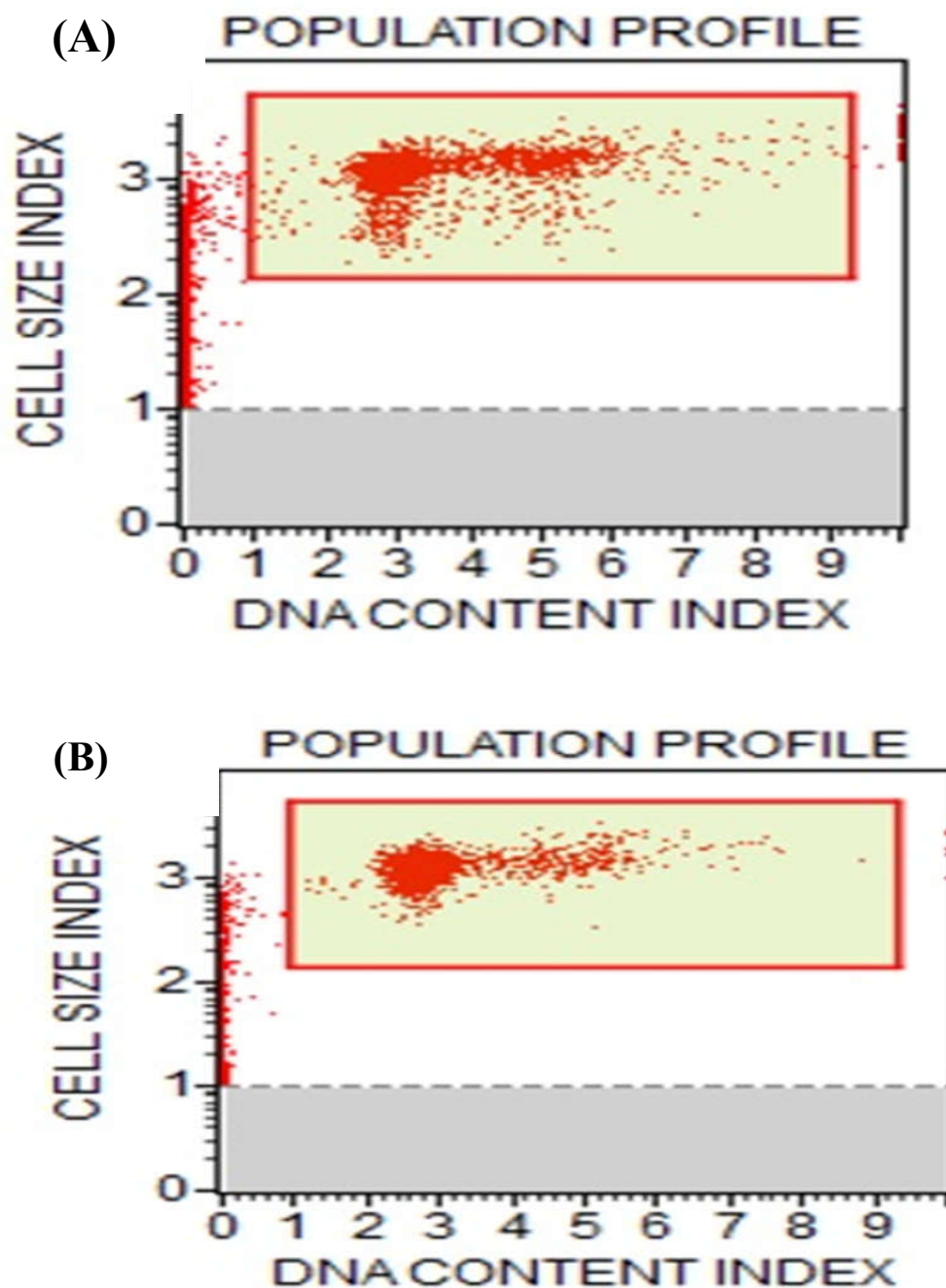
Using flow cytometry, it was determined how the methanolic plant extract of *E. deccanensis* affected the cell cycle's progression and DNA content profile. This plant extract was found to be the most potent one among the three chosen extracts. For this purpose, the MCF-7 cell line was treated with LC<sub>50</sub> concentration of the test sample and incubated for a duration of 24 hours. Both DNA and RNA have the ability to bind to nuclear DNA intercalating stains such as propidium iodide (PI), and emit fluorescence. There is a direct correlation between the amount of DNA and the fluorescence emitted by PI. If RNAase is present, PI can distinguish between cells at different phases of the cell cycle based on the differences in DNA content, increasing the specificity of DNA staining at each stage.

Regarding the population profile, it was observed that the distribution of cells in the treated cells was almost aggregating, whereas, in the control cells, the distribution of cells was more scattered. Thus, during a particular phase of the cell cycle, the progression of the cycle might become inhibited.

The cell cycle stage at which the cell cycle arrest takes place was investigated using the DNA content profile. At the G<sub>0</sub>/G<sub>1</sub>, S, and G<sub>2</sub>/M phases, the treated cells' cell count was found to be 88.2, 5, and 5.8%, respectively. From the histogram of both the treated and control cells (**Fig. 36**), it was observed that treated cells (88.2%) had a higher percentage of cell count at the G<sub>0</sub>/G<sub>1</sub> stage than the control (71.1%). Moreover, it was noted that the cell count in the S phase had decreased by a percentage of 5 in treated and by a percentage of 10 in control cells respectively.

It was demonstrated that cell cycle arrest occurs during the G<sub>0</sub>/G<sub>1</sub> phase in treated cells, as evidenced by the high cell count observed in the G<sub>0</sub>/G<sub>1</sub> phase and subsequent decrease in cell count in the S phase. Considerable apoptosis occurs in the treated cells, which was indicated by the markedly lower cell count in treated cells relative to control. As a consequence, the flow cytometry results of the cell cycle analysis demonstrated that the test sample's ability to cause apoptosis inhibited

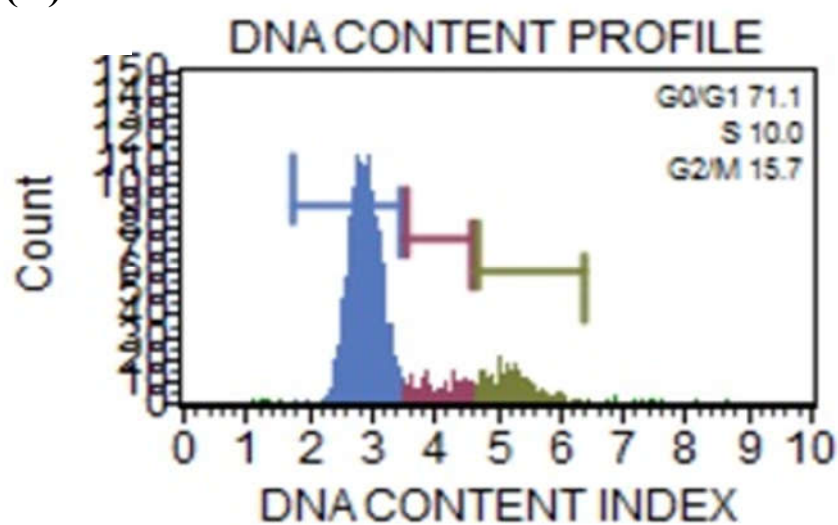
the MCF-7 cancer cell line's cells' ability to move from the G0/G1 phase to the S phase and thereby inhibited the cell cycle from continuing.



**Figure. 35: Determination of cell cycle arrest by FACS; (A) Population profile of untreated control cells (B) Population profile of *E. deccanensis* plant extract treated MCF-7 cells**



(C)



(D)

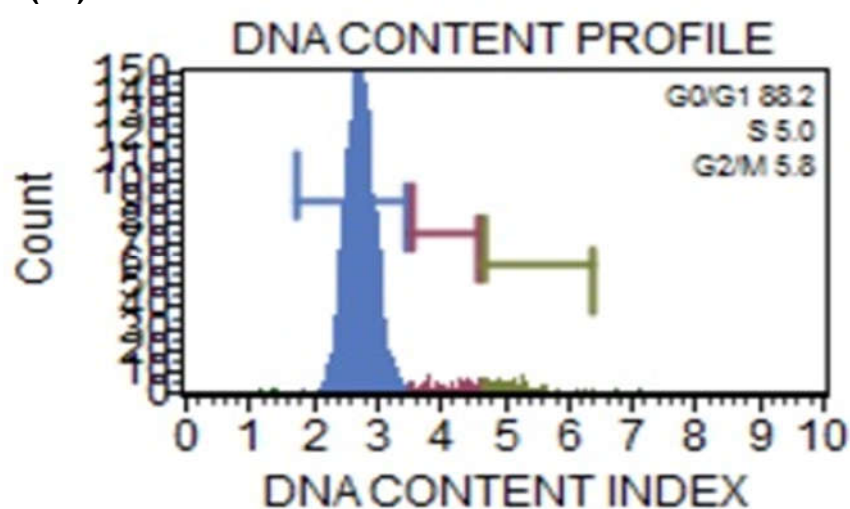
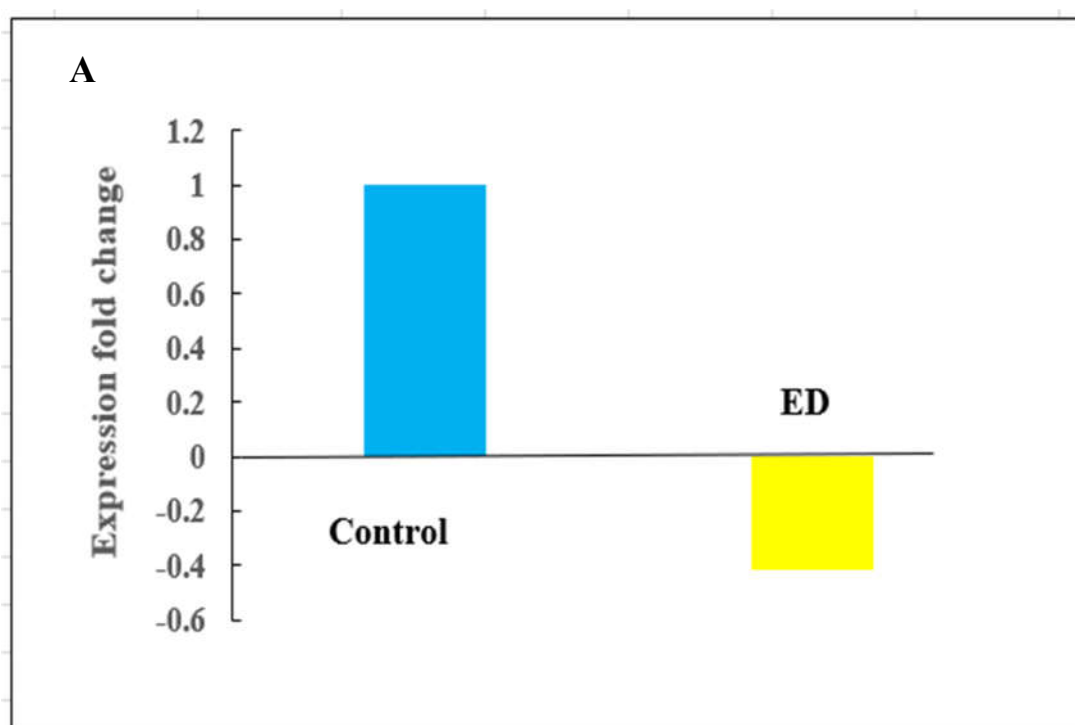


Figure. 36: Determination of cell cycle arrest by FACS; (C) DNA content profile of untreated control (D) DNA content profile of *E. deccanensis* plant extract treated MCF-7 cells

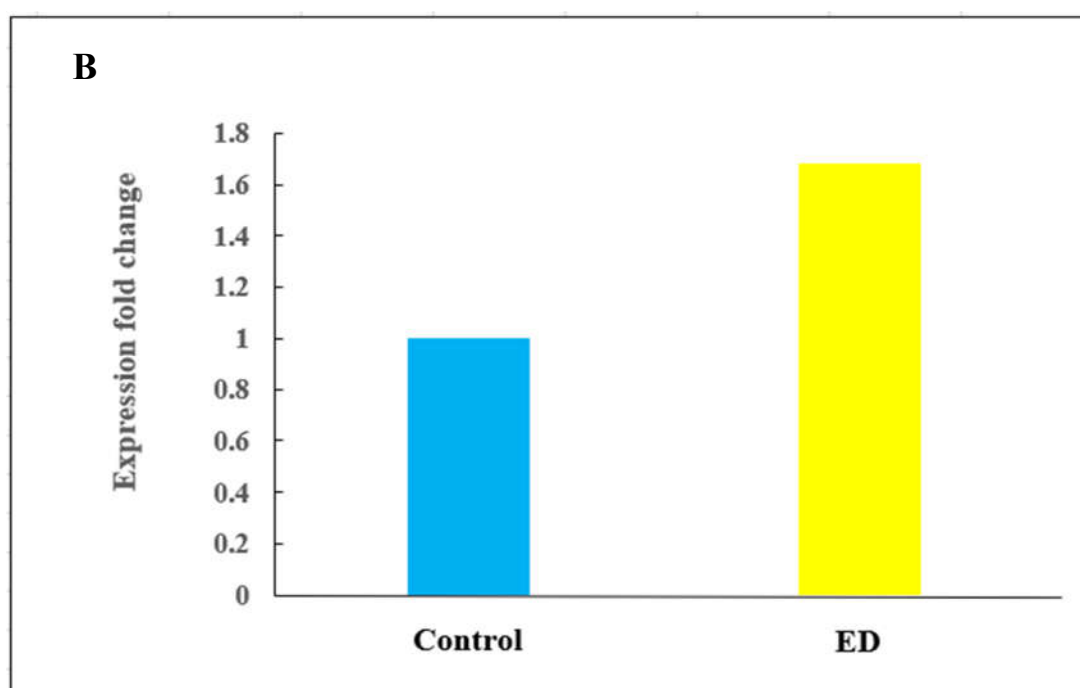
### v) Gene expression studies

To determine how *E. deccanensis* methanolic extract affected the expression of various genes, two genes related to apoptosis, namely p53 and STAT-3, were employed in RT-qPCR procedures that were followed by gel electrophoresis with GAPDH serving as the housekeeping gene. The MCF-7 cell lines were selected for the work after being treated with *E. deccanensis* methanolic extract at an LC<sub>50</sub> concentration of 93.21 µg/ml and incubated for 24 hours and untreated cells were taken as the control. The results of the experiment are given in the **Fig. 37 (A, B) and 38 (A, B)**. As compared to the control, it is evident from the data that there is a significant upregulation in p53 gene expression and downregulation of STAT-3 gene expression in the plant extract-treated samples. In comparison to the control, the expression of the p53 gene has increased by 1.68 fold (**Fig. 37 B**), which indicates that the p53 gene's expression fold has nearly doubled. When examining the case of gene STAT-3, a fold of 0.4142 reduction was detected, which in indicates that the downregulation has occurred (**Fig. 37 A**).

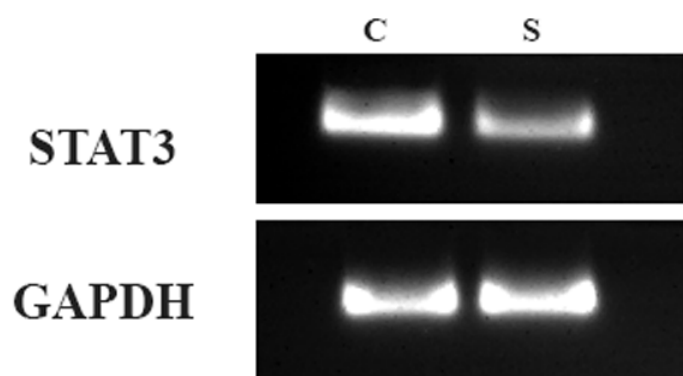
The outcomes demonstrated how effectively the *E. deccanensis* methanolic extract could downregulate the STAT-3 gene and upregulate the expression of the p53 gene in comparison to the control. The results of the gel image analysis of both genes supported this. Examining the sample-treated wells, it is possible to see that the fluorescence they release is comparatively higher than the control in the case of gene p53, indicating increased expression of the gene (**Fig. 38 B**). When it comes to gene STAT-3, the fluorescence released by the sample treated well is comparatively lower than that of the control, which suggests that the gene's expression is declining (**Fig. 38 A**). Therefore, it was evident from the investigation that the methanolic extract of *E. deccanensis* possesses the ability to upregulate the p53 gene and downregulate the gene STAT-3, both of which play important roles in the apoptosis pathways linked to cancer.



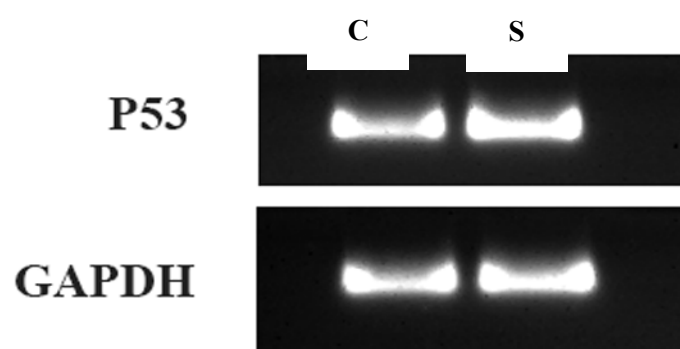
**Figure. 37 A:** Expression fold change of the gene STAT-3 in MCF-7 cells treated with *E. deccanensis* methanolic extract



**Figure. 37 B:** Expression fold change of the gene p53 in MCF-7 cells treated with *E. deccanensis* methanolic extract



**Fig. 38 A:** Expression analysis of the gene STAT-3 using Real-Time PCR (C= Control; S= Sample)



**Fig. 38 B:** Expression analysis of the gene p53 using Real-Time PCR (C= Control; S= Sample)

### PART III

#### GREEN SYNTHESIS OF SILVER NANOPARTICLES

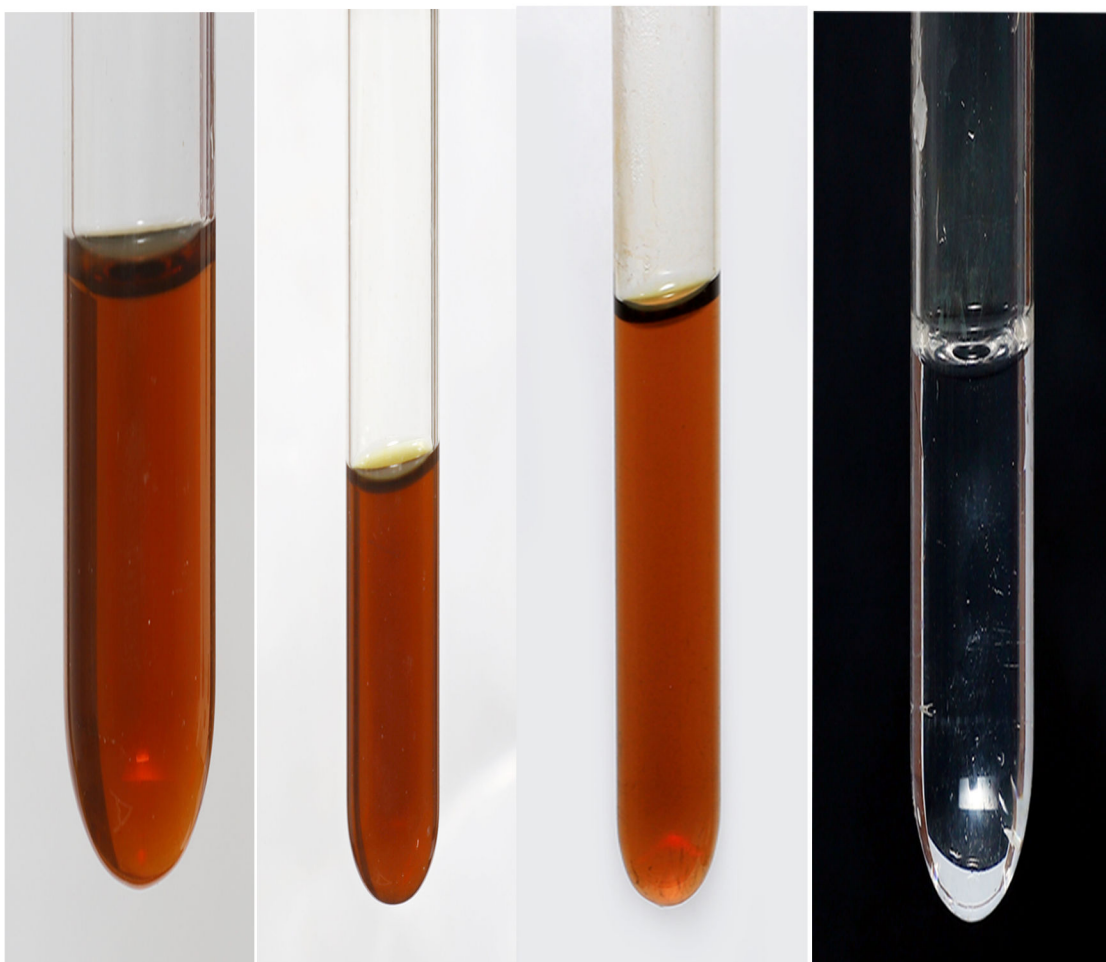
The ability of methanolic extracts of selected *Euphorbia* plant species to synthesize silver nanoparticles was determined. The interaction of the methanolic plant extract and the silver nitrate solution results in the formation of silver nanoparticles. To analyse the detailed description of biosynthesized silver nanoparticles, a variety of physicochemical characterization techniques were used, such as UV–visible spectroscopy (UV-Vis spectroscopy), Field emission scanning electron microscopy (FE SEM) and Fourier transform infrared spectroscopy (FTIR). The ideal parameters required for creating nanoparticles were:

- i) Temperature - 60°C temperature for 15 minutes
- ii) Volume ratio of plant extracts to silver nitrate - 2 ml : 8 ml
- iii) Silver nitrate concentration - 2 mM
- iv) pH of the reaction mixture - 8

The development of silver nanoparticles as a result of the reduction of silver nitrate is indicated visually by the gradual change in the colour of the reaction mixture.

#### Visual observation of colour change

In the current work, the presence of a yellowish-brown or reddish-brown colour indicates the fabrication of silver nanoparticles. Concerning *E. deccanensis*, the reaction mixture's colour changes from pale yellow to dark reddish brown as a result of a reaction between the plant's methanolic extract and the 2 mM silver nitrate solution. A deep reddish-brown colour was also produced in the case of *E. rothiana* as a result of the plant extract's interaction with the silver nitrate solution at a temperature of 60°C. However, in the instance of *E. thymifolia*, the combining actions of the solutions at the same temperature resulted in the development of a comparatively pale reddish-brown colour (**Plate 17**).



**Plate 17:** Visual colour indication of silver nanoparticle formation in the order of ED, ER, ET along with the colourless silver nitrate solution

## ii) CHARACTERISATION TECHNIQUES

### a) UV–visible spectroscopy (UV-Vis spectroscopy)

The formation of silver nanoparticles was verified through UV-Vis spectral analysis within the range of 200-800 nm wavelength. Silver nanoparticle absorption spectra unambiguously demonstrate the interaction between silver ions and phytometabolites present in methanolic plant extracts. The range of 380 - 470 nm was the  $\lambda$  max taken into consideration for the occurrence of silver nanoparticles. In *E. deccanensis*, *E. rothiana*, and *E. thymifolia*, a sharp, noticeable peak was detected at 416 nm, 432 nm, and 443 nm, respectively (**Figure 39 a, b, c**). Thus, every plant extract that was chosen for the current study showed a distinct peak that fell between these ranges. Hence, this spectral analysis confirmed the formation of silver nanoparticles in the chosen plant's of methanolic extract.

### b) FE-SEM analysis (Field emission scanning electron microscopy)

Using FE-SEM analysis, the morphological details of Ag NPs were evaluated. The surface characteristics of the generated silver nanoparticles, including their size and shape, are detailed in SEM images. It was noted that all of the selected plant extracts (*E. deccanensis*, *E. rothiana*, *E. thymifolia*) have nearly spherical-shaped silver nanoparticles (**Plate 18**). The results showed that the average particle size of the silver nanoparticles formed in the selected species ranged from 23 to 44 nm, based on the SEM images. It was found that the silver nanoparticles obtained for *E. deccanensis* ranges between 23.24 nm to 43.75 nm with an average particle size of 33.4 nm and 36 nm in the case of *E. rothiana*. With an average particle size of 37.6 nm, relatively large spherical-shaped nanoparticles were found in *E. thymifolia*.

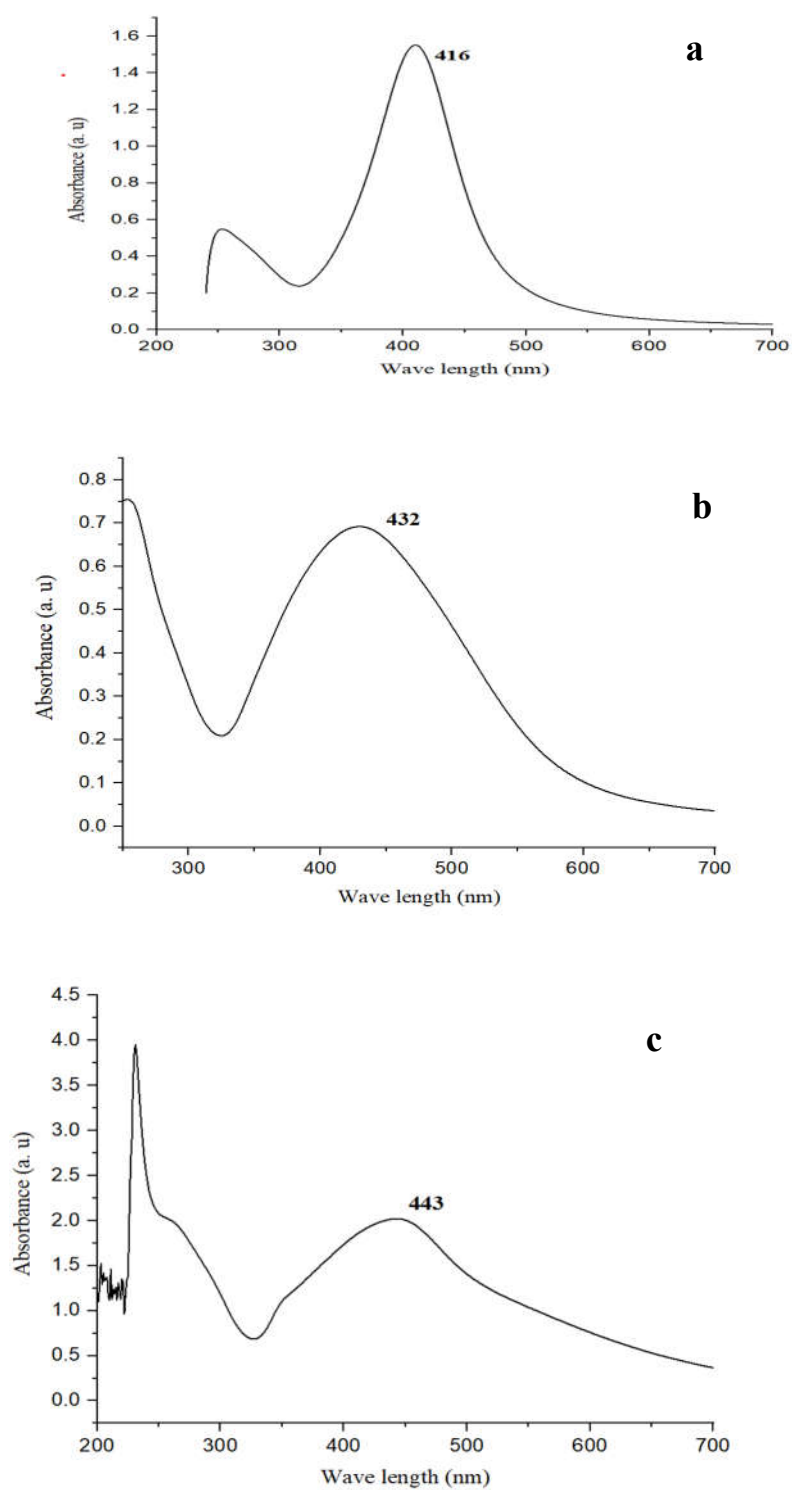
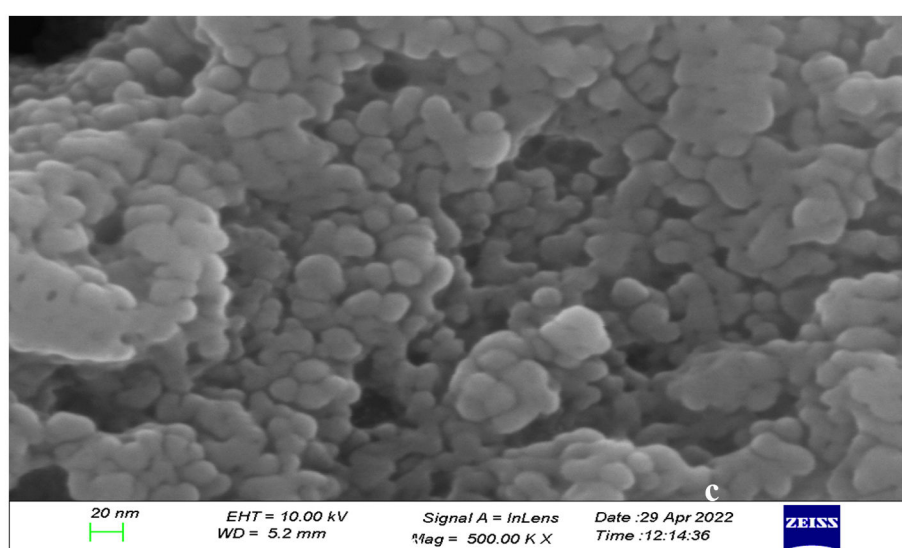
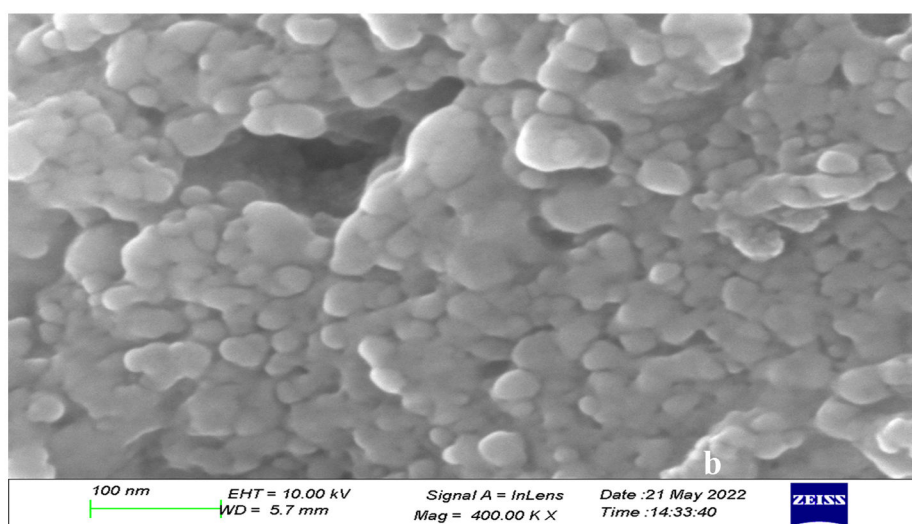
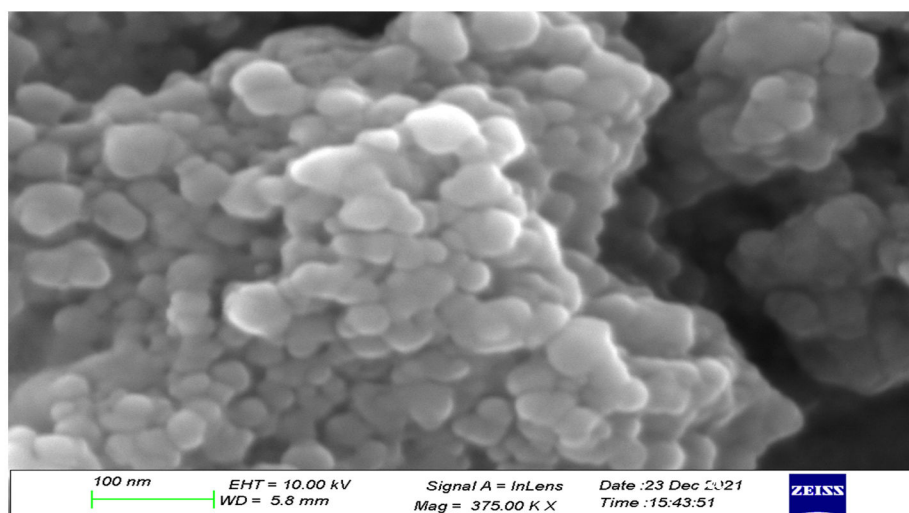


Figure. 39: UV-Vis spectra of silver nanoparticles synthesised by selected species of *Euphorbia* a) *E. deccanensis*, b) *E. rothiana*, c) *E. thymifolia*



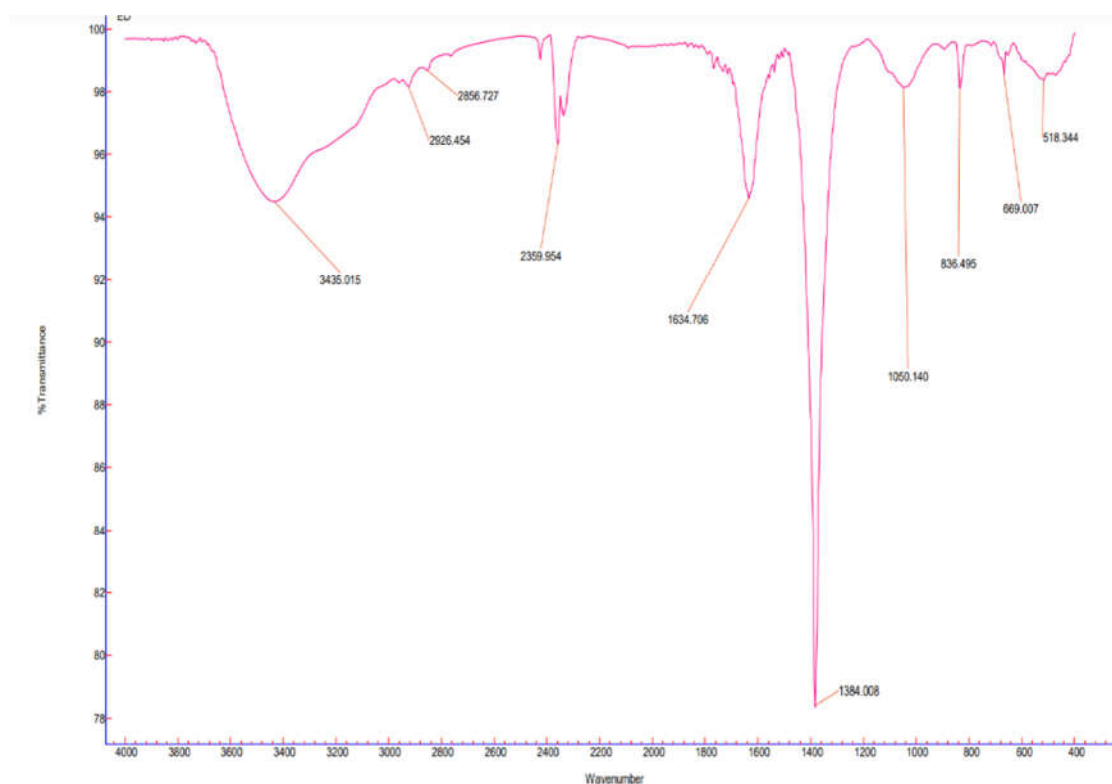


**Plate 18:** SEM images of silver nanoparticles synthesised from **a.** *E. deccanensis*; **b.** *E. rothina*; **c.** *E. thymifolia*

### c) Fourier transform infrared spectroscopy (FTIR) Analysis

To ascertain the presence of distinct functional groups found in biomolecules that are crucial to the production of Ag NPs via the reduction of silver ions, Fourier transform infrared spectroscopy (FTIR) analysis was done. It is also useful to understand the chemical structure of Ag NPs and the biomolecules that act as capping and stabilising agents during the nanoparticle synthetic process. In this analysis, the obtained intense bands were compared with standard values to determine the functional groups. The different functional groups of secondary metabolites were represented by these spectra.

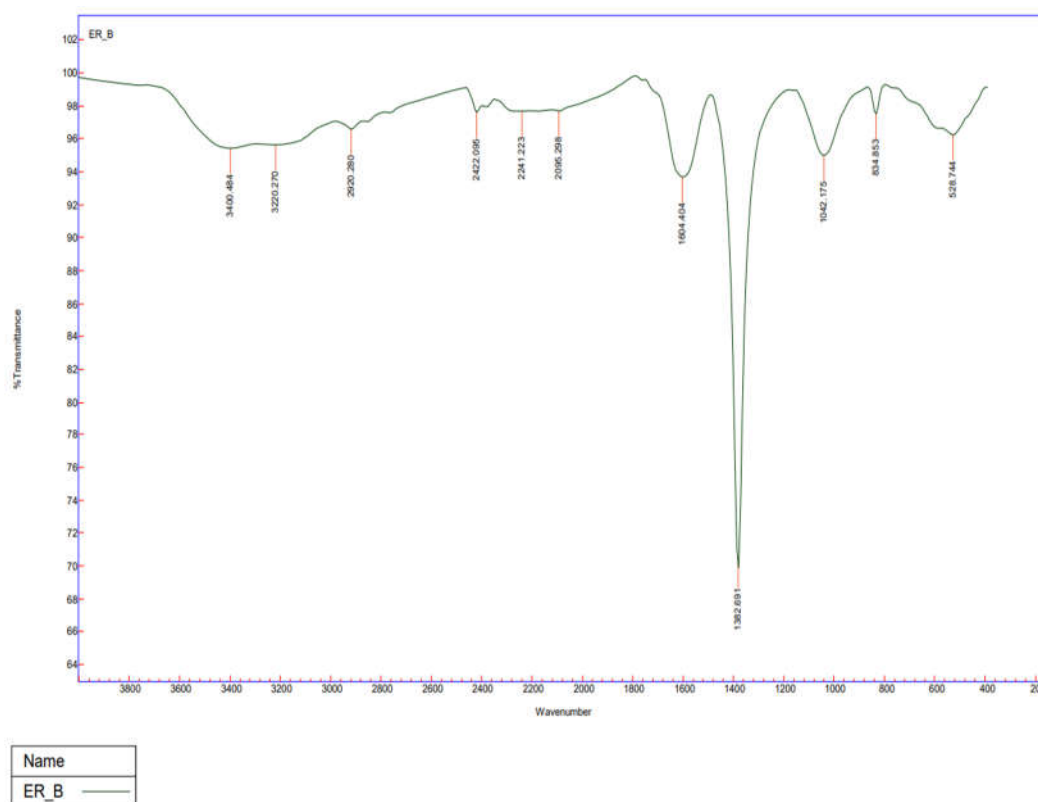
The major vibration modes of *E. deccanensis* were identified by the FTIR spectrum at 3435, 2926, 2856, 2359, 1634, 1384, 1050, 836, 669, and 518  $\text{cm}^{-1}$  (**Fig. 40 a**). In the case of *E. rothiana*, major vibration modes were observed at 3400, 3220, 2920, 2422, 2412, 2095, 1604, 1382, 1042, 834 and 528  $\text{cm}^{-1}$  (**Fig. 40 b**). In *E. thymifolia*, major vibration modes were obtained at 3774, 3409, 2918, 2424, 2092, 1618, 1382, 1038, 596 and 528  $\text{cm}^{-1}$  (**Fig. 40 c**), which indicate the existence of phytochemicals serving as capping agents. There are 10 major vibration peaks observed in the cases of *E. deccanensis* and *E. thymifolia*, and 11 vibration peaks in the case of *E. rothiana*. Phenols and halo compounds were found to be common in the three chosen species of *Euphorbia*. According to the current research, all three plant species have notable functional groups that correlate to significant phytoconstituents. It is therefore reasonable to assume that these functional groups play a key role in the production, stabilisation, and reduction of silver nanoparticles. The major functional groups obtained from FTIR analysis that significantly contributed to the development of Ag NPs were tabulated in **Table 12, 13 and 14**.



**Fig. 40 a: FTIR spectra of *E. deccanensis***

**Table 12: Functional groups observed in the FTIR analysis of *E. deccanensis* methaolic extract**

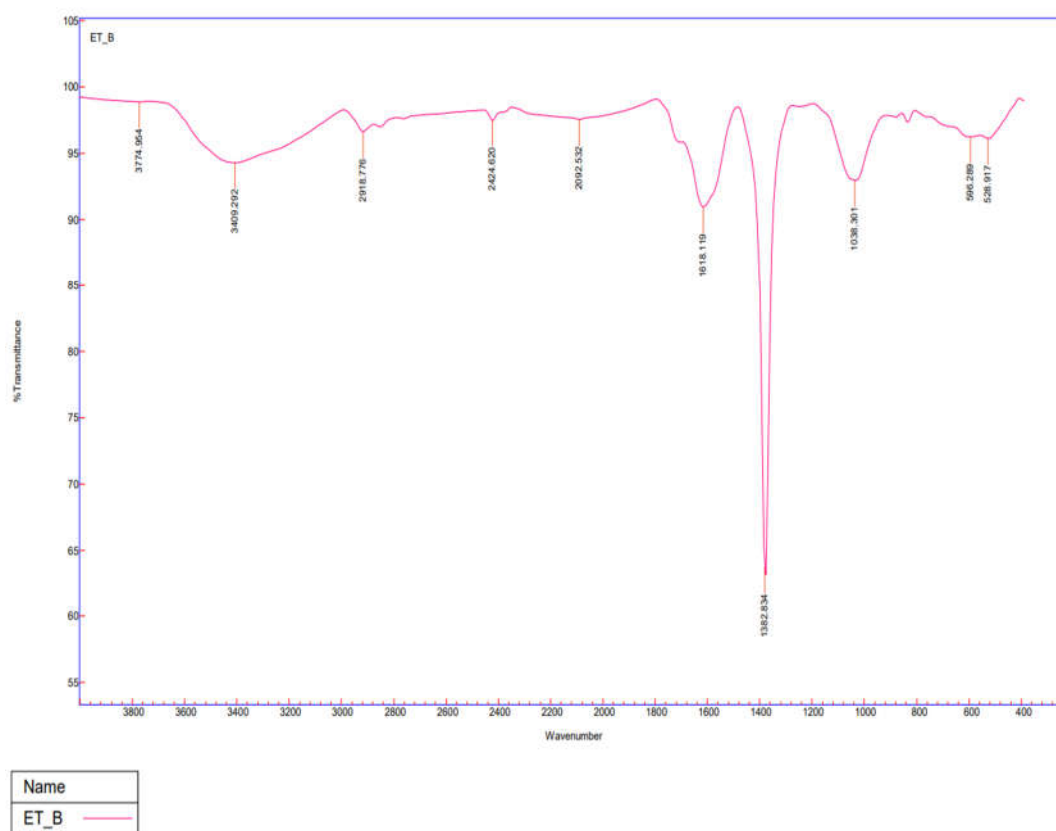
| Sl. No. | Frequency range (cm <sup>-1</sup> ) | Functional group                  |
|---------|-------------------------------------|-----------------------------------|
| 1       | 3435                                | Alcohols and phenols              |
| 2       | 2926                                | Methyl group                      |
| 3       | 2856                                | Aromatic compounds                |
| 4       | 2359                                | C=O stretching in ketone group    |
| 5       | 1634                                | Diketones                         |
| 6       | 1384                                | C-H bending of aldehyde           |
| 7       | 1050                                | Polysaccharide                    |
| 8       | 836                                 | Aromatic compounds                |
| 9       | 669                                 | Aliphatic bromo compounds         |
| 10      | 518                                 | Halogen compounds, iodo compounds |



**Fig. 40 b: FTIR spectra of *E. rothiana***

**Table 13: Functional groups observed in the FTIR analysis of *E. rothiana* methanolic extract**

| Sl. No. | Frequency range (cm <sup>-1</sup> ) | Functional group                    |
|---------|-------------------------------------|-------------------------------------|
| 1       | 3400                                | Aliphatic primary amine             |
| 2       | 3220                                | O-H (hydroxyl) functional group     |
| 3       | 2920                                | Aliphatic hydrocarbons              |
| 4       | 2422                                | Carboxylic acid                     |
| 5       | 2412                                | Carboxylic acid                     |
| 6       | 2095                                | Nitriles (C≡N stretching vibration) |
| 7       | 1604                                | Amino acid                          |
| 8       | 1382                                | Phenol                              |
| 9       | 1042                                | Polysaccharide                      |
| 10      | 834                                 | Alkene                              |
| 11      | 528                                 | Halo compound                       |



**Fig. 40 c: FTIR spectra of *E. thymifolia***

**Table 14: Functional groups observed in the FTIR analysis of *E. thymifolia* methanolic extract**

| Sl. No. | Frequency range (cm <sup>-1</sup> ) | Functional group                            |
|---------|-------------------------------------|---------------------------------------------|
| 1       | 3774                                | Hydroxyl group (OH)                         |
| 2       | 3409                                | O-H alcohol group                           |
| 3       | 2918                                | C-H bonds in alkanes, aliphatic hydrocarbon |
| 4       | 2424                                | Carboxylic acid                             |
| 5       | 2092                                | Nitrile functional group                    |
| 6       | 1618                                | $\alpha$ , $\beta$ -unsaturated ketone      |
| 7       | 1382                                | Phenol                                      |
| 8       | 1038                                | Anhydride                                   |
| 9       | 596                                 | Alkyl halide                                |
| 10      | 528                                 | Halo compound                               |

### iii) Antimicrobial activity of synthesised silver nanoparticles

#### a) Antibacterial activity of Ag NPs

The potential of antibacterial activity of synthesised silver nanoparticles against various human pathogens such as Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and Gram-positive bacteria (*Streptococcus mutans*, and *Enterococcus faecalis*) was determined. To ascertain the antibacterial property, the disc diffusion assay was employed. Different concentrations of Ag NPs such as (250 µg/ml, 500 µg/ml, and 1000 µg/ml) were extracted from a 10 mg/ml stock solution. The **Plates 19, 20, 21 & 22** displayed the antibacterial effects of plant-produced Ag NPs with a diameter of <100 nm at various concentrations (250–1000 µg/ml) against the Gram-positive bacteria *S. mutans*, and *E. faecalis*, as well as the Gram-negative bacteria *P. aeruginosa* and *E. coli*. Results show that, *in vitro*, bacterial growth can be effectively inhibited by Ag NPs produced from methanolic plant extracts when compared to untreated bacteria and the negative control.

The developed silver nanoparticles effectively and significantly inhibited the growth of all four of the selected bacteria. The zone of inhibition developed by the action of silver nanoparticles against the growth of gram-negative bacteria ranged from 10 – 19 mm for *E. Coli* and 10 – 13 mm for *P. aeruginosa* (**Fig. 41**). When compared to the other test plant extracts, *E. deccanensis*- derived nanoparticles showed a prominent suppression on the growth of *E. coli* bacteria.

In the case of Gram-positive bacteria, the zone of inhibition that resulted from the activity of silver nanoparticles ranged from 10 - 20 mm (**Fig. 42**). Here also the activity of silver nanoparticles derived from *E. deccanensis* caused the prominent inhibition on the growth of both pathogenic bacteria *E. faecalis* and *S. mutans*. While the other two plant species exhibited comparable antibacterial activity.

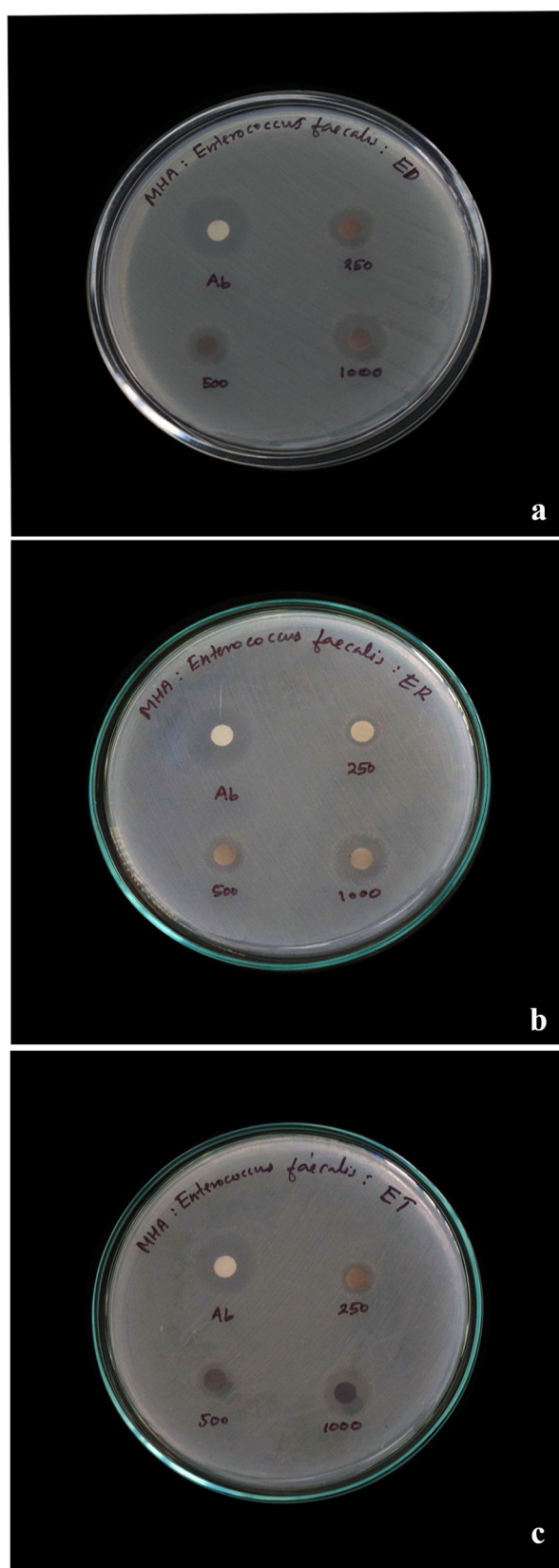
All of the plant-derived nanoparticles were found to suppress the growth of chosen bacterial strains in a dose-dependent manner, in both Gram-positive and

Gram-negative strains of bacteria. There was a discernible rise in antibacterial activity as concentration increased from lower to higher values. There was a positive correlation found between the concentration of silver nanoparticles and their antibacterial activity. Therefore, the derived nanoparticles' antibacterial efficacy was found to be dose-dependent.

When bacteria are taken into account, the ability of silver nanoparticles to inhibit the growth of Gram-positive bacteria was found to be much better than the inhibition of the growth of Gram-negative bacteria. Methanolic plant extracts contain phytometabolites that function as capping and stabilising agents during the nanofabrication process. Additionally, they have the power to strengthen the antibacterial properties of the formed silver nanoparticles. Thus, it can be concluded that the antibacterial property observed is largely dependent on the phytometabolites present in the tested extracts. In addition, perhaps the most compelling explanation for the intense activity of nanoparticles is their extraordinary capacity to enter bacterial cells and kill them due to their high surface-to-volume ratio. Because of these plant species' strong antibacterial effects, this study found that the green synthesis of Ag NPs using the selected plant species is a practical method for stopping the spread of these pathogens.

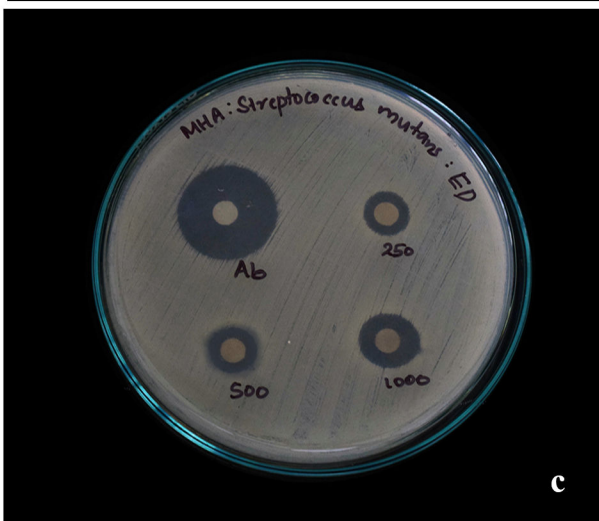
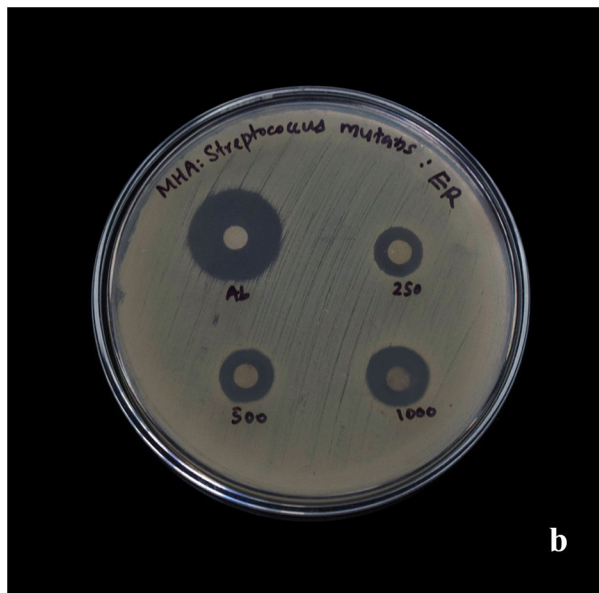
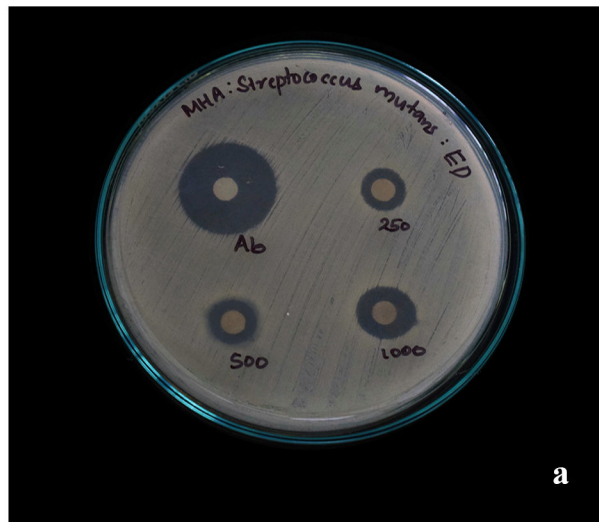
**Table 15: The diameter of the zone of inhibition (mm) produced by plant extract-derived silver nanoparticles against selected Gram-positive bacteria**

| Plant Species                    | Zone of inhibition (mm)           |           |           |                             |           |           |
|----------------------------------|-----------------------------------|-----------|-----------|-----------------------------|-----------|-----------|
|                                  | Bacterial strains (Gram-positive) |           |           |                             |           |           |
|                                  | <i>Enterococcus faecalis</i>      |           |           | <i>Streptococcus mutans</i> |           |           |
| Concentrations of Ag NPs (µg/ml) | 250                               | 500       | 1000      | 250                         | 500       | 1000      |
| <i>E. deccanensis</i>            | 11 ± 0.2                          | 12 ± 0.15 | 14 ± 0.27 | 12 ± 0.25                   | 16 ± 0.35 | 20 ± 0.25 |
| <i>E. rothiana</i>               | 10 ± 0.26                         | 11 ± 0.35 | 13 ± 0.26 | 12 ± 0.26                   | 14 ± 0.25 | 17 ± 0.27 |
| <i>E. thymifolia</i>             | 10 ± 0.11                         | 11 ± 0.41 | 13 ± 0.37 | 12 ± 0.29                   | 14 ± 0.26 | 16 ± 0.18 |

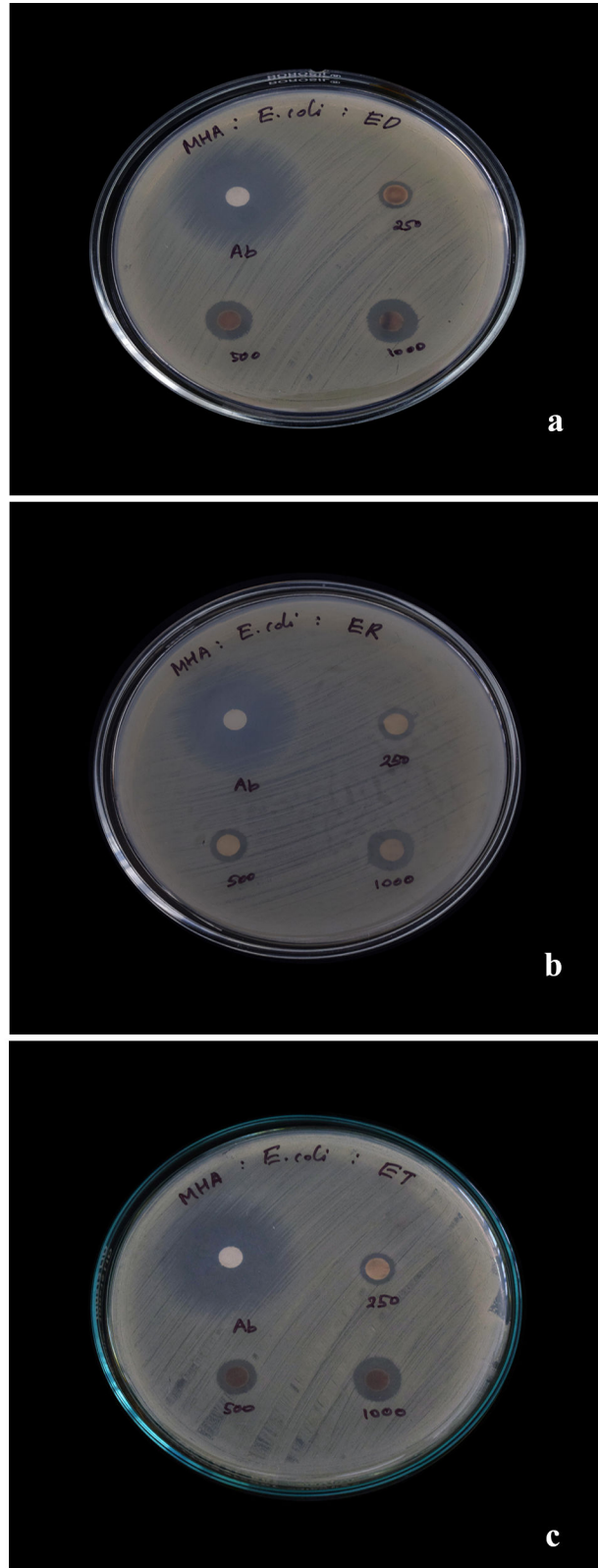


**Plate 19:** Antibacterial activity of silver nanoparticles derived from plant extracts against *Enterococcus faecalis* **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*

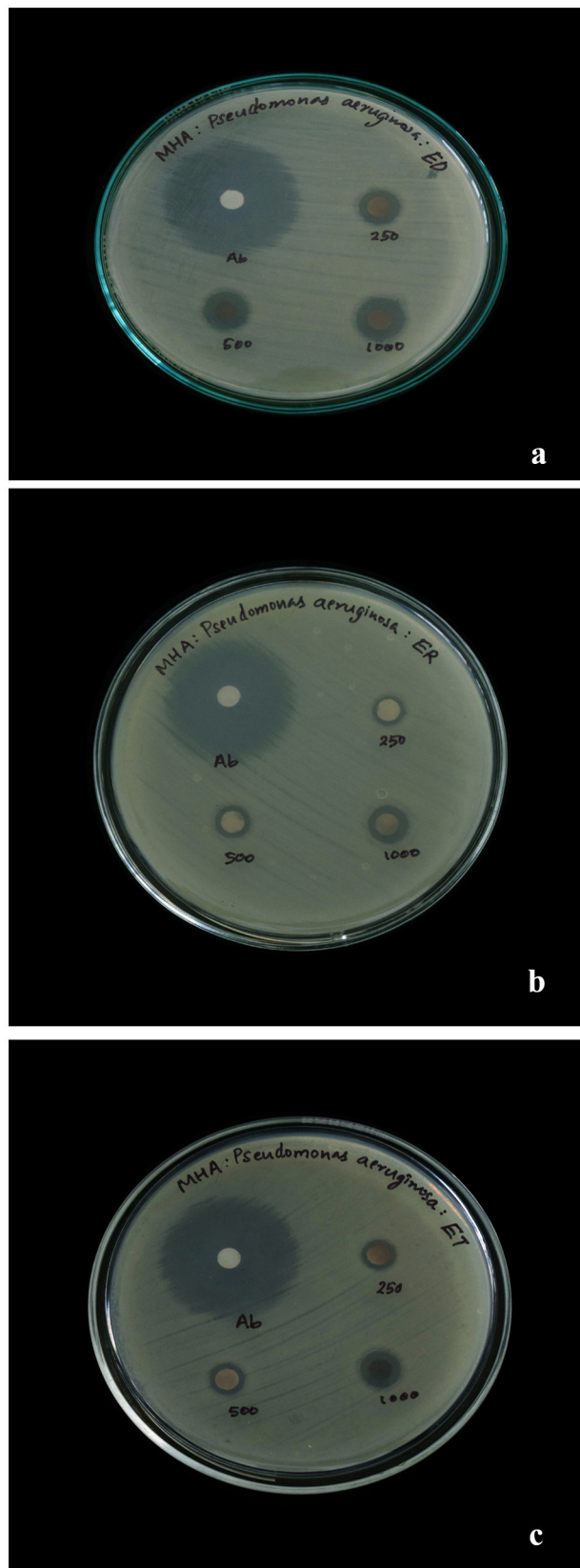




**Plate 20:** Antibacterial activity of silver nanoparticles derived from plant extracts against *Streptococcus mutans* **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*



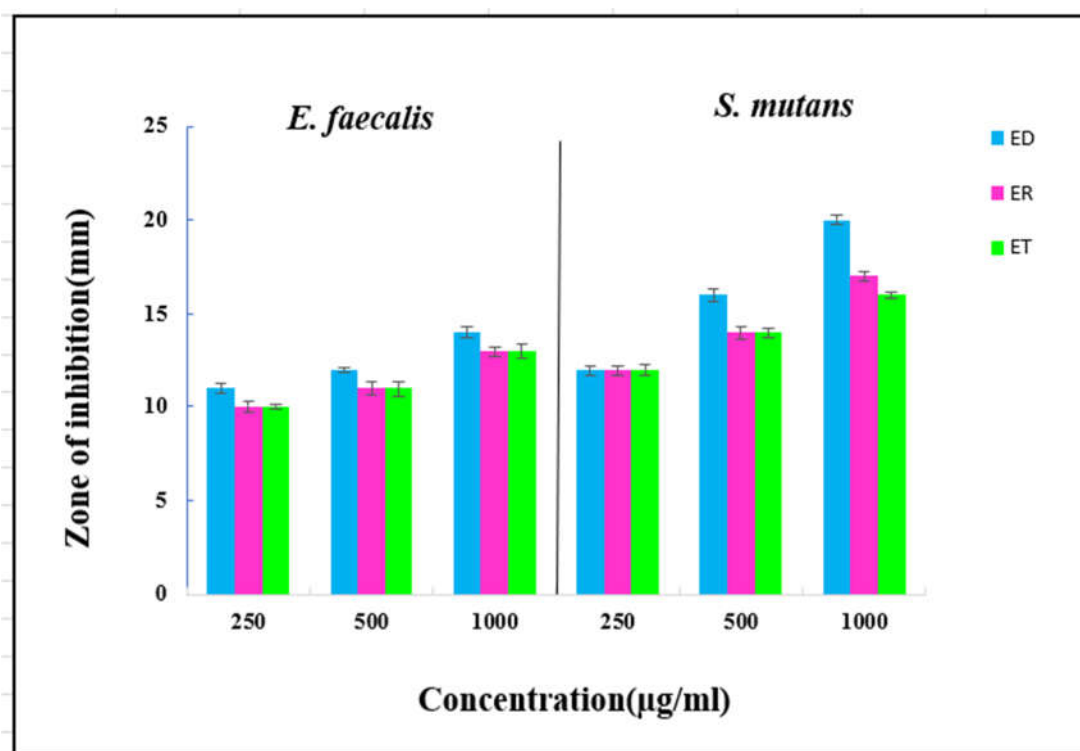
**Plate 21:** Antibacterial activity of silver nanoparticles derived from plant extracts against *E. coli* **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*



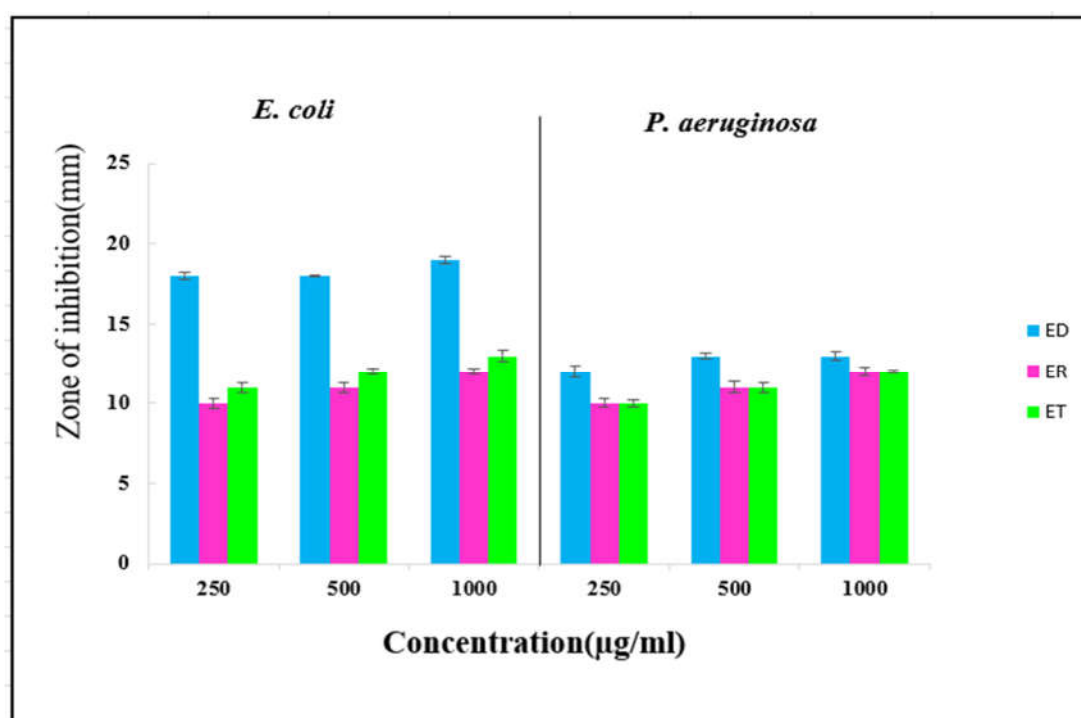
**Plate 22:** Antibacterial activity of silver nanoparticles derived from plant extracts against *Pseudomonas aeruginosa* **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*

**Table 16: The diameter of the zone of inhibition (mm) produced by plant extract-derived silver nanoparticles against selected Gram-negative bacteria**

| Plant Species                 | Zone of inhibition (mm)<br>Bacterial strains (Gram-negative) |           |           |                               |           |           |
|-------------------------------|--------------------------------------------------------------|-----------|-----------|-------------------------------|-----------|-----------|
|                               | <i>Escherichia coli</i>                                      |           |           | <i>Pseudomonas aeruginosa</i> |           |           |
| Concentrations Ag NPs (µg/ml) | 250                                                          | 500       | 1000      | 250                           | 500       | 1000      |
| <i>E. deccanensis</i>         | 18 ± 0.26                                                    | 18 ± 0.07 | 19 ± 0.21 | 12 ± 0.37                     | 13 ± 0.21 | 13 ± 0.23 |
| <i>E. rothiana</i>            | 10 ± 0.34                                                    | 11 ± 0.33 | 12 ± 0.15 | 10 ± 0.26                     | 11 ± 0.37 | 12 ± 0.26 |
| <i>E. thymifolia</i>          | 11 ± 0.31                                                    | 12 ± 0.15 | 13 ± 0.36 | 10 ± 0.26                     | 11 ± 0.31 | 12 ± 0.10 |



**Fig. 42: Zone of inhibition produced by biosynthesized silver nanoparticles against tested Gram-positive bacteria (ED = *E. deccanensis*, ER = *E. rothiana*, ET = *E. thymifolia*)**



**Fig. 41: Zone of inhibition produced by biosynthesized silver nanoparticles against tested Gram-negative bacteria (ED = *E. deccanensis*, ER = *E. rothiana*, ET = *E. thymifolia*)**

#### **b) Antifungal activity of AgNPs**

Antifungal resistance is a major public health concern, particularly in light of the toxicity and increasing resistance of different antifungal agents, as well as the limitations of antifungal treatments. In the present work, AgNPs biosynthesised from the three chosen plant species were tested for their ability to inhibit two strains of fungus such as *Candida albicans* and *Aspergillus niger*. Using the Agar disc diffusion method, the fungicidal property was ascertained. For this purpose, various concentrations, including 250, 500, and 1000 µg/ml, were extracted from the stock solution. The test organisms are freshly seeded onto a petriplate and the antifungals in the samples are allowed to diffuse out into the medium and interact with the organisms. Due to the confluent lawn of growth, the resulting zones of inhibition will be uniformly circular. Millimetres can be used to express the diameter of the zone of inhibition. **Plates 23 and 24** displayed the inhibition zone produced by Ag NPs activity at various concentrations. Clotrimazole, a well-known antifungal medication was used as positive control.

The results of the current study showed that, when applied to the two tested fungal strains, all of the biofabricated Ag NPs demonstrated notable antifungal properties. Ag NPs inhibit pathogen growth in a dose-dependent manner. In addition, at the maximum concentration of 1000 µg/ml selected, Ag NPs synthesised from all plant species demonstrated significant inhibition on the chosen pathogens.

When Ag NPs derived from *E. deccanensis* were used, the growth of *A. niger* and *C. albicans* was inhibited at concentrations of 1000 µg /ml, with corresponding zones of inhibition measuring 20 mm and 14 mm. Ag NPs mediated by *E. rothiana* showed similar results in terms of their antifungal properties. In this instance as well, the growth of *A. niger* was inhibited with a zone of inhibition measuring 20 mm at the highest concentration selected, and that of *C. albicans* with a zone of inhibition measuring 14 mm. Likewise, Ag NPs synthesised from *E. thymifolia* possess fungicidal properties; when compared to *C. albicans*, Ag NPs significantly inhibit the growth of *A. niger*. The zone of inhibition produced at the highest concentration (1000 µg/ml) was measured to be 19 mm in *A. niger* and 16 mm in *C. albicans*, respectively.

**Table 17: Antifungal activity exhibited by Ag NPs developed from *E. deccanensis* methanolic extract**

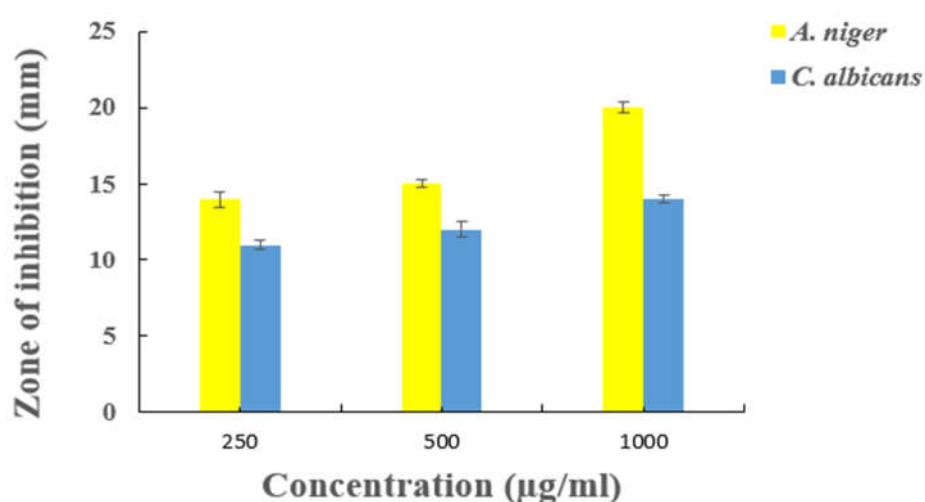
| Sl No. | Fungus             | Zone of inhibition (mm)<br>Concentrations of Ag NPs |                |                 | Positive control<br>Clotrimazole |
|--------|--------------------|-----------------------------------------------------|----------------|-----------------|----------------------------------|
|        |                    | 250<br>(µg/ml)                                      | 500<br>(µg/ml) | 1000<br>(µg/ml) |                                  |
| 1      | <i>A. niger</i>    | 14 ± 0.51                                           | 15 ± 0.26      | 20 ± 0.35       | 21                               |
| 2      | <i>C. albicans</i> | 11 ± 0.28                                           | 12 ± 0.51      | 14 ± 0.26       | 26                               |

**Table 18: Antifungal activity exhibited by Ag NPs developed from *E. rothiana* methanolic extract**

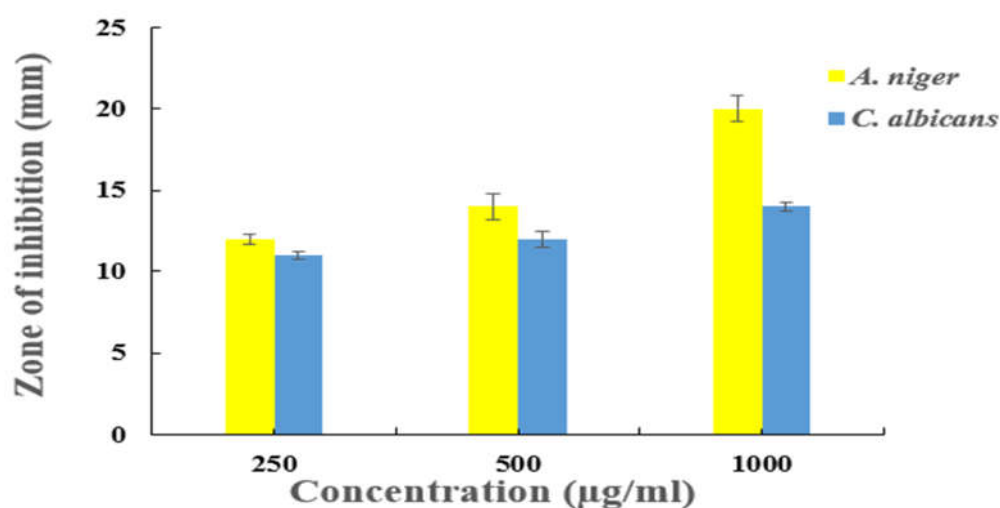
| Sl No. | Fungus             | Zone of inhibition (mm)<br>Concentrations of Ag NPs |                |                 | Positive control<br>Clotrimazole |
|--------|--------------------|-----------------------------------------------------|----------------|-----------------|----------------------------------|
|        |                    | 250<br>(µg/ml)                                      | 500<br>(µg/ml) | 1000<br>(µg/ml) |                                  |
| 1      | <i>A. niger</i>    | 12 ± 0.27                                           | 14 ± 0.78      | 20 ± 0.78       | 21                               |
| 2      | <i>C. albicans</i> | 11 ± 0.26                                           | 12 ± 0.51      | 14 ± 0.26       | 26                               |

**Table 19: Antifungal activity exhibited by Ag NPs developed from *E. thymifolia* methanolic extract**

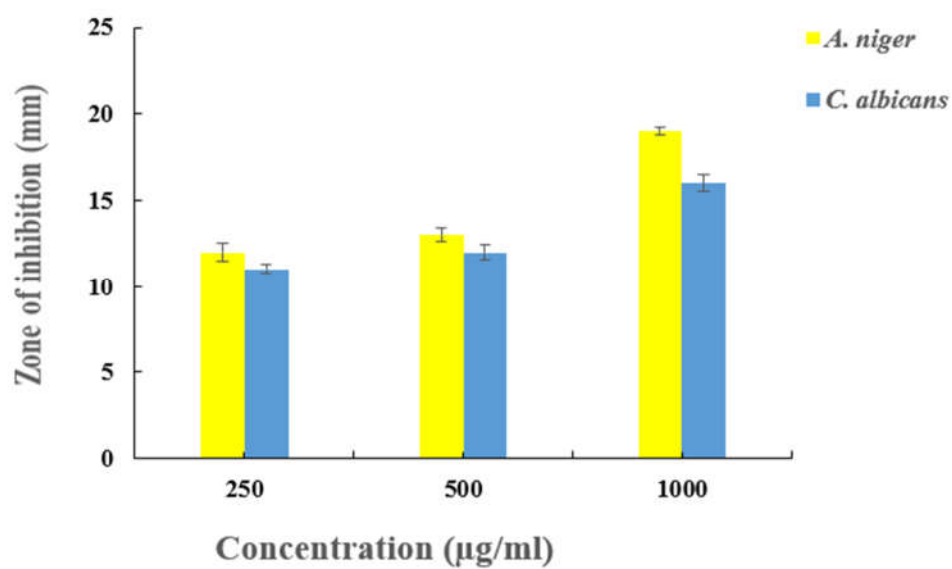
| Sl No. | Fungus             | Zone of inhibition (mm)<br>Concentrations of Ag NPs |                             |                              | Positive control<br>Clotrimazole |
|--------|--------------------|-----------------------------------------------------|-----------------------------|------------------------------|----------------------------------|
|        |                    | 250<br>( $\mu\text{g/ml}$ )                         | 500<br>( $\mu\text{g/ml}$ ) | 1000<br>( $\mu\text{g/ml}$ ) |                                  |
| 1      | <i>A.niger</i>     | $12 \pm 0.51$                                       | $13 \pm 0.37$               | $19 \pm 0.24$                | 21                               |
| 2      | <i>C. albicans</i> | $11 \pm 0.26$                                       | $12 \pm 0.41$               | $16 \pm 0.46$                | 26                               |



**Fig. 43: Zone of inhibition produced by silver nanoparticles biosynthesized from *E. deccanensis* against selected fungal strains**

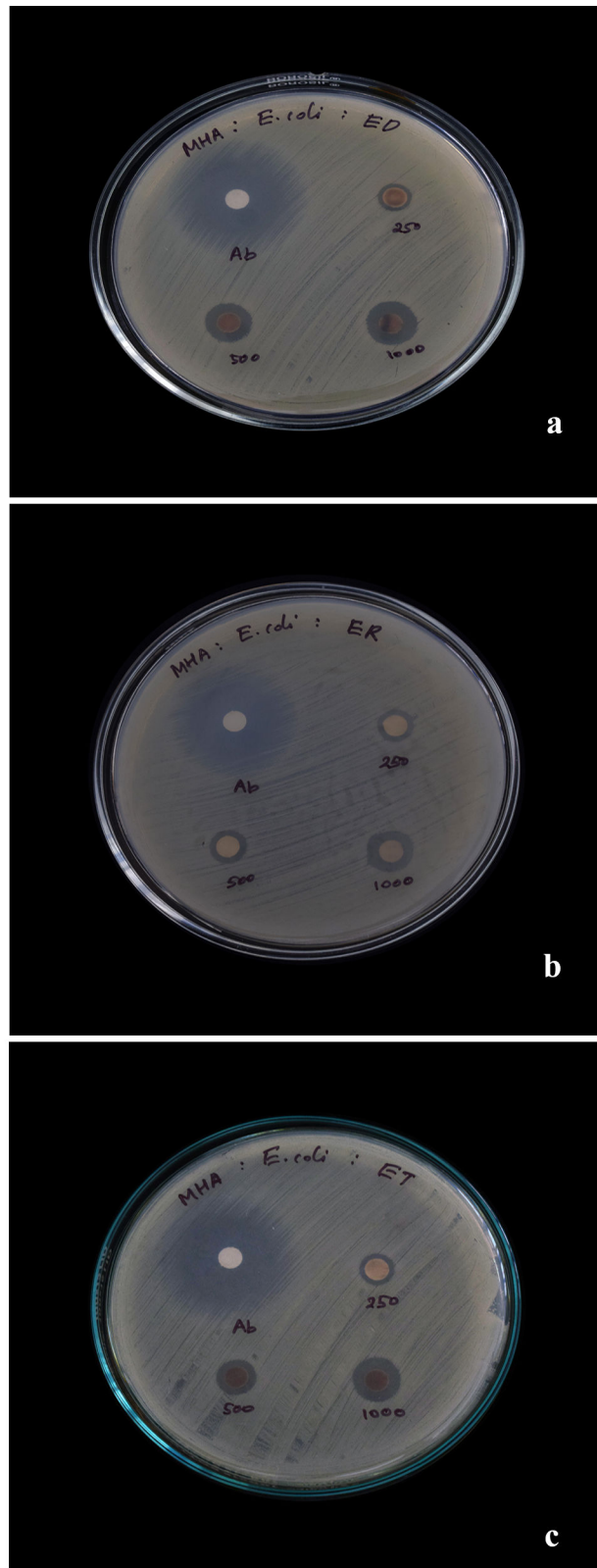


**Fig. 44: Zone of inhibition produced by silver nanoparticles biosynthesized from *E. rothiana* against selected fungal strains**

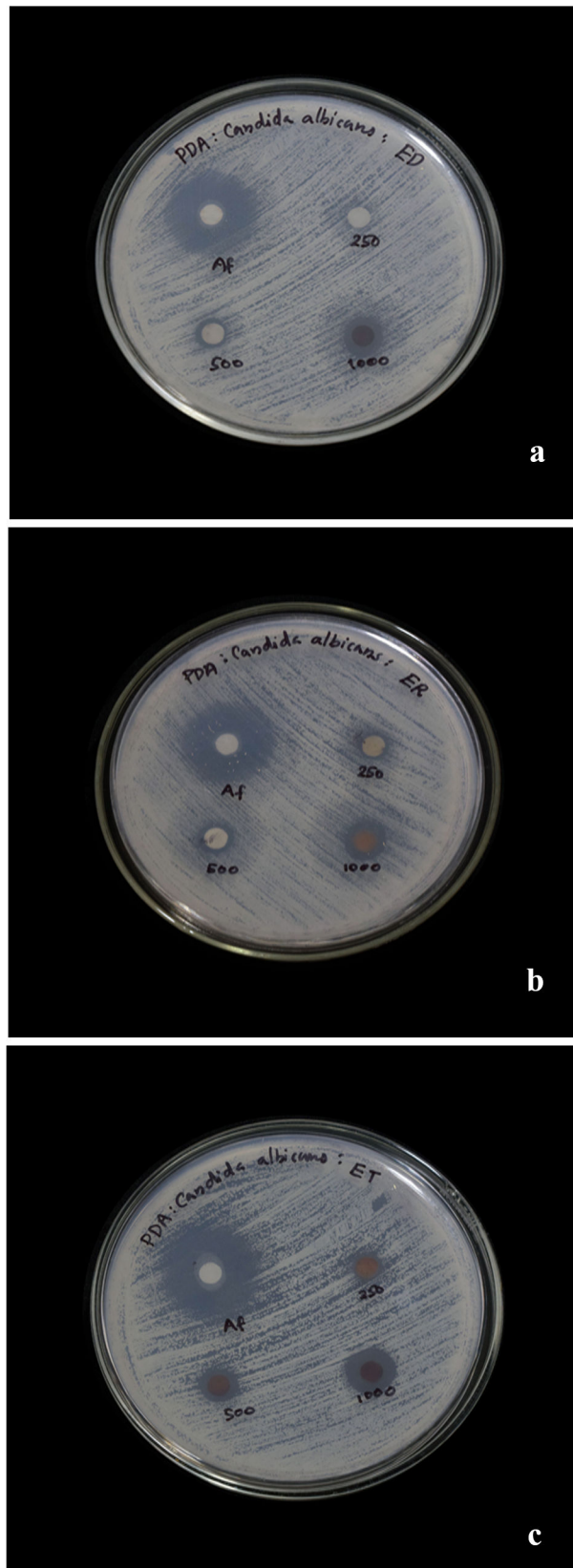


**Fig. 45: Zone of inhibition produced by silver nanoparticles biosynthesized from *E. thymifolia* against selected fungal strains**





**Plate 23:** Antifungal activity exhibited by silver nanoparticles against fungi *Aspergillus niger* **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*



**Plate 24:** Antifungal activity exhibited by silver nanoparticles against fungi *Candida albicans*. **a.** *E. deccanensis*; **b.** *E. rothiana*; **c.** *E. thymifolia*



## DISCUSSIONS

### PART I – PROFILING OF PHYTOCONSTITUENTS

In developing nations, medicinal plants serve as the main source of healthcare for approximately 70 - 95 percent of the population (Heredia et al., 2022). Plants and non-pathogenic endophytic microorganisms living inside the plants produce potential chemical constituents, which are structurally diverse secondary metabolites. Polyphenols, flavonoids, steroidal saponins, organosulfur compounds, and vitamins are just a few of the many chemical entities that make up phytochemicals found in natural products. Dietary consumption of phytochemicals may promote health benefits and protect against chronic degenerative diseases like cancer, cardiovascular, and neurodegenerative diseases (Prakash et al., 2012). These phytochemicals have a great deal of therapeutic potential for treating a wide range of diseases, either individually or in combination. Determining the phytochemical components of medicinal plants is crucial because doing so will help us understand their pharmacological significance and potential health risks. The current study primarily focuses on the assessment of the distribution of phytometabolites present in the chosen species of *Euphorbia* using qualitative phytochemical analysis as well as analytical techniques like GC-MS, HR LC-MS, and quantitative measurement of key metabolites.

#### a) Qualitative screening of phytochemicals

Since ancient times, various human illnesses have been treated using traditional medical knowledge. The bioactive phytochemical components of the plant, which have a variety of physiological effects on the human body, are what give the plant its medicinal value. Thus, through phytochemical screening, one could find various significant compounds that could serve as the foundation for contemporary medications to treat a variety of diseases (Sheikh et al., 2013). The majority of herbal medications and the products that are derived from them are frequently made from unrefined plant extracts, which contain a complex blend of

various phytochemical components. The main factors influencing the continued use of herbal remedies are their efficacy, accessibility, low cost, and moderately low or non-existent toxic properties. Among the various species, there are significant differences in the chemical characteristics of these constituents. Even identical herbal extracts can differ depending on the harvest season, plant origin, drying process, and other elements (Yang et al., 2009). Since several traditional medicinal plants have been found to contain a variety of phytochemicals with pharmacological activity, it is important to look into how these plants can be used therapeutically.

In the present study, the phytochemicals were examined using three different solvents to determine which one had the largest variety of metabolites using conventional methods and the results are tabulated in **Table 5**. The visual colour change reaction or precipitate formation is used as the primary indicator of the presence of a particular phytochemical compound in all of these qualitative determinations. The presence of phytoconstituents such as flavonoids, terpenoids, phenolics, and tannins were found in all three of the examined species. It was also observed that all of the chosen species were completely devoid of phlobatannins. Flavonoids and phenols were found in the ethanolic extract of the entire *E. thymifolia* plant in a prior study by Jayaveera et al. (2010). Similar to this, in a study on three species of *Euphorbia*, a variety of phytochemicals including alkaloids, flavonoids, tannins, saponins, and carbohydrates were found in the methanolic extract of the leaves (Gapuz et al., 2018). Similarly, it was discovered that the leaves, stem, and fruits of *E. cotinifolia* contained a variety of secondary metabolites, including amino acids, anthraquinones, glycosides, saponins, steroids, polyphenols and tannins, triterpenoids and flavonoids in five different extracts, including ethanol, methanol, chloroform, benzene, and aqueous (Tadesse et al., 2016).

Because they treat illnesses without endangering people, phytochemicals are also known as "man-friendly medicines." Phenolic compounds and flavonoids are well-known natural antioxidants and are potential candidates for bioactive agents in the pharmaceutical and medical sectors to improve human health, prevent disease,

and treat various illnesses (Tungmunthum et al., 2018). Dietary foods that are high in phenolics have been shown to be protective against the onset and management of conditions such as diabetes, cancer, osteoporosis, cardiovascular diseases, and neurodegenerative diseases (Mutha et al., 2021). Therefore, it is crucial to conduct a phytochemical analysis to assess a plant's potential as a medicine and to identify the active ingredients that are responsible for the known biological behaviours displayed by plants. Additionally, it offers the foundation for targeted compound isolation and the ability to carry out more exact investigations that will be used as tools for quality control of drugs in the future for the treatment of a variety of disease conditions.

The type of solvent is a crucial consideration when identifying the potential biomolecule in plant materials. And the choice of solvent depends on a variety of factors, including polarity, availability, boiling point, ease of evaporation, and low toxicity (Nortjie et al., 2022). Based on their polarity, three solvents were chosen for the current study, with water being polar, methanol being semi-polar, and n-hexane being non-polar. It was observed that methanolic extracts of all the chosen species contain more compounds and produce precipitate at a higher rate than the other two. There have also been a lot of reports on methanolic extract that discuss a higher extraction yield and class of compounds. In addition, methanol has a slight dissolution of some non-polar compounds (Dhawan & Gupta, 2017; Akbar & Soekamto 2021). Although numerous solvents were employed in other *Euphorbia* species to test the suitability of those with higher phytometabolites, methanolic extract was found to be the most effective. As a result, the methanolic extract was thus considered for additional analysis.

#### **b) Quantitative phytochemical estimation**

Terpenoids, phenols, and flavonoids were identified as three prominent ones through preliminary screening of secondary metabolites. As a result, it needs to be quantified so that the appropriate amount of that particular compound can be determined. The quality and quantity of the active phytoconstituents determine the therapeutic effectiveness of any medicinal plant. The relevant pharmacological function may be brought about by the synergistic effects of bioactive

phytoconstituents. The development and marketing of drugs based on medicinal plants and their practical and scientific value have undergone a recent trend. The efficacy and safety of these plant-derived products have been established for a given application and have undergone stringent standardisation. The precise measurement of phytometabolite concentrations is thus an unavoidable evil. The findings of the major metabolite quantification in the selected species of *Euphorbia* are shown in **Figs. 10, 11 and 12.**

Because of their potent biological activity, plant phenols and flavonoids must be studied carefully. The secondary metabolites known as phenolic compounds are found in plants and share an aromatic ring with one or more hydroxyl groups. To date, more than 8000 organic phenolic compounds have been discovered (Anantharaju et al., 2016). They guard the plants from pathogens as well as abiotic stressors like temperature changes, water content changes, UV light exposure, and mineral nutrient deficiencies (Patay et al., 2016). Due to their numerous advantages for human health and well-being, they make up a significant portion of the average diet. Consuming phenolic-rich fruits and vegetables, like berries and spinach, is now more frequently advised (Balasundram et al., 2006). Likewise, phenolics are strong antioxidants and are more effective than carotenoids, vitamins E and C. Their capacity to donate a hydrogen atom or an electron, as well as to delocalize the unpaired electron within the aromatic structure, is what gives them their antioxidant activity. Biological molecules can also be shielded from oxidation by them. Hence, the precise phenol concentration must be analysed to determine the ability of the chosen plant species to function as an antioxidant (Fernandez-Panchon et al., 2008).

Phenol is one of the well-known secondary metabolites found in the genus *Euphorbia*, which is also well known for having more types of isolated phenolic compounds, flavonoids, and terpenoids. Taib et al. (2022) studied the phenolic compounds of nine different species of *Euphorbia* that were found in northern and central Iraq, and they found significant amounts of phenolic compounds like quercetin, myricetin, kaempferol, and chlorogenic acid in all of the species that were part of the study. The phenolic content of *Euphorbia royleana*'s methanol extract

( $276.41 \pm 64$  mg GAE/g dry extract wt.) was found to be the highest in a recent study. They also mentioned the significant antioxidant potential as a result of this (Anees-ur-Rehman et al., 2022). Likewise, Mahomoodally et al. (2020) examined the total phenolic content of three different *Euphorbia* species, including *E. heterophylla*, *E. hirta*, and *E. convolvuloides*, using a variety of extracts including methanol, ethyl acetate, and aqueous. All the three species of *Euphorbia*'s methanolic and aqueous extracts showed a significant amount of total phenolic content. Among the chosen plants, they observed that *E. hirta* had the highest TPC and displayed the highest antioxidant activity, acting as a phyto-antioxidant agent. Similarly, response surface methodology (RSM) was used to analyse the total phenolic compounds present in the leaf extract of *E. tirucalli* and found a significant amount of total phenolic content (Vuong et al., 2014). The total phenol concentration of crude extracts of ethanol, ethyl acetate, water, and hexane made from *E. tithymaloides* was calculated using the Folin-Ciocalteu method and gallic acid as the standard. Hexane extract ( $10.26 \pm 1.77$  mg At E/g) had the highest overall phenolic content of the four crude extracts, followed by ethyl acetate ( $7.11 \pm 0.36$  mg At E/g), ethanol ( $6.3 \pm 1.75$  mg At E/g), and water ( $3.18 \pm 0.67$  mg At E/g)(Patil et al., 2023).

In support of this, all three of the plant species used in the current investigation showed promising and significant amounts of phenols. However, the methanolic extract of *E. rothiana* ( $229 \pm 3.11$ mg GAE/g DW) had the highest total phenol content, followed by *E. deccanensis* ( $139 \pm 2.25$  mg GAE/g DW) and *E. thymifolia*. As a result, the findings point towards the presence of phenols in the selected species as a clue to its antioxidant properties.

The natural substance flavonoids, which range in colour from white to yellow, are almost exclusively responsible for the pink and violet colour and are widely found in leaves, flowers, bark, grain, root, stem and seeds of a variety of plants, fruits and vegetables. Common sources of them include olive oil, tea, citrus fruits, and seeds. For a long time before their isolation, they were significant in their positive effects on health. There are over 4000 different types of flavonoids known.



They are regarded as an essential component in a wide range of nutraceutical, pharmaceutical, medicinal, and cosmetic applications mainly due to their ability to modulate important cellular enzyme functions in addition to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties (Panche et al., 2016). These substances shield plants from pathogens, herbivores, and harmful ultraviolet radiation. Flavonoids' chelating and antioxidant properties are thought to be the main causes of their positive health effects. They have a prominent substitution structure of hydroxyl groups, which gives them their antioxidant properties (Lin et al., 2016). The flavonoids function as an anti-inflammatory agent by obstructing a variety of enzymes, protein kinases, and transcription factors that are important in inflammation. While some types of flavonoids act as anti-inflammatory agents by blocking cAMP phosphodiesterase, it is infact a crucial messenger molecule that controls a variety of cellular processes during inflammation (Guo et al., 2018).

In the genus *Euphorbia*, flavonoids are widely present. The most common forms of these compounds are neoflavonoids, isoflavonoids, flavonol, flavanone, anthocyanidins, and chalcones. The pharmacological effects of the flavonoids from the genus *Euphorbia* are diverse and include antioxidant, anti-malaria, anti-urease, cytotoxic, DNA damage, apoptosis promoter, antiproliferative, and anti-inflammatory properties. They have excellent therapeutic potential as a result of the wide variety of pharmaceutical activities (Magozwi et al., 2021). From various species of the genus *Euphorbia*, a large number of flavonoids with outstanding medicinal value were discovered. For instance, Awaad et al. (2013) isolated four flavonoids from the alcoholic extract of *E. cuneata* that showed notable antiulcerogenic activity including apigenin, naringenin, 4'-O-methoxy-luteolin-7-O-rhamnoglucoside, and aromadendrin. Among the isolated ones, 4'-O-methoxy-luteolin-7-O-rhamnoglucoside showed scavenging potential as well. Consequently, it is crucial to ascertain the total flavonoid content of chosen *Euphorbia* species due to the wide range of applicability of flavonoids.

The results of the current study significantly demonstrated the presence of a noteworthy amount of flavonoid content in each of the selected *Euphorbia* plant species. Every one of the three plant species displayed a significant amount of flavonoids, with *E. deccanensis* and *E. rothiana* both exhibiting nearly comparable amounts. In the methanolic extract of *E. retusa*, higher antioxidant properties are associated with higher total flavonoid content ( $19.95 \pm 0.96 \mu\text{g QEs.mg}^{-1}$  extract) and total phenol content ( $105.33 \pm 7.75 \mu\text{g GAE.mg}^{-1}$  extract) (Lahmadi et al., 2020). Likewise, the highest flavonoid content in the present study's methanolic extracts of *E. deccanensis* and *E. rothiana* both highlight the plants' strong antioxidant properties. The least amount of flavonoids was observed in the methanolic extract of *E. thymifolia*. In a prior study, Sisiodiya & Srivastava (2018) determined the flavonoid content of *E. thymifolia* using the aluminium chloride colourimetric method. Flavonoids were present in extracts at a rate of 2.12 mg/100 mg extract weight. Therefore, the current study unequivocally shows the presence of flavonoids in *E. thymifolia*.

There are so many *Euphorbia* species that were subjected to the determination of flavonoid content. To obtain the best extract with the highest flavonoid content, four extracts were developed from the entire *E. echinus* plant using various extraction techniques, including decoction, aqueous maceration, methanolic maceration, and methanolic hot continuous extraction. It was found that compared to other extracts, those made by decoction and methanolic hot continuous extraction both had higher levels of total flavonoid content (Lahlou et al., 2014). In a similar study, Dehghani et al. (2022) evaluated the total flavonoid content in the root and shoot of *E. serpens* using extracts of ethanol, methanol, petroleum ether, and dichloromethane. When comparing these solvent extracts, the methanolic extract of the shoot had the highest total flavonoid content (34 mg RE/g), while the ethanolic extract of the root had the lowest (1.204 mg q/g). Additionally, they observed that the plant's aerial parts contained more flavonoids than their underground counterpart. Thus, the aerial parts' abundant flavonoid content indicates a high potential for antioxidant properties.

Terpenoids are a prominent class of natural compounds that are present in numerous genera of plants, sponges, algae, and fungi. They attracted a lot of pharmaceutical attention in ancient times because of their wide range of medical applications. They defend the plants against outside threats like pathogens, competitors, and pathogens. Sesqui-terpenoids and monoterpenoids, in particular, act as pollinator attractants in flowering plants, promoting pollination (Das et al., 2022). Terpenoids have pharmacological advantages, such as antibacterial, anti-inflammatory, immunoregulating properties antitumor, and antioxidative, and can be used to prevent cardiovascular diseases (Jeon et al., 2022).

In the genus *Euphorbia*, terpenoids are found in the greatest abundance. The distribution of different terpenoids in the genus *Euphorbia* was therefore thoroughly documented in numerous reports. Daphnanes, jatrophone, tiglanes, ingenanes, myrsinols, abietanes, and cembranes are just a few of the major skeletal structures found in diterpenoids, which are dominant among other terpenoids due to their structural diversity. Furthermore, the majority of the reported diterpenoids from *Euphorbia* displayed notable pharmacological activities like anti-inflammatory, anti-HIV, and cytotoxic properties. For instance, the important medicinal plant *E. maculata* contains bioactive triterpenoids that are particularly effective at reducing inflammation (Sun et al., 2018). Natural diterpenes are extremely useful in the development of drugs. For instance, two of the most well-known terpene-based medications are the anticancer drug Taxol® and the antimalarial drug Artemisinin. A recent release of ingenol metabutate (picato), a diterpene isolated from *E. peplus* for the treatment of actinic keratosis has also rekindled interest in the phytochemistry of *Euphorbia* species (Kemboi et al., 2021). Therefore, terpenoid-rich medicinal plants play a crucial role in the treatment of cancer and other diseases. Therefore, it is crucial to determine the terpenoid content of the three plant species used in the current study.

A recent study by Sudha & Padmini (2023) using an ethanolic extract made from *E. hirta* leaves to quantitatively determine the total terpenoid content found a significant amount of terpenoid of  $38.59 \pm 2.70$  mg/gm. Likewise, in another study,

Basu et al. (2019) discovered that the latex extract of *E. rigida* contains more terpenoid and phenol than flavonoid content. They also mentioned that these phytochemicals are useful for reducing acute or chronic pain that is brought on by nociceptor activation, oxidative stress, and/or inflammation. In a similar manner, the chemical profile of latex extracted from the stem of *E. peplus* was examined, and it was discovered that there were about 13 terpenoids, which served as constitutive defense metabolites and showed potential antifeedant activity. Additionally, acyclic triterpenoid peplusol demonstrated antifungal activity (Hua et al., 2017). The total terpenoid content of *E. thymifolia* was low, supporting the low total flavonoid content. Only  $24.12 \pm 3.28$  mg equivalent linalool of total terpenes are present in it. The other two species, in contrast, have a six-fold increase. In *E. deccanensis*, the observed terpenoid content was  $135.51 \pm 2.67$  mg LE/g DW and in *E. rothiana*, it was  $134.4 \pm 3.34$  mg LE/g DW. The research thus revealed that the selected species possess a rich diversity of terpenoids.

The quantitative screening revealed the prevalence and type of secondary metabolite distribution in the chosen species, in which all three plant species showed notable amounts of metabolites. However, in terms of flavonoids and terpenoids, *E. deccanensis* was observed to contain the most. The abundance of these chemical components in selected *Euphorbia* species makes them an excellent source of therapeutic natural products with a range of pharmacological properties.

### **c) HRLC-MS Analysis**

The non-volatile and polar compounds present in the methanolic extract of the three chosen species of *Euphorbia* were identified using the analytical method HR LC-MS. It is a potent analysis and detection method for non-volatile compound observation.

A total of 83 compounds were discovered by using liquid chromatography-mass spectrometry on the methanolic extracts of the three *Euphorbia* plant species. *E. deccanensis* among these showed a higher number of compounds (43) followed by *E. rothiana* (41), and *E. thymifolia* (29) respectively. Compounds are analysed in

the current investigation using both positive and negative ionisation modes. The list of compounds identified by HR LC-MS analysis was tabulated in **Table 6**. All three-plant species' liquid chromatograms are shown in **Fig. 13 (i, ii, iii)**.

Flavonoids are the most significant class of compounds that were observed in appreciable numbers in all three extracts chosen. There were 17 flavonoids in total, of which 10 compounds were detected in *E. deccanensis*, 7 compounds detected in *E. rothiana*, and 6 compounds noted in *E. thymifolia*. The flavonoid, Rhamnazin 3- sophoroside with an RT value of 14.89 was common in all three plant extracts. The next class of compound found in the extracts was phenolic compounds, which consist of 10 compounds. Comparing the three plant extracts, *E. rothiana* showed more phenolic compounds. All three plant extracts showed the presence of gallic acid, a potent phenolic compound. In selected plant species, terpenoids are also prominently observed. Six terpenoids in total were identified, with triterpenoids standing out the most. All of the identified terpenoids were found in the methanolic extract of *E. deccanensis*. On the other hand, *E. thymifolia* do not have any terpenoids present in them. In methanolic extracts of both *E. deccanensis* and *E. rothiana*, an important diterpenoid euphorbin, found in many other species of *Euphorbia*, was found. Quinic acid, a cyclohexane carboxylic acid with a molecular mass of 192.0622, was the first compound to be eluted. It was found in the methanolic extract of both *E. rothiana* and *E. thymifolia*. The last eluted compound was 25-Methylgramisterol, a steroid found in the methanolic extract of *E. thymifolia*.

Rutin, a common dietary flavonoid, has drawn a lot of attention in literature because of its pharmacological properties, which include antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects. This compound is abundantly found in a number of foods and beverages, including wine and black tea, as well as berries, peaches, plums, tomatoes, onions, oranges, lemons, grapes, and limes. A significant source of natural rutin is reported to be buckwheat from the Polygonaceae family (Gullon et al., 2017). It has immense potential to act as a powerful inhibitor of the main protease (Mpro) of the SARS-CoV-2 (COVID-19) virus and prevent the spread of viral replications. The binding of rutin with Mpro

was studied using molecular dynamics simulation and docking studies, which showed promise for the drug's future use. Furthermore, traditional viral medications that contain this compound were prescribed to patients infected with mild COVID-19 symptoms in China (Agrawal et al., 2021). This gives an insight into the compound's potential as a repurposed bioactive metabolite against SAR-CoV-2. Likewise, the review of the biological properties of this compound demonstrated its anticancer potential, which suggested that it might be achieved by reducing cell growth, triggering apoptosis, and preventing angiogenesis and metastasis. This compound could stop the growth of cancer cells at a number of cell cycle checkpoints, including the G1, G2/M, and S phases. This substance did this by altering cell cycle regulatory proteins like cyclins, CDKs, and CDK inhibitors, which are involved in controlling cell cycle progression. Thus, the compound can be used as an alternative cancer treatment (Farha et al., 2022). In addition, the molecular mechanisms underlying this compound's anticancer potential and its ability to control numerous cellular signalling pathways, including p53, PI3K/Akt, JAK/STAT, MAPK, NF- $\kappa$ B signalling, Wnt/-catenin, and p53-independent pathways, were determined (Imani et al., 2021). Hence, this phytochemical present in *E. rothiana* is a potent one in respect of cancer studies.

The natural flavonoid quercetin was abundant in edible vegetables and fruits. Because of its health benefits, quercetin was consumed in large quantities on a daily basis in the Western diet. They were used to prevent a variety of chronic diseases, including cardiovascular and neurodegenerative diseases, as well as cancer (Lesjak et al., 2018). It has anticancer activity by inducing cell apoptosis through inhibiting Bcl-2 and Bcl-xL proteins, which are important apoptosis regulators (Primikyri et al., 2014). Some of the proven bioactivities of this compound include antioxidant, anticancer, antiaging, antiviral, and anti-inflammatory properties (Wang et al., 2022). It has chemo-preventive activity against several types of cancer, including blood, lung, and prostate cancer. In addition, it exhibited anti-inflammatory properties by decreasing the level of Cox-2 (Lotfi et al., 2023).

Myricetin is a natural flavonoid found in fruits, vegetables, tea, and medicinal plants. From the standpoint of cancer, it works by modulating angiogenesis, inflammation, cell cycle arrest, and induction of apoptosis. In addition, cancer prevention was achieved by inhibiting inflammatory markers such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox-2) (Rahmani et al., 2023). The compound was capable of improving antioxidant status in cancer cells (Agraharam et al., 2022). Other medicinal potentialities of this compound include antiobesity, antidiabetic, cardiovascular protection, osteoporosis protection, anti-inflammatory, and hepatoprotective activity (Imran et al., 2021). Only *E. deccanensis* contains this compound. Previous reports on LC-MS analysis of *E. peplus* revealed the presence of myricetin. Myricitrin is another flavonoid compound found in *E. deccanensis* and *E. thymifolia*. This compound was also detected in *E. hirta* recently (Nugroho et al., 2020). The compound has a variety of biological activities, including sedation, myorelaxation, and analgesia. Similarly, the compound's potential anxiolytic effect was also noteworthy (Fernandez et al., 2009).

Natural flavonoid kaempferol is widely found in genera like *Camellia*, *Delphinium*, and *Citrus*. It is widely present in a variety of foods and beverages, including teas, apples, broccoli, citrus fruits, strawberries, beans and onions. It was observed that the compound had hepatoprotective properties against alcohol-induced liver damage in mice by inhibiting the important microsomal enzyme cytochrome 2E1 (CYP2E1) and by boosting the function of the body's antioxidant defence system (Wang et al., 2015). The compound's antioxidant ability to neutralise DPPH and ABTS radicals was documented by Wang et al. (2018). This compound has proven cardioprotective, renoprotective, gastroprotective, anti-diabetic, anti-arthritic, and neuroprotective properties (Periferakis et al., 2022). Thus, the formulation of effective and secure nutraceutical products can make use of this compound's multitarget and multi-effect action mode (Bangar et al., 2022). Kaempferol's promising anti-cancer activity has been reported in earlier studies against a variety of cancer cell lines, including those from the colon, ovary, pancreas, stomach, and bladder. By preventing cell growth and inducing apoptosis, this compound slows the growth of bladder cancer tumours (Dang et al., 2015). Likewise, in breast cancer

cells, kaempferol was able to downregulate the expression of bcl2 proteins, causing apoptosis and thus inhibiting cell proliferation (Yi et al., 2016). In addition, kaempferol along with other fruit peel polyphenols including myricetin, rutin and quercetin exhibited excellent activity against breast cancer cell lines of MCF-7 and MDA-MB-231 (Yasuda et al., 2017). In the current study, *E. deccanensis* extract demonstrated excellent anticancer activity against MCF-7 breast cancer cell lines by inducing apoptosis, which might be because of the impact of kaempferol present in it.

Euphornin, a diterpenoid, was found in both *E. deccanensis* and *E. rothiana*. This substance was initially discovered in the related plant *E. helioscopia*. Previous reports mentioned the compound's capacity to induce apoptosis and cell cycle arrest at the G2/M phase in order to inhibit the proliferation of human cervical adenocarcinoma HeLa cells (Li et al., 2018). Additionally, significant cytotoxic activity exhibited by euphornin against mice lung adenocarcinoma cells (LA795) was also investigated (Chen et al., 2012).

Gallic acid, a tri-phenolic substance that is found in nature and has a low molecular weight, has been found to be a powerful apoptosis inducer. It can be found in plants in the form of hydrolysable tannins, free acids, esters, catechin derivatives, and esters. It offers effective defence against oxidative damage brought on by reactive species frequently found in biological systems, such as hydroxyl, superoxide, and peroxy, as well as the non-radicals hydrogen peroxide and hypochlorous acid (Badhani et al., 2015). Therefore, gallic acid's ability to act as an antioxidant is one of its most notable activity. In the current study, gallic acid was detected in the methanolic extract of all three plant species using HR LC-MS analysis. These findings suggest that the presence of gallic acid in selected species might be responsible for their high antioxidant potential, which was discovered during studies on free radical quenching. It has strong antioxidant activity in addition to antimicrobial, antiulcer, anti-inflammatory, anticancer, and anti-HIV properties. It also serves as a precursor in the manufacturing of the antibiotic trimethoprim. Additionally, it is used as a preservative to prevent the oxidation of



foods and beverages due to its ability to quench free radicals (Pal et al., 2018; Wianowska & Olszowy-Tomczyk, 2023). For example, during the ensiling of *Moringa oleifera* leaves, a recent study by He et al. (2020) demonstrated the effectiveness of gallic acid in improving protein preservation and antioxidant capacity.

In-depth research has been done by Bai et al. (2021) on gallic acid's role in inflammation-related diseases and its potential use as an anti-inflammatory agent. They emphasised that the role of gallic acid was to inhibit NF- $\kappa$ B and MAPK signalling pathways, which in turn inhibited the release of inflammatory mediators like COX-2 and NO as well as inflammatory factors like TNF- and IL-1/6. Additionally, it reduces inflammatory cell infiltration, which enhances the inflammatory response. Consequently, it lessens the inflammatory response by decreasing the release of inflammatory cytokines, chemokines, adhesion molecules, and cell infiltration. This is consistent with the current research, in which methanolic extract of *E. deccanensis* inhibited the inflammatory enzymes Cox, Lox and iNOS on LPS-induced RAW 264.7 cells, exhibiting significant anti-inflammatory activity. The decrease in Cox-2 enzyme level measured using the ELISA technique further supported this. Enzymes such as Cox-2 and iNOS are both controlled by the NF- $\kappa$ B pathway, one of the major signalling pathways involved in inflammation. Therefore, the decrease in these two enzymes lends credence to the idea that the plant extract inhibits the NF- $\kappa$ B signalling pathway. Here, the plant extract's observed anti-inflammatory potential might be due to the presence of gallic acid along with other phytoconstituents.

Gentisic acid, a phenolic acid initially isolated from the root of the genus *Gentiana*, was present in the extracts of *E. rothiana* and *E. thymifolia*. Many plants, including gentian, batoko plum, kiwi, grapes, citrus, apple, and bitter melon, commonly contain gallic acid. This compound has positive effects on human health, including antimicrobial, anti-inflammatory, hepatoprotective, and neuroprotective effects, with antioxidant properties predominating among them (Abedi et al., 2020). In addition, it has high cell proliferation activity and the ability to heal wounds. It

displayed greater healing activity when compared to allantoin, a positive control. Additionally, gentisic acid therapy may promote cell division. By examining proteins involved in the mitogen-activated protein (MAP) kinase signalling pathway using Western blot analysis, it can be determined that gentisic acid treatment increases the phosphorylation of ERK1/2, which in turn promotes cell proliferation (Kim et al. (2020). Due to its ability to heal wounds, gentisic acid, which is derived from *Alchornea cordifolia* plant extract, has been used as a traditional medicine in Africa (Mechchate et al., 2021).

Cryptochlorogenic acid isolated from *Ageratina adenophora* was found to have anti-inflammatory properties by preventing NF- $\kappa$ B protein phosphorylation (Ma et al., 2022). Likewise, the compound had anti-inflammatory activity on LPS-induced RAW264.7 cells by inhibiting NF- $\kappa$ B activation and MAPK phosphorylation (Zhao et al (2020). Hence, the compound has the potential to be a therapeutic agent for the management of inflammation. In the current study, only *E. rothiana* was found to possess the compound.

Caffeic acid is a phenolic acid eluted from *E. rothiana*. The effectiveness of the compound in preventing premature ageing and boosting collagen production has been noted in the review on the potential use of this compound in medication. The compound also has antimicrobial, antioxidant, and anticancer properties against oral and colon cancer (Magnani et al., 2014). This compound exhibits activity against a significant form of cancer, hepatocarcinoma, which is thought to be causing significant mortality worldwide. It accomplishes this by preventing the excessive production of ROS, promoting angiogenesis, and aiding in the DNA oxidation-mediated death of tumour cells (Espíndola et al., 2019).

Extracts of *Euphorbia rothiana* and *E. thymifolia* contain quinic acid. Several medicinal plants contain this cyclohexane carboxylic acid. The reported bioactivities of this substance include antioxidant, anti-diabetic, anti-cancer, anti-microbial, antiviral, and analgesic effects (Benali et al., 2022). Quinic acid was also noted for its antibacterial properties. The compound showed significant antibacterial activity and acted as an antibiofilm agent against clinically resistant *Pseudomonas*

*aeruginosa* and associated infections by controlling the transcription of specific target genes (Lu et al., 2021).

Ellagic acid, identified in the methanolic extract of both *E. rothiana* and *E. thymifolia*, is a polyphenolic compound found in a wide range of plant species. This compound is found in other *Euphorbia* species such as *E. antisyphilitica*, *E. hirta*, and *E. hylonoma* (Ascacio-Valdés et al., 2010; Mekam et al., 2019; Guo et al., 2011). The potential of this compound to inhibit microbes such as bacteria, viruses, and fungi has been highlighted in a review of its applications. Anti-inflammatory, hepato- and cardioprotective, chemopreventive, neuroprotective, anti-diabetic, gastroprotective, antihyperlipidemic, and antidepressant-like properties are also demonstrated by the compound (Evtuyugin et al., 2020). When administered to mice, the compound showed anti-arthritic activity by upregulating anti-inflammatory cytokines and downregulating pro-inflammatory cytokines (Allam et al., 2016). In addition, ellagic acid significantly inhibited the growth of breast cancer cell lines. A crucial enzyme involved in the development of cancer, cyclin-dependent kinase, was expressed less when the compound was present. Thus, the compound's quality was further improved by its inhibitory effect on breast cancer cell lines (Yousuf et al., 2020). Another study discovered that ellagic acid has the potential to inhibit lung cancer cell proliferation. The ability to inhibit tumour growth by reducing cell growth, inducing apoptosis, and damaging mitochondria revealed its anticancer properties (Duan et al., 2020). According to Chen et al. (2015), ellagic acid causes cell cycle arrest at G0/G1 phase, inhibits MCF-7 breast cancer cell proliferation, and induces apoptosis in cancer cells.

Isodiospyrin, a dimeric naphthoquinone was detected in the extracts of all three plant species chosen for the current investigation. This compound has potent antibacterial activity against a wide range of bacteria (Adeniyi et al., 2000) and was found to have significant cytotoxic effects (Kuo et al., 1997). Isodiospyrin's role in preventing human DNA topoisomerase I activity was also noted by Ting et al. (2003).

Only the methanolic extract of *E. deccanensis* contained triterpenoid beta-boswellic acid acetate. Zhao et al. (2003) reported that this compound can cause fibrosarcoma and metastatic melanoma cells to undergo apoptosis and differentiation. Similar characteristics of this phytoconstituent were found in leukaemia cell lines (Jing et al., 1999). *E. thymifolia* contains the flavonoid guajavarin. The compound, which was previously isolated from *Psidium guajava*, is a powerful inhibitor of *Streptococcus mutans* growth (Prabu et al., 2006).

In addition to the aforementioned phytoconstituents, the chosen plant species also contained numerous steroids, tannins, coumarins, and fatty acids. This extensive collection of non-volatile phytocompounds revealed through HR LC-MS analysis showed significant biological properties. Therefore, the presence of these compounds and their potential benefits helped to highlight the importance of using the selected *Euphorbia* species for bioactivity screening.

#### **d) GC-MS analysis**

One of the best methods for identifying non-volatile compounds is GC-MS, which combines the abilities of mass spectrometry and gas chromatography to separate chemical mixtures and identify each of their constituents. It is an effective way to gain comprehensive knowledge of the low-molecular-weight phytoconstituents. In the present study, the bioactive volatile phytoconstituents present in the chosen *Euphorbia* species were discovered through the analysis of them using gas chromatography-mass spectrometry.

The analysis revealed a total of 21 phytocompounds, with *Euphorbia thymifolia* showing higher compounds. While *Euphorbia deccanensis* and *E. rothiana* displayed six compounds in each sample. The retention time for the first compound eluted was 4.169, and the retention time for the last compound eluted was 30.692 (**Table 7**). Alkanes and hydrocarbons were the predominant class of compounds identified by this analysis. No compound was found to be as prevalent in the three plants. But O-decyl hydroxylamine was observed in *E. rothiana* and *E. deccanensis*, both of which had a peak area percentage of 24.2 and 15.76,

respectively. The peak with the highest area percentage was found in *E. thymifolia* and measured 67.5% for 1,2,3-Benzenetriol, a o phenolic compound.

1,2,3-Benzenetriol, commonly known as pyrogallol, was the highly intensified phytoconstituent noted in *E. thymifolia* at a retention time (RT) of 12.495. A variety of edible plant species, including fruit peel, vegetables, nuts, and medicinal plants, contain the compound pyrogallol. Due to its powerful quenching property, pyrogallol consumption helps to prevent oxidative damage to proteins, nucleic acids, and lipids. It also provides protection from a variety of diseases, such as cardiovascular, autoimmune, and brain disorders like Alzheimer's and Parkinson's disease (Gupta et al., 2021). Its presence could increase the value of this species across a range of industries, particularly in the food industry. Yang et al. (2019) reported on both its antioxidant activity and its use as an antioxidant agent. Mendes et al. (2015) made a similar observation regarding yeast's resistance to oxidative stress as a result of the presence of pyrogallol in them. Pyrogallol was well known for having potent anti-inflammatory and anti-cancer properties. Through *in vivo* studies, Revathi et al. (2019) identified the compound's anti-colon cancer activity. Additionally, by blocking the AKT and PI3K signalling pathways and inducing cell cycle arrest at S-phase, pyrogallol exerts antitumor activity against hepatocellular carcinoma cells (Ahn et al., 2019)). Additionally, the pyrogallol present in *Phyllanthus emblica* demonstrated anticancer activity against gastric and lung cancer cell lines (Zhao et al., 2015). Because the compound has antibacterial and antioxidant activities, it has been used to treat a variety of microbial diseases (Ehaimir et al., 2023).

Palmitic acid, which is extremely abundant in the human body and can be obtained through food or produced internally through de novo lipogenesis, was one of the most studied saturated fatty acids from the perspective of cancer (Carta et al., 2017). Palmitic acid has emerged as a potent anticancer agent against a variety of malignancies, including colorectal, cervical, breast, and gastric cancer. Through the inhibition of tumour cell proliferation and apoptosis induction, it exhibited antitumor activity (Wang et al., 2023). The compound was found to be cytotoxic to the MCF-7

cell line based on the observation of the drop in viable cells. The DAPI staining method was used to identify the relevant apoptotic mechanism and noted that in contrast to the anti-apoptotic protein Bcl-2, the expression of apoptosis-related proteins caspase-3, 9, Bax, and p53 was found to be upregulated by palmitic acid (Zafaryab et al., 2019). Through controlling the STAT-3 signalling pathway, palmitic acid has been found to significantly reduce the risk of gastric cancer (Yu et al., 2023). In a similar manner, Zhu et al. (2021) reported the anticancer property of the compound against prostate cancer. The anticancer property was accomplished through G1 phase arrest, which also includes the inhibition of the crucial molecules of the PI3K/AKT pathway to stop the spread of prostate cancer. The presence of palmitic acid was discovered through GC-MS analysis of *Kigelia pinnata* leaves, which also demonstrated significant anticancer effects against HCT-116 cells with an IC<sub>50</sub> value of 0.8 µg ml<sup>-1</sup> (Ravi & Krishnan, 2017). Thus, the compound's cytotoxicity was established. By increasing the expression of C-reactive protein, tumour necrosis factor and inducible nitric oxide synthase, palmitic acid has been shown to have pro-inflammatory effects on vascular smooth muscle cells (Wu et al., 2014). Aparna et al. (2012) looked into the potential role of the naturally occurring dietary fatty acid palmitic acid in acting as an anti-inflammatory agent. They learned that palmitic acid was a competitive inhibitor of phospholipase A2, which is one way to control inflammation. This validates the significance of using medicated oil rich in palmitic acid for treating rheumatism in India's traditional Ayurvedic medicine. Similarly, palmitic acid isolated from *Pentanisia prunelloides*, whose presence in the plant was discovered using GC-MS technology, displayed significant antibacterial activity against both Gram-negative and Gram-positive bacteria. This supports the view held by Zulu traditional medicine that this plant has the ability to effectively treat bacterial and viral infections, inflammation, and stimulate uterine contraction (Yff et al., 2002). Previous reports suggested that several other *Euphorbia* species, including *E. hirta* and *E. milii*, contained palmitic acid (Rautela et al., 2020). With a peak area percentage of 33.03, palmitic acid was discovered to be the primary component in *E. thymifolia* (Prasad & Bisht, 2011). In the present

study, however, palmitic acid was only discovered in the methanolic extract of *E. thymifolia*, which had a peak area percentage of 4.64.

*Euphorbia* plants are a plentiful source of latex and hydrocarbons (Jassbi, 2006). Different types of hydrocarbons have been found in various *Euphorbia* species. For instance, the quantification of hydrocarbons found in *E. macroclada* revealed the presence of potent hydrocarbons (Özcan & Özcan, 2004). In a study on the hydrocarbons found in the genus *Euphorbia*, from Nigeria, using GC-MS analysis, it was found that aliphatic hydrocarbons were present in many of the species (Ekeke & Ndukwu, 2014). O-decyl hydroxylamine is an important hydrocarbon found in many plant species. In the present study, it was the only volatile compound discovered to be prevalent in both the methanolic extract of *E. deccanensis* and *E. rothiana*. The presence of this compound was detected as the major volatile component in the hexane fraction of Rock Jasmine, with a peak area percentage of 30 (Zaheer et al., 2021). The cytotoxic, antileishmanial and immunomodulatory potential of the compound was determined by Okokon et al. (2012). In an attempt to analyse the various phytochemical compounds produced by endophytic fungi derived from the aquatic plant *Nelumbo nucifera*, Techaoei et al. (2020) discovered the presence of the compound, O-decyl hydroxylamine. They also discovered that the compound has significant antibacterial activity along with other phytoconstituents.

The main volatile plant component identified in the methanolic extract of *E. deccanensis* was the alkane, decane. It was resolved at 6.025 retention time with a peak area percentage of 35.80. The decane was identified as one of the prominent phytocompounds with a peak area percentage of 8.96 by Khan & Javaid (2021) after conducting a GC-MS analysis of the chloroform fraction of neem leaves. Similarly, GC-MS analysis of the Mediterranean aroma plant *Foeniculum vulgare*, which is used for antimicrobial, anti-fungal, and favouring applications, revealed decane as the major compound (Bahreini et al., 2015). Given that microalgae are currently viewed as one of the promising alternatives to producing biodiesel, the *Chlorella* species were treated with phytohormones like indole-3-butyric acid in order to

enhance the lipid oil formation within them. The GC-MS analysis of lipid oil revealed the distribution of decane, ethylbenzene, and o-xylene as significant compounds for the production of biofuels (Bhuyar et al., 2020). Decane found in seagrass and marine red seaweed, has recently come to light as having antioxidant and antibacterial properties (Das et al., 2023).

The dihydropyranone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), with a retention time of 6.975 is present in the methanolic extract of *E. thymifolia*. The complete absence of this compound is noted in the methanolic extract of the other two species selected. It was produced during the Maillard reaction's intermediary phase. From powdered orange juice kept in storage, DDMP was first discovered. It was also found in foods like prunes, roasted tigernut oil, wheat bread, and natural extract (Chen et al., 2021). DDMP was made from maltose rather than glucose to determine whether it has a bitter taste or not, and it was found to be tasteless (Chen et al., 2021). The GC-MS analysis of *Vitex negundo* leaves revealed the presence of DDMP in them (Kumar et al., 2010). The antioxidant potential of this substance has been mentioned in numerous earlier reports. Due to the presence of the compound, Echovská et al. (2011) noted the antioxidant potential of plums and prunes. Similarly, Yu et al. (2013) examined this substance as a potent antioxidant in glucose-histidine Maillard reaction products. This compound, isolated from culture supernatants of *Lactobacillus pentosus* Strain S-PT84, has been shown to stimulate autonomic nerve activity in rats (Beppu et al., 2012). In addition, cadmium chloride-induced male reproductive toxicity in Wistar rats was lessened by the protective properties of DDMP found in the medicinal plant *Plukenetia conophora*. Thus, it strengthens the notion that *Plukenetia conophora* is a fertility-promoting plant used in Nigerian traditional medicine (Olaniyan et al., 2018). By using the headspace solid-phase microextraction with gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS) method, the amount of DDMP present in five cultivars of baked red jujube, one of the common food ingredients used to enrich the nutritional value and enhance the aroma, was quantitatively measured. The DDMP content measured ranged from 4.55 to 46.50 µg/g (Qiao et al., 2022). The antifungal activity of DDMP isolated from *Schizophyllum commune* was



discovered by Teoh & Mashitah (2016). They identified DDMP as a promising antifungal alternative with long-term effectiveness against fungal attack on rubberwood. When made from *Pavetta crassicaulis* fruit extract, DDMP showed potent anti-inflammatory properties. The compound's ability to reduce inflammation was thus demonstrated (Ashwathanarayana & Naika 2018).

The methanolic extract of *E. thymifolia* contains the compound desulphosinigrin, a glucosinolate with a peak area percentage of 2.73. It had a retention time of 17.859 when eluted. The presence of this compound has been previously identified in the other two species of the same genus under investigation, *E. lathyrus* and *E. hirta*, in their respective methanolic seed extracts and ethyl acetate leaf extracts (Sosa et al., 2016; Sudhan et al., 2021). Additionally, its occurrence was noted in a number of plant species, including the methanolic extract of *Descurainia sophia* (Sadegh et al., 2022) and the ethanol stem bark extract of *Trilepisium madagascariense* (Olajuyigbe et al., 2018). Desulphosinigrin, which was discovered by GC-MS, was extracted from the seeds of *Soymida febrifuga* and used as a ligand to investigate its inhibitory activity towards cyclin-dependent kinase through docking and simulation studies. The observed ability of the compound to inhibit cyclin-dependent kinase 2 significantly increases its anticancer potency. Desulphosinigrin could therefore be used as the best therapeutic drug for the treatment of cancer (Krishnaveni, 2015). In the same way, Sari et al. (2023) investigated bioactive substances like desulphosinigrin and chimanine D using molecular dynamics simulations to highlight their importance as anti-diabetic agents. They noticed that both compounds had the same drug-like properties in their ability to inhibit alpha-glucosidase activity. Moreover, Sophia et al. (2021) noted the ability of this compound to act as an anti-inflammatory agent. The presence of the compound desulphosinigrin in the *M. malabathricum* leaves' ethanolic extract was discovered by GC-MS, and these compounds showed potential use in issues related to inflammation. Among the 12 isolated compounds, desulphosinigrin was identified as one of the principal phytochemicals with strong anti-inflammatory activity. It accomplished this by preventing the secretory phospholipase A2 enzyme from working properly, which is crucial for promoting inflammation.

There was a 9.78 peak area percentage of (1-methyl dodecyl)-benzene in the methanolic extract of *E. deccanensis*. It was also discovered in Marine Angiosperms *Enhalus acoroides*, where it reached a peak area of 4.43% (Selvam et al., 2022). It was also discovered to be a significant compound in the fixed oil of *Cucumis melo* seed. It is a known anti-inflammatory agent because it lowers proinflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- $\alpha$ ). Antimicrobial and immunomodulatory activities are just two of this compound's many functions (El-anssary et al., 2021).

The number of compounds discovered through GC-MS analysis was discovered to be lower than that of HR LC-MS analysis. Nevertheless, the eluted compound, which belonged to various classes, displayed notable bioactivities. Even though the quantity and number of compounds were found to be extremely low, they may have made a significant contribution to the various bioactivities noted in the current investigation, such as free radical scavenging, antiproliferative efficacy, and anti-inflammation potential. Thus, the phytoconstituents eluted by the GC-MS analysis further validate the importance of the chosen species' traditional value.

## **PART II- BIOACTIVITY SCREENING**

### **A) Antioxidant activities**

All living things require oxygen, which is found in the triplet state. Although it has weak reactivity, oxygen can produce a number of highly reactive and aggressive particles, including the singlet state superoxide anion ( $O_2^-$ ), oxygen ( $^1O_2$ ), hydroxyl radicals (OH), hydroperoxides ( $H_2O_2$ ), a number of different alkoxyl radicals ( $LO^\bullet$ ), and peroxide ( $LO_2^-$ ). Free radicals, also known as reactive oxygen species (ROS), are highly reactive and have unpaired electrons (Pande & Chanda, 2020). Free radicals and antioxidants in the body are in an unbalanced state, which causes oxidative stress and may harm cells and tissues. More than 100 diseases, including cancer, hypertension, diabetes, neurodegenerative disorders, and aging, have been found to be linked to oxidative stress (Islam, 2017).

Antioxidants are molecules that are necessary for avoiding the harmful effects of reactive oxygen species. They are the primary factors that can maintain the balance between oxidation and antioxidation, thereby ensuring system smoothness. Exogenous and endogenous antioxidants both play important roles in neutralising the effects of free radicals by scavenging free radical scavengers, preventing the radical chain reaction of oxidation, and inhibiting the oxidation process. Antioxidants can be both synthetic and natural. Synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertbutylhydroquinone (TBHQ), 2-tert-butyl-4-methylphenol (TBMP), and propyl gallate (PG). However, some synthetic antioxidants have reportedly been linked to health risks for people (Monika et al., 2016).

Plants have historically been a significant source of natural antioxidants. It is noted that roughly two-thirds of plants in the world have their own medicinal properties, the majority of which have been noted to have strong antioxidant potential (Krishnaiah et al., 2011). There have been Ayurvedic formulations of some Rasayanas with clear antioxidant properties (Gupta & Sharma, 2006). BHA, one of the synthetic antioxidants, is not as effective as some plant-derived antioxidants like carnosol, rosmariquinone, rosmanol, and androsmaridiphenol (Carvalho et al., 2005). Hence, the demand for natural antioxidants is rising as a result of their widespread use and high therapeutic efficacy with few side effects.

The effectiveness of substances with antioxidant activity is supposed to be assessed through a variety of *in vitro* assays. Conducting *in vitro* antioxidant tests by trapping dangerous radicals is a quick, easy, and affordable procedure (Romulo, 2020). Additionally, because it is challenging to fully compare one method with that of the other method, it is not possible to estimate the antioxidant potential of a test sample based on a single antioxidant test model. The antioxidant properties are therefore assessed using a variety of *invitro* antioxidant test methods. Therefore, five different assays were used in the current study to evaluate the antioxidant activity of the selected species, including the DPPH scavenging assay, the hydroxyl radical assay, the nitric oxide assay, the superoxide assay, and the ABTS scavenging assay.

DPPH radical scavenging ability is a milestone in assessing the antioxidant potential of isolated compounds or crude extracts of plants. It is a reliable and easy method to check antioxidant properties (Ul-Haq et al., 2012). This assay provides insightful data regarding the antioxidant activity of plant extracts. Numerous studies have examined the DPPH antioxidant capacity of plant extracts and what they might mean for health benefits. This method can be used to measure antioxidants present in complex biological systems, for instance, in either solid or liquid samples, such as fruit juices, vegetables, milk, and edible seed oils (Kedare & Singh, 2011).

The antioxidant assay conducted *in vitro* using the DPPH method is based on the observation that the DPPH solution, which is typically violet or purple, is converted by the antioxidants' scavenging power into the yellow-coloured product diphenyl picryl hydrazine. Hence, antioxidants are necessary for this method because they quench DPPH and lessen its coloration. Additionally, because this assay only needs a short amount of time, this analysis has been used very effectively to predict scavenging ability (Rahman et al., 2015). The peak absorbance of the DPPH radical occurs at 517 nm. Any substance that can scavenge DPPH reduces this wavelength's absorbance (Karagöz et al., 2015). DPPH's absorbance at 517 nm was noted in the current study. In this instance, a decrease in absorbance at 517 nm was observed as the concentration of test compounds was increased from 12.5 to 100 µg/ml, indicating that the DPPH radical was being inhibited by the activity of antioxidant molecules present in the chosen plant extract. Since all of the chosen species have the ability to donate hydrogen, which is necessary for the reduction of DPPH, the methanolic extracts of the selected species of *Euphorbia* showed significant potency to inhibit the DPPH radical in a dose-dependent manner.

*E. deccanensis* showed the highest percentage of inhibition (81.17%) at the highest concentration taken among all the concentrations. When *E. trigona*'s methanolic extract of (stem) was used, it showed strong antiradical activity against DPPH with an inhibition percentage of 83.25% at 300 µg/ml by the studies of Salar et al. (2015). Additionally, they noted that an ascorbic acid standard used in their study was found to have an inhibition percentage of 81.30% at 300 µg/ml. This

inhibition percentage was discovered to be comparable to the inhibition percentage of *E. deccanensis* noted in the current study, which further strongly supports the high potential of scavenging activity of *E. deccanensis*.

When IC<sub>50</sub> values were taken into account, each of the three chosen species displayed a value that was close to the value of the standard. *E. deccanensis*, however, displayed more similar values than the other two when compared. Previous research by Kamalanathan et al. (2015) discovered that the methanolic extract of *E. fusiformis*, one of the closely related species for the current study, demonstrated significant DPPH quenching ability with an IC<sub>50</sub> value of  $48.99 \pm 1.06$  µg/ml, which was roughly similar to the IC<sub>50</sub> values of all the selected species. So, it was highlighted that selected *Euphorbia* species had significant scavenging potential.

Previous studies on various *Euphorbia* species have demonstrated their capacity to scavenge free radicals and subsequently serve as antioxidants. The dichloromethane and methanol extracts of the aerial and root parts of *Euphorbia milli* showed a significant ability to scavenge DPPH (Saleem et al., 2019). It was also demonstrated that *E. helioscopia* has enormous DPPH scavenging potential, with an IC<sub>50</sub> value of  $0.06 \pm 0.02$  mg/ml (Mustafa et al., 2021).

The distribution of phenolic compounds and flavonoids, which occur naturally in plants, is mainly attributed to their antioxidant properties (Ilahi et al., 2013). In support of this, it was found that *E. deccanensis* had the highest levels of flavonoid and phenolic content when total phenolic and flavonoid content were estimated for the selected species. The total content of both these flavonoid and phenolic compounds and their scavenging potential are positively correlated. A similar trend was discovered by Sasikuma et al. (2020) in their studies, which correlated the total phenolic content of the test samples with the ability of the extracts to inhibit DPPH. Additionally, Tantary et al. (2017) observed this relationship between the total phenolic content derived from *E. wallichii* and the different extracts' capacity to scavenge free radicals. They found that methanolic extracts had the greatest scavenging effects, followed by ethyl acetate and aqueous extract. They also discovered the total phenolic content in a similar order. Thus, a

strong correlation between scavenging potential and phenolic content has been established. The redox properties of phenolic compounds, which enable them to act as singlet oxygen quenchers, reducing agents and hydrogen donors are responsible for their antioxidant capacity.

The highly reactive free radicals known as hydroxyl radicals are produced in biological systems and have a short lifespan. Hydroxyl radicals can cause negative effects in a variety of significant macromolecules, including proteins and nucleic acids, due to their highly reactive nature as well as their simplicity in crossing cell membranes. This radical causes DNA strand damage, which results in carcinogenesis, mutagenesis, and cytotoxicity. Furthermore, hydroxyl radicals are a powerful reactive species that can start the process of lipid peroxidation by obtaining hydrogen atoms from unsaturated fatty acids. Therefore, inhibiting the hydroxyl radical is crucial for the proper maintenance of biological systems.

The degree to which the Fenton reaction inhibits the breakdown of 2 deoxyribose is measured in the hydroxyl radical scavenging assay. The activity was assessed by using different concentrations of both the standard and methanolic plant extracts such as 125, 250, 500, 1000 and 2000 µg/ml and all the test samples demonstrated the lowest activity at 125 µg/ml and maximum activity at 2000 µg/ml. According to the present investigation, it is clear that, among the test samples, the extract of *E. deccanensis* effectively prevents the deoxyribose cleavage brought on by the hydroxyl radical in a concentration-dependent manner, and the highest inhibition percentage was found to be  $85.42 \pm 0.615$  at the highest concentration of 2000 µg/ml. These results are supported by the findings of Asha et al. (2016), which found that the antioxidant potential of *E. hirta* ethanolic extract showed concentration-dependent scavenging effects when compared with that of the standard against hydroxyl radicals. In addition, Pavithra & Vadivukkarasi (2015) found a comparable trend in the capacity to scavenge hydroxyl radicals. While, in the case of *E. rothiana* and *E. thymifolia* extracts, scavenging activity was moderate.

In a previous investigation, it was found that methanol extracts of grapefruit and pomegranate peel also exhibited hydroxyl radical scavenging activity, which

could be attributed to the presence of phenolic compounds (Loganayak et al., 2013). This was further supported by the current study, which showed that *E. deccanensis*, the plant with the strongest hydroxyl quenching properties, contained a high concentration of polyphenolic compounds. This was revealed through quantitative analysis of phytochemicals as well as HR LC-MS analysis.

*In vitro* nitric oxide radical scavenging is a way to measure antioxidant activity. Nitric oxide (NO) is a free radical produced when NO interacts with oxygen or reactive oxygen species. Because of the unpaired electron in nitric oxide, which makes it a free radical, this substance reacts strongly with some proteins as well as other free radicals like superoxide (Boora et al., 2014). Nitric oxide is produced by mammalian cells and is involved in the control of several physiological processes. However, nitric oxide production that is out of control is linked to a number of illnesses (Venkatachalam & Muthukrishnan, 2012). The creation of agents to stop the overproduction of nitric oxide has emerged as a fresh research area for the treatment of chronic inflammatory diseases (Shen et al., 2002). The nitric oxide radical scavenging assay works on the basis that sodium nitroprusside produces nitric oxide in aqueous solution. The generated nitric oxide reacts with oxygen to form nitrite ions, the amount of which can be calculated using the Griess reagent. A purple azo dye was produced as a result of the nitrite ions' interaction with the Griess reagent. If the test compound has a scavenging potential, the amount of nitrite and the azo dye will decrease, proving the scavenging potential (Preethamol & Thoppil, 2022). Through their effective NO scavenging activity, plants can play a significant role in reducing the amount of NO. Therefore, the plant extract may be used to treat a range of diseases caused by nitric oxide damage.

The ability of selected *Euphorbia* species to inhibit nitric oxide radicals was tested using the nitric oxide free radical scavenging assay, and it was discovered that all test samples did so in a dose-dependent manner, as shown in the **Fig. 19**. In the current study, the reference compound ascorbic acid was found to have the highest nitric oxide scavenging activity ( $IC_{50} = 226.83 \mu\text{g/ml}$ ), followed by the plant extract

of *E. deccanensis* ( $IC_{50} = 410.976 \mu\text{g/ml}$ ). However, the three extracts'  $IC_{50}$  values were found to be somewhat closer.

According to earlier reports by Wesley et al. (2022), *E. nivulia* ethanolic extracts ( $IC_{50}$  value  $250 \mu\text{g/ml}$ ) have a much greater capacity for nitric oxide scavenging than the standard ascorbate ( $IC_{50}$  value  $410 \mu\text{g/ml}$ ). Additionally, another study by Gomathi et al. (2015) using an ethanolic extract of *Evolvulus alsinoides* revealed a significant inhibition of nitric oxide radicals that was found to be comparable to that of standard ascorbic acid. They deduced that the antioxidant activity may be due to the occurrence of flavonoids, phenolics, and other phytochemical constituents. In support of this, it was observed that *E. deccanensis* contained a high concentration of polyphenolic compounds, which had a stronger anti-free radical effect. Accordingly, these results are consistent with the studies about the scavenging potential that were mentioned in earlier reports.

Superoxide, one of the less potent oxidants, can act as a precursor to hazardous reactive species like hydroxyl radicals and singlet oxygen, which lead to oxidative stress. Lipid peroxidation is accelerated by it, thereby, acting as a catalyst. In addition, xanthine oxidase, one of the oxidative enzymes generates superoxide in living things (Alam et al., 2013). Hence, superoxide radicals must be neutralised in order to protect the cells from their harmful effects.

One of the most important antioxidant defence enzymes, superoxide dismutase, reduces the toxicity of the superoxide anion by converting it to hydrogen peroxide. In the riboflavin-NADH system, the superoxide anions are created by the oxidation of NADH, and their production is detected by the reduction of NBT (yellow colour), which leads to the formation of a blue formazan product (Arika et al., 2019). The reduction of NBT, a measure of superoxide anion scavenging activity that is indicated by a decrease in absorbance at  $560 \text{ nm}$ , is decreased by drugs with superoxide scavenging activity. Hence, the antioxidant activity of the extracts was indicated by a drop in absorbance, which may have been caused by the inactivation or consumption of superoxide anion radicals produced in the reaction mixture.



The methanolic extract of all the selected species showed a dose-dependent inhibition of the superoxide radicals (**Fig. 20**). Among the chosen species, *E. deccanensis* exhibited maximum inhibition percentage ( $80.59 \pm 0.67\%$ ) at the highest concentration (2000  $\mu\text{g/ml}$ ) considered for the study. This was almost close to that of the standard ( $94.29 \pm 0.39\%$ ). A similar trend was also detected in the case of  $\text{IC}_{50}$  values. Previous studies suggest that the presence of phenolic chemicals, especially flavonoids, contributes to the scavenging of superoxide radicals in plant extracts because of their chemical structure. By transferring protons and hydrogen atoms, they can neutralise the superoxide (Cañas et al., 2023). All three extracts in the current study had a considerable amount of flavonoid content revealed from quantitative estimation. However, when compared, *E. deccanensis* showed a higher amount. The flavonoid content of *E. deccanensis* may account for its potent ability to inhibit superoxide radicals. Previous reports mentioned how well *Euphorbia* species can inhibit superoxide radicals. Tantary et al. (2017), for instance, found that the methanolic extract of *E. wallichii* had a significant capacity to neutralise superoxide, with an  $\text{IC}_{50}$  value of  $34.05 \pm 0.65 \mu\text{g/ml}$ . At a concentration of 100  $\mu\text{g/ml}$ , *E. heterophylla* exhibited a maximum inhibition percentage (97.01%) with an  $\text{IC}_{50}$  value of 425.8  $\mu\text{g/ml}$  (Keerthana et al., 2014). They also discussed the role of phenolic compounds and flavonoids in inhibiting superoxide radicals.

ABTS radical cation is formed when ABTS is oxidised with potassium persulfate. These radicals are reduced when antioxidants with the ability to donate hydrogen are present. The blue ABTS radical cation was decolorised during this time. The absorbance of ABTS, is characteristic at 734 nm and decreases with scavenging capacity. The ABTS assay is a fantastic tool for determining the antioxidant activity of both chain-breaking and hydrogen-donating antioxidants (Nitha et al., 2010).

The ABTS radical scavenging ability of selected *Euphorbia* species followed a similar pattern as seen in other assays. A dose-dependent scavenging activity was also observed here. Similarly, the methanolic extract of *E. deccanensis* demonstrated efficient radical inhibition when compared to the reference, with an inhibition

percentage of  $80.54 \pm 0.9472$  at a concentration of 2000  $\mu\text{g/ml}$  (**Fig. 21**). In the case of reference, it was found to be  $93.44 \pm 0.5575$ . Both *E. rothiana* and *E. thymifolia* had  $\text{IC}_{50}$  values that differed significantly from the value of the standard. So, these two extracts were found to have a moderate ability to scavenge ABTS radicals. Three species of *Euphorbia* such as *E. terracina*, *E. biumbellata*, and *E. dendroides*, have demonstrated ABTS radical scavenging efficacy in previous studies (Zeghad et al., 2016).

The evaluation of *in vitro* free radical scavenging assays yielded encouraging results. All of the selected species demonstrated remarkable potential that might be helpful in reducing the detrimental effects of oxidative stress and free radical damage. *E. deccanensis* was a top scavenger in all the assays and displayed significant inhibition against all the chosen free radicals. It was particularly notable for its ability to scavenge hydroxyl, superoxide, and DPPH radicals; in these cases, it had a nearly comparable ability to inhibit radicals as the standard. Furthermore, both *E. rothiana* and *E. thymifolia* also exhibited significant scavenging potential to scavenge free radicals, which was determined from the  $\text{IC}_{50}$  values. It is obvious from their comparison that both had a particular scavenging ability on particular reactive oxygen species. Thus it demonstrated the necessity of using a variety of antioxidant assays to accurately assess scavenging effects.

The antioxidant potential is primarily caused by polyphenolic compounds, particularly phenols and flavonoids. High-phenolic bioactive herbal extracts can have potent antioxidant effects. Similarly, flavonoids can quench singlet oxygen to scavenge various oxidising species, such as superoxide anion, hydroxyl, or peroxy radicals, and thereby act as potent antioxidants (Haida & Hakiman, 2019). Recent studies have shown, however, that the antioxidant properties of plant extracts can be attributed to the synergistic interaction of the phytochemical constituents that are present in them (Oyebode et al., 2019). According to the phytochemical analysis, *E. deccanensis* has the most bioactive compounds, followed by *E. rothiana* and *E. thymifolia*. The difference in antioxidant activity might be influenced by this variation. Among the numerous metabolites with antioxidant properties, notable

examples include pyrogallol, quercetin, myricetin, gallic acid, quinic acid, caffeic acid, kaempferol and gentisic acid.

The phytochemical profiling of selected *Euphorbia* species revealed their radical scavenging ability. It was determined from the preliminary analysis that the chosen species were abundant in different classes of secondary metabolites. This was also supported by the fact that the major metabolites in them had been distributed evenly after being studied through quantitative measurement. The same has been verified by GC-MS and HR LC-MS results. In light of these results, selected species of *Euphorbia* can be used to lessen the negative effects of reactive oxygen species.

## **B) Anti-inflammatory activities**

Inflammation is a type of defense mechanism the body employs to ward off dangerous conditions and also takes action to heal the tissues. However, chronic inflammation leads to several diseases such as cancer, asthma, obesity and autoimmune disorders. The use of medicinal plants in conventional medicine to treat inflammatory conditions appears to be a viable and logical alternative when looking for effective and safe anti-inflammatory agents. Additionally, the phytochemical analysis of the selected plant species showed the presence of a number of phytoconstituents, including flavonoids, phenols, terpenoids, tannins, etc. Moreover, metabolites with strong anti-inflammatory properties were found in the compounds discovered through GC-MS and HRLC-MS analysis of these selected species. Hence, to ascertain its activity in terms of anti-inflammatory potential, the methanolic extract of chosen plant species was screened. As a result, the plant extract was initially subjected to a quick and efficient *in-vitro* biochemical assay to demonstrate the ability of chosen species of *Euphorbia* to reduce inflammation.

### **i) Evaluation of Anti-inflammatory activity using *In vitro* biochemical assays**

#### **a) Protein denaturation inhibition**

The denaturation of tissue proteins is one of the well-studied causes of arthritic and inflammatory diseases. Protein denaturation is a phenomenon where

proteins lose their fundamental secondary and tertiary structures as a result of external stress or other substances such as strong bases or acids, organic solvents, or heat. This occurs as a result of variations in the hydrogen, hydrophobic, and disulfide bonds found in protein structures. As a result, the majority of proteins fail to perform their functions as intended (dev Sharma et al., 2023). Therapeutic anti-inflammatory drugs have focused primarily on bioactive compounds with the ability to prevent these changes and also have the ability to suppress protein denaturation (Basyal et al., 2021). Therefore, using biochemical assays to detect protein denaturation inhibition is an appropriate way to demonstrate the anti-inflammatory properties of plant extracts. A significant protein denaturation activity has been reported for NSAIDS like diclofenac sodium used to treat arthritic conditions (Anokwah et al., 2022). Therefore, in the current study, the inhibition of protein denaturation activity of the plant extracts was evaluated using diclofenac sodium as standard.

According to the results of the protein denaturation assay, plant extracts effectively prevented the protein from becoming denaturized. It was discovered that the effects of the extracts on the inhibition of protein denaturation were comparable to those of conventional diclofenac sodium and were dependent on concentration. *E. deccanensis* displayed the greatest inhibition of protein denaturation, with an  $IC_{50}$  value of  $119.77 \pm 0.35$   $\mu\text{g/ml}$ . In their study using four ethnomedicinal plants found in Pakistan, Naz et al. (2017) noted a concentration-dependent activity of inhibition of protein denaturation, as found in the current study. Protein hydrolysate from *Artocarpus altilis* was found to have significant effects on inhibiting protein denaturation and was comparable to the standard they used in their research (Dada et al., 2023).

The ability of plant extracts to inhibit protein denaturation may be due to the synergistic effects of flavonoids and other compounds (Mendez-Encinaset al., 2023). Apart from that, the anti-denaturation effect was brought on by the interaction of tannins, alkaloids, and flavonoids (Heendeniya et al., 2018). Among the chosen species, *E. deccanensis* showed the best inhibition on protein denaturation,

supporting this claim with the highest flavonoid content, revealed through estimation as well as other classes of compounds obtained from HRLC-MS analysis.

#### **b) Inhibition of proteinase activity**

Proteinases are involved in the development of arthritic symptoms. Neutrophils, which are found in lysosomes, are known to contain large amounts of serine proteinase. The development of tissue damage during the inflammatory process is significantly influenced by leukocyte proteinases. By indirectly inhibiting neutral proteinases or by suppressing infiltration into inflammatory loci, proteinase inhibition activity also leads to the reduction of inflammation. Several biologically active substances found in plants have been shown in the literature to be able to inhibit the activity of these enzymes (Gunathilake et al., 2018; Michalak et al., 2023). The potential for proteinase inhibition was therefore also evaluated in this work.

The current study examined the selected plant species' methanolic extracts to see if they could inhibit proteinases in action. The proteinase activity was inhibited in a dose-dependent manner in each of the test samples. However, *E. deccanensis* showed more activity than the other two plant extracts, with an inhibition percentage of  $72.86 \pm 0.40\%$  and an  $IC_{50}$  value of  $198.78 \pm 0.46 \mu\text{g/ml}$ . Additionally, the plant extract displayed comparable activity to that of the standard diclofenac sodium in terms of inhibiting proteinase activity. This is corroborated by the previous study by Saleem et al. (2020) that the methanolic extract of *Moringa oleifera* inhibited the proteinase activity in a promising manner, with an inhibition percentage of  $88.33 \pm 0.18\%$  at a concentration of  $1600 \mu\text{g/ml}$ , which was also dose-dependent. In addition, at concentrations of  $100 \mu\text{g/ml}$ , the *E. hirta* leaf and root extracts significantly exhibited antiproteinase activity with significant inhibition percentages of  $97.30 \pm 0.58\%$  and  $54.21 \pm 0.11\%$ , respectively (Das et al., 2022).

For plant extracts to have antiproteinase activity, they must contain phytoconstituents like flavonoids, tannins, phenols, and saponins (Kommu et al., 2023). In addition, the presence of flavonoids, polyphenols, and terpenoids in plant extracts is linked to antiproteinase activity (Njoya et al., 2022). This highlights the

importance of secondary metabolites, particularly flavonoids and phenolic compounds, in plant extract. This explains the antiproteinase activity found in the *E. deccanensis* plant extract.

In the *in vitro* biochemical assays used to evaluate the anti-inflammatory potential, *E. deccanensis* performed better than the other test samples in terms of preventing protein denaturation and repressing proteinase activity. Therefore *E. deccanensis* plant extract was used to study its impact on various proinflammatory mediators in RAW 264.7 macrophage cells in order to advance research.

## **ii) anti-inflammatory activity on RAW 264.7 macrophage cells**

An inflammatory response involves the activation and release of inflammatory mediators like nitric oxide (NO), pro-inflammatory cytokines like tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, and inflammatory enzymes like lipoxygenase (LOX), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) (Marques et al., 2022). It has been reported that the production of prostaglandins and nitric oxide by the proinflammatory mediators COX-2 and inducible nitric oxide synthase is a major cause of many inflammatory diseases. Due to the presence of phytochemicals in medicinal plants, enzyme inhibitory assays like COX and LOX were extensively studied to ascertain the efficacy of plants in treating inflammatory diseases (Abu Bakar et al., 2018). Therefore, the methanolic extract of *E. deccanensis* was tested to see if it had any effect on these inflammatory enzymes. Additionally, its effects on the level of cellular nitrite were assessed in LPS-treated RAW 264.7 macrophage cells.

When examining the bioactivity of isolated natural products and extracts from plants, RAW 264.7 macrophage models are frequently used. By assessing the decline of inflammatory mediators in stimulated RAW 264.7 cells, numerous studies have documented the anti-inflammatory potential of various plant groups and their metabolites. Because of this, the current study uses RAW 264.7 macrophages for anti-inflammatory screening experiments.

**a) Cyclooxygenase (COX) activity**

Prostaglandins (PGs), which are lipid molecules involved in many physiological processes, are crucial in the emergence of inflammatory responses. They work by controlling how the body reacts to tissue damage or inflammation. Among them, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is the predominant prostaglandin that causes the typical symptoms of inflammation, such as fever, tumour, pain, and anaphylactic reaction. The expression of the cyclooxygenase enzyme, which is present in inflammatory tissues, is essential for the production of these prostaglandins (Ju et al., 2022).

There are two isoforms of cyclooxygenase, COX-1 and COX-2, based on their structure and function. Most cells contain a significant amount of COX-1, a constitutive enzyme. Contrarily, COX-2, an induced enzyme, serves as the primary source of prostaglandins. As a result, COX-2 is thought to be the pathological enzyme that is fundamentally to blame for inflammation. NSAIDs work to reduce inflammation by selectively inhibiting COX activity and preventing the release of prostaglandins at sites of injury. The most efficient method of treating inflammation remains the discovery of new NSAIDs with improved safety profiles. Furthermore, substances that inhibit the development of cyclooxygenase enzymes, particularly COX-2, are known to be effective anti-inflammatory agents (Gandhi et al., 2017).

In the current study, the methanolic plant extract of *E. deccanensis* demonstrated a dose-dependent inhibition of the enzyme cyclooxygenase in LPS-induced RAW cells. From lower (62.5 µg/ml) to higher (25 µg/ml) concentrations, the percentage of inhibition also rises, reaching a percentage of  $57.21 \pm 0.74\%$  at the highest concentration. In addition, the IC<sub>50</sub> value of plant extract (21.939 µg/ml) was found to be very close to the value of standard diclofenac sodium (15.7813 µg/ml). Because of this, it implies that the plant extract possessed potent anti-inflammatory properties compared with those of diclofenac sodium. The ethanolic extract of *Careya arborea* inhibits COX activity in RAW 264.7 cell lines induced by lipopolysaccharide with an IC<sub>50</sub> value of 5.30 µg/ml, supporting the current study. *Careya arborea* has strong anti-inflammatory properties, as evidenced by the fact

that the standard, diclofenac used in their study inhibited COX with an IC<sub>50</sub> value of 2.84 µg/ml (Bindu & Aleykutty, 2022).

Numerous studies have looked at plant extracts' ability to inhibit the COX enzyme. For instance, *Filipendula ulmaria* has proven to have strong anti-inflammatory properties by inhibiting COX activity. The anti-inflammatory impact was dose-dependent, with an IC<sub>50</sub> value of 90.26 µg/ml (Van der Auwera et al., 2023). In addition, *Premna integrifolia*, a traditional medicinal plant with a significant role in Ayurveda, Siddha, and Unani systems of medicine, demonstrated significant inhibition of COX-2 activity with an IC<sub>50</sub> value of 6.15 µg/ml (Azad et al., 2018). Maleki et al. (2019) found that the main compounds responsible for the anti-inflammatory effects are flavonoids. The plant extract's observed anti-inflammatory potential is explained by the wide variety of flavonoids present in it.

#### **b) Lipoxygenase (LOX) activity**

Lipoxygenase enzymes, which are crucial for inflammation, catalyse the conversion of polyunsaturated fatty acids into bioactive lipid mediators like leukotrienes and lipoxins. Along with other aspects of the inflammatory response, these lipid mediators regulate the recruitment and activation of immune cells (Serhan & Levy, 2018). Additionally, LOX transforms arachidonic acid into several leukotrienes, such as LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, and others. LTB<sub>4</sub> is the most significant leukotriene in inflammation, as demonstrated by the high levels of LTB<sub>4</sub> in people with inflammatory bowel disease and rheumatoid arthritis. It has been suggested that the pathogenesis of inflammatory diseases is influenced by the dysregulation of lipoxygenase activity (Abu Bakar et al., 2018). Lipoxygenase enzymes have been identified as promising targets for the development of anti-inflammatory therapies.

This study showed that the plant extract had a potent inhibitory effect on lipoxygenase activity. In comparison to standard diclofenac sodium, plant extract significantly inhibited enzyme activity less efficiently at the lowest concentration (6.25 µg/ml). At a concentration of 12.5 µg/ml, the inhibition potential of the plant extract increased dramatically and suddenly, rising from 14.75 ± 0.682 to 64.48 ± 0.935. At the highest concentration selected for the study, the inhibitory effect of



plant extract on LOX was comparable to that of the standard and appeared to be very promising. This is in line with the observation of Andrade et al. (2019), who made investigations in order to know how the methanolic *Ficus curtipes* leaf and stem extract affected LOX activity. Significant inhibitory activity was seen in both extracts, and it was dose-dependent. With significant inhibition percentage of  $91.72 \pm 4.51\%$ , the stem bark extract was, however, much more effective at inhibiting LOX activity than the leaf extract ( $59.57 \pm 16.80\%$ ).

The inhibition of LOX activity by different plant extracts has recently been reviewed by Lončarić et al. (2021). They concluded that plant extracts from the family including Asteraceae, Clusiaceae, Apiaceae, Lamiaceae, and Euphorbiaceae had the potential to inhibit LOX. In addition, when different fractions of *Serevenia buxifolia* bark extract were screened for its LOX activity, appreciable effects on the percentage of LOX were seen. With an IC<sub>50</sub> value of 90.45 µg/ml, the ethyl acetate fraction in particular had the highest LOX activity (Truong et al., 2021). The metabolites, including flavonoids, phenolics, terpenoids, and other metabolites, work synergistically to inhibit LOX activity (Bhat et al., 2019).

### **c) Inducible nitric oxide synthase activity**

The nitric oxide synthase (NOS) enzyme is responsible for converting L-Arginine into nitric oxide (NO). In general, there are three types of nitric oxide synthase. Which includes i) neuronal NOS (nNOS), ii) inducible NOS (iNOS), and iii) endothelial NOS (eNOS). The expression of inducible nitric oxide synthase, a Ca<sup>2+</sup> dependent enzyme, rises in inflammatory cells in response to various stimuli. In the presence of LPS, macrophages have the capacity to produce large amounts of NO via iNOS. Inflammation, immune responses, and oxidative stress are just a few of the physiological and pathological processes in which NO produced by iNOS plays a significant role. Abnormalities in iNOS activity have been associated with the onset and progression of several diseases. *In-vitro* assays were used by Asanuma et al. (2001) to show that non-steroidal anti-inflammatory medications have the capacity to scavenge nitric oxide and inhibit inducible nitric oxide synthase.

Nitric oxide (NO) production has been consistently inhibited by the effects of medicinal plants. Plants or compounds derived from plants that have strong inhibitory activity against NO production have shown promising therapeutic potential for the treatment of inflammation (Balaji & Ganesan, 2021). In the current study, the plant extract showed significant suppressive effects on iNOS activity at each of the three selected concentrations which were comparable to those of diclofenac sodium. The plant extract had an inhibition percentage of  $67.13 \pm 0.51\%$  at the highest concentration of 25  $\mu\text{g/ml}$ , while the standard had an inhibition percentage of  $73.52 \pm 0.69\%$  at the same concentration. An earlier investigation found that the ethanolic extract of *Artemisia montana* leaves decreased NO production in a concentration-dependent manner by reducing the activity of iNOS in RAW 264.7 cells after LPS stimulation (Jeong et al., 2018). Similar to this, *Pleurospermum benthamii* extract significantly reduces LPS-induced NO production (Pandey et al., 2020).

#### **d) Level of cellular nitrite**

Nitric oxide (NO) is a key signalling molecule involved in many physiological and pathological processes, including inflammation. Its numerous functions in inflammation range from controlling vascular tone to modifying immune reactions. When formed nitric oxide reacts with oxygen, nitrite ions are produced. The level of nitrite will therefore decrease as a result of the inhibition of inducible nitric oxide synthase (Preethamol & Thoppil, 2020).

In the present work, the level of cellular nitrite significantly decreased from  $718.74 \pm 1.25 \mu\text{g/ml}$  to  $464.80 \pm 0.827 \mu\text{g/ml}$ . For diclofenac, the drop was from  $737.055 \pm 0.636774 \mu\text{g/ml}$  to  $377.19 \pm 0.59039 \mu\text{g/ml}$ . The decrease in cellular nitrite level is justified by the previous assay's findings that plant extract has the immense ability to inhibit iNOS activity. Nitrite levels were significantly reduced, indicating that plant extracts might have the ability to decrease inflammation.

Analysis of the effects of methanolic plant extract on the inflammatory enzymes on LPS-treated RAW cells revealed significant results indicating its potent anti-inflammatory properties. The plant extract has the potential to be a strong

candidate for the creation of anti-inflammatory drugs. The plant extract *Euphorbia* demonstrated nearly equivalent activity to that of the standard, diclofenac sodium, on all four assays that were performed. In addition to its therapeutic effects, diclofenac sodium use has a number of side effects, such as gastropathy and inflammatory liver changes. Therefore, it is emphasised how important it is to develop anti-inflammatory drugs.

### **iii) Evaluation of cytotoxicity using MTT assay on RAW 264.7 cells**

MTT assay was conducted to ensure that the inhibitory effects of plant extract observed in *in vitro* assays were caused by the plant extract's anti-inflammatory potential and not by its cytotoxic effects. The plant extract's LC<sub>50</sub> value, which was found to be 139.63 µg/ml, was barely toxic to RAW 264.7 cells, so it was taken into consideration for further analysis.

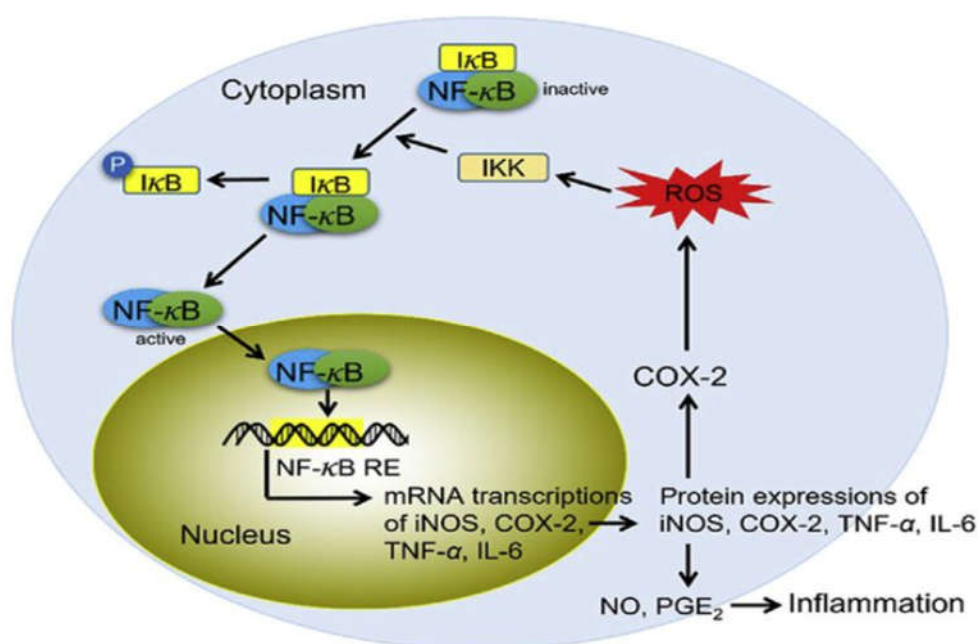
### **iv) Estimation of inflammatory mediator using ELISA**

Proinflammatory mediators are the primary causes of inflammatory processes. The enzyme cyclooxygenase-2 (COX-2), which is involved in the production of pro-inflammatory prostaglandins, is crucial for processes connected to inflammation. Enzyme-linked immunosorbent assays (ELISA) are essential for estimating cyclooxygenase-2 (COX-2) levels in anti-inflammatory assays because they allow for the quantification of COX-2 expression and provide information on the efficacy of anti-inflammatory interventions. Recent studies have emphasised the significance of COX-2 estimation using ELISA in assessing an agent's capacity to reduce inflammation (Shaikh et al., 2016).

In the current study, COX-2 levels in LPS-stimulated RAW macrophage cells were assessed using an ELISA. In order to accomplish this, 139.63 µg/ml of the extract was treated with RAW cells, and the COX-2 level was measured. It was noted that the plant extract had the ability to reduce COX-2 levels substantially from  $0.841 \pm 0.08$  to  $0.505 \pm 0.062$  U/ml of cell lysate. As a result, the results obtained were consistent with the earlier assays.

Recently, the anti-inflammatory effects of red ginseng oil on lipopolysaccharide-stimulated macrophages were examined. The researchers used ELISA to measure COX-2 levels, and red ginseng oil showed promising results in inhibiting COX-2 expression. It was also found that the oil could alter the NF- $\kappa$ B signalling pathway, which validates its anti-inflammatory qualities (Liu et al., 2021). Likewise, the present investigation's ELISA findings, which demonstrate a significant reduction in COX-2 levels, emphasise the plant extract's anti-inflammatory properties even more.

The NF- $\kappa$ B signalling pathway plays a critical role in LPS-induced models of inflammation. NF $\kappa$ B-binding sites have been identified in the promoter regions of the COX-2 and iNOS genes. Because of this, NF- $\kappa$ B is crucial among the various pathways to keep the production of COX-2 and iNOS in check (Bak et al., 2012). Therefore, the decreased level of COX-2, which is supported by the decreased activity of the iNOS enzyme and the cellular nitrite level, suggests that the plant extract exhibited a strong suppression on the NF- $\kappa$ B pathway. The anti-inflammatory mechanism of COX-2 inhibitor in RAW 264.7 macrophage cells is represented in Fig. 46.



**Fig. 46: Mechanism of anti-inflammatory action exerted by COX-2 inhibitor** (Ju et al., 2022)

A well established connection is there between the initiation and modulation of inflammatory disorders and free radical species (superoxide, hydroxyl radicals, nitric oxide, peroxynitrite, and the free radical-derived product hydrogen peroxide) (Shaikh et al., 2016). The NF- $\kappa$ B pathway's signalling can also be moderated by the oxidative stress brought on by ROS and NO (Akanda et al., 2018). Numerous studies have shown that the flavonoids and polyphenolic compounds found in plants are what give them their ability to resist oxidative stress. Plant extracts with strong antioxidant activity have been found to have anti-inflammatory qualities (Ravipati et al., 2012).

This was supported by the current study's apparent direct correlation between antioxidant activity and anti-inflammatory activity. The extract's ability to mop up reactive oxygen species, which could act as a trigger for an inflammatory response, could be a critical factor in reducing inflammation. Thus, the antioxidant activity of the *E. deccanensis* extract observed may corroborate with its anti-inflammatory potential by influencing the NF- $\kappa$ B signalling pathway.

Because many medicinal plants contain secondary compounds that impede the key steps of the inflammatory pathway, such as lipoxygenase, cyclooxygenase, and nuclear factor NF- $\kappa$ B, herbal medicine remains an important part of treating a wide range of health conditions. Numerous studies have discussed the possible ability of herbal remedies to reduce inflammation (Chouhan & Guleria, 2020). Among the secondary metabolites, polyphenols, terpenoids, and flavonoids have potent anti-inflammatory properties. Through the inhibition of enzymes such as cyclooxygenase, lipoxygenase, prostaglandin, ATPase, and the metabolism of arachidonic acid, the flavonoids exhibited their anti-inflammatory properties (Nunes et al., 2020). In the same way, the terpenoid  $\beta$ -amyrin demonstrated anti-inflammatory effects. It functions by preventing the iNOS protein from functioning and the production of nitric oxide in LPS-induced RAW cells (Meda et al., 2023). It has been discovered in the literature that quercetin, gallic acid, myricetin, and kaempferol significantly influence the NF- $\kappa$ B signal pathway and the expression of genes involved in inflammation (Amri et al., 2018).

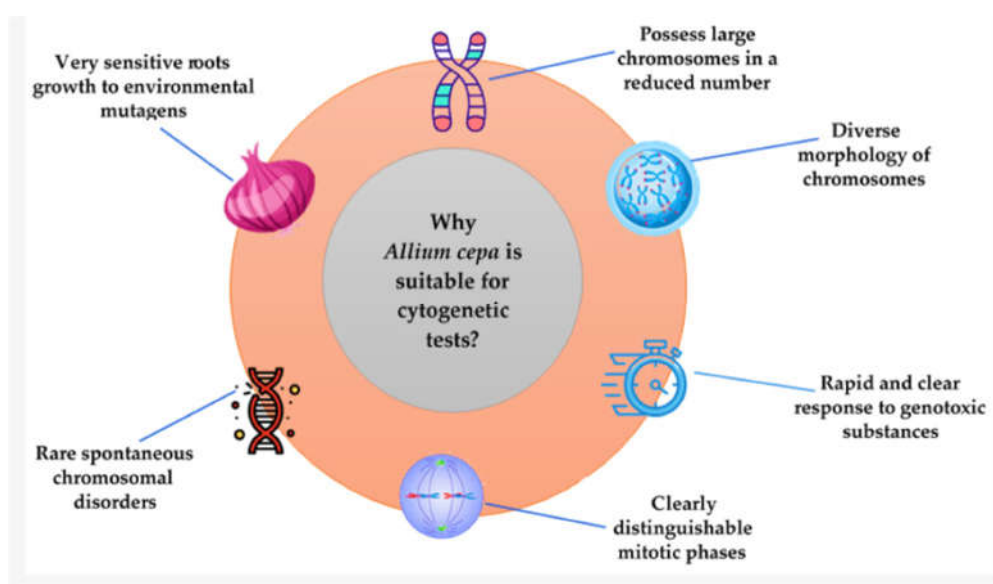
The multitude of anti-inflammatory compounds found in the methanolic extract of *E. deccanensis* and their combined effects are responsible for lowering inflammation. Through GC-MS and HRLC-MS analyses, several strong anti-inflammatory compounds with documented anti-inflammatory activity, such as gallic acid, vanillic acid, punicaortein B, herbacetin, and kaempferol, were identified from the plant extract. Even though there are some reports on the anti-inflammatory properties of different *Euphorbia* species, this study is the first to address anti-inflammatory property in the chosen species. So, the current study demonstrates the selected species' powerful anti-inflammatory potential and suggests it as a viable option for an anti-inflammatory medication.

### **C) Cytotoxicity study using *Allium cepa***

*Allium cepa* is a well-known and frequently used model to determine environmental risk factors and evaluating the cytotoxicity potential of bioactive compounds dispersed in plant extracts. By examining chromosomal abnormalities and changes that occur during the mitotic cycle, the *A. cepa* test is used to identify DNA damage, including clastogenic and aneugenic damage, which results from the action of different mutagenic substances. Levan (1938) was the first to use *A. cepa* bioassay to investigate the effect of colchicine on onion mitosis. The development of this assay was greatly aided by Grant (1994) and Fiskesjö (1985). Rank & Nielsen (1994) discovered that this bioassay had a high sensitivity, making it suitable for environmental monitoring as a standard procedure. In assessing the genotoxicity of medicinal plants, this assay is also considered as a warning bioindicator. When exposed to various particles, onion roots may exhibit cytotoxic or genotoxic effects, which is why they are used in this test. Onions are selected because the spontaneous growth of its root and the meristematic cells found in their tips in various stages of cell division, showing a spontaneous response towards the cytotoxic particles. It is accomplished mainly due to the contact of both actively dividing cells found in the root tips with that of substances having cytotoxic property. Thus, it enables the evaluation of its toxic effects on various phases of mitosis and chromosomal abnormalities. Furthermore, the low number of chromosomes ( $2n = 16$ ) in *A. cepa* makes it easier to determine whether any genetic variations in chromosomes are caused by the effects of toxic compounds. This test makes it possible to learn more

about how chromosomal structure variations are caused by toxic compounds either alone or in combination. Furthermore, for the first time, this assay yields a better alternative to animal-based toxicological evaluation of novel compounds. Many researchers use the *A. cepa* assay to identify cytotoxic and genotoxic variations because of all these factors (Alias et al., 2023; Arumuganathan & Earle, 1991).

A parameter determining the total number of cells in a cell cycle is made possible by the mitotic index or it is a measure of the percentage of cells that are actively dividing in each stage of mitosis relative to the total number of cells. The phases of mitosis are interphase, prophase, metaphase, anaphase, telophase and cytokinesis. It takes longer for the cell cycle to replicate genetic material during interphase, and many cells are typically seen during this stage. Information about any cytotoxicity that the sample under study may have is provided by this parameter. Variations in the cell cycle, delays in cell division, and cell death are indicators of any decrease in the mitotic index and an increase in extract concentration. High MI value means that cells are growing actively and that there are no disruptions to their division or growth. Furthermore, the low MI value denotes cell death brought on by the plant extract's cytotoxicity action (Nicuță et al., 2025).



**Fig. 47: Properties of the onion that make it suitable for cytogenetic tests** (Nicuță et al., 2025).

*A. cepa* was shown to be cytotoxically affected by the methanolic extracts of *E. deccanensis*, *E. rothiana*, and *E. thymifolia* in the current investigation. The

mitotic index and plant extract concentration were found to be negatively correlated. Additionally, a positive correlation between the concentration and the percentage of abnormalities was noted. One possible explanation for the observed decrease in mitotic activity is the inhibition of DNA synthesis, which prevents the cells from going through mitosis. Accordingly, the study's findings suggest that different concentrations of methanolic extracts from every plant species have mitodepressive effects on *A. cepa*. The study took into account concentrations of each extract of 25, 50, 75, and 100 µg/ml. At its lowest concentration, *E. rothiana* had the highest mitotic index ( $64.35 \pm 0.51\%$ ), followed by *E. thymifolia* and *E. deccanensis*, respectively. The cell division of *A. cepa* was significantly inhibited by the various concentrations of *E. hirta* methanolic extract, and the mitotic index gradually decreased in comparison to the control, according to Ping et al. (2012). Additionally, at all concentrations, methanolic extract induces a variety of chromosomal abnormalities, such as C-mitosis, stickiness, and chromosomal bridges. Shilpa et al. (2023) found a decrease in the mitotic index in a dose-dependent manner of extract, which is consistent with the current work. In addition, anomalies such as stickiness, gigantic cells, nuclear lesions, C-metaphase, and aberrant metaphase and anaphase were noted.

In the present work, the plant extracts caused a wide variety of chromosomal abnormalities in all stages of mitosis in addition to the mitotic index. The dose dependent increase in the percentage of abnormalities shows that the action of plant extracts alters the structure of chromosomes. **Plates 4, 5, 6, 7, 8, 9 and 10** document the abnormalities found in different stages of mitotic division. And the **Plate 3** shows the typical stages of mitosis. At each concentration, the three extracts showed a wide variety of aberrations. A few of these include chromosome vagrants, laggards, nuclear erosion and lesion, micro and macro cells, micronucleus and C-metaphase.

Chromosome abnormalities may be classified as either clastogenic or non-clastogenic. Any kind of variation in the chromosome's structure can cause clastogenic aberrations, but disruptions to the mitotic apparatus can cause non-



clastogenic aberrations, also known as aneugenic aberrations, which typically result in numerical aberrations rather than structural ones. Both of these types are found in the current work. In this study, one of the most noticeable clastogenic aberrations found was chromosome stickiness. Both metaphase and anaphase are affected; here, different types of chromosome abnormalities involving their sticky nature are seen, including disturbed sticky metaphase and sticky anaphase with narrow chromosome bridges. DNA depolymerisation and chromosome condensation are typically linked to stickiness. This is usually irreversible, resulting in cell death (Türkoğlu, 2007). The most often reported cytotoxic effects of medicinal plant extracts on the meristematic cells of *A. cepa* roots are stickiness of the chromosomes and anaphase bridge, which arises as a result of the spindle mechanism being suppressed (Akwu et al., 2019). In *A. cepa* treated with methanolic extracts of selected *Euphorbia* species, these aberrations were found, and it was observed that the percentage of aberration increases with concentration.

The presence of nuclear lesion provides the evidence of the cytotoxicity activity of plant extract on *A. cepa* and inhibition on DNA synthesis. Nuclear lesion is the indication of nuclear poison. This is accomplished mainly through two ways including avoiding the incorporation of precursor into DNA or through effecting the DNA precursor biosynthesis (Akaneme & Iyioka, 2008). Whatever, it may be this inhibition leads to the complete stoppage of mitosis. The current study found both single and double nuclear lesions, which are primarily found during prophase, interphase, and cytokinesis. Therefore, the potential of plant extracts to inhibit DNA synthesis may be the cause of the observed nuclear lesions. In the work, chromosomal bridges are also noted prominently in anaphase. Bridges are formed when homologous and non-homologous chromosomes exchange or when sister chromatids longitudinally separate incorrectly during anaphase (Bonciu et al., 2018). Cell cycle arrest, polyploidy, and aneuploidy are other abnormalities that arise from this. The existence of bridges indicates a moderate cytotoxic potential of the plant extract. The study also found an aberration in the chromosome having a pulverised condition, which may have resulted from premature chromosomal condensation.

Along with all of these abnormalities, the current study also found a number of aneugenic abnormalities, with the majority of the aberrations occurring in both the anaphase and metaphase. C-metaphase is one of the prominent ones observed after the plant extract *Euphorbia* treatment on *A. cepa* meristematic cells. This resulted from the spindle apparatus not being organised. The presence of toxic substances in the extract is indicated by the occurrence of C-metaphase in *A. cepa*. Therefore, it demonstrated that the plant extracts of *Euphorbia* spp. being studied contained cytotoxicity inducing compounds. Among the numerous severe abnormalities found in this work, binucleate cells were also found. The development of cells with a binucleate condition results from the inhibition of phragmoplast formation during cytokinesis (Giménez-Abián et al., 2004). In this case, the different phytoconstituents present in the plant extracts might function as an inhibitor of phragmoplast formation by changing the regular cytokinesis process. According to Bonciu et al. (2018), binucleate cells arise because of variations in spindle division in early anaphase or after telophase, which inhibits the cytokinesis process. Ball shaped chromosome, which is clearly visible in the work in the metaphase stage of cell division, and is formed when chromosomes combine to form a compact group in the centre of the cell. This might have occurred as a result of the plant extract's cytotoxicity action. Chromosomal laggards are typically observed when inhibiting the activity of cytoskeleton proteins or suppressing tubulin polymerisation. The plant also induces the vagrant chromosome, which is another abnormality. This abnormality is primarily caused by the chromosomes' early movement in the spindle poles. Interphase nuclei of varying sizes are thus created (El-Ghamery et al., 2003). Additionally, micronucleus along with nuclear disintegration was found in prophase of cell cycle in the current study. The formation of micronucleus due to the effect of plant extracts on *A. cepa* was reported by many previous studies. When lagging chromosomes fail to participate in daughter nuclei during the telophase of mitotic division, or when acentric fragments of chromosomes occur, these are typically formed (Sajitha & Thoppil, 2018).

Chromosome misorientation is caused by abnormal mitotic apparatus functioning, which includes diagonal metaphase, diagonal sticky anaphase, diagonal

coagulated anaphase, disturbed metaphase exhibiting vagrants, and disturbed metaphase in a polyploid cell. In addition to all these abnormalities, hypoploid cell, polyploid cell, tropokinesis, stathmo-anaphase and chromosome coagulation were revealed from the study. Here, the majority of identified aberrations were with multiple anomalies, in which sometimes both clastogenic and non-clastogenic aberrations were detected together. Some of them include abnormal cytokinesis showing nuclear erosion and lesion, chromatin disintegration with a micronucleus at the cell plate in cytokinesis, nuclear and cytoplasmic lesion at cytokinesis, single nuclear lesion and erosion at prophase in a binucleate cell, nuclear peak in a strap cell at prophase, sticky anaphase with narrow chromosome bridges, nuclear lesion in a ball metaphase, sticky metaphase showing chromosome erosion and chromosome erosion in a ball metaphase.

Methanolic plant extracts have been shown to induce apoptosis by causing cell death through the development of different abnormalities such as nuclear disintegration, nuclear peak formation, chromosome fragmentation and condensation, etc. The bioactive compounds in the plant extracts might have caused the clastogenic and aneugenic abnormalities. Hence, the initial screening of plant extracts for their antiproliferative effectiveness on actively dividing cells was made possible by the cytotoxic studies employing *A. cepa*.

#### **D)Antiproliferative activity**

Currently, even in developed nations, cancer has a high death rate and is one of the most dangerous diseases that is progressing alarmingly. A study on cancer case records for 2020 showed that, out of 29.8 million cases, there were roughly 19.3 million new cases and 10 million cancer-related deaths. Breast cancer (11.7%), lung cancer (11.6%), colorectal cancer (10%), and prostate cancer (7.3%) were the most common cancers diagnosed (Jiménez-González et al., 2023). Currently, treatment strategies such as radiation, chemotherapy, and surgery are used to cure different types of cancer. Chemotherapeutic agents are only temporary relief providers, and many anticancer drugs have negative side effects. This highlights the need for the creation of more potent anticancer agents (Chandra et al., 2023).

The most common type of cancer that is diagnosed in women and the main cause of cancer-related deaths in this population is breast cancer (BC). Breast cancer cell lines most commonly used for *in vitro* studies are MCF-7, MDA-MB-231, MDA-MB-468, and T47B (Lutoti et al., 2023). Thus, a normal L929 cell line and the breast cancer cell line MCF-7 were chosen for the current study.

Phytomedicines have gained a lot of attention due to the use of plant extracts, and they have significantly fewer side effects when compared to chemotherapy drugs. While there is no known natural treatment for breast cancer, maintaining a healthy diet, getting enough sleep, and exercising regularly are crucial. Eating foods high in flavonoids, such as tomatoes, onions, potatoes, apples, oranges, and aromatic herbs, can help reduce the risk of breast cancer to some extent. One of the primary sources of anticancer compounds is the plant kingdom. By suppressing cancer cell lines through DNA destruction and the activation of apoptosis-inducing enzymes, secondary metabolites such as isoflavones, flavones, flavonoids, coumarins, anthocyanins, catechins, lignans, and iso-catechins present in plant extracts can lessen the negative effects of conventional cancer treatments. Therefore, prospective anticancer compounds should be treated with appropriate care (Shrihastini et al., 2021).

Since many of the *Euphorbia* species produces a wide variety of secondary metabolites, including diterpenoids and triterpenoids, it has been reported that various parts of the species have been used internally or externally to treat a variety of diseases, including cancer. Its importance in traditional medicines as anticancer agents stems from the distribution of different phytoconstituents. The cytotoxic phytometabolites present in *Euphorbia* members are involved in multiple mechanisms, including cell differentiation and proliferation, apoptosis, excessive production of reactive oxygen species, and inhibition of metastasis (Kemboi et al., 2020).

### **Cytotoxicity determination using MTT assay**

Cytotoxic assays are among the first *in vitro* bioassay for determining a substance's toxicity to different tissues. The MTT assay is one of the widely used

assays to identify the metabolically active cells. It was developed by Mosmann et al. in 1983. The reduction of the mono tetrazolium salt, or MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide), produces formazan, a purple-coloured molecule that is insoluble in water. In living cells, a mitochondrial dehydrogenase enzyme converts yellow tetrazolium MTT salt to purple MTT formazan (Vajrabhaya & Korsuwannawong, 2018). Therefore, the chromogenic nature of this redox reaction makes it possible to analyse intracellular formazan formation using a colorimetric approach, which in turn allows for the assessment of cell states, including viability (Ghasemi et al., 2021).

**i) Anticancer assay on normal L929 cell line & MCF-7 cell line**

The cytotoxicity effects of selected plant species' methanolic extracts were first examined in this study using the normal L929 cell line using MTT assay. Every plant species was treated on the normal cell line for 24 hours at different concentrations, ranging from 6.25 to 100 µg/ml, and the LC<sub>50</sub> value of each species was ascertained. A dose-dependent cytotoxicity effects of each test extract on the normal L929 cell line are observed (**Fig. 32**). **Table 11** shows the tabulation of the LC<sub>50</sub> values for each species.

In a similar way, the cytotoxic effects of selected species were evaluated using the breast cancer MCF-7 cell line. Here, too, all chosen species exhibited cytotoxic effects that were dependent on concentration (**Figure 33**). Furthermore, apoptosis features like condensed nucleus, apoptotic bodies, membrane blebbing, budding, and cell shrinkage were observed in the microscopic images of the treated MCF-7 cell line (**Plates 14, 15, 16**). This result suggests that the induction of apoptosis was associated with the cytotoxic properties of the tested extracts.

*E. deccanensis* was found to substantially inhibit the proliferation of the MCF-7 cell line and to exhibit strong antiproliferative potential with an LC<sub>50</sub> value of  $93.21 \pm 0.58$  µg/ml when the percentage of viable cells and the LC<sub>50</sub> value of each plant species was examined in both cell lines. However, the LC<sub>50</sub> value for the other two plant species was higher than that of this species. Moreover, when *E. deccanensis* was compared to the other two species, the nature and extent of toxicity

it displayed on a normal L929 cell line was found to be minimal or very low. Therefore, the methanolic extract of *E. deccanensis* was discovered to have toxicity specific to cancer cells out of the three tested extracts. Thus, the MTT assay indicated that *E. deccanensis* is a better candidate than the other two plant species, so it was selected for further study on apoptosis detection, cell cycle analysis, and gene expression.

An impressive antiproliferation effect against the breast cancer MCF-7 cell line was shown by *E. granulata*, with an  $IC_{50}$  value of  $25.17 \pm 0.95 \mu\text{g/ml}$  (Mahdavi et al., 2022). A study on the cytotoxic effects of *E. hirta* plant extract against normal Vero cells and a variety of cancer cell lines, including cervical, colon, and breast cancer cell lines was done by Kwan et al. (2016). The plant extract's cytotoxic effects, which were dose- and time-dependent, significantly reduced the survival and growth of the breast cancer MCF-7 cell line than the other cell lines.

The effects of one of the well-known medicinal herbs, *E. hirta*, on the viability of human breast adenocarcinoma cell lines, such as MDA-MB-231 and MCF-7, were also examined using the aqueous and methanolic extracts of the plant. Compared to the aqueous extract, the methanolic extract demonstrated a more promising cytotoxic potential. Upon considering cell lines, it was found that both extracts were more effective in disrupting the MCF-7 cell line than the MDA-MB-231. When tested on MDA-MB-231 and MCF-7 cells, the methanolic extract had lower  $IC_{50}$  values ( $82.81$  and  $24.31 \mu\text{g/ml}$ ) than the aqueous extract's  $IC_{50}$  values ( $92.86$  and  $53.08 \mu\text{g/ml}$ ). This focuses on the plant's possible ability to slow down the growth of ER-positive MCF-7 breast cancer cells (Elankanni et al., 2021). Moreover, by utilising the MTT assay, Sodde et al. (2015) assessed the cytotoxic potential of a methanolic extract obtained from the stem of *Macrosolen parasiticus* against MCF-7. They observed a noteworthy inhibition on MCF-7, with an  $IC_{50}$  value of  $97.33 \pm 3.75 \mu\text{g/ml}$ , and an increase in the percentage of cytotoxicity from a lower concentration  $31.25 \mu\text{g/ml}$  to higher concentration  $250 \mu\text{g/ml}$ . This was consistent and validated with the current investigation, wherein the methanolic extract of the most effective plant species *E. deccanensis*, demonstrated an  $LC_{50}$

value ( $93.21 \pm 0.58$   $\mu\text{g/ml}$ ) that was nearly close to the value noted in the previous study. Therefore, the MTT assay that was carried out can be verified as the most effective and significant, and it will guide the choice of *E. deccanensis* for additional research.

In an investigation into the anticancer properties of root and aerial extracts from *E. grandicornis*, seven compounds were isolated from this plant, and the toxicity was tested against triple-negative breast cancer (HCC70), hormone receptor-positive breast cancer (MCF-7), and non-tumorigenic mammary epithelial (MCF-12A) cell lines. The results showed that on all the cell lines, the aerial extract had non-specific, significant cytotoxic potential impacts. As opposed to non-cancerous cell lines, root extract demonstrated significant effects on cancerous cell lines. Additionally, in the case of the seven pure compounds that were isolated, the compound “hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl) -2-propenoate (**7**)” showed greater suppression of the growth and survival of all three cell lines (Magozwi et al., 2021). Additionally, a study by Salman et al. (2024) examined the cytotoxic potential of flavonoids isolated from *E. milii* against breast and prostate cancer cells (PC3 and MCF-7). Against PC3 (67%) and MCF-7 (71.66%), respectively, flavonoids were found to exhibit strong cytotoxic potential. By triggering apoptosis, flavonoids can prevent cancer cells from invading cancer cell lines and diminish their rate of growth. In the present investigation, the results of HR LC-MS analysis demonstrated prominent flavonoid distribution in the methanolic extract of *E. deccanensis*. Thus, the broad distribution of flavonoids in the plant extract might be one of the potential explanations for its anticancer efficacy observed against the MCF-7 cell line.

### **Apoptosis detection**

Programmed cell death is an essential part of many cell mechanisms, such as embryogenesis, tissue growth regulation, and other biological processes. The most widely used mechanism of cell death is apoptosis; it is primarily used in cancer research to identify cellular mechanisms and signalling pathways (Kari et al., 2022). Apoptosis-related alterations to the cell membrane are analysed using dual acridine

orange/ethidium bromide fluorescent staining. This approach additionally guarantees an accurate and unambiguous differentiation between the various stages of apoptosis (Liu et al., 2015). Thus, the acridine orange/ethidium bromide staining method was used to ascertain the plant extract's capacity to induce apoptosis in the MCF-7 cell line.

In the current investigation, control cells showed green-coloured nuclei. In contrast, in treated cells, early apoptosis is indicated by a yellowish colour, and late apoptosis is indicated by a bright orange colour. Additionally, most of the early apoptotic cells displayed the typical morphological traits associated with apoptosis, including blebbing of the plasma membrane (**Fig. 34**). Acridine orange is a necessary stain that can stain a cell's cytoplasm as well as its nucleus and gives the appearance of green fluorescent material. Ethidium bromide, on the other hand, readily enters the nucleus of apoptotic cells and fluoresces red when it intercalates with DNA minor grooves. Because of the co-localization of AO and EtBr, a cell that is going through the emerging stage of apoptosis will therefore appear yellow in colour (Yashaswee & Trigun, 2020). In a different study using AO/EB staining, it was discovered that the DNA of late apoptotic cells was fragmented and stained orange, while the DNA of necrotic cells stained red (Lakshmi et al., 2017). AO/Et Br staining was used in a prior study to assess the impact of *E. milii* plant extract on changes in the nuclear morphology of MCF-7 cells. They discovered that the morphology of the MCF-7 cells treated with plant extract differed from that of control cells. They discovered early apoptotic cells, characterised by yellow-tinted nuclei and broken chromatin. Additionally, condensed chromatin and orange-coloured nuclei were visible in the late apoptotic cells (Salman et al., 2024). In light of these reports, the double staining techniques revealed that the methanolic extract of *E. deccanensis* induced apoptosis and DNA fragmentation.

Numerous prior studies have documented the ability of flavonoids to trigger apoptosis in cancer cells by interacting with various signalling pathways linked to apoptosis (Jeong et al., 2022). As per the present research, the plant *E. deccanensis* possesses an abundance of flavonoids, which could potentially play a significant part in inducing apoptosis and causing cytotoxic effects on MCF-7 cells.



### Analysis of cell cycle using flow cytometry

The cell cycle is a vital and significant concept in the field of research. The cell cycle consists of five distinct phases: quiescence (G0 phase), synthesis (S phase), interphase (G2 phase), and mitosis (M phase). According to reports, cancer is typically related to the cell cycle and results from a loss of cell cycle regulation. Therefore, cell cycle analysis is crucial and plays a major role in cancer research, anticancer drug screening, and cytokinetic studies (Qiu et al., 2013). Determining the distribution of cells in different stages of the cell cycle is aided by cell cycle analysis. It is a sign of the distribution of DNA present at various cell cycle stages. A highly regulated process, the cell cycle regulates both the growth and differentiation of cells. Any variations in the distribution of cells at any point during the cell cycle may be connected to differentiation and indirectly to the phenomenon of apoptosis (Yedjou et al., 2016).

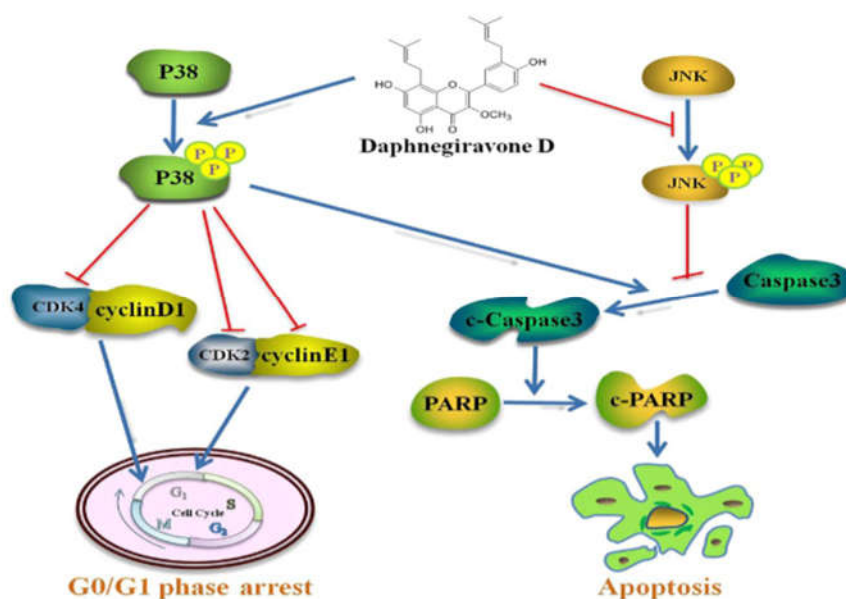
Flow cytometry has been used extensively in cell cycle analysis because of its accuracy and speed. And it is mostly dependent on measurements of DNA content. Cell distribution in each phase is calculated using this method. DNA-intercalating fluorescent dye is used to stain each cell. A common nuclear staining dye used to measure cell cycle is propidium iodide (PI). Before staining, the cells are fixed with ethanol or methanol because the dye cannot penetrate live cells. Next, every cell is stained. As a cell gets ready to divide, its fluorescence will increase in proportion to its DNA content. Using variations in fluorescence intensity, one can calculate the proportion of cells in each cell cycle phase (Nair & Manohar, 2021). Plotting the DNA content at each phase yields a frequency histogram that illustrates the proportion of cells in each cell cycle phase.

The present investigation, determined whether *E. deccanensis*'s methanolic extract could interfere with any phase of the cell cycle in MCF-7 cells. It was observed that the treated and untreated control cells had significant differences in their DNA compositions. In the treated cells, the percentage of G0/G1 phase cells is higher (88.2%) than in the control cells (71.1%), as shown by the histogram (**Fig. 36**). Likewise, the proportion of S-phase cells dropped from 10.0% in control cells

to 5.0% in treated cells. The extract causes a decrease in the percentage of cells in the S (DNA synthesis) phase and an increase in the percentage of cells in the G0/G1 phase of the cell cycle. In treated cells, relatively higher DNA content was discovered in the G0/G1 phase, indicating that the cells were retained in this phase and were not moving on to the subsequent stages of the cell cycle. It can be inferred from this observation that the extract causes cell cycle arrest during the G0/G1 phase. This observation was in close accordance with earlier reports by de La Cruz et al. (2015), wherein MCF-7 cells were subjected to an arrest at G0/G1 phase due to the application of *Glehnia littoralis* root extract. Additionally, it backs up the research conducted by Lai et al. (2021) that found that a *Juniperus communis* extract caused colorectal adenocarcinoma to be arrested at the G0/G1 phase.

The sequence and timing of cell cycle events, such as G1/G0 boundaries, S-phase, and G2/M phases, are tracked by cell cycle checkpoints. Checkpoints are a set of regulatory mechanisms that only allow proliferation to occur when stimulatory signals are present. In addition, DNA damage and misaligned chromosomes at the mitotic spindle initiate the checkpoints. In this case, checkpoint-induced growth arrest enables the cell to repair the damage. Once the damage is fixed, the cell cycle continues. When a repair is not feasible, the cell is eliminated by apoptosis (Pucci et al., 2000).

Cell cycle regulatory mechanisms tightly regulate the characteristics of a cell, including its growth, proliferation, differentiation, and survival. Any uncontrolled conditions that emerge during these processes lead to the development of cancers. Cell cycle progression is controlled by several factors, including cyclin-dependent kinases (CDKs) inhibitors and cyclins. Furthermore, the most prevalent complex involved in the progression of the cell cycle is the cyclin-CDKs complex. Thus, inhibitors that target CDKs are used to treat various cancers in the modern world. One well-known inhibitor of CDK4 and CDK6 specificity, Palbociclib, has been demonstrated to be effective in treating a number of breast cancers (Wang et al., 2017). The connection between the cell cycle and apoptosis is represented in **Fig. 48**.



**Figure. 48: The connection between the mechanism of cell cycle and apoptosis** (Wang et al., 2017)

Compounds with anticancer qualities are abundant in herbal medicines and are generally safe, non-toxic, and easily accessible. Sixty percent of medications used to treat cancer are plant-based, according to FDA reports. Notable anticancer properties were shown by several phytochemicals. Remedies for cancer can be achieved with compounds like taxanes, vinca alkaloids, and derivatives of camptothecin (Garcia-Oliveira et al., 2021). The flavonoid quercetin exhibits notable anti-cancer properties against a range of cancer types, such as ovarian, breast, and colon cancer. In addition to inducing apoptosis by activating proapoptotic factors and decreasing the activity of antiapoptotic factors, it can also cause the cell cycle to be arrested by controlling the cyclin D1 and p53 pathways (Tang et al., 2020). Likewise, kaempferol, a well-known flavonoid, showed potent anticancer effects. It can alter various signalling cascades associated with cell cycle arrest, apoptosis, and metastasis (Rajendran et al., 2014). This supports the results of the current investigation, which found quercetin and kaempferol in the methanolic extract of *E. deccanensis* and suggests that they may be involved in the inhibition of cell cycle progression.

By preventing cells from moving from G0/G1 to S-phase, the *E. tirucalli* plant extract significantly reduced the proliferation of MCF-7 cells. They observed that this activity is caused by the presence of terpenoids and flavonoids (Choene & Motadi, 2016). Phytochemical analysis revealed that *E. deccanensis* contained a large number of flavonoids and terpenoids. Thus, in MCF-7 cells treated with the plant's methanolic extract, the inhibition of cell cycle progression from G0/G1 to S-phase can be attributed to the presence of these potent compounds along with other compounds and their synergistic actions.

In this work, the double staining method using acridine orange/ethidium bromide has revealed the apoptosis effect exhibited by the methanolic extract of *E. deccanensis* on the proliferation of breast cancer MCF-7 cells. Cell cycle analysis using flow cytometry subsequently demonstrated the potential of plant extract to induce apoptosis by causing cell cycle arrest at the G0/G1 phase and the inhibition of progression to S-phase in breast cancer MCF-7 cells. Hence, p53 and STAT-3, two important genes involved in signaling pathways leading to apoptosis, were subjected to quantitative expression analysis using RT-qPCR followed by gel electrophoresis using GAPDH as the housekeeping gene in order to explore the possible genetic mechanism underlying this in the plant extract-treated MCF-7 cells. Housekeeping genes are essential for cells to survive, regardless of the precise way in which they are employed within an organism or tissue. They are continuously expressed irrespective of the type of tissue, developmental stage, cell cycle stage, or outside cues. They are conserved across species and carry out cell maintenance and perform basic cellular functions. One of the common housekeeping gene is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). It is frequently utilised as an internal control in studies involving proteins, mRNA, and DNA. For Q-RT-PCR to be accurate, it must be normalised to a housekeeping gene, an internal control. This is why quantitative TR-PCR techniques were used in the present study, as its expression remains constant over time and experiment manipulation.

In the current study, MCF-7 cells were incubated for 24 hours after being treated with an LC<sub>50</sub> concentration (93.21 µg/ml) of *E. deccanensis* methanolic

extract. An obvious and significant upregulation of the p53 gene's expression was found in the results (**Fig. 37 a & b**). The expression fold change was 1.68. The STAT-3 gene expression was also significantly downregulated by the plant extract at the same time, with a fold change of 0.4. The gel images of both genes corroborated this as well (**Fig 38 a & b**). In comparison to the control, the sample treated well exhibits a comparatively higher intensity of fluorescence in the p53 gene. Relatively less fluorescence was observed in the STAT-3 gene when compared to the control. These two demonstrate the capacity of the methanolic extract to influence the mRNA expression of both genes. Thus, it is evident from the current work that plant extract has the ability to markedly downregulate gene STAT-3 and upregulate gene p53 at the same time.

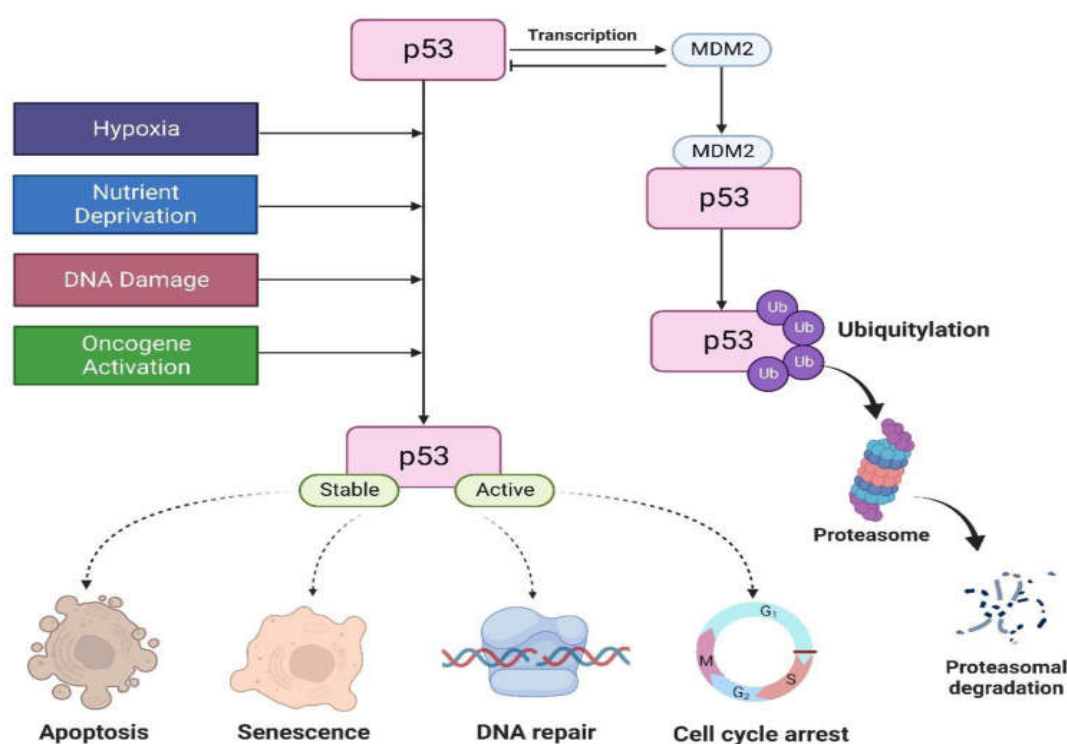
One of the vital genes, p53, also referred to as the guardian of the genome, is essential for various cellular mechanisms like DNA repair, cell cycle arrest, and the induction of apoptosis in response to cellular stress. More than 40 years ago, p53 signaling was identified. p53 status plays a critical role in clinical settings in predicting a patient's response to chemotherapy. When treating basal like breast cancers, the p53 mutant can be utilised as a marker for chemotherapy. The p53 protein consists of 393 amino acids in addition to its transactivation, DNA binding, nuclear localization, and nuclear export domains (Li et al., 2022).

The p53 gene encodes the transcription factor protein p53. Tumour suppression is the primary role of the p53 protein. An estimated 50% of human cancers, including those of the breast, colon, prostate, skin, liver, and lungs, are caused by mutations in the p53 gene (Marei et al., 2021). A group of ubiquitin ligases, including Cip1, CHIP, and MDM2, normally regulate the stability of the p53 protein. Among them, MDM2 is well-known. p53 activation triggers the expression of MDM2, which then interacts with p53 to cause its destruction and ubiquitination. The p53-MDM2 loop is capable of efficiently controlling both homeostasis and the appropriate reaction to different stimuli (Li et al., 2022). The regulation of numerous distinct cellular processes was significantly influenced by the transcription factor p53. p53 is involved in the inhibition of cell proliferation in cancer cells under

various conditions, including hypoxia, DNA damage, nutrient deprivation, and hyperproliferative signals. In reaction to cellular stress, p53 is susceptible to a range of post-translational modifications, such as transactivation, where p53 controls the expression of genes related to the cell cycle, DNA repair, and the induction of apoptosis (Marvalim et al., 2023).

Numerous studies have demonstrated that p53 has the ability to initiate apoptosis and regulate a number of apoptotic-related genes. p53 can mediate apoptosis through both intrinsic and extrinsic mechanisms. By inducing the expression of the cell death receptors, Fas and Death Receptor 5, p53 can activate the extrinsic apoptotic pathway. As a result, a signalling complex known as death-inducing complex forms, which in turn activates a cascade of caspases, including caspase-8 and caspase-3. p53 can start apoptosis in intrinsic pathways by transactivating proapoptotic genes from the Bcl-2 family, including BAX, Noxa, PUMA, and BID (Chen, 2016).

Numerous reports demonstrated the effectiveness of p53 as a tumour suppressor. Through inducing apoptosis and cell cycle arrest, the upregulated p53 gene has been shown to assist in the destruction of triple-negative breast cancers, including MDA-MB-231 and MDA-MB-436 (Fouzat et al., 2022). This is in line with recent research, in which the p53 gene suppresses the growth of MCF-7 breast cancer cells by inducing apoptosis and cell cycle arrest by mediating a variety of signaling pathways. Furthermore, after being treated with different species of *Euphorbia*, including *E. ingens* and *E. pulcherrima* (El-Hallouty et al., 2022), previous studies have shown high levels of p53 or upregulated expression of p53 followed by the induction of apoptosis. This indicates more about the impact of the plant extract *E. deccanensis* on the upregulation of the p53 gene. The role of p53 in activation of genes involved in cell cycle regulation, DNA repair, and apoptosis induction is represented in **Fig. 49**.



**Fig. 49: Involvement of p53 in activation or repression of target genes involved in the control of cell cycle, DNA repair, and induction of senescence and apoptosis (Marvalim et al., 2023).**

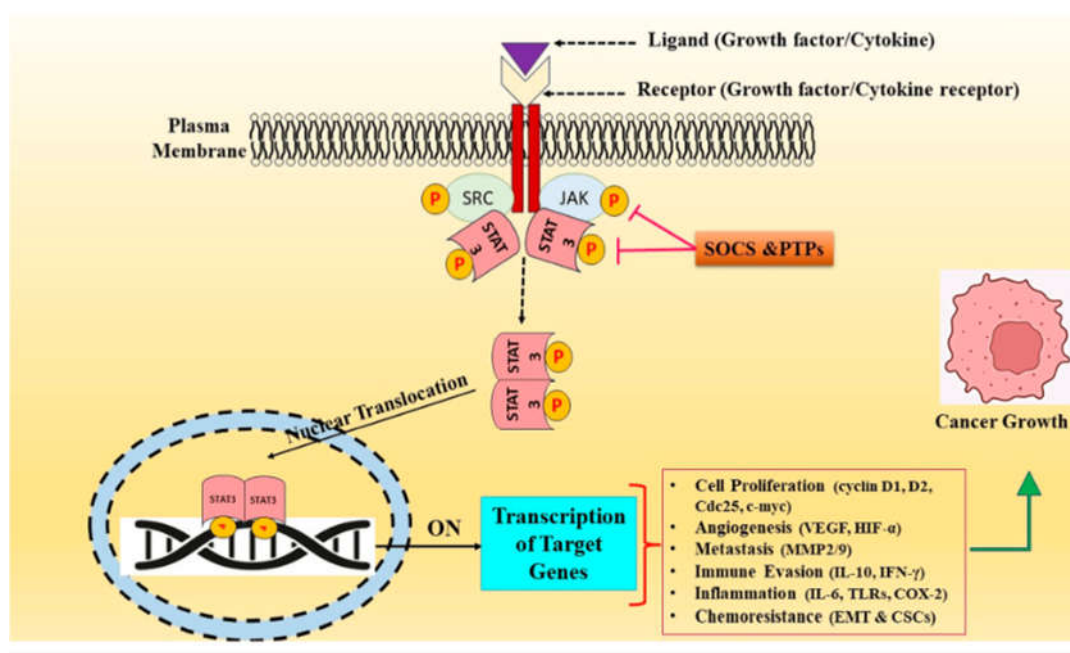
The activator of transcription and signal transducer (STAT-3) is a member of the STAT family. This group is primarily composed of seven transcription factors: STAT1, 2, 3, 4, 5a, 5b, and 6. A number of vital processes, including angiogenesis, apoptosis, differentiation, cell cycle, and metastasis, are thought to be regulated by STAT3. Furthermore, STAT-3 is constitutively active in several tumour cell lines, including those from the colon, breast, melanoma, pancreas, prostate, esophagus, and stomach. Because of constitutive activation, STAT-3 is able to promote the proliferation of cancer cells (Zhou et al., 2018). Research on STAT-3, an oncogenic transcription factor, has shown strong correlations with immune evasion, chemoresistance, metastasis, and the start of cancer (Qin et al., 2019). Angiogenesis, differentiation, cell cycle progression, immune suppression, and chemotherapy resistance are all aided by STAT3's regulation of its downstream target genes, which include Bcl-2, Bcl-xL, cyclin D1, c-Myc, and survivin. Researchers considered STAT3 to be a top candidate for cancer treatment because of its crucial role in the development of cancer (Khan et al., 2024).

Phosphorylation by growth factors and cytokines initiates the activation of the STAT-3 signalling cascade system. This is followed by a variety of modifications, including translocation into the nucleus and dimerization. Once inside the nucleus, it initiates the transcription of target genes that regulate angiogenesis, invasion, migration, autophagy, chemoresistance, immune escape, anti-apoptosis, cell survival and cell progression (Qin et al., 2019). In addition, multiple investigations have demonstrated that STAT-3 upregulates several genes linked to angiogenesis (HGF, VEGF, bFGF, IL-17, and IL-23), metastasis (MMP-1/2/3/9, fascin, vimentin, and ICAM-1), cell cycle progression (cyclin D1, c-Myc, and Pim1/2), cell proliferation (IL-6, CXCL12, and HIF-1 $\alpha$ ) and antiapoptosis (Bcl-2, Bcl-xL, survivin, and Mcl-1). It may also lessen the expression of transcription factors that inhibit cancer growth, such as p53 (Arshad et al., 2020).

In most cells, apoptosis can prevent STAT-3 from being activated. Genes for cell survival are expressed in tumour cells as a result of constitutive STAT-3 activation. Apoptosis is exacerbated by suppressing the expression of genes involved in cell survival, which is accomplished by blocking the function of STAT-3 genes. Through the expression of FAS proteins, apoptosis can also facilitate the downregulation of STAT-3 (Konnikova et al., 2003; Arshad et al., 2020). This supports the findings of the present investigation, which indicate that the main cause of the apoptosis observed in breast cancer MCF-7 cells is the suppression of STAT-3 and its subsequent downregulation.

Phytochemicals have the ability to kill cancer cells by a variety of means, including changing signaling pathways and triggering apoptosis. The regulation of signaling pathways governs the metabolic pathways in various forms of cancer. Terpenoids are a powerful class of phytochemicals that can inhibit STAT-3 activation by blocking STAT-3 phosphorylation, inhibiting STAT-3 dimerization, or preventing activated STAT-3 from translocating into the nucleus (Khan et al., 2024). **Fig. 50** revealed the involvement of STAT3 in various cancer related mechanisms such as proliferation of cells, angiogenesis, metastasis and invasion. The wide range of terpenoids found in the *E. deccanensis* extract is responsible for the plant's ability to inhibit the STAT-3 gene.





**Fig. 50: Phosphorylation of STAT3 stimulates STAT3 target genes implicated in cell proliferation, angiogenesis, metastasis, invasion, and drug resistance (Khan et al., 2024).**

By causing cell cycle arrest and apoptosis, p53 shields the cells from cancer. On the other hand, STAT-3 promotes the growth and progression of cancer cells by being constitutively expressed in a variety of cancer cells. Therefore, in the regulation of cellular pathways, STAT-3 and p53 play opposing roles (Pham et al., 2020). Phosphorylation of STAT-3 in breast and prostate cancer cells is reported to be decreased by p53 in another study (Lin et al., 2002). This aligned with the current investigation, wherein the suppression of cell cycle arrest during the G0/G1 phase, succeeded by apoptosis, happened due to the activation of the p53 gene and the suppression of STAT-3 gene activity. The p53 activity causes the breast cancer MCF-7 cells to undergo apoptosis in this instance, but the anti-apoptotic activity of STAT-3 is inhibited.

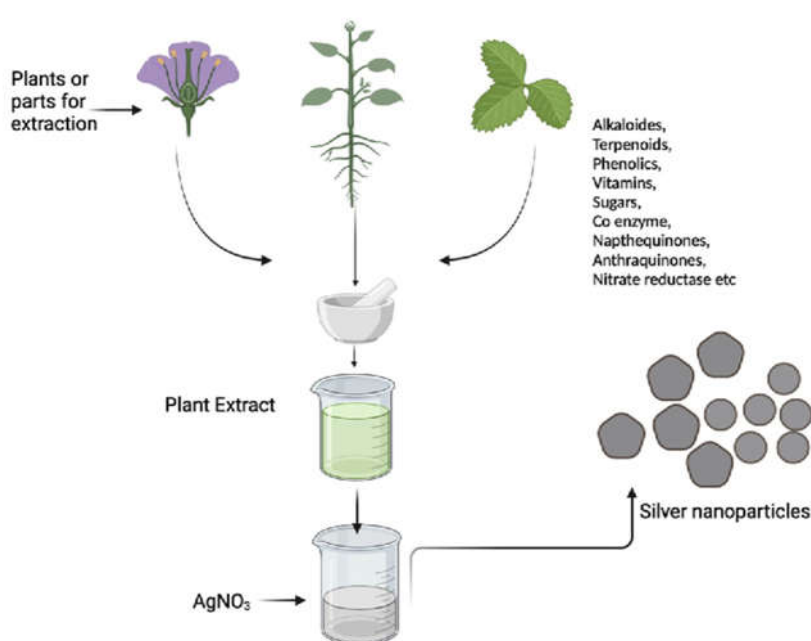
### PART III

#### BIOSYNTHESIS OF SILVER NANOPARTICLES

The literature contains a plethora of reports on *Euphorbia* species' potential as potent candidates for the synthesis of Ag NPs. Significant biological characteristics were demonstrated by the Ag NPs that were derived from different

parts of the plant species, including the stem, leaf, root, latex, and flowers. In the current work, the methanolic extracts of the three selected plant species demonstrated enormous potential to easily and sustainably develop Ag NPs using green strategies without the need for any physical or chemical factors. Ag NPs were created by the reaction of the methanolic extract of the selected test samples with the silver nitrate solution.

One of the most important processes in science is thought to be the production of Ag NPs using plant components. Since the different biomolecules present in plant extracts, including phenols, polyphenols, flavonoids, ascorbic acid, and citric acid, act as reducing agents with capping abilities, using phytobiomass for nanoparticle formation is an important strategy (Abada et al., 2023). **The Fig. 49** describes the involvement of various biomolecules found in the plant extracts in the formation of silver nanoparticles. The development of a reddish-brown colour formation indicates the formation of Ag NPs visually. A number of physicochemical techniques were used to confirm the formation of Ag NPs, including UV- vis spectroscopy (UV-visible spectroscopy), FE-SEM analysis (Field emission scanning electron microscopy), and FTIR spectroscopy (Fourier Transform Infrared Spectroscopy) to understand the specific and properties of synthesised Ag NPs.



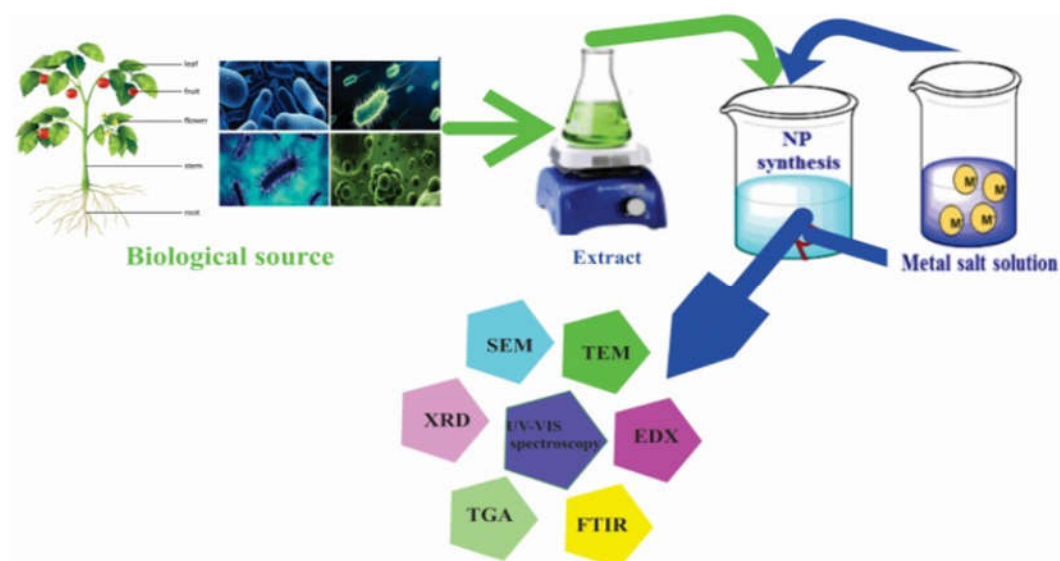
**Fig. 49: Interaction of biomolecules found in the plants and silver nitrate solution leads to the synthesis of silver nanoparticles (Wahab et al., 2021)**

### **Visual observation of silver nanoparticles**

The reduction of silver ions to silver metal nanoparticles by the action of plant extract was initially detected visually by observing the colour change of the reaction mixture. When different leaf extracts were added to silver nitrate solution, the colour of the solution changed, showing Ag NP production from weak light to yellowish-brown to reddish-brown to colloidal brown (Banerjee et al., 2014). Likewise, the distinctive brownish colour of the final slurry indicates the colloidal Ag metal nanoparticles which are developed as a result of the simultaneous mixing of silver nitrate and plant extract solutions (Saeb et al., 2014). In this study, similar colour changes were seen, indicating the formation of Ag NPs. In all three tested extracts, the colour of the reaction mixture was changed into yellowish brown or reddish brown after the interaction between the plant extract and the 2 mM silver nitrate solution at a temperature of 60<sup>0</sup>C. The variation in colour change was caused by the surface plasmon resonance (SPR). The interaction of conducting electrons in metal nanoparticles' outer orbitals with an electric field causes the phenomenon known as surface plasmon resonance (SPR), in which the particles collectively oscillate in resonance at specific wavelengths. The colour and absorbance that form in a colloidal solution of Ag NPs are caused by the excitation of SPR (Vanlalveni et al., 2021).

### **CHARACTERISATION TECHNIQUES**

So many characterisation methods are available to detect the presence of Ag NPs such as UV–visible spectroscopy, SEM, FTIR, TEM, DLS, etc. Among them, analyses like UV–visible spectroscopy, SEM and FTIR were conducted in the present investigation. Schematic representations of various characterisation tools are represented in **Fig. 50**.



**Fig. 50: Various characterisation techniques employed in nanoparticles identification** (Ijaz et al., 2022)

### UV–visible spectroscopy (UV-Vis spectroscopy)

One of the fundamental analytical techniques, UV-visible spectral analysis proved to be very reliable for the initial characterization of developed metal nanoparticles. Using this technique, the stability of Ag NPs can be ascertained. There is no need for calibration for particle characterization of colloidal suspensions using this quick, sensitive, and straightforward technique to monitor the formation of Ag NPs (Ashraf et al., 2016). Ag NPs have a large optical property that enables them to work closely with specific light frequencies. In Ag NPs, the valence band and the conduction band are close to one another, allowing free electrons to move freely and causing surface plasmon resonance (SPR) (Patil et al., 2021). The characteristic formation of colloidal silver nanoparticles is usually indicated by sharp surface plasmon resonances (SPRs) in the 350 – 600 nm range (Bardania et al., 2020). Furthermore, several earlier studies found that absorption spectra of Ag NPs range between 410 and 450 nm as a result of surface plasmon resonance (Mahendran & Kumari, 2016).

The formation of silver nanoparticles is monitored in the present work using UV-visible spectral analysis. Every extract put into the experiment produced a distinct, sharp significant peak in the 400 – 500 nm range, which is characteristic of

the UV–Vis signal resulting from the surface plasmon resonance of silver nanoparticles. *E. rothiana* and *E. thymifolia* had peaks at 432 and 443 nm, respectively. While *E. deccanensis*' absorption peak was formed at 416 nm, which was specific for the occurrence of Ag NPs (**Fig. 39 a**). This was consistent with the earlier reports by Mahendran & Kumari (2016). For Ag NPs produced using *Nothapodytes nimmoniana* fruit extract, they observed a distinct SPR peak at 416 nm. Additionally, they highlighted the potential of phytochemicals such as steroids, flavonoids, terpenoids, and alkaloids that were discovered in fruit extract and were thought to be in charge of accelerating the reduction process and thereby enhancing the formation of Ag NPs. Hence, the absorption spectra of silver nanoparticles obtained from the current study revealed the interaction between silver ions and phytochemicals found in methanolic extracts of tested samples. Thus, the development of silver nanoparticles in all of the tested samples was confirmed.

Numerous studies reported the efficacy of various *Euphorbia* species to produce Ag NPs by the reduction of silver nitrate solution. The ability of *E. deccanensis* and *E. tortilis* methanolic extracts to reduce silver nitrate to silver nanoparticles was found by Sameena &Thoppil (2023). It was previously reported that the ethanolic extract of *E. helioscopia* produced Ag NPs when it reacted with a 1 mM silver nitrate solution. A sharp peak at 442 nm was discovered by UV-VIS spectrum analysis (Nirmala et al., 2023). Similarly, an experiment by Santhosh et al. (2019) produced Ag NPs through the combined action of *E. geniculata* leaf extract and 0.05 M aqueous silver nitrate solution. At 430 nm, they noticed a sharp, intense peak indicating the development of Ag NPs. Ag NP synthesis by *E. rothaiana* was demonstrated in a previous study conducted by Banumathi et al. (2017). For this, 0.01 M silver nitrate solution and aqueous leaf extract were used to create Ag NPs, and they were allowed to react with one another and kept for the nanosynthesis in the dark for five hours. A sharp silver peak at 428 nm was visible in the UV-Vis spectrum, and they observed the colour change of the solution from yellowish to reddish brown.

**FE-SEM analysis (Field emission scanning electron microscopy)**

To gain a better understanding of nanomaterials, researchers in the fields of nanotechnology and nanoscience are currently looking for motivation to develop highly resolved microscopic techniques. SEM is an advanced surface imaging technique that can resolve a wide range of particle sizes, shapes, and surface characteristics of nanomaterials at both the micro and nanoscales, among other electron microscopy techniques (Zhang et al., 2016). In the current work, SEM analysis was utilised to obtain detailed information about the size and shape of the synthesised Ag NPs. Here, the three Ag NPs produced from plant extract showed a spherical shape. It was consistent with earlier reports by Ibrahim (2015). Using banana peel extract as a capping and reducing agent, they were able to produce spherical-shaped Ag NPs with a particle size of 23.7 nm in their experiment. They differ in size despite having a similar shape. It was discovered that the Ag NPs produced from *E. deccanensis* were substantially smaller in size than the Ag NPs produced from the other two test extracts. Ag NPs produced from *E. deccanensis* had an average particle size of 33.4 nm. For *E. rothiana* it was 36 nm, and for *E. thymifolia*, it was 37.6 nm. Hence, using this method, the differences in Ag NP sizes were revealed. The Ag NPs, synthesised from *Convolvulus arvensis* leaf extract, had a spherical shape and an average particle size of 60 nm (Rasheed et al., 2018). Therefore, the Ag NPs that are synthesised from *E. deccanensis* may be a promising option for creating smaller-sized silver nanoparticles.

Previous studies by Bardania et al. (2020) clarified the connection between Ag NP size and spectral peak. They observed that if the concentration of Ag ions and plant extract rises during the synthetic mechanism, the spectral peak of Ag NPs shifts to lower wavelengths. This ultimately results in the formation of nanoparticles with smaller sizes. Moreover, it has been shown that Ag NPs of smaller sizes exhibit their SPR peaks at lower wavelengths than Ag NPs of larger sizes (Neri et al., 2016). This was true in this experiment also. Here, the extract of *E. deccanensis* developed the Ag NPs with smaller sizes, which also exhibited SPR peaks at shorter wavelengths.

A reaction mixture's pH, temperature, and extract concentration all affect the size and shape of silver nanoparticles. Ag NP's morphology varies from species to species and can be spherical, triangular, cubic, round or rod shaped and the most notable among them is spherical (Dawadi et al., 2021). In addition, the number of SPR peaks decreases with an increase in Ag NPs symmetry. Moreover, there was just one peak visible in the spherical Ag NPs. In contrast, two or more peaks were visible in the nanoparticles with disc and triangular forms (Ahsan et al., 2020).

### **Fourier transform infrared spectroscopy (FTIR) Analysis**

The material is exposed to infrared light during Fourier-transform infrared spectroscopy (FTIR) analysis, and the radiation that is transmitted is recorded. For every sample, the spectrum produced using this method is distinct. This technique can be used to determine the sample's polarity, different bond types, and degree of oxidation (Deepty et al, 2019). FTIR is a useful tool for identifying the functional groups involved in the stabilisation of synthesised nanoparticles. The FTIR spectra of the green synthesised Ag NPs clearly show peaks throughout the selected range. This method is used to detect the presence of biomolecules. To identify the best matches, the organic material in the sample's obtained FTIR spectrum was compared to material libraries that had been catalogued.

Ten significant absorption bands were detected in the FTIR spectrum of *E. deccanensis*, with one deep band located in the absorption peak at  $1384\text{ cm}^{-1}$ . The broadband at  $3435\text{ cm}^{-1}$  is caused by phenols and alcohols. The absorption band at  $2856\text{ cm}^{-1}$  and  $2926\text{ cm}^{-1}$  is sharp and is caused by aromatic compounds and alkanes, respectively. Additional noteworthy peaks detected at  $1384\text{ cm}^{-1}$  signify the C-H bending of aldehyde groups. The presence of peaks at  $836\text{ cm}^{-1}$  and  $669\text{ cm}^{-1}$ , respectively, indicates the presence of aromatic compounds and (C-Br) aliphatic bromo compounds. Functional groups that correspond to biomolecules were found by FTIR analysis of this plant species, which was consistent with previous research by (Satheesh et al., 2012; Wan Mat Khalir et al., 2020). They also got comparable absorption peaks in their experiment to that we observed in the current investigation.

They explained that during the formation of nanoparticles, these biomolecules play a strong role as capping and stabilising agents.

Eleven notable absorption peaks were visible in the FTIR spectrum that was obtained from *E. rothiana*. The bands at  $3400\text{ cm}^{-1}$  and  $3220\text{ cm}^{-1}$ , respectively, stand for the O-H (hydroxyl) group and the aliphatic primary amine. The absorption band at  $2422\text{ cm}^{-1}$  indicates the presence of carboxylic acid, while the band at  $1604\text{ cm}^{-1}$  indicates the occurrence of amino acids. The strong, deep peak at  $1382\text{ cm}^{-1}$  is indicative of phenols. The presence of alkene and halo compounds were indicated by the small peaks seen at  $834\text{ cm}^{-1}$  and  $528\text{ cm}^{-1}$ . These bands agree with the earlier reports of Lingegowda et al. (2012).

Different functional groups involved in the formation of nanoparticles were revealed by the alteration in peak intensity. Ag NP-derived *E. thymifolia* showed ten distinct absorption bands in the FTIR analysis. The absorption spectra at  $3774\text{ cm}^{-1}$  represent O-H stretching vibrations of the hydroxyl group. Similarly, the spectra at  $3409\text{ cm}^{-1}$  correspond to the O-H stretch of the alcohol group. In addition, the other prominent bands at  $1382\text{ cm}^{-1}$  and  $2424\text{ cm}^{-1}$ , respectively, suggest the presence of carboxylic acids and phenols. The tiny bands at  $596\text{ cm}^{-1}$  and  $528\text{ cm}^{-1}$  are due to the occurrence of halo compounds. This was in agreement with the earlier study conducted by Lingegowda et al. (2012). In all three of the plant-derived Ag NPs, significant phytoconstituents corresponding to prominent functional groups were detected. It is therefore reasonable to believe that these functional groups play a critical role in the formation, stabilisation, and reduction of silver nanoparticles.

### **Antimicrobial activity of synthesised silver nanoparticles**

#### **Antibacterial activity of Ag NPs**

Today, one of the main challenges in treating many diseases is the growing resistance of bacteria and fungi to antimicrobial drugs. In a similar way, more recent bacterial strains, both Gram-positive and Gram-negative, have been found to possess a high degree of resistance. To counteract bacterial resistance, efforts must be made to develop novel antimicrobial agents as well as to prevent the formation and



dissemination of multi-resistant bacterial strains. Therefore, it is imperative to find a future solution. Silver has stronger, more potent, and more significant antimicrobial properties than any other metal. Silver salts were employed as a potent antimicrobial agent before the development of silver nanoparticles. Nonetheless, the excessive application of silver agents has diminished their potency as antimicrobial agents. With the development of nanotechnology in recent years, interest in using silver nanoparticles as antibacterial agents has increased, and more people are now considering using nanoparticles to treat microbial infections of the skin. Silver nanoparticles' antibacterial activity is one of their most well-known bioactivities. Due to silver's antibacterial qualities, *Staphylococcus aureus* and *Pseudomonas aeruginosa*-related bacterial skin infections are frequently treated with it. The effectiveness of Ag NPs' antibacterial qualities against bacteria that are resistant to multiple drugs has been examined in a great deal of research, and a wealth of reports have demonstrated this.

In the current study, the antibacterial properties of Ag NPs derived from selected plant extracts were evaluated. This was accomplished using the disc diffusion method with both Gram-positive and Gram-negative bacterial strains. *Pseudomonas aeruginosa* and *Escherichia coli* were taken as negative strains, and *Enterococcus faecalis* and *Streptococcus mutans* were taken as positive strains. The presence of a visible zone formed around the Ag NP disc indicated that all three of the synthesised Ag NPs exhibited significant antibacterial activity against the selected bacterial strains, according to the results.

Gram-positive and Gram-negative bacteria were effectively targeted by all of the produced Ag NPs in this instance. Nonetheless, it was noted that Ag NPs were most effective against Gram-positive *S. mutans* among other harmful bacteria. Upon considering the plant extracts used in the study, Ag NPs obtained from the methanolic extract of *E. deccanensis* demonstrated a noteworthy suppression on the growth as well as the development of selected bacteria. As a result, this plant is regarded as one of the best options for antibacterial applications.

The two most important types of Gram-negative bacteria that form biofilms are *P. aeruginosa* and *E. coli* (Lavery et al., 2014). Furthermore, these two species are uropathogenic in nature (Giannattasio-Ferraz et al., 2022). The antibacterial effects exerted by Ag NPs were found to be effective against *E. coli* in two previous studies: Kemala et al. (2022) reported a zone of inhibition of  $11.29 \pm 0.45$  mm, and Urnukhsaikhon et al. (2021) reported a zone of inhibition ranging from  $5.5 \pm 0.2$  mm to  $6.5 \pm 0.3$  mm. This highlights the present work, where, all of the synthesised Ag NPs produced an inhibition zone that was found to be larger than that of earlier research. Thus, the ability of Ag NPs derived from methanolic plant extract to inhibit the growth and spread of *E. coli* was achieved and revealed.

The pathogen, *P. aeruginosa*, with opportunistic behaviour, which is present in aqueous solutions, causes deaths in patients who have cancer, traumatic injuries, and immunosuppression. The increase in multidrug-resistant *P. aeruginosa* (MRPA) is a critical public health problem that raises morbidity and mortality in hospitalized patients (Al-Momani et al., 2023). The ability of Ag NPs made from a water extract of *Pyrus communis* flesh and peel to stop the growth of *P. aeruginosa* and *E. coli* was previously described by Simon et al. (2021). Likewise, the Ag NPs that were synthesised from *Cinnamomum tamala* leaves demonstrated efficacious activity against *P. aeruginosa*, displaying a zone of inhibition measuring  $17.67 \pm 0.577$  mm (Al Mashud et al., 2022). The current study provides evidence for the efficacy of all three biogenic Ag NPs in preventing *P. aeruginosa* from growing, thereby preventing the spread of the infection and the development of diseases linked to it.

The bacterias *S. mutans* was taken into consideration for the current investigation in the case of Gram-positive bacteria. *S. mutans* has been identified as a potential dental caries pathogen (Zhang et al., 2022). Here, a prominent inhibition zone was observed in *E. deccanensis* (20 mm), *E. rothiana* (17 mm), and *E. thymifolia* (16 mm) for each of the selected Ag NPs, indicating that they all had a significant impact on *S. mutans*. This was consistent with the previous studies, which demonstrated the effectiveness of green synthesised Ag NPs against *S. mutans*. In a study by Mohamad Hanafiah et al. (2023), *Strobilanthes crispus*-

mediated Ag NPs demonstrated promising activity against *S. mutans*, with a zone of inhibition measuring  $11.00 \pm 0.33$  mm. This was also true in the reports of Mahmood et al. (2021), who observed a significant suppression of *S. mutans* activity.

*E. faecalis* can spread genes linked to antibiotic resistance in addition to its virulence through horizontal gene transfer. This nosocomial pathogen has characteristics of a conventional generalist microbe. A discernible 15 mm inhibition zone was seen in the suppression of *E. faecalis* by Ag NPs derived from the medicinal plant *Cestrum nocturnum* (Keshari et al., 2020). Concurrently, Arsène et al. (2021) reported that grapefruit peel extract-mediated Ag NPs were able to evade *E. faecalis* activity as well as that of other pathogens. They clarified that the reason for the fight against the pathogens could be the antibacterial compound found in the peel extract. The methanolic plant extract-mediated Ag NPs showed encouraging results against *E. faecalis* in this investigation as well (**Tab.15**).

Ag NPs synthesised from plant extracts have been shown in numerous studies to exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. Ag NPs have been found to cause greater sensitivity in Gram-negative bacteria than in Gram-positive bacteria in several studies (Sharma et al., 2022). In the current study, however, conflicting findings were noted. Apart from the Gram-negative strains, the phytofabricated Ag NPs showed significant inhibition on Gram-positive strains. The size and shape of the silver nanoparticles, along with the structural differences between the two bacterial species, could be the cause of their varying susceptibilities (Almatroudi, 2020).

Ag NPs' antibacterial activity is known to be influenced by a wide range of variables, including concentration, size, shape, charge, and stability. Prior research has revealed that the capacity of Ag NPs to impede bacterial growth varies with their concentration (Bruna et al., 2021). In the current investigation, dose-dependent antibacterial activity was observed against all of the selected harmful pathogens. The zone of inhibition that forms around the sterile disc increases as the concentration rises from lower to higher concentrations, suggesting an increase in antibacterial

activity. Our results are in line with earlier studies by Choi et al. (2021) and Harsha et al. (2022).

Similarly, when comparing the antibacterial activity of smaller-sized spherical Ag NPs to larger spherical Ag NPs, the smaller Ag NPs showed superior results (Raza et al., 2016). In addition, smaller nanoparticles dissolve more quickly in various media and release silver ions during the process, which may play a significant role in the antibacterial activity of nanoparticles (Bruna et al., 2021). These claims were supported by the results of the current study. In this case, Ag NPs made from *E. deccanensis* showed the strongest antibacterial activity; SEM data showed that these NPs have a smaller spherical shape (33.4 nm). Due to their reduced size, *E. deccanensis* Ag NPs exhibit superior bacterial barrier penetration, thereby augmenting their antibacterial efficacy. Although the other two plant extract-mediated Ag NPs showed a notable antibacterial effect as well, it's possible that this was caused by their spherical shape and nearly smaller sizes (36 nm in *E. rothaina* and 37.6 nm in *E. thymifolia*, respectively), which were discovered to be closer to the most effective one's size.

The production of Ag NPs via green nanotechnology is a simple and reliable process. It assists in avoiding the problems with other traditional methods, which result in the production of hazardous byproducts. It helps to avoid the disadvantages of plant-extractable antimicrobials, such as the fact that a lot of bacteria have developed resistance to them. Thus, the Ag NPs produced from the methanolic extract of the current study may be useful in the development of novel approaches and in halting the growth of pathogens, thereby preventing the spread of illnesses.

### **Antifungal activity of Ag NPs**

Fungal infections have been a major public health concern in recent decades. Around 2 million people have died from fungal diseases like mycoses, which have caused more than 300 million people to suffer from serious health problems. Consequently, fungal diseases are becoming the primary cause of death worldwide. Moreover, the lack of antifungal medications is caused by the fact that fungi exhibit molecular and cellular biology that is strikingly similar to that of human cells,

making it extremely challenging to identify specific therapeutic targets against fungi (Mussin & Giusiano, 2022). This prompted researchers to look for an alternative to antifungal drugs, of which metals predominate, particularly silver given its lengthy history of use as an antimicrobial agent in medicine.

This work demonstrated the fungicidal efficacy of Ag NPs against *A. niger* and *C. albicans*. Since nanoparticles can adhere to the surface of fungal hyphae, they can stop the growth of dangerous fungi (Skłodowski et al., 2023). People with weakened immune systems are more vulnerable to fungal infections, and the current lack of antifungal medication options makes treating fungal-related diseases a laborious process. Because of this, the development of antifungal drugs that are biocompatible, non-toxic, and environmentally friendly seems to be an essential and urgent need (Habeeb Rahuman et al., 2022). Ag NPs currently have a major effect on antifungal drugs that are used to treat various infections caused by fungi. A multitude of investigations postulated that the antifungal activity of Ag NPs resulted from their influence on membrane-bound enzymes, like those in the respiratory chain, or from the degradation of cellular membrane integrity (Kim et al., 2012).

The fungicidal property of Ag NPs is currently ascertained using the disc diffusion method. The sterile disc was found to have a noticeable zone of inhibition surrounding it. This zone clearly demonstrated the antifungal potential of the material against specific fungal pathogens. Ag NPs developed from the methanolic extracts of ED, ER, and ET, effectively inhibit both fungal species. When fungi were taken into account, Ag NPs from all three plant species considerably inhibited *A. niger* more than *C. albicans*. The zone of inhibition developed at the highest concentration (1000 µg/ml) in each of these Ag NPs was found to be nearly comparable to that of the well-known antifungal drug Clotrimazole, which served as the positive control.

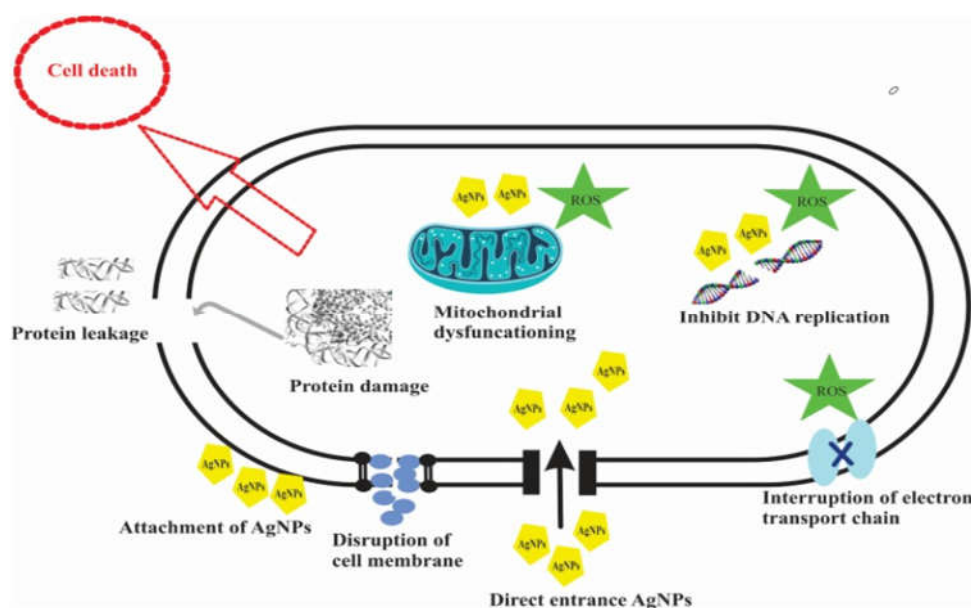
The most prevalent fungi that can produce mycotoxins, which are extremely dangerous to humans and animals, are *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., and *Candida* sp. (Haque et al., 2020). Among them, *Aspergillus* species are common fungi that account for up to 22% of the air spore samples. Most of their infections

target the respiratory system. Among the *Aspergillus* species, *A. niger* has been identified as the most common infection that causes mycosis in people (Miller & Wilmott, 2019).

In the current study, Ag NPs of all the three *Euphorbia* species significantly suppressed the growth of *A. niger* at all concentrations, including 250, 500, and 1000 µg/ml, in a promising way. Moreover, the highest concentration of Ag NPs demonstrated a zone of inhibition that was noted to be extremely similar to the zone of inhibition displayed by the positive control that was chosen. This finding aligns with earlier research. Hashem et al. (2022) for instance, evaluated the effectiveness of biogenic Ag NPs against *A. niger* and other *Aspergillus* species to ascertain their fungicidal properties. They observed notable fungicidal activity against *A. niger*, exhibiting a 16 mm zone of inhibition at 500 µg/ml. In the present study, Ag NPs from *E. deccanensis* showed a zone of inhibition of 15 mm at the same concentration, followed by those from *E. rothiana* with 14 mm and *E. thymifolia* with 13 mm respectively. This emphasises the importance of Ag NPs mediated by *E. deccanensis*, *E. rothiana* and *E. thymifolia* in terms of their capacity to impede the growth and dissemination of *A. niger*.

*Candida albicans* is the most frequent opportunistic fungal pathogen belonging to the genus *Candida*. It is challenging to treat this infection clinically since there are few low-toxicity, effective antifungal medications available (Zhou et al., 2021). Arsène et al. (2023) assessed the antifungal activity of Ag NPs derived from *Aloe vera* extract against *Candida albicans* and observed a concentration-dependent inhibition on the fungal organism's activity. Similar to this, Beldjilali et al. (2020) employed *Thymus algeriensis* extract-mediated Ag NPs to investigate the fungal action of the extract and found a potent anticandidal activity with a 20 mm zone of inhibition. A similar finding was observed in this investigation as well. In this case, all three methanolic plant extract-mediated Ag NPs showed significant dose-dependent inhibition of *C. albicans*. Thus, it is demonstrated how important the current study is and how applicable it is to the biomedical industries.

Many researchers have provided explanations regarding the mechanism of Ag NPs' antimicrobial action. The microorganism's cell wall and membrane both accumulate with Ag ions that are released from the Ag NPs, which allows the nanoparticles to enter into the cytoplasm of the cell. Researchers explained how the negatively charged cell membrane surface and positively charged silver ions' electrostatic attraction helps to bind Ag NPs to the cell wall and induce structural variation in the membrane, increasing the permeability of Ag NPs to enter the cell more readily. As soon as Ag ions enter a cell, they release reactive oxygen free radicals, which lead to oxidative stress and are one of the primary agents behind the antimicrobial action. Reactive oxygen species have the ability to affect every other mechanism, including lipid peroxidation, damage to DNA, disruption of protein synthesis, inhibition of the electron transport chain, destruction of cell membranes, and leakage of constituents from the cell. All of these factors work together to cause microbes' cell to undergo apoptosis (Jain et al., 2021; Górka & Kubiński, 2023).



**Fig. 51: Mechanism of antimicrobial action exhibited by the silver nanoparticles** (Ijaz et al., 2022)

## SUMMARY AND CONCLUSION

Plants play an important role in the ecosystem by performing a variety of essential functions. Plants are essential for the survival of humans and other living beings. Plants, specifically therapeutic herbs, have been used to assess the health of an ecosystem. Due to their strong medicinal properties and numerous health advantages, members of the genus *Euphorbia* have long been used as traditional medicines in Europe, China, America, Turkey, and India. Various parts of the plant, including the stem, leaves, root, and latex, are widely used around the world to treat a variety of ailments, including migraines, intestinal parasites, body pain, and sensory disturbances. In India, several species are used as part of traditional medicines and are used to treat a variety of illnesses such as kidney infections, snakebite, skin disorders, and infertility. Due to their extensive use in traditional medicine, a large number of phytochemicals and pharmacological effects exhibited by them have been studied. The current study thus focuses on analysing the distribution of phytochemicals and their possible biological activities of the three *Euphorbia* species, of which two species—*E. deccanensis* and *E. rothiana*—have not been the subject of any significant and noticeable research, and the third is the widely recognised medicinally valuable *E. thymifolia*. This research also looks at how environmentally friendly chosen plant species can synthesize silver nanoparticles.

The active substances that are produced and stored in the plants' bodies are called phytochemicals. While primary metabolites aid in energy metabolism and the development of plant cell structure, secondary metabolites play a role in how plants interact with their surroundings. The pharmacological effects of these phytochemicals were numerous and included anti-allergic, anti-inflammatory, antibacterial, antispasmodic, neuroprotective, and antioxidant activities. The primary source of traditional plant species' medicinal potential is their phytochemicals, which have a variety of pharmacological effects on the human body.



Preliminary examination was performed using three different solvents based on their polarity index, with water being polar, methanol being semi-polar and n-hexane being non-polar. Using standard procedures, the phytochemicals were first screened to determine their distribution. It was observed that methanolic extracts of all the chosen *Euphorbia* species contain more compounds and produce precipitate at a higher rate than the other two solvents. Thus, methanol was chosen as the solvent for the present study. The various compounds analysed include flavonoids, alkaloids, terpenoids, tannins, saponins, triterpenoids, phenols, and glycosides. None of the three plant species had cardiac glycosides or carotenoids found in them. The quantitative analysis was used to determine the total amount of phytoconstituents present in the methanolic extract. The total amount of flavonoids, phenols, and terpenoids was ascertained. Equal amounts of total terpenoid and flavonoid content were found in the extracts from *E. deccanensis* and *E. rothiana*. While the total phenolic content of *E. rothiana* was found to be high, the other two extracts had nearly equal amounts. Analytical techniques like GC-MS for volatile compounds and HR LC-MS for non-volatile compounds were used to conduct a thorough phytochemical investigation. The HR LC-MS analysis identified 83 compounds, while the GC-MS analysis identified 21 compounds. Among the powerful phytochemicals identified are gallic acid, o-methyl somniferine, gentisic acid, chlorogenic acid, and others. Both *E. deccanensis* and *E. rothiana* extracts contained Euphornin, a well-known diterpenoid found in other species of *Euphorbia*. The compounds found in this study are important because they have several documented biological effects. The presence of these strong phytoconstituents may be the cause of the bioactivities displayed by the plant extracts.

Free radicals, including reactive nitrogen and oxygen species, are by-products of normal cellular functions. Under balanced conditions, it plays a significant role in differentiation, growth, and metabolism. On the other hand, oxidative damage results from excessive production. Antioxidants are substances that can neutralise the harmful effects caused by free radicals and quench them. Plants are a rich source of phytochemicals that have antioxidant properties. It is extremely important to uncover the antioxidant potential of plant species. Five *in*

*vitro* assays were performed to better understand the free radical scavenging potential of selected *Euphorbia* species. This includes the DPPH radical, hydroxyl radical, superoxide radical, nitric oxide radical, and ABTS radical scavenging assays. When compared to the standard used in the study, *E. deccanensis* had the highest scavenging potential of the three plant species. It demonstrated strong activity against the DPPH, hydroxyl, and superoxide radicals. In contrast, all of the tested radicals were moderately affected by the other two extracts. Polyphenols and terpenoid present in the methanolic extracts of *E. deccanensis* are responsible for the strong antioxidant activity that has been observed.

Inflammation is the first line of defence of the immune system against infections, stress, wounds, and pathogens of all kinds. Atherosclerosis and cancer are among the many illnesses brought on by chronic inflammation. Therefore, addressing inflammation in a timely manner is essential in continuing treatment for other related conditions. Conventional anti-inflammatory medications typically have a lot of negative effects on the health and safety of the patient. A practical solution with strong anti-inflammatory activities is therefore required. Numerous researchers have reported the anti-inflammatory properties of various phytochemicals. Using two *in vitro* biochemical assays—the protein denaturation assay and the proteinase inhibition assay—the anti-inflammatory potential of all three extracts was first evaluated. Regarding the inhibition of protein denaturation and proteinase activity, *E. deccanensis* exhibited better anti-inflammatory potential than the other two species under investigation. So, for further analysis, *E. deccanensis* was taken. Next, using an LPS-induced RAW 264.7 macrophage cell line, the effects of *E. deccanensis* methanolic extract on the activity of specific proinflammatory mediators such as cyclooxygenase, lipoxygenase, and inducible nitric oxide synthase, as well as its effects on the level of cellular nitrite, were assessed. Comparing the plant extract to the diclofenac sodium, this served as the standard in every assay, revealed observable effects on every enzyme taken into consideration. The ability of the plant extract to lower the amount of cellular nitrite further suggests that it has anti-inflammatory properties. Additionally, RAW 264.7 macrophage cells were tested for viability using the MTT assay after being treated with different

concentrations of plant extract. The  $LC_{50}$  value obtained was  $139.63 \pm 0.68 \mu\text{g/ml}$ , which was used to estimate COX-2, one of the main inflammatory mediators, using the ELISA. A notable reduction in COX-2 levels adds credence to the methanolic plant extract's anti-inflammatory activities.

*Allium cepa* is considered a significant model because of its wide range of applications in the assessment of cytotoxicity and genotoxicity. The three chosen plants' methanolic extracts were tested for cytotoxicity using the *Allium cepa* root tip assay. A 24-hour treatment period was chosen to comprehend the cytotoxic effects and different plant extract concentrations-6.2, 12.5, 25, 50, and 100  $\mu\text{g/ml}$ -was used. With regard to dosage, all three plant extracts showed cytotoxic effects, causing a decrease in the mitotic index and an increase in the percentage of aberrations. All three extracts of *Euphorbia* showed both clastogenic and non-clastogenic aberrations. Therefore, the study aids in evaluating the plant extract's cytotoxic impact on cells that are actively dividing.

When cells in the body grow abnormally, it can lead to cancer. There are numerous distinct forms of cancer, each exhibiting distinct behaviour and reaction to treatment. It can be malignant, which is characterised by metastases, or benign, which stays at the original location. Breast cancer is one of the most common and enduring types of cancer and the primary cause of death for women around the world. About 2.3 million cases of invasive breast cancer were reported in 2020, making it a common disease. Breast cancer is caused by both modifiable factors, like smoking, drinking alcohol, and being obese, as well as non-modifiable factors, like genetic mutations and breast tissue density. Finding compounds with anticancer properties in plants to treat cancer may be a significant step towards treating breast cancer. Using the MCF-7 breast cancer cell line, the current study examines the selected *Euphorbia* species' *in vitro* anticancer potential. Both modifiable factors, such as obesity, alcohol intake, smoking and non-modifiable factors, such as genetic mutations, density of breast tissue, contribute to breast cancer. Determination of compounds with anticancer activity from plants for the treatment of cancer might be the stepping stone in breast cancer therapy. The present work focuses on the *in vitro*

anticancer potential of chosen *Euphorbia* species in the MCF-7 breast cancer cell line. According to the study, the extract from *E. deccanensis* had the strongest anticancer properties out of the three plant extracts that were chosen. With a noteworthy  $LC_{50}$  value of  $93.21 \pm 0.58 \mu\text{g/ml}$ , this extract was able to specifically inhibit the growth of breast cancer cells without harming the normal cell line L929, which has a high  $LC_{50}$  value of  $218.04 \pm 0.40 \mu\text{g/ml}$ . Acridine orange and ethidium bromide are two dyes used in the double staining method, which confirms that the cell death seen in breast cancer cells was caused by apoptosis. To determine which phase of the cell cycle is arrested by the effect of the plant extract, flow cytometry was used. It was found that the cell cycle arrest happens at the G0/G1 phase. The methanolic plant extract of *E. deccanensis* demonstrated anticancer properties through apoptosis and cell cycle inhibition at the G0/G1 phase. Analysis of the expression of two significant genes associated with apoptosis, p53 and STAT-3, further supported this. The upregulation of p53 and downregulation of STAT-3 demonstrated the effect of the methanolic extract of *E. deccanensis* on the breast cancer cells under investigation.

Molecules that have a diameter of 1–100 nm are called nanoparticles. It has many uses in various fields because of its high surface to volume ratio. There are numerous adverse effects from conventional techniques, including chemical and physical ones. Given the availability of plants and the presence of bioactive compounds that are involved in nano formation, the green synthesis of nanoparticles using plant extracts is a feasible option for this. In addition, it is economical and environmentally beneficial. In the current study, the three chosen extracts underwent synthesis of silver nanoparticles and were characterised using FTIR analysis, FE-SEM, and UV-visible spectroscopy. Silver nitrate was significantly reduced to match silver nanoparticles in all of the chosen extracts. When the plant extract and 2 mM silver nitrate solution interacted, a reddish-brown colour was produced, signifying the formation of silver nanoparticles. The development of nanoparticles was further validated by UV-visible spectroscopy, which produced a sharp absorption peak between 380 and 470 nm. Additionally, the synthesised nanoparticles' morphology was assessed using FE-SEM analysis, which showed that

they were roughly spherical in shape. FTIR analysis was used to identify the different phytochemicals that were involved in the reduction of the silver nitrate solution. Four distinct bacterial species and two fungal species were used to test the produced silver nanoparticles' antimicrobial activity. Since every organism reacts differently, it is impossible to identify one nanoparticle as the best one, even though all three plant-derived nanoparticles demonstrated both antibacterial and antifungal activity.

### **Deliverables**

- The phytochemical analysis showed that the three plant species that were chosen (*E. rothiana*, *E. deccanensis*, and *E. thymifolia*) had a distribution of strong bioactive compounds with potential medical uses.
- The three medicinally valued species of *Euphorbia* have shown significant antioxidant potential.
- Anti-inflammatory efficacy of the plant extracts was determined using LPS-treated RAW 264.7 macrophage cell line.
- Cytotoxicity assay using *Allium cepa* bioassay revealed the cytotoxic nature of plant extracts, which directly acts as leading point to the antiproliferative activity.
- The methanolic extract of *E. deccanensis* displayed significant selective antitumor potential on the breast cancer MCF-7 cell line.
- The plant *E. deccanensis* triggered apoptosis and cell cycle arrest in a notable manner.
- All selected *Euphorbia* extracts revealed the potential for the green synthesis of silver nanoparticles in an effective and eco-friendly method and showed notable antimicrobial activities.

### Recommendations

- Identification and isolation of bioactive compounds for further bioactivity screening that may prove to be useful for the development of curative medicines.
- Confirmation of anticancer activity using *in vivo* animal models to ensure plant extracts potential.
- Molecular docking to get detailed information regarding the mode of action of the therapeutically active compounds among the phytochemicals in *Euphorbia*.
- Exploration of biofabricated nanoparticles in different biological domains.
- Conservation of endemic plant *E. deccanensis*, which exhibited significant biological activities along with the occurrence of potent phytocompounds revealed from this study.



## REFERENCES

- Abada, E., Mashraqi, A., Modafar, Y., Al Abboud, M. A., & El-Shabasy, A. (2023). Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity. *Saudi Journal of Biological Sciences*, 31, 103877- 103888. <https://doi.org/10.1016/j.sjbs.2023.103877>
- Abbas, J. F., AL-Amery, S. M. H., & Al-Hussaini, I. M. (2023). A comparative study between the two *Euphorbia* L. species by used chemical and antioxidant evidence. *Journal of Survey in Fisheries Sciences*, 10(3S), 4049-4061. <https://doi.org/10.17762/sfs.v10i3S.1405>
- Abdel-Aty, A. M., Hamed, M. B., Salama, W. H., Ali, M. M., Fahmy, A. S., & Mohamed, S. A. (2019). *Ficus carica*, *Ficus sycomorus* and *Euphorbia tirucalli* latex extracts: Phytochemical screening, antioxidant and cytotoxic properties. *Biocatalysis and Agricultural Biotechnology*, 20, 101199. <https://doi.org/10.1016/j.bcab.2019.101199>
- Abdel-Sattar, E., Abou-Hussein, D., & Petereit, F. (2015). Chemical constituents from the leaves of *Euphorbia ammak* growing in Saudi Arabia. *Pharmacognosy Research*, 7(1), 14. <https://doi.org/10.4103/0974-8490.147136>
- Abdulhafiz, F., Reduan, M. F. H., Hisam, A. H., Mohammad, I., Abdul Wahab, I. R., Abdul Hamid, F. F., Mohammed, A., Nordin, M. L., Shaari, R., Bakar, L. A., Kari, Z. A., Wei, L. S., Goh, K. W., & Ahmad Mohd Zain, M. R. (2022). LC–TOF-MS/MS and GC-MS based phytochemical profiling and evaluation of wound healing activity of *Oroxylum indicum* (L.) Kurz (Beka). *Frontiers in Pharmacology*, 13, 1050453. <https://doi.org/10.3389/fphar.2022.1050453>
- Abdullah, M. Z., Mohd Ali, J., Abolmaesoomi, M., Abdul-Rahman, P. S., & Hashim, O. H. (2017). Anti-proliferative, *in vitro* antioxidant, and cellular antioxidant activities of the leaf extracts from *Polygonum minus* Huds: Effects of solvent polarity. *International Journal of Food Properties*, 20, 846-862. <http://dx.doi.org/10.1080/10942912.2017.1315591>
- Abedi, F., Razavi, B. M., & Hosseinzadeh, H. (2020). A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: Comprehensive pharmacology, toxicology, and some pharmaceutical aspects. *Phytotherapy Research*, 34(4), 729-741. <https://doi.org/10.1002/ptr.6573>
- Abu Bakar, F. I., Abu Bakar, M. F., Abdullah, N., Endrini, S., & Rahmat, A. (2018). A review of Malaysian medicinal plants with potential anti-inflammatory activity. *Advances in Pharmacological and Pharmaceutical Sciences*, 2018, 1-6. <https://doi.org/10.1155/2018/8603602>
- Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, 12(1), 1-10. [https://doi.org/10.4103%2Fjpbs.JPBS\\_175\\_19](https://doi.org/10.4103%2Fjpbs.JPBS_175_19)
- Adam, O. A. O., Abadi, R. S. M., & Ayoub, S. M. H. (2020). Antioxidant activity, total phenolic and flavonoid contents and cytotoxic activity of *Euphorbia aegyptiaca*. *Journal of Drug Delivery and Therapeutics*, 10(2), 37-41. <https://doi.org/10.22270/jddt.v10i2.3911>



- 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.
- Adeniyi, B. A., Fong, H. H. S., Pezzuto, J. M., Luyengi, L., & Odelola, H. A. (2000). Antibacterial activity of diospyrin, isodiospyrin and bisisodiospyrin from the root of *Diospyros piscatoria* (Gurke)(Ebenaceae). *Phytotherapy Research*, 14(2), 112-117. [https://doi.org/10.1002/\(sici\)1099-1573\(200003\)14:2%3C112::aid-ptr488%3E3.0.co;2-t](https://doi.org/10.1002/(sici)1099-1573(200003)14:2%3C112::aid-ptr488%3E3.0.co;2-t)
- Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, 46(1), 1-22. <http://dx.doi.org/10.1186/s42269-022-00770-8>
- Agraharam, G., Girigoswami, A., & Girigoswami, K. (2022). Myricetin: A multifunctional flavonol in biomedicine. *Current Pharmacology Reports*, 8 (1), 48-61. <https://doi.org/10.1007/s40495-021-00269-2>
- Agrawal, P. K., Agrawal, C., & Blunden, G. (2021). Rutin: A potential antiviral for repurposing as a SARS-CoV-2 main protease (Mpro) inhibitor. *Natural Product Communications*, 16(4), 1934578X2199172. <https://doi.org/10.1177/1934578X21991723>
- Ahmad, N., Jabeen, M., Haq, Z. U., Ahmad, I., Wahab, A., Islam, Z. U., Islam, Z. U., Ullah, R., Bari, A., Abdel-Daim, M. M., El-Demerdash, F. M., & Khan, M. Y. (2022). Green fabrication of silver nanoparticles using *Euphorbia serpens* Kunth aqueous extract, their characterization, and investigation of its *in vitro* antioxidative, antimicrobial, insecticidal, and cytotoxic activities. *BioMed Research International*, 2022. <https://doi.org/10.1155/2022/5562849>
- Ahmed, S., Al-Rehaily, A. J., Alam, P., Alqahtani, A. S., Hidayatullah, S., Rehman, M. T., Mothana, R. A., Abbas, S. S., Khan, M. U., Khalid, J. M., & Siddiqui, N. A. (2019). Antidiabetic, antioxidant, molecular docking and HPTLC analysis of miquelianin isolated from *Euphorbia schimperi* C. Presl. *Saudi Pharmaceutical Journal*, 27(5), 655-663. <https://doi.org/10.1016%2Fj.jsps.2019.03.008>
- Ahmed, S., Mothana, R. A., Yousaf, M., & Al-Rehaily, A. J. (2017). Activity guided isolation of chemical constituents from the biologically active methanol extract of *Euphorbia schimperi* C. Presl. *Bulletin of the Chemical Society of Ethiopia*, 31(3), 471-479. <http://dx.doi.org/10.4314/bcse.v31i3.11>
- Ahn, H., Im, E., Lee, D. Y., Lee, H. J., Jung, J. H., & Kim, S. H. (2019). Antitumor effect of pyrogallol via miR-134 mediated S phase arrest and inhibition of PI3K/AKT/Skp2/cMyc signaling in hepatocellular carcinoma. *International Journal of Molecular Sciences*, 20(16), 3985. <https://doi.org/10.3390%2Fijms20163985>
- Ahsan, A., Farooq, M. A., Ahsan Bajwa, A., & Parveen, A. (2020). Green synthesis of silver nanoparticles using *Parthenium hysterophorus*: Optimization, characterization and *in vitro* therapeutic evaluation. *Molecules*, 25(15), 3324-3338. <https://doi.org/10.3390/molecules25153324>
- Akanda, M. R., Kim, I. S., Ahn, D., Tae, H. J., Nam, H. H., Choo, B. K., Kim, K., & Park, B. Y. (2018). Anti-inflammatory and gastroprotective roles of *Rabdosia inflexa* through downregulation of pro-inflammatory cytokines and MAPK/NF-κB

- signaling pathways. *International Journal of Molecular Sciences*, 19(2), 584-596. <https://doi.org/10.3390/ijms19020584>
- Akaneme, F. I., & Iyioke, I. V. (2008). Mutagenic potentials of the sterilizing fluid–Purtil on root tip mitosis of *Allium cepa*. *Bio-Research*, 6(1), 293-297. <https://doi.org/10.4314/br.v6i1.28623>
- Akbar, A., & Soekamto, N. H. (2021). Antioxidant of n-hexane, ethyl acetate and methanol extracts of *Padina* sp. with DPPH method. In *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, 800 (1), 012019. <http://dx.doi.org/10.1088/1755-1315/800/1/012019>
- Akgül, H., Mohammed, F. S., Kına, E., Uysal, İ., Sevindik, M., & Doğan, M. (2022). Total antioxidant and oxidant status and DPPH free radical activity of *Euphorbia eriophora*. *Turkish Journal of Agriculture-Food Science and Technology*, 10(2), 272-275. <http://dx.doi.org/10.24925/turjaf.v10i2.272-275.4685>
- Akinboro, A., & Bakare, A. A. (2007). Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *Journal of Ethnopharmacology*, 112(3), 470-475. <https://doi.org/10.1016/j.jep.2007.04.014>
- Akwu, N. A., Naidoo, Y., & Singh, M. (2019). Cytogenotoxic and biological evaluation of the aqueous extracts of *Grewia lasiocarpa*: An *Allium cepa* assay. *South African Journal of Botany*, 125, 371-380. <http://dx.doi.org/10.1016/j.sajb.2019.08.009>
- Al Mashud, M. A., Moinuzzaman, M., Hossain, M. S., Ahmed, S., Ahsan, G., Reza, A., ... & Jamal, M. A. H. M. (2022). Green synthesis of silver nanoparticles using *Cinnamomum tamala* (Tejpata) leaf and their potential application to control multidrug resistant *Pseudomonas aeruginosa* isolated from hospital drainage water. *Heliyon*, 8(7), 1-9. <https://doi.org/10.1016/j.heliyon.2022.e09920>
- Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152. <https://doi.org/10.1016/j.jsps.2012.05.002>
- Al-Faifi, Z. I., Masrahi, Y. S., Aly, M. S., Al-Turki, T. A., & Dardeer, T. (2017). Evaluation of cytotoxic and genotoxic effects of *Euphorbia triaculeata* Forssk. extract. *Asian Pacific Journal of Cancer Prevention: APJCP*, 18(3), 771. <https://doi.org/10.22034/2FAPJCP.2017.18.3.771>
- Al-Hamoud, G. A., Fantoukh, O. I., Amina, M., Nasr, F. A., Musayeib, N. M. A., Ahmed, M. Z., Noman, O. M., Al-sharidah, R. E., Alasmari, F., & Alqahtani, A. S. (2022). Unprecedented insights on chemical and biological significance of *Euphorbia cactus* growing in Saudi Arabia. *Plants*, 11(5), 681. <https://doi.org/10.3390/plants11050681>
- Alias, C., Feretti, D., Viola, G. V., Zerbini, I., Bisceglie, F., Pelosi, G., Zani, C., Buschini, A., Carcelli, M., Rogolino, D., & Degola, F. (2023). *Allium cepa* tests: A plant-based tool for the early evaluation of toxicity and genotoxicity of newly synthesized antifungal molecules. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 889, 503654.
- Aljeldah, M. M., Yassin, M. T., Mostafa, A. A. F., & Aboul-Soud, M. A. (2023). Synergistic antibacterial potential of greenly synthesized silver nanoparticles with fosfomycin

- against some nosocomial bacterial pathogens. *Infection and Drug Resistance*, 125-142. <https://doi.org/10.2147/idr.s394600>
- Aljubiri, S. M., El-Shwiniy, W. H., Younes, A. A., Alosaimi, E. H., & El-wahaab, B. A. (2023). Use of *Euphorbia balsamifera* extract in precursor fabrication of silver nanoparticles for efficient removal of bromocresol green and bromophenol blue toxic dyes. *Molecules*, 28(9), 3934-3955. <https://doi.org/10.3390/molecules28093934>
- Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, 27(9), 2901. <https://doi.org/10.3390/molecules27092901>
- Allam, G., Mahdi, E. A., Alzahrani, A. M., & Abuelsaad, A. S. (2016). Ellagic acid alleviates adjuvant induced arthritis by modulation of pro-and anti-inflammatory cytokines. *Central European Journal of Immunology*, 41(4), 339-349. <https://doi.org/10.5114/ceji.2016.65132>
- Almatroudi, A. (2020). Silver nanoparticles: Synthesis, characterisation and biomedical applications. *Open Life Sciences*, 15(1), 819-839. <https://doi.org/10.1515/biol-2020-0094>
- Al-Momani, H., Almasri, M., Al Balawi, D. A., Hamed, S., Albiss, B. A., Aldabaibeh, N., ... & Ward, C. (2023). The efficacy of biosynthesized silver nanoparticles against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Scientific Reports*, 13(1), 8876.
- Al-Otibi, F., Al-Ahaidib, R. A., Alharbi, R. I., Al-Otaibi, R. M., & Albasher, G. (2020). Antimicrobial potential of biosynthesized silver nanoparticles by *Aaronsohnia factorovskyi* extract. *Molecules*, 26(1), 130. <https://doi.org/10.3390/molecules26010130>
- Al-Robai, S. A., Ahmed, A. A., Ahmed, A. A., Zabin, S. A., Mohamed, H. A., & Alghamdi, A. A. (2023). Phenols, antioxidant and anticancer properties of *Tagetes minuta*, *Euphorbia granulata* and *Galinsoga parviflora*: *In vitro* and *in silico* evaluation. *Journal of Umm Al-Qura University for Applied Sciences*, 9(1), 15-28. <http://dx.doi.org/10.1007/s43994-022-00017-2>
- Al-Saraireh, Y. M., Youssef, A. M., Alsarayreh, A. Z., Al Hujran, T. A., Al-Sarayreh, S., Al-Shuneigat, J. M., & Alrawashdeh, H. M. (2021). Phytochemical and anti-cancer properties of *Euphorbia hierosolymitana* Boiss. crude extracts. *Journal of Pharmacy and Pharmacognasy Research*, 9, 13-23.
- Altammar, K. A. (2023). A review on nanoparticles: Characteristics, synthesis, applications, and challenges. *Frontiers in Microbiology*, 14, 1155622. <https://doi.org/10.3389/fmicb.2023.1155622>
- Alur, İ. (2023). Low-grade inflammation: A familiar factor in cardiovascular diseases. *JACC: Basic to Translational Science*, 8(11), 1475. <https://doi.org/10.1016/j.jacbts.2023.09.010>
- Al-Yousef, H. M., Alqahtani, A. S., Ghani, A. E. A., El-Toumy, S. A., El-DougDoug, W. I., Hassan, W. H., & Hassan, H. M. (2020). Nephroprotective and antioxidant activities of ethyl acetate fraction of *Euphorbia geniculata* Ortega family

- Euphorbiaceae. *Arabian Journal of Chemistry*, 13(11), 7843-7850. **Error! Hyperlink reference not valid.**
- Amri, O., Zekhnini, A., Bouhaimi, A., Tahrouch, S., & Hatimi, A. (2018). Anti-inflammatory activity of methanolic extract from *Pistacia atlantica* Desf. leaves. *Pharmacognosy Journal*, 10(1), 71-76. <http://dx.doi.org/10.5530/pj.2018.1.14>
- Amtaghri, S., Akdad, M., Slaoui, M., & Eddouks, M. (2022). Traditional uses, pharmacological, and phytochemical studies of *Euphorbia*: A review. *Current Topics in Medicinal Chemistry*, 22(19), 1553-1570. <https://doi.org/10.2174/1568026622666220713143436>
- An, L., Liang, Y., Yang, X., Wang, H., Zhang, J., Tuerhong, M., Li, D., Wang, C., Lee, D., Xu, J., Shuai, L., & Guo, Y. (2019). NO inhibitory diterpenoids as potential anti-inflammatory agents from *Euphorbia antiquorum*. *Bioorganic Chemistry*, 92, 103237. <https://doi.org/10.1016/j.bioorg.2019.103237>
- Anantharaju, P. G., Gowda, P. C., Vimalambike, M. G., & Madhunapantula, S. V. (2016). An overview on the role of dietary phenolics for the treatment of cancers. *Nutrition Journal*, 15(1), 1-16. <https://nutritionj.biomedcentral.com/articles/10.1186/s12937-016-0217-2>
- Andrade, C., Ferreres, F., Gomes, N. G., Duangsrisai, S., Srisombat, N., Vajrodaya, S., Pereira, D. M., Gil-Izquierdo, A., Andrade, P. B., & Valentão, P. (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 RAW 264.7 macrophages. *Biomolecules*, 9(9), 400-411. <https://doi.org/10.3390/biom9090400>
- Anees-ur-Rehman, M., Tariq, M., & Ali, N. (2022). Assessment of phenolic contents, cytotoxic, and anti-oxidant potential of *Euphorbia royleana*. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, 10(1), 3-9. <https://doi.org/10.37962/jpps.v10i1.627>
- Anju, V., & Rameshkumar, K. B. (2022). Phytochemical investigation of *Euphorbia trigona*. *Journal of the Indian Chemical Society*, 99(1), 100253. <http://dx.doi.org/10.1016/j.jics.2021.100253>
- Anokwah, D., Kwatia, E. A., Amponsah, I. K., Jibira, Y., Harley, B. K., Ameyaw, E. O., ... & Mensah, A. Y. (2022). Evaluation of the anti-inflammatory and antioxidant potential of the stem bark extract and some constituents of *Aidia genipiflora* (DC.) Dandy (Rubiaceae). *Heliyon*, 8(8), 1-9. <https://doi.org/10.1016/j.heliyon.2022.e10082>
- Ansari, P., Uddin, M. J., Rahman, M. M., Abdullah-Al-Mamun, M., Islam, M. R., Ali, M. H., & Reza, A. A. (2017). Anti-inflammatory, anti-diarrheal, thrombolytic and cytotoxic activities of an ornamental medicinal plant: *Persicaria orientalis*. *Journal of Basic and Clinical Physiology and Pharmacology*, 28(1), 51-58. <https://doi.org/10.1515/jbcpp-2016-0023>
- Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology & Drug Design*, 80(3), 434-439. <https://doi.org/10.1111/j.1747-0285.2012.01418.x>

- Arif, M., Ullah, R., Ahmad, M., Ali, A., Ullah, Z., Ali, M., Al-Joufi, F. A., Zahoor, M., & Sher, H. (2022). Green synthesis of silver nanoparticles using *Euphorbia wallichii* leaf extract: Its antibacterial action against citrus canker causal agent and antioxidant potential. *Molecules*, 27(11), 3525. <https://doi.org/10.3390/molecules27113525>
- Arif, R., & Uddin, R. (2021). A review on recent developments in the biosynthesis of silver nanoparticles and its biomedical applications. *Medical Devices & Sensors*, 4(1), e10158. <https://doi.org/10.1002/mds3.10158>
- Arika, W., Kibiti, C. M., Njagi, J. M., & Ngugi, M. P. (2019). *In vitro* antioxidant properties of dichloromethanolic leaf extract of *Gnidia glauca* (Fresen) as a promising antiobesity drug. *Journal of Evidence-Based Integrative Medicine*, 24, 2515690X19883258. <https://doi.org/10.1177%2F2515690X19883258>
- Arsène, M. M. J., Viktorovna, P. I., Alla, M., Mariya, M., Nikolaevitch, S. A., Davares, A. K. L., ... & Milana, D. (2023). Antifungal activity of silver nanoparticles prepared using *Aloe vera* extract against *Candida albicans*. *Veterinary World*, 16(1), 18. <https://doi.org/10.14202%2Fvetworld.2023.18-26>
- Arsène, M. M., Podoprighora, I. V., Davares, A. K., Razan, M., Das, M. S., & Senyagin, A. N. (2021). Antibacterial activity of grapefruit peel extracts and green-synthesized silver nanoparticles. *Veterinary World*, 14(5), 1330.
- Arshad, S., Naveed, M., Ullia, M., Javed, K., Butt, A., Khawar, M., & Amjad, F. (2020). Targeting STAT-3 signaling pathway in cancer for development of novel drugs: Advancements and challenges. *Genetics and Molecular Biology*, 43, e20180160. <https://doi.org/10.1590/1678-4685-GMB-2018-0160>
- Arumuganathan, K., & Earle, E. D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*, 9(3), 208-218.
- Asanuma, M., Nishibayashi-Asanuma, S., Miyazaki, I., Kohno, M., & Ogawa, N. (2001). Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. *Journal of Neurochemistry*, 76(6), 1895-1904. <https://doi.org/10.1046/j.1471-4159.2001.00205.x>
- Ascacio-Valdés, J., Aguilera-Carbó, A., Martínez-Hernández, J., Rodríguez-Herrera, R., & Aguilar, C. (2010). *Euphorbia antisiphilitica* residues as a new source of ellagic acid. *Chemical Papers*, 64(4), 528-532. <http://dx.doi.org/10.2478/s11696-010-0034-6>
- Asha, S., Thirunavukkarasu, P., Mani, V. M., & Sadiq, A. M. (2016). Antioxidant activity of *Euphorbia hirta* Linn. leaves extracts. *European Journal of Medicinal Plants*, 14(1), 1-14. <http://dx.doi.org/10.9734/EJMP/2016/24952>
- Ashfaq, K., Rehman, M. A., Ghaffari, M. A., Ali, N., & Sohail, N. (2022). *Euphorbia milii*: Phenolic contents, cytotoxic, and antioxidant Prospect. *Tropical Journal of Natural Product Research*, 6(8).
- Ashraf, A., Sarfraz, R. A., Rashid, M. A., & Shahid, M. (2015). Antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant (*Euphorbia royleana*) from Pakistan. *Journal of Food and Drug Analysis*, 23(1), 109-115. <https://doi.org/10.1016/j.jfda.2014.05.007>

- Ashraf, J. M., Ansari, M. A., Khan, H. M., Alzohairy, M. A., & Choi, I. (2016). Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques. *Scientific Reports*, 6(1), 20414-20424. <https://doi.org/10.1038%2Fsrep20414>
- Ashwathanarayana, R., & Naika, R. (2018). Anti-inflammatory properties of *Pavetta crassicaulis* Bremek. leaf and flower crude extracts and its pure compounds collected from Western Ghats, Karnataka, India. *Asian Journal of Pharmaceutical and Clinical Research*, 11 (9), 72-90. <http://dx.doi.org/10.22159/ajpcr.2018.v11i9.21885>
- Aşkin Çelik, T., & Aslantürk, Ö. S. (2010). Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test. *BioMed Research International*, 2010(1), 189252. <https://doi.org/10.1155/2010/189252>
- Atoyebe, S. M., Oyeyemi, I. T., Dauda, B. A., & Bakare, A. A. (2015). Genotoxicity and anti-genotoxicity of aqueous extracts of herbal recipes containing *Luffa cylindrica* (L.), *Nymphaea lotus* (L.) and *Spondias mombin* (L.) using the *Allium cepa* (L.) assay. *African Journal of Pharmacy and Pharmacology*, 9(15), 492-499. <http://dx.doi.org/10.5897/AJPP2014.4219>
- Awaad, A. S., Al-Jaber, N. A., Moses, J. E., El-Meligy, R. M., & Zain, M. E. (2013). Antiulcerogenic activities of the extracts and isolated flavonoids of *Euphorbia cuneata* Vahl. *Phytotherapy Research*, 27(1), 126-130. <https://doi.org/10.1002/ptr.4872>
- Axelrod, B., Cheesebrough, T. M., & Laakso, S. (1981). Lipoxygenase from soyabeans. *Methods in Enzymology*, 71, 441-451.
- Azad, R., Babu, N. K., Gupta, A. D., & Reddanna, P. (2018). Evaluation of anti-inflammatory and immunomodulatory effects of *Premna integrifolia* extracts and assay-guided isolation of a COX-2/5-LOX dual inhibitor. *Fitoterapia*, 131, 189-199. <https://doi.org/10.1016/j.fitote.2018.10.016>
- Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *RSC Advances*, 5(35), 27540-27557. <https://doi.org/10.1039/C5RA01911G>
- Bahreini, M., Bondarian, F., Gholibegian, M., & Larijani, K. (2015). Evaluation of cell suspension chemical profile of Iranian fennel by TLC and GC-MS. *American Journal of Biological Chemistry*, 3(6), 74-77.
- Bai, J., Zhang, Y., Tang, C., Hou, Y., Ai, X., Chen, X., Zhang, Y., Wang, X., & Meng, X. (2021). Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. *Biomedicine & Pharmacotherapy*, 133, 110985. <https://doi.org/10.1016/j.biopha.2020.110985>
- Bak, M. J., Hong, S. G., Lee, J. W., & Jeong, W. S. (2012). Red ginseng marc oil inhibits iNOS and COX-2 via NFκB and p38 pathways in LPS-stimulated RAW 264.7 macrophages. *Molecules*, 17(12), 13769-13786. <https://doi.org/10.3390/molecules171213769>
- Balaji, S., & Ganesan, K. K. (2021). Inhibition of inducible nitric oxide production by *Caryota urens* and its active constituents umbelliferone and rutin. *Journal of*

- Ayurveda and Integrative Medicine*, 12(2), 369-372. <https://doi.org/10.1016/j.jaim.2020.09.002>
- Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1), 191-203. <http://dx.doi.org/10.1016/j.foodchem.2005.07.042>
- Banerjee, P., Satapathy, M., & Mukhopahayay, A. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour Bioprocess*, 1(1): 1–10.
- Bangar, S. P., Chaudhary, V., Sharma, N., Bansal, V., Ozogul, F., & Lorenzo, J. M. (2022). Kaempferol: A flavonoid with wider biological activities and its applications. *Critical Reviews in Food Science and Nutrition*, 63 (28), 9580-9604. <https://doi.org/10.1080/10408398.2022.2067121>
- Banumathi, B., Vaseeharan, B., Chinnasamy, T., Vijayakumar, S., Govindarajan, M., Alharbi, N. S., ... & Benelli, G. (2017). *Euphorbia rothiana*-fabricated Ag nanoparticles showed high toxicity on *Aedes aegypti* larvae and growth inhibition on microbial pathogens: A focus on morphological changes in mosquitoes and antibiofilm potential against Bacteria. *Journal of Cluster Science*, 28, 2857-2872. <https://link.springer.com/article/10.1007/s10876-017-1263-4>
- Bardania, H., Mahmoudi, R., Bagheri, H., Salehpour, Z., Fouani, M. H., Darabian, B., ... & Akrami, M. (2020). Facile preparation of a novel biogenic silver-loaded nanofilm with intrinsic anti-bacterial and oxidant scavenging activities for wound healing. *Scientific Reports*, 10(1), 6129.
- Basu, P., Hornung, R. S., Averitt, D. L., & Maier, C. (2019). *Euphorbia bicolor* (Euphorbiaceae) latex extract reduces inflammatory cytokines and oxidative stress in a rat model of orofacial pain. *Oxidative Medicine and Cellular Longevity*, 2019, 8594375. <https://doi.org/10.1155/2019/8594375>
- Basyal, D., Neupane, A., Pandey, D. P., & Pandeya, S. (2021). Phytochemical screening and *in vitro* antioxidant and anti-inflammatory activities of aerial parts of *Euphorbia hirta* L. *Journal of Nepal Chemical Society*, 42(1), 115-124. <http://dx.doi.org/10.3126/jncs.v42i1.35362>
- Bautista-Hernández, I., Aranda-Ledesma, N. E., Rojas, R., Tafolla-Arellano, J. C., & Martínez-Ávila, G. C. (2021). Antioxidant activity of polyphenolic compounds obtained from *Euphorbia antisiphilitica* by-products. *Heliyon*, 7(4), 1-12. <https://doi.org/10.1016/j.heliyon.2021.e06734>
- Beldjilali, M., Mekhissi, K., Khane, Y., Chaibi, W., Belarbi, L., & Bousalem, S. (2020). Antibacterial and antifungal efficacy of silver nanoparticles biosynthesized using leaf extract of *Thymus algeriensis*. *Journal of Inorganic and Organometallic Polymers and Materials*, 30(6), 2126-2133.
- 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 and Genetic Engineering Reviews*, 19, 1-30. <https://doi.org/10.1080/02648725.2022.2122303>

- Benjamaa, R., Moujanni, A., Kaushik, N., Choi, E. H., Essamadi, A. K., & Kaushik, N. K. (2022). *Euphorbia* species latex: A comprehensive review on phytochemistry and biological activities. *Frontiers in Plant Science*, 13, 1008881. <https://doi.org/10.3389/fpls.2022.1008881>
- Benmerache, A., Alabdul Magid, A., Labed, A., Kabouche, A., Voutquenne-Nazabadioko, L., Hubert, J., Morhani, H., & Kabouche, Z. (2017). Isolation and characterisation of cytotoxic compounds from *Euphorbia clementei* Boiss. *Natural Product Research*, 31(18), 2091-2098.
- Bennet Rohan, D., Raji, P., Divya Kumar, M., Kripu Sharma, V., Keerthana, D., Karishma, S., & Prakash, P. (2020). Green synthesis and antibacterial activity studies of silver nanoparticles from the aqueous extracts of *Euphorbia hirta*. *Journal of Pure & Applied Microbiology*, 14(1), 301-306. <http://dx.doi.org/10.22207/JPAM.14.1.31>
- Benrahou, K., El Guourrami, O., Mrabti, H. N., Cherrah, Y., & Faouzi, M. E. A. (2022). Investigation of antioxidant, hypoglycemic and anti-obesity effects of *Euphorbia resinifera* L. *Journal of Pharmacopuncture*, 25(3), 242-249. <https://doi.org/10.3831%2FKPI.2022.25.3.242>
- Beppu, Y., Komura, H., Izumo, T., Horii, Y., Shen, J., Tanida, M., Nakashima, T., Tsuruoka, N., & Nagai, K. (2012). Identification of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4 H-pyran-4-one isolated from *Lactobacillus pentosus* strain S-PT84 culture supernatants as a compound that stimulates autonomic nerve activities in rats. *Journal of Agricultural and Food Chemistry*, 60(44), 11044-11049. <https://doi.org/10.1021/jf302355e>
- Bharathi, S. V., & Das, M. (2022). Cytotoxicity effect of nanoparticles of *Euphorbia antiquorum* on breast cancer cell line. *South African Journal of Botany*, 151, 410-416. <http://dx.doi.org/10.1016/j.sajb.2022.10.017>
- Bhat, M. Y., Gul, M. Z., Husain, M. K., & Ghazi, I. A. (2019). *In vitro* evaluation of antiproliferative, lipoxxygenase and xanthine oxidase inhibitory activities of *Artemisia nilagirica* (CB Clarke) Pamp. leaf extracts. *Indian Journal of Pharmaceutical Sciences*, 81(2), 389-395. <http://dx.doi.org/10.4172/pharmaceutical-sciences.1000522>
- Bhat-Nakshatri, P., Khatpe, A. S., Chen, D., Batic, K., Mang, H., Herodotou, C., McGuire, P. C., Xuei, X., Erdogan, C., Gao, H., Liu, Y., & Nakshatri, H. (2024). Signaling pathway alterations driven by BRCA1 and BRCA2 germline mutations are sufficient to initiate breast tumorigenesis by the PIK3CAH1047R oncogene. *Cancer Research Communications*, 4(1), 38-54. <https://doi.org/10.1158%2F2767-9764.CRC-23-0330>
- Bhuyar, P., Yusoff, M. M., Ab Rahim, M. H., Sundararaju, S., Maniam, G. P., & Govindan, N. (2020). Effect of plant hormones on the production of biomass and lipid extraction for biodiesel production from microalgae *Chlorella* sp. *The Journal of Microbiology, Biotechnology and Food Sciences*, 9(4), 671-674. <http://dx.doi.org/10.15414/jmbfs.2020.9.4.671-674>
- Bigoniya, P., & Rana, A. (2008). A comprehensive phyto-pharmacological review of *Euphorbia nerifolia* Linn. *Pharmacognosy Reviews*, 2(4), 57-66.
- Bijauliya, R. K., Alok, S., Singh, M., & Mishra, S. B. (2017). A comprehensive review on cancer and anticancer herbal drugs. *International Journal of Pharmaceutical*



- Sciences and Research*, 8(7), 2740- 2761. [http://dx.doi.org/10.13040/IJPSR.0975-8232.8\(7\).2740-61](http://dx.doi.org/10.13040/IJPSR.0975-8232.8(7).2740-61)
- Bindu, A. R., & Aleykutty, N. A. (2022). *In vitro* models on inhibition of inflammatory mediators by ethanolic extract of *Careya arborea* Roxb. bark in lipopolysaccharide-induced RAW 264.7 cell lines. In *Natural Product Experiments in Drug Discovery* (pp. 231-248). New York, NY: Springer US. [http://dx.doi.org/10.1007/978-1-0716-2683-2\\_16](http://dx.doi.org/10.1007/978-1-0716-2683-2_16)
- Binojkumar, M. S. (1993). *A taxonomic revision of the genus Euphorbia L. Euphorbiaceae in India*. (Doctoral dissertation) Shodhganga.
- Binojkumar, M. S., & Balakrishnan, N. P. (2010). *The genus Euphorbia L (Euphorbiaceae) in India: A taxonomic revision*. Bishen Singh Mahendra Pal Singh. Dehradun, India.
- Bonciu, E., Firbas, P., Fontanetti, C. S., Wusheng, J., Karaismailoğlu, M. C., Liu, D., Menicucci, F., Pesnya, D. S., Popescu, A., Romanovsky, A.V., Schiff, S., Ślusarczyk, J., de Souza, C. P., Srivastava, A., Sutan, A., & Papini, A. (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia*, 71(3), 191-209. <https://doi.org/10.1080/00087114.2018.1503496>
- Boora, F., Chirisa, E., & Mukanganyama, S. (2014). Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. *Journal of Food Processing*, 2014 (2), 1-7. <http://dx.doi.org/10.1155/2014/918018>
- Brown, D. C. (2016). Resiniferatoxin: The evolution of the “molecular scalpel” for chronic pain relief. *Pharmaceuticals*, 9(3), 47-58. <https://doi.org/10.3390%2Fph9030047>
- Bruna, T., Maldonado-Bravo, F., Jara, P., & Caro, N. (2021). Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, 22(13), 7202. <https://doi.org/10.3390%2Fijms22137202>
- Cañas, S., Rebollo-Hernanz, M., Bermúdez-Gómez, P., Rodríguez-Rodríguez, P., Braojos, C., Gil-Ramírez, A., Benítez, V., Aguilera, Y., & Martín-Cabrejas, M. A. (2023). Radical scavenging and cellular antioxidant activity of the cocoa shell phenolic compounds after simulated digestion. *Antioxidants*, 12(5), 1007. <https://doi.org/10.3390/antiox12051007>
- Carta, G., Murru, E., Banni, S., & Manca, C. (2017). Palmitic acid: Physiological role, metabolism and nutritional implications. *Frontiers in Physiology*, 8, 902. <https://doi.org/10.3389/fphys.2017.00902>
- Carter, S., Egli, U., & CITES Plants Committee. (1997). *The CITES checklist of succulent Euphorbia taxa (Euphorbiaceae)*. German Federal Agency for Nature Conservation. Bonn, Germany.
- Carvalho Jr, R. N., Moura, L. S., Rosa, P. T., & Meireles, M. A. A. (2005). Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): Kinetic data, extract's global yield, composition, and antioxidant activity. *The Journal of Supercritical Fluids*, 35(3), 197-204. <http://dx.doi.org/10.1016/j.supflu.2005.01.009>
- Casals, E., Gusta, M. F., Bastus, N., Rello, J., & Puentes, V. (2025). Silver nanoparticles and antibiotics: A promising synergistic approach to multidrug-resistant

- infections. *Microorganisms*, 13(4), 952. <https://doi.org/10.3390/microorganisms13040952>
- Cayona, R., & Creencia, E. (2022). Phytochemicals of *Euphorbia hirta* L. and their inhibitory potential against SARS-CoV-2 Main Protease. *Frontiers in Molecular Biosciences*, 8, 801401. <https://doi.org/10.3389/fmolb.2021.801401>
- Čechovská, L., Cejpek, K., Konečný, M., & Velišek, J. (2011). On the role of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(4 H)-pyran-4-one in antioxidant capacity of prunes. *European Food Research and Technology*, 233, 367-376. <http://dx.doi.org/10.1007/s00217-011-1527-4>
- Chamkhi, I., Hnini, M., & Aurag, J. (2022). Conventional medicinal uses, phytoconstituents, and biological activities of *Euphorbia officinarum* L.: A systematic review. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022, 1-9. <https://doi.org/10.1155/2022/9971085>
- Chandimali, N., Bak, S. G., Park, E. H., Lim, H. J., Won, Y. S., Kim, E. K., Park, S. I., & Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: A review. *Cell Death Discovery*, 11(1), 19. <http://dx.doi.org/10.1038/s41420-024-02278-8>
- Chandra, S., Gahlot, M., Choudhary, A. N., Palai, S., de Almeida, R. S., de Vasconcelos, J. E. L., dos Santos, F. A. V., de Farias, P. A. M., & Coutinho, H. D. M. (2023). Scientific evidences of anticancer potential of medicinal plants. *Food Chemistry Advances*, 2, 100239. <https://doi.org/10.1016/j.focha.2023.100239>
- Chang, S. T., Wu, J. H., Wang, S. Y., 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.
- Chaudhary, P., & Janmeda, P. (2022). Quantification of phytochemicals and *in vitro* antioxidant activities from various parts of *Euphorbia neriifolia* Linn. *Journal of Applied Biology and Biotechnology*, 10(2), 133-145. <http://dx.doi.org/10.7324/JABB.2022.100217>
- Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, 11, 1158198. <https://doi.org/10.3389/fchem.2023.1158198>
- Chaudhry, G. E. S., Md Akim, A., Sung, Y. Y., & Sifzizul, T. M. T. (2022). Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. *Frontiers in Pharmacology*, 13, 842376. <https://doi.org/10.3389/fphar.2022.842376>
- Chen, H. S., Bai, M. H., Zhang, T., Li, G. D., & Liu, M. (2015). Ellagic acid induces cell cycle arrest and apoptosis through TGF- $\beta$ /Smad3 signaling pathway in human breast cancer MCF-7 cells. *International Journal of Oncology*, 46(4), 1730-1738. <https://doi.org/10.3892/ijo.2015.2870>
- Chen, H., Wang, Z., & Yang, L. (2012). Analysis of euphornin in *Euphorbia helioscopia* L. and its cytotoxicity to mice lung adenocarcinoma cells (LA795). *Natural Product Research*, 26(22), 2112-2116. <https://doi.org/10.1080/14786419.2011.622275>

- Chen, J. (2016). The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. *Cold Spring Harbor Perspectives in Medicine*, 6(3), 1-6. <https://doi.org/10.1101/cshperspect.a026104>
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), 7204. <https://doi.org/10.18632/oncotarget.23208>
- Chen, Z., Liu, Q., Zhao, Z., Bai, B., Sun, Z., Cai, L., Fu, Y., Ma, Y., Wang, Q., & Xi, G. (2021). Effect of hydroxyl on antioxidant properties of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4 H-pyran-4-one to scavenge free radicals. *RSC Advances*, 11(55), 34456-34461. <https://doi.org/10.1039%2Fd1ra06317k>
- Chen, Z., Xi, G., Fu, Y., Wang, Q., Cai, L., Zhao, Z., Liu, Q., Bai, B., & Ma, Y. (2021). Synthesis of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one from maltol and its taste identification. *Food Chemistry*, 361, 130052. <https://doi.org/10.1016/j.foodchem.2021.130052>
- Chirumamilla, P., & Taduri, S. (2023). Assessment of in vitro anti-inflammatory, antioxidant and antidiabetic activities of *Solanum khasianum* Clarke. *Vegetos*, 36(2), 575-582.
- Choene, M., & Motadi, L. (2016). Validation of the antiproliferative effects of *Euphorbia tirucalli* extracts in breast cancer cell lines. *Molecular Biology*, 50, 98-110. <https://doi.org/10.7868/s0026898416010043>
- Choi, H. M., & Lim, S. Y. (2014). Effect of *Euphorbia supina* extract on antioxidant activity. *The Open Nutraceuticals Journal*, 7(1), 28-32. <http://dx.doi.org/10.2174/1876396001407010028>
- Choi, J. S., Jung, H. C., Baek, Y. J., Kim, B. Y., Lee, M. W., Kim, H. D., & Kim, S. W. (2021). Antibacterial activity of green-synthesized silver nanoparticles using *Areca catechu* extract against antibiotic-resistant bacteria. *Nanomaterials*, 11(1), 205. <https://doi.org/10.3390/nano11010205>
- Choodej, S., Hanthanong, S., Aree, T., & Pudhom, K. (2020). Diterpenoids from the aerial parts of *Euphorbia antiquorum* and their efficacy on nitric oxide inhibition. *Phytochemistry*, 180, 112523. <https://doi.org/10.1016/j.phytochem.2020.112523>
- Chouhan, S., & Guleria, S. (2020). Anti-inflammatory activity of medicinal plants: Present status and future perspectives. *Botanical Leads for Drug Discovery*, 67-92. [http://dx.doi.org/10.1007/978-981-15-5917-4\\_4](http://dx.doi.org/10.1007/978-981-15-5917-4_4)
- Chun, J., Mah, S. Y., & Kim, Y. S. (2023). Anti-inflammatory effect of ebractenoid F, a major active compound of *Euphorbia ebracteolata* Hayata, through inhibition of nuclear factor- $\kappa$ B activation. *Plants*, 12(15), 2845. <https://doi.org/10.3390/plants12152845>
- Clusan, L., Ferrière, F., Flouriot, G., & Pakdel, F. (2023). A basic review on estrogen receptor signaling pathways in breast cancer. *International Journal of Molecular Sciences*, 24(7), 6834. <https://doi.org/10.3390/ijms24076834>
- Comşa, Ş., Cimpean, A. M., & Raica, M. (2015). The story of MCF-7 breast cancer cell line: 40 years of experience in research. *Anticancer Research*, 35(6), 3147-3154.

- Crisan, C. M., Mocan, T., Manolea, M., Lasca, L. I., Tăbăran, F. A., & Mocan, L. (2021). Review on silver nanoparticles as a novel class of antibacterial solutions. *Applied Sciences*, 11(3), 1120-1136. <https://doi.org/10.3390/app11031120>
- Dada, S. O., Ehie, G. C., Osukoya, O. A., Anadozie, S. O., Adewale, O. B., & Kuku, A. (2023). *In vitro* antioxidant and anti-inflammatory properties of *Artocarpus altilis* (Parkinson) Fosberg (seedless breadfruit) fruit pulp protein hydrolysates. *Scientific Reports*, 13(1), 1493-1502. <https://doi.org/10.1038/s41598-023-28684-z>
- Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, 7, 1831 <https://doi.org/10.3389/fmicb.2016.01831>
- Dang, Q., Song, W., Xu, D., Ma, Y., Li, F., Zeng, J., Zhu, G., Wang, X., Chang, L. S., He, D., & Li, L. (2015). Kaempferol suppresses bladder cancer tumor growth by inhibiting cell proliferation and inducing apoptosis. *Molecular Carcinogenesis*, 54(9), 831-840. <https://doi.org/10.1002/mc.22154>
- Dar, R. A., Shahnawaz, M., & Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 6(6), 349-351. <http://dx.doi.org/10.31254/phyto.2017.6608>
- Das, D., Arulkumar, A., Paramasivam, S., Lopez-Santamarina, A., del Carmen Mondragon, A., & Miranda Lopez, J. M. (2023). Phytochemical constituents, antimicrobial properties and bioactivity of marine red Seaweed (*Kappaphycus alvarezii*) and seagrass (*Cymodocea serrulata*). *Foods*, 12(14), 2811. <https://doi.org/10.3390/foods12142811>
- Das, K., Asdaq, S. M. B., Khan, M. S., Amrutha, S., Alamri, A., Alhomrani, M., ... & Harshitha, P. (2022). Phytochemical investigation and evaluation of *in vitro* anti-inflammatory activity of *Euphorbia hirta* ethanol leaf and root extracts: A comparative study. *Journal of King Saud University-Science*, 34(7), 102261-102269. <http://dx.doi.org/10.1016/j.jksus.2022.102261>
- Das, S., Goswami, M., Yadav, R. N. S., & Bandyopadhyay, T. (2022). Quantitative estimation of terpenoid content in some tea cultivars of northeast India and their *in vitro* cell cultures using an optimized spectrophotometric method. *Journal of Advanced Scientific Research*, 13(03), 112-117. <http://dx.doi.org/10.55218/JASR.202213318>
- Da-Song, Y. A. N. G., Qiu-Xia, H. E., Yong-Ping, Y. A. N. G., Ke-Chun, L. I. U., & Xiao-Li, L. I. (2014). Chemical constituents of *Euphorbia tibetica* and their biological activities. *Chinese Journal of Natural Medicines*, 12(1), 38-42. [https://doi.org/10.1016/s1875-5364\(14\)60007-8](https://doi.org/10.1016/s1875-5364(14)60007-8)
- Dawadi, S., Katuwal, S., Gupta, A., Lamichhane, U., Thapa, R., Jaisi, S., Lamichhane, G., Bhattarai, D. P., & Parajuli, N. (2021). Current research on silver nanoparticles: Synthesis, characterization, and applications. *Journal of Nanomaterials*, 2021(1), 6687290. <https://doi.org/10.1155/2021/6687290>
- De Albuquerque, A., de Araújo Ramos, R. F., Abreu, L. S., do Nascimento, Y. M., Tavares, J. F., & de Oliveira Costa, V. C. (2018). Phenolic compounds isolated from *Euphorbia phosphorea*. In *Proceedings of MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition* (p. 1). MDPI. <http://dx.doi.org/10.3390/mol2net-04-05554>

- de La Cruz, J. F., Vergara, E. J., Cho, Y., Hong, H. O., Oyungerel, B., & Hwang, S. G. (2015). *Glehnia littoralis* root extract induces G0/G1 phase cell cycle arrest in the MCF-7 human breast cancer cell line. *Asian Pacific Journal of Cancer Prevention*, 16(18), 8113-8117. <https://doi.org/10.7314/APJCP.2015.16.18.8113>
- de Lima, M. D. F. R., Cavalcante, L. A., de Araújo Costa, E. C. T., de Veras, B. O., da Silva, M. V., Cavalcanti, L. N., & Araújo, R. M. (2021). Bioactivity flavonoids from roots of *Euphorbia tirucalli* L. *Phytochemistry Letters*, 41, 186-192. <http://dx.doi.org/10.1016/j.phytol.2020.10.017>
- de Souza, L. S., Puziol, L. C., Tosta, C. L., Bittencourt, M. L., Santa Ardisson, J., Kitagawa, R. R., Filgueiras, P. R., & Kuster, R. M. (2019). Analytical methods to access the chemical composition of an *Euphorbia tirucalli* anticancer latex from traditional Brazilian medicine. *Journal of Ethnopharmacology*, 237, 255-265. <https://doi.org/10.1016/j.jep.2019.03.041>
- Deepty, M., Srinivas, C., Kumar, E. R., Mohan, N. K., Prajapat, C. L., Rao, T. C., ... & Sastry, D. L. (2019). XRD, EDX, FTIR and ESR spectroscopic studies of co-precipitated Mn-substituted Zn-ferrite nanoparticles. *Ceramics International*, 45(6), 8037-8044. <https://doi.org/10.1016/j.ceramint.2019.01.029>
- Dehghani, M., Beyzaei, H., & Ebrahimnezhad, Z. (2022). Determination of phenolic and flavonoid contents of roots and shoots of *Euphorbia serpens* Kunth. using different solvents. *Current Applied Sciences*, 2 (1), 59-66. <https://doi.org/10.22034/cas.2022.354096.1026>
- Desai, S. J., Prickril, B., & Rasooly, A. (2018). Mechanisms of phytonutrient modulation of cyclooxygenase-2 (COX-2) and inflammation related to cancer. *Nutrition and Cancer*, 70(3), 350-375. <https://doi.org/10.1080%2F01635581.2018.1446091>
- dev Sharma, A., Kaur, I., Chuhan, A., Kumar, S., & Singh, N. (2023). Phytochemical profile, *in-vitro* antioxidant, anti-diabetic and anti-inflammatory activities of traditionally used *Euphorbia hirta* (L.) growing under wild conditions of Northern Punjab. *Drug Analytical Research*, 7(1), 27-40.
- Dhaka, A., Mali, S. C., Sharma, S., & Trivedi, R. (2023). A review on biological synthesis of silver nanoparticles and their potential applications. *Results in Chemistry*, 101108. <https://doi.org/10.1016/j.rechem.2023.101108>
- Dhawan, D., & Gupta, J. (2017). Research article comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. *International Journal of Biological Chemistry*, 11(1), 17-22. <http://dx.doi.org/10.3923/ijbc.2017.17.22>
- Duan, J., Li, Y., Gao, H., Yang, D., He, X., Fang, Y., & Zhou, G. (2020). Phenolic compound ellagic acid inhibits mitochondrial respiration and tumor growth in lung cancer. *Food & Function*, 11(7), 6332-6339. <https://doi.org/10.1039/D0FO01177K>
- Dumanović, J., Nepovimova, E., Natić, M., Kuča, K., & Jačević, V. (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in Plant Science*, 11, 552969. <https://doi.org/10.3389/fpls.2020.552969>

- Dvorakova, M., & Landa, P. (2017). Anti-inflammatory activity of natural stilbenoids: A review. *Pharmacological Research*, 124, 126-145. <https://doi.org/10.1016/j.phrs.2017.08.002>
- Dzoyem, J. P., Tchuengem, R. T., Iqbal, J., Yameen, M. A., Mannan, A., Shahzadi, I., Ismail, T., Fatima, N., & Murtaza, G. (2022). Anticandidal activity of green synthesised silver nanoparticles and extract loaded chitosan nanoparticles of *Euphorbia prostata*. *Artificial Cells, Nanomedicine, and Biotechnology*, 50(1), 188-197. <https://doi.org/10.1080/21691401.2022.2088546>
- 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.
- EFSA panel on food additives and nutrient sources added to food (ANS). (2012). Scientific opinion on the revaluation of *Candelilla wax* (E 902) as a food additive. *EFSA Journal*, 10(11), 2946-2973. <https://doi.org/10.2903/j.efsa.2012.2946>
- Egbuna, C., Ifemeje, J. C., Maduako, M. C., Tijjani, H., Udedi, S. C., Nwaka, A. C., & Ifemeje, M. O. (2018). Phytochemical test methods: Qualitative, quantitative and proximate analysis. In *Phytochemistry* (pp. 381-426). Apple Academic Press.
- Ehaimir, S. Y., Ibrahim, M. A., Abdalhalim, A. M., Ibrahim, S. O., Alzain, A. A., Alameen, A. A., Hassan, S. H., Ahmed, S. H., Ahmed, E. M., & Abdalrahman, M. A. (2023). Antibacterial, antioxidant activities, GC-MS analysis and docking studies of *Guiera senegalensis* (L.) ethanol leaves extract. *Tropical Journal of Natural Product Research*, 7(1), 2162-2167. <https://www.tjnpr.org/>
- Ekeke, C., & Ndukwu, B. C. (2014). Taxonomic values of secondary metabolites (hydrocarbons and flavonoids) in the genus *Euphorbia* L. from Niger Delta, Nigeria. *Research Journal of Botany*, 9(1), 1-8. <http://dx.doi.org/10.3923/rjb.2014.1.8>
- El Hejjoui, B., Bouguenouch, L., Melhouf, M. A., El Mouhi, H., & Bennis, S. (2023). Clinical evidence of circulating tumor DNA application in aggressive breast cancer. *Diagnostics*, 13(3), 470-482. <https://doi.org/10.3390/diagnostics13030470>
- Elagdi, C., Bouaouda, K., Rahhal, R., Hsaine, M., Badri, W., Fougrach, H., & Hajjouji, H. E. (2023). Phenolic compounds, antioxidant and antibacterial activities of the methanolic extracts of *Euphorbia resinifera* and *Euphorbia echinus*. *Scientific African*, 21 (10) e01779. <http://dx.doi.org/10.1016/j.sciaf.2023.e01779>
- Elankanni, R., Kumar, D. R. N., & Kumar, R. A. (2021). Annihilating efficacy of *Euphorbia hirta* L. extracts on *Artemia salina nauplii* and human breast adenocarcinoma cells (mcf-7 & mda-mb-231). *Journal of Phytology*, 13, 91-94.
- El-Anssary, A. A., Raoof, G. F. A., Saleh, D. O., & El-Masry, H. M. (2021). Bioactivities, physicochemical parameters and GC/MS profiling of the fixed oil of *Cucumis melo* L seeds: A focus on anti-inflammatory, immunomodulatory, and antimicrobial activities. *Journal of Herbmmed Pharmacology*, 10(4), 476-485. <http://dx.doi.org/10.34172/jhp.2021.55>
- Elgamal, A. M., El Raey, M. A., Gaara, A., Abdelfattah, M. A., & Sobeh, M. (2021). Phytochemical profiling and anti-aging activities of *Euphorbia retusa* extract: *In silico* and *in vitro* studies. *Arabian Journal of Chemistry*, 14(6), 103159. <http://dx.doi.org/10.1016/j.arabjc.2021.103159>

- El-Ghamery, A. A., El-Kholy, M. A., & Abou El-Yousser, M. A. (2003). Evaluation of cytological effects of Zn<sup>2+</sup> in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 537(1), 29-41.
- El-Hallouty, S. M., Gohar, L. H., & Elgazzar, E. M. (2022). Enhancement of radiation-induced cytotoxicity in hepatocellular carcinoma cells by some plant extracts *in vitro*. *Egyptian Journal of Radiation Sciences and Applications*, 35(1), 39-47. <https://doi.org/10.21608/ejrsa.2022.158279.1138>
- ELhassan, G. O., Adhikari, A., Abdalla, O. M., Shukrulla, A., Khalid, A., Choudhary, M. I., Mesaik, M. A., & Yagi, S. (2015). Chemical constituents of *Euphorbia polyacantha* Boiss. and their immunomodulatory properties. *Records of Natural Products*, 9(1), 146.
- Elizabeth, K., & Rao, M. N. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, 58, 237-240.
- Elshamy, A. I., Abd-ElGawad, A. M., El Gendy, A. E. N. G., & Assaeed, A. M. (2019). Chemical characterization of *Euphorbia heterophylla* L. essential oils and their antioxidant activity and allelopathic potential on *Cenchrus echinatus* L. *Chemistry & Biodiversity*, 16(5), e1900051. <https://doi.org/10.1002/cbdv.201900051>
- Elumalai, E. K., Prasad, T. N. V. K. V., Venkata, K., Nagajyothi, P. C., & David, E. (2010). Green synthesis of silver nanoparticle using *Euphorbia hirta* L. and their antifungal activities. *Archives of Applied Science Research*, 2(6), 76-81.
- Ensenyat-Mendez, M., Llinàs-Arias, P., Orozco, J. I., Íñiguez-Muñoz, S., Salomon, M. P., Sesé, B., DiNome, M. L., & Marzese, D. M. (2021). Current triple-negative breast cancer subtypes: Dissecting the most aggressive form of breast cancer. *Frontiers in Oncology*, 11, 681476. <https://doi.org/10.3389/fonc.2021.681476>
- Ernst, M., Grace, O. M., Saslis-Lagoudakis, C. H., Nilsson, N., Simonsen, H. T., & Rønsted, N. (2015). Global medicinal uses of *Euphorbia* L. (Euphorbiaceae). *Journal of Ethnopharmacology*, 176, 90-101. <https://doi.org/10.1016/j.jep.2015.10.025>
- Ertas, A., Yilmaz, M. A., & Firat, M. (2015). Chemical profile by LC–MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two *Euphorbia* species. *Natural Product Research*, 29(6), 529-534. <https://doi.org/10.1080/14786419.2014.954113>
- Espíndola, K. M. M., Ferreira, R. G., Narvaez, L. E. M., Silva Rosario, A. C. R., Da Silva, A. H. M., Silva, A. G. B., Vieira, A. P. O., & Monteiro, M. C. (2019). Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Frontiers in Oncology*, 9, 541. <https://doi.org/10.3389/fonc.2019.00541>
- Evans, W. C., & Evans, D. (2002). *Trease and evans's pharmacognosy*. 15th edition, Elsevier Health Sciences, New york (pp. 21-24).
- Evtugin, D. D., Magina, S., & Evtugin, D. V. (2020). Recent advances in the production and applications of ellagic acid and its derivatives: A review. *Molecules*, 25(12), 2745. <https://doi.org/10.3390/molecules25122745>

- Fais, A., Delogu, G. L., Floris, S., Era, B., Medda, R., & Pintus, F. (2021). *Euphorbia characias*: Phytochemistry and biological activities. *Plants*, 10(7), 1468. <https://doi.org/10.3390%2Fplants10071468>
- Farha, A. K., Gan, R. Y., Li, H. B., Wu, D. T., Atanasov, A. G., Gul, K., Zhang, J. R., Yang, Q. Q., & Corke, H. (2022). The anticancer potential of the dietary polyphenol rutin: Current status, challenges, and perspectives. *Critical Reviews in Food Science and Nutrition*, 62(3), 832-859. <https://doi.org/10.1080/10408398.2020.1829541>
- Farozzi, A., Banday, J. A., & Shah, S. A. (2015). Genicunolide A, B and C: Three new triterpenoids from *Euphorbia geniculata*. *Beilstein Journal of Organic Chemistry*, 11(1), 2707-2712. <https://doi.org/10.3762/bjoc.11.291>
- Fayed, A. A. A., Ahamed, M. S., Faried, A. M., & Mohamed, M. H. (2020). Leaf morphology and venation patterns of *Euphorbia* L. (Euphorbiaceae) in Egypt with special notes on their taxonomic implications. *Jordan Journal of Biological Sciences*, 13(2), 165-176.
- Fernandez, S. P., Nguyen, M., Yow, T. T., Chu, C., Johnston, G. A., Hanrahan, J. R., & Chebib, M. (2009). The flavonoid glycosides, myricitrin, gossypin and naringin exert anxiolytic action in mice. *Neurochemical Research*, 34, 1867-1875. <https://doi.org/10.1007/s11064-009-9969-9>
- Fernandez-Arche, A., Saenz, M. T., Arroyo, M., De la Puerta, R., & Garcia, M. D. (2010). Topical anti-inflammatory effect of tirucallol, a triterpene isolated from *Euphorbia lactea* latex. *Phytomedicine*, 17(2), 146-148. <https://doi.org/10.1016/j.phymed.2009.05.009>
- Fernandez-Panchon, M. S., Villano, D., Troncoso, A. M., & Garcia-Parrilla, M. C. (2008). Antioxidant activity of phenolic compounds: From *in vitro* results to *in vivo* evidence. *Critical Reviews in Food Science and Nutrition*, 48(7), 649-671. <https://doi.org/10.1080/10408390701761845>
- Ferreira, A. M. V., Carvalho, M. J. M., Sequeira, M. M., Silva, A. M., & Carvalho, L. H. (2013). Chemical constituents of *Euphorbia hyberna* L. (Euphorbiaceae). *Natural Product Research*, 27(3), 282-285. <https://doi.org/10.1080/14786419.2012.668688>
- Fiskesjö, G. (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas*, 102(1), 99-112.
- Fouzat, A., Hussein, O. J., Gupta, I., Al-Farsi, H. F., Khalil, A., & Al Moustafa, A. E. (2022). *Elaeagnus angustifolia* plant extract induces apoptosis via p53 and signal transducer and activator of transcription 3 signaling pathways in triple-negative breast cancer cells. *Frontiers in Nutrition*, 9, 871667. <https://doi.org/10.3389%2Ffnut.2022.871667>
- Frazão, R., Catarino, S., Goyder, D., Darbyshire, I., Magalhães, M. F., & Romeiras, M. M. (2020). Species richness and distribution of the largest plant radiation of Angola: *Euphorbia* (Euphorbiaceae). *Biodiversity and Conservation*, 29, 187-206. <https://link.springer.com/article/10.1007/s10531-019-01878-6>
- Frezza, C., Venditti, A., Sciubba, F., Tomai, P., Antonetti, M., Franceschin, M., Di Cocco, M. E., Gentili, A., Delfini, M., Serafini, M., & Bianco, A. (2018). Phytochemical profile of *Euphorbia peplus* L. collected in Central Italy and NMR semi-quantitative



- analysis of the diterpenoid fraction. *Journal of Pharmaceutical and Biomedical Analysis*, 160, 152-159. <https://doi.org/10.1016/j.jpba.2018.07.059>
- Gandhi, J., Khera, L., Gaur, N., Paul, C., & Kaul, R. (2017). Role of modulator of inflammation cyclooxygenase-2 in gamma herpes virus mediated tumorigenesis. *Frontiers in Microbiology*, 8, 538-542. <https://doi.org/10.3389/fmicb.2017.00538>
- Gandhi, Y., Kumar, R., Grewal, J., Rawat, H., Mishra, S. K., Kumar, V., ... & Acharya, R. (2022). Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review. *Food Chemistry Advances*, 1 (1), 100085. <http://dx.doi.org/10.1016/j.focha.2022.100085>
- Gao, J., & Aisa, H. A. (2017). Terpenoids from *Euphorbia soongarica* and their multidrug resistance reversal activity. *Journal of Natural Products*, 80(6), 1767-1775. <https://doi.org/10.1021/acs.jnatprod.6b01099>
- Gapuz, M. C. D., & Besagas, R. L. (2018). Phytochemical profiles and antioxidant activities of leaf extracts of *Euphorbia* species. *Journal of Biodiversity and Environmental Sciences*, 12(4), 59-65.
- Garcia-Oliveira, P., Otero, P., Pereira, A. G., Chamorro, F., Carpena, M., Echave, J., ... & Prieto, M. A. (2021). Status and challenges of plant-anticancer compounds in cancer treatment. *Pharmaceuticals*, 14(2), 157. <https://doi.org/10.3390%2Fph14020157>
- Geng, D., Shi, Y., Da Min, Z., & Liang, J. Y. (2010). A new diterpenoid from *Euphorbia helioscopia*. *Chinese Chemical Letters*, 21(1), 73-75. <http://dx.doi.org/10.1016/j.cclet.2009.09.013>
- George, N., Joy, J., Mathew, B., & Koshy, E. P. (2024). Green mediated synthesis of silver nanoparticle using *Euphorbia maculate* leaf extract and their catalytic reduction and antibacterial properties. *Materials Today: Proceedings*, 1-7. <http://dx.doi.org/10.1016/j.matpr.2024.01.013>
- Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. (2021). The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *International Journal of Molecular Sciences*, 22 (23), 12827. <https://doi.org/10.3390/ijms222312827>
- Ghauri, M. A., Iqbal, L., Raza, A., Hayat, U., Atif, N., & Javeed, A. (2021). *In vivo* anti-inflammatory, antipyretic, analgesic activity and *in vitro* anti-proliferative activity of aqueous methanolic extract of *Euphorbia granulata* Forssk. *Future Journal of Pharmaceutical Sciences*, 7, 1-10. <http://dx.doi.org/10.1186/s43094-021-00184-9>
- 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](https://doi.org/10.1016/S0039-9140(03)00238-8)
- Ghorai, N., Chakraborty, S., Gucchait, S., Saha, S. K., & Biswas, S. (2012). Estimation of total terpenoids concentration in plant tissues using a monoterpene, linalool as standard agent. *Protocol Exchange*, 5(10), 1038-1043.
- Ghosh, P., Ghosh, C., Das, S., Das, C., Mandal, S., & Chatterjee, S. (2019). Botanical description, phytochemical constituents and pharmacological properties of

- Euphorbia hirta* Linn: A review. *International Journal of Health Sciences and Research*, 9(3), 273-286.
- Ghosh, R., Singha, P. S., Das, L. K., Ghosh, D., & Firdaus, S. B. (2023). Anti-inflammatory activity of natural coumarin compounds from plants of the Indo-Gangetic plain. *AIMS Molecular Science*, 10(2), 79-98. <https://doi.org/10.3934/molsci.2023007>
- Giannattasio-Ferraz, S., Ene, A., Gomes, V. J., Queiroz, C. O., Maskeri, L., Oliveira, A. P., ... & Barbosa-Stancioli, E. F. (2022). *Escherichia coli* and *Pseudomonas aeruginosa* isolated from urine of healthy bovine have potential as emerging human and bovine pathogens. *Frontiers in Microbiology*, 13, 764760. <https://doi.org/10.3389/fmicb.2022.764760>
- Giménez-Abián, M. I., Giménez-Abián, J. F., Utrilla, L., & De la Torre, C. (2004). Nuclear ploidy is contingent on the microtubular cycle responsible for plant cytokinesis. *Protoplasma*, 224(1), 41-47.
- Gomathi, D., Ravikumar, G., Kalaiselvi, M., Vidya, B., & Uma, C. (2015). *In vitro* free radical scavenging activity of ethanolic extract of the whole plant of *Evolvulus alsinoides* (L.) L. *Chinese Journal of Integrative Medicine*, 21(6), 453-458. <https://doi.org/10.1007/s11655-014-1763-0>
- Gopi, K., Renu, K., Vishwanath, B. S., & Jayaraman, G. (2015). Protective effect of *Euphorbia hirta* and its components against snake venom induced lethality. *Journal of Ethnopharmacology*, 165, 180-190. <https://doi.org/10.1016/j.jep.2015.02.044>
- Górka, K., & Kubiński, K. (2023). Antifungal activity against human and plant mycopathogens, and green synthesis of silver nanoparticles exhibiting such activity. *Applied Sciences*, 14(1), 115. <https://doi.org/10.3390/app14010115>
- Goyal, M., Sasmal, D., & Nagori, B. P. (1970). GCMS analysis and antimicrobial action of latex of *Euphorbia caducifolia*. *Journal of Complementary Medicine Research*, 1(2), 119-123. <http://dx.doi.org/10.5455/jice.20120618045914>
- Grant, W. F. (1994). The present status of higher plant bioassays for the detection of environmental mutagens. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 310(2), 175-185. [https://doi.org/10.1016/0027-5107\(94\)90112-0](https://doi.org/10.1016/0027-5107(94)90112-0)
- Grimm, D. (2023). Recent advances in breast cancer research. *International Journal of Molecular Sciences*, 24(15), 11990. <https://doi.org/10.3390/ijms241511990>
- Grosso, G., Yang, J., Marventano, S., Micek, A., Galvano, F., & Kales, S. N. (2015). Nut consumption on all-cause, cardiovascular, and cancer mortality risk: A systematic review and meta-analysis of epidemiologic studies. *The American Journal of Clinical Nutrition*, 101(4), 783-793. <https://doi.org/10.3945/ajcn.114.099515>
- Gulcin, İ. (2025). Antioxidants: A comprehensive review. *Archives of Toxicology*, 99(5), 1893-1997. <https://doi.org/10.1007/s00204-025-03997-2>
- Gullon, B., Lú-Chau, T. A., Moreira, M. T., Lema, J. M., & Eibes, G. (2017). Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology*, 67, 220-235. <http://dx.doi.org/10.1016/j.tifs.2017.07.008>

- Gunathilake, K. D. P. P., Ranaweera, K. K. D. S., & Rupasinghe, H. V. (2018). *In vitro* anti-inflammatory properties of selected green leafy vegetables. *Biomedicines*, 6(4), 107. <https://doi.org/10.3390%2Fbiomedicines6040107>
- Guo, Y. Q., Tang, G. H., Lou, L. L., Li, W., Zhang, B., Liu, B., & Yin, S. (2018). Prenylated flavonoids as potent phosphodiesterase-4 inhibitors from *Morus alba*: Isolation, modification, and structure-activity relationship study. *European Journal of Medicinal Chemistry*, 144, 758-766. <https://doi.org/10.1016/j.ejmech.2017.12.057>
- Guo, Z., Xu, Y., Han, L., Bo, X., Huang, C., & Ni, L. (2011). Antioxidant and cytotoxic activity of the acetone extracts of root of *Euphorbia hylonoma* and its ellagic acid derivatives. *Journal of Medical Plants Research*, 5(23), 5584-5589. <https://doi.org/10.5897/JMPR.9000214>
- Gupta, A., Jeyakumar, E., & Lawrence, A. R. (2021). Pyrogallol: A competent therapeutic agent of the future. *Biotech Environmental Science*, 23(2), 213-217.
- Gupta, C. (2023). Bioactive phytochemicals on humanoid endurance and advancement of herbal medicine. *Journal of Microbiology and Experimentation*, 11(5), 125-135. <http://dx.doi.org/10.15406/jmen.2023.11.00400>
- Gupta, S. C., Prasad, S., Kim, J. H., Patchva, S., Webb, L. J., Priyadarsini, I. K., & Aggarwal, B. B. (2011). Multitargeting by curcumin as revealed by molecular interaction studies. *Natural Product Reports*, 28(12), 1937-1955. <https://doi.org/10.1039/c1np00051a>
- Haida, Z., & Hakiman, M. (2019). A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. *Food Science & Nutrition*, 7(5), 1555-1563. <https://doi.org/10.1002%2Ffsn3.1012>
- Hajhashemi, V., Ghanadian, M., Palizaban, A., Mahnam, K., Eshaghi, H., Gheisari, B., & Sadeghi-Aliabadi, H. (2022). Cycloart-23-ene-3 $\beta$ , 25-diol a pentacyclic steroid from *Euphorbia spinidens*, as COX inhibitor with molecular docking, and *in vivo* study of its analgesic and anti-inflammatory activities in male swiss mice and wistar rats. *Prostaglandins & Other Lipid Mediators*, 150, 106473. <https://doi.org/10.1016/j.prostaglandins.2020.106473>
- Haque, M. A., Wang, Y., Shen, Z., Li, X., Saleemi, M. K., & He, C. (2020). Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. *Microbial Pathogenesis*, 142 (2020) 104095-104107. <https://doi.org/10.1016/j.micpath.2020.104095>
- Harborne, A. J. (1998) *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd edition. Springer Pvt. Ltd., New Delhi.
- Harsha, L., Jain, R. K., & Prasad, A. S. (2022). Synthesis, characterization, and antimicrobial activity of silver nanoparticles derived from *Mentha X piperita*+ *Ocimum tenuiflorum*: An *in vitro* study. *Journal of Advanced Pharmaceutical Technology & Research*, 13(Suppl 1), S272-S276.
- Hashem, A. H., Saied, E., Amin, B. H., Alotibi, F. O., Al-Askar, A. A., Arishi, A. A., ... & Elbahnasawy, M. A. (2022). Antifungal activity of biosynthesized silver nanoparticles (AgNPs) against *Aspergilli* causing aspergillosis: Ultrastructure Study. *Journal of Functional Biomaterials*, 13(4), 242. <https://doi.org/10.3390/jfb13040242>

- Hassan, A., Yaqoob, U., Nawchoo, I. A., Gulzar, S., Mohi-Ud-Din, G., Nazir, S., & Ashraf, A. (2016). Conspectus of phytochemical constituents of *Euphorbia wallichii* Hook. f.: A review. *Research & Reviews Journal of Botany*, 5, 24-31.
- Hassan, H. M. A. (2015). A short history of the use of plants as medicines from ancient times. *Chimia*, 69(10), 622-622. 10.2533/chimia.2015.
- 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>
- He, L., Lv, H., Chen, N., Wang, C., Zhou, W., Chen, X., & Zhang, Q. (2020). Improving fermentation, protein preservation and antioxidant activity of *Moringa oleifera* leaves silage with gallic acid and tannin acid. *Bioresource Technology*, 297, 122390. <https://doi.org/10.1016/j.biortech.2019.122390>
- Heendeniya, S. N., Ratnasooriya, W. D., & Pathirana, R. N. (2018). *In vitro* investigation of anti-inflammatory activity and evaluation of phytochemical profile of *Syzygium caryophyllatum*. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 1759-1763.
- Heredia, D., Green, I., Klaasen, J., & Rahiman, F. (2022). Importance and relevance of phytochemicals present in *Galenia africana*. *Scientifica*, 2022., 1-12. <https://doi.org/10.1155/2022/5793436>
- Hosseingholian, A., Gohari, S. D., Feirahi, F., Moammeri, F., Mesbahian, G., Moghaddam, Z. S., & Ren, Q. (2023). Recent advances in green synthesized nanoparticles: From production to application. *Materials Today Sustainability*, 24, 100500. <https://doi.org/10.1016/j.mtsust.2023.100500>
- Hu, X., Shao, P., Liu, X., Han, L., Gui, L., Cai, Z., ... & Dai, C. (2022). Study on the anti-inflammatory effect and mechanism of yuxuebi tablet based on network pharmacology. *ACS Omega*, 7(36), 32784-32794. <https://doi.org/10.1021%2Facs.omega.2c04641>
- Hua, J., Liu, Y. C., Jing, S. X., Luo, S. H., & Li, S. H. (2015). Macrocyclic diterpenoids from the latex of *Euphorbia helioscopia*. *Natural Product Communications*, 10(12), 2037-2039. <http://dx.doi.org/10.1177/1934578X1501001206>
- Hua, J., Liu, Y., Xiao, C. J., Jing, S. X., Luo, S. H., & Li, S. H. (2017). Chemical profile and defensive function of the latex of *Euphorbia peplus*. *Phytochemistry*, 136, 56-64. <https://doi.org/10.1016/j.phytochem.2016.12.021>
- Huang, D., Pan, Y. H., Yuan, F. Y., Li, W., Yan, X. L., Zou, M. F., Tang, G. H., & Yin, S. (2021). Euphohyrinoids A and B, two highly rearranged lathyrane diterpenoids from *Euphorbia lathyris*. *Organic Letters*, 23(24), 9602-9605. <https://doi.org/10.1021/acs.orglett.1c03812>
- Huang, P. Z., Yang, H. Y., Zhang, L. J., Sun, Y., Li, Y., & Gao, K. (2023). Anti-inflammatory activities of triterpenoids from *Euphorbia humifusa* Willd. *Phytochemistry Letters*, 54, 129-132.
- Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*, 15(1), 1-23.
- Hue, B. T. B., & Tram, N. T. T. (2020). Chemical constituents from n-hexane and ethyl acetate extracts of *Euphorbia hirta* L. grown in Vietnam. In *IOP Conference Series*:

- Materials Science and Engineering* (Vol. 736, No. 2, p. 022083). IOP Publishing. <http://dx.doi.org/10.1088/1757-899X/736/2/022083>
- Hujon, F., & Saral, A. M. (2022). Chemical investigation of epicuticular wax obtained from *Euphorbia milii* leaves. *SN Applied Sciences*, 4(4), 122. <http://dx.doi.org/10.1007/s42452-022-05002-2>
- Ibrahim, H. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*, 8(3), 265-275. <https://doi.org/10.1016/j.jrras.2015.01.007>
- Ibrahim, M. B., Sowemimo, A. A., & Odukoya, O. A. (2014). Chromosomal aberrations induced by *Markhamia tomentosa* (Benth.) K. Schum. ex Engl. ethanolic leaf extract on *Allium cepa* root cells. *Nigerian Journal of Natural Products and Medicine*, 18, 12-17. <http://dx.doi.org/10.4314/njnp.v18i1.3>
- Ijaz, I., Bukhari, A., Gilani, E., Nazir, A., Zain, H., & Saeed, R. (2022). Green synthesis of silver nanoparticles using different plants parts and biological organisms, characterization and antibacterial activity. *Environmental Nanotechnology, Monitoring & Management*, 18, 100704. <https://doi.org/10.1016/j.enmm.2022.100704>
- Ilahi, I., Samar, S., Khan, I., & Ahmad, I. (2013). *In vitro* antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. *Pakistan Journal of Pharmaceutical Sciences*, 26(5), 949-952.
- Imani, A., Maleki, N., Bohlouli, S., Kouhsoltani, M., Sharifi, S., & Maleki Dizaj, S. (2021). Molecular mechanisms of anticancer effect of rutin. *Phytotherapy Research*, 35(5), 2500-2513. <https://doi.org/10.1002/ptr.6977>
- Imran, M., Saeed, F., Hussain, G., Imran, A., Mehmood, Z., Gondal, T. A., El-Ghorab, A., Ahmad, I., Pezzani, R., Arshad, M. U., Bacha, U., Shariarti, M. A., Rauf, A., Muhammad, N., Shah, Z. A., Zengin, G., & Islam, S. (2021). Myricetin: A comprehensive review on its biological potentials. *Food Science & Nutrition*, 9(10), 5854-5868. <https://doi.org/10.1002/2Ffsn3.2513>
- Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Science*, 9(6), 385-406.
- Islam, M. T. (2017). Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurological Research*, 39(1), 73-82. <https://doi.org/10.1080/01616412.2016.1251711>
- Ismail, A., Mohamed, M., Kwei, Y. F., & Yin, K. B. (2019). *Euphorbia hirta* methanolic extract displays potential antioxidant activity for the development of local natural products. *Pharmacognosy Research*, 11(1), 78. [http://dx.doi.org/10.4103/pr.pr\\_113\\_18](http://dx.doi.org/10.4103/pr.pr_113_18)
- Jacob, A., & Sathiyajith, J. N. (2022). Phytochemical profiling, *in vitro* antioxidant activity of *Euphorbia hirta* extracts and *in silico* study targeting human peroxiredoxin 5 receptor. *Current Trends in Biotechnology and Pharmacy*, 16(3), 344-356.
- Jain, A. S., Pawar, P. S., Sarkar, A., Junnuthula, V., & Dyawanapelly, S. (2021). Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial

- applications. *International Journal of Molecular Sciences*, 22(21), 11993. <https://doi.org/10.3390/ijms222111993>
- James, O., & Friday, E. T. (2010). Phytochemical composition, bioactivity and wound healing potential of *Euphorbia heterophylla* (Euphorbiaceae) leaf extract. *International Journal on Pharmaceutical and Biomedical Research*, 1(1), 54-63.
- Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, 7(1), 1-7. <http://dx.doi.org/10.15171/jhp.2018.01>
- Jan, I., Andrabi, N. I., Arfath, Y., Akram, T., Kotra, T., Ahmed, Z., & Kumar, M. (2022). Anti-inflammatory potential of *Euphorbia helioscopia* extracts against RAW264. 7 macrophages. *Pharmacognosy Research*, 14(3), 338-341. <http://dx.doi.org/10.5530/pres.14.3.50>
- Jassbi, A. R. (2006). Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. *Phytochemistry*, 67(18), 1977-1984. <https://doi.org/10.1016/j.phytochem.2006.06.030>
- Jayaveera, K. N., Yoganandham, R. K., Govindarajula, Y., & Kumanan, R. (2010). Phytochemical screenings, antibacterial activity and physico chemical constants of ethanolic extract of *Euphorbia thymifolia* Linn. *International Journal of Pharmacy and Pharmaceutical Science*, 2(3), 81-82.
- Jeon, M. J., Roy, N. S., Choi, B. S., Oh, J. Y., Kim, Y. I., Park, H. Y., Um, T., Kim, N. S., Kim, S., & Choi, I. Y. (2022). Identifying terpenoid biosynthesis genes in *Euphorbia maculata* via full-length cDNA sequencing. *Molecules*, 27(14), 4591. <https://doi.org/10.3390/molecules27144591>
- Jeong, S. H., Kim, H. H., Ha, S. E., Park, M. Y., Bhosale, P. B., Abusaliya, A., ... & Kim, G. S. (2022). Flavones: Six selected flavones and their related signaling pathways that induce apoptosis in cancer. *International Journal of Molecular Sciences*, 23(18), 10965-10977. <https://doi.org/10.3390/ijms231810965>
- Jeong, S. H., Kim, J., & Min, H. (2018). *In vitro* anti-inflammatory activity of the *Artemisia montana* leaf ethanol extract in macrophage RAW 264.7 cells. *Food and Agricultural Immunology*, 29(1), 688-698. <https://doi.org/10.1080/09540105.2018.1439454>
- Jian, B., Zhang, H., Han, C., & Liu, J. (2018). Anti-cancer activities of diterpenoids derived from *Euphorbia fischeriana* Steud. *Molecules*, 23(2), 387. <https://doi.org/10.3390/molecules23020387>
- Jiménez-González, V., Kowalczyk, T., Piekarski, J., Szemraj, J., Rijo, P., & Sitarek, P. (2023). Nature's green potential: Anticancer properties of plants of the Euphorbiaceae family. *Cancers*, 16(1), 114. <https://doi.org/10.3390/cancers16010114>
- Jing, Y., Nakajo, S., Xia, L., Nakaya, K., Fang, Q., Waxman, S., & Han, R. (1999). Boswellic acid acetate induces differentiation and apoptosis in leukemia cell lines. *Leukemia Research*, 23(1), 43-50. [https://doi.org/10.1016/s0145-2126\(98\)00096-4](https://doi.org/10.1016/s0145-2126(98)00096-4)

- Joshi, T., & Juyal, V. (2021). Evaluation of antioxidant activity of some medicinal plants and their combination. *Pharmacognosy Journal*, 13(2), 596-599. <http://dx.doi.org/10.5530/pj.2021.13.75>
- Jothi, G. J., Benniamin, A., & Manickam, V. S. (2008). Glimpses of tribal botanical knowledge of Tirunelveli hills, Western ghats, India. *Ethnobotanical Leaflets*, 2008(1), 14.
- Ju, Z., Li, M., Xu, J., Howell, D. C., Li, Z., & Chen, F. E. (2022). Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. *Acta Pharmaceutica Sinica B*, 12(6), 2790-2807. <https://doi.org/10.1016/j.apsb.2022.01.002>
- Kadam, S. T., & Pawar, A. D. (2020). Conservation of medicinal plants: A review. *International Ayurvedic Medicine Journal*, 8, 3890-3895. **Error! Hyperlink reference not valid.**
- Kainsa, S., & Singh, R. (2016). Flavan-3-ol isomers isolated from *Euphorbia thymifolia* Linn. *Pharmacognosy Communications*, 6(1), 28. <http://dx.doi.org/10.5530/pc.2016.1.5>
- Kalaivani, S., Jayanthi, S., Revathi, K., & Chandrasekaran, R. (2023). Phytochemical profile of *Euphorbia hirta* plant extract and its *in vitro* anticancer activity against the liver cancer HepG2 cells. *Vegetos*, 37 (2), 1-8.
- Kamal, N., Ilowefah, M. A., Hilles, A. R., Anua, N. A., Awın, T., Alshwyeh, H. A., Aldosary, S. K., Jambocus, N. G. S., Alosaimi, A. A., Rahman, A., Mahmood, S., & Mediani, A. (2022). Genesis and mechanism of some cancer types and an overview on the role of diet and nutrition in cancer prevention. *Molecules*, 27(6), 1794. <https://doi.org/10.3390/molecules27061794>
- Kamalanathan, D., Srinivasan, R., Pratheeba, T., Yuvarajan, R., & Natarajan, D. (2015). Antioxidant activities of leaf extracts of *Euphorbia fusiformis* Buch.-Ham. ex D. Don (Euphorbiaceae). *Free Radicals and Antioxidants*, 5(2), 83-89. <http://dx.doi.org/10.5530/fra.2015.2.6>
- Kamurthy, H., & Dontha, S. (2014). Phytochemical screening on *Euphorbia milii* red flowers—Isolation of terpenoids, flavone and phenols. *American Journal of Ethnomedicine*, 2, 322-332.
- Karagöz, A., Artun, F. T., Özcan, G., Melikoğlu, G., Anıl, S., Kültür, Ş., & Sütülpınar, N. (2015). *In vitro* evaluation of antioxidant activity of some plant methanol extracts. *Biotechnology & Biotechnological Equipment*, 29(6), 1184-1189. <http://dx.doi.org/10.1080/13102818.2015.1080600>
- Kari, S., Subramanian, K., Altomonte, I. A., Murugesan, A., Yli-Harja, O., & Kandhavelu, M. (2022). Programmed cell death detection methods: A systematic review and a categorical comparison. *Apoptosis*, 27(7), 482-508. <https://doi.org/10.1007/s10495-022-01735-y>
- Karpuz, M., Silindir-Gunay, M., & Ozer, A. Y. (2018). Current and future approaches for effective cancer imaging and treatment. *Cancer Biotherapy & Radiopharmaceuticals*, 33(2), 39-51. <https://doi.org/10.1089/cbr.2017.2378>
- Kaur, N., Bhatia, A., & Sidhu, S. (2017). Evaluation of antibacterial activity of plant extracts of *Euphorbia thymifolia* against human pathogenic bacteria. *International Journal*

- of *Emerging Research in Management and Technology*, 6(8), 335-338. <http://dx.doi.org/10.23956/ijermt.v6i8.162>
- Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48, 412-422. <https://doi.org/10.1007%2Fs13197-011-0251-1>
- Keerthana, K., Deepa, A., Shobana, G., Jothi, G., & Sridharan, G. (2014). Preliminary phytochemical screening and *in vitro* antioxidant potential of *Euphorbia heterophylla* L. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(8), 549-553.
- Kefayati, Z., Motamed, S. M., Shojaii, A., Noori, M., & Ghods, R. (2017). Antioxidant activity and phenolic and flavonoid contents of the extract and subfractions of *Euphorbia splendida* Mobayen. *Pharmacognosy Research*, 9(4), 362-365. [https://doi.org/10.4103/pr.pr\\_12\\_17](https://doi.org/10.4103/pr.pr_12_17)
- Kemala, P., Idroes, R., Khairan, K., Ramli, M., Jalil, Z., Idroes, G. M., ... & Nasution, R. (2022). Green synthesis and antimicrobial activities of silver nanoparticles using *Calotropis gigantea* from Ie Seu-Um Geothermal area, Aceh Province, Indonesia. *Molecules*, 27(16), 5310. <https://doi.org/10.3390%2Fmolecules27165310>
- Kemboi, D., Peter, X., Langat, M., & Tembu, J. (2020). A review of the ethnomedicinal uses, biological activities, and triterpenoids of *Euphorbia* species. *Molecules*, 25(17), 4019-4028. <https://doi.org/10.3390%2Fmolecules25174019>
- Kemboi, D., Siwe-Noundou, X., Krause, R. W., Langat, M. K., & Tembu, V. J. (2021). *Euphorbia* diterpenes: An update of isolation, structure, pharmacological activities and structure–activity relationship. *Molecules*, 26(16), 5055. <https://doi.org/10.3390/molecules26165055>
- Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B., & Nath, G. (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *Journal of Ayurveda and Integrative Medicine*, 11(1), 37-44. <https://doi.org/10.1016/j.jaim.2017.11.003>
- Khade, O. S. K., Sonkar, R. M., Gade, P. S., & Bhatt, P. (2023). Plant secondary metabolites: Extraction, screening, analysis and their bioactivity. *International Journal of Herbal Medicine*, 11(2), 1-17. <http://dx.doi.org/10.22271/flora.2023.v11.i2a.855>
- Khan, F., Pandey, P., Verma, M., & Upadhyay, T. K. (2024). Terpenoid-mediated targeting of STAT3 signaling in cancer: An overview of preclinical studies. *Biomolecules*, 14(2), 200-215. <https://doi.org/10.3390/biom14020200>
- Khan, I., & Javaid, A. (2021). Identification of biologically important compounds in neem leaves through GC-MS analysis. *Jordan Journal of Pharmaceutical Sciences*, 14(3), 359-366.
- Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K. (2020). Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*, 10(1), 47. <https://doi.org/10.3390/biom10010047>



- Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K. (2019). Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*, 10(1), 47. <https://doi.org/10.3390/biom10010047>
- Khattak, U., Ullah, R., Khan, S. A., Jan, S. A., Rauf, A., & Ramadan, M. F. (2019). Synthesis, characteristics and biological activities of silver nanoparticles from *Euphorbia dracunculoides*. *Eurasian Journal of BioSciences*, 13(2), 2249–2260.
- Khursheed, A., & Jain, V. (2022). *Euphorbia hirta* as a gold mine of high-value phytochemicals: A comprehensive review of its pharmacological activities and possible role against SARS-CoV-2. *Biomedical Research and Therapy*, 9(2), 4930–4949. <https://doi.org/10.15419/bmrat.v9i2.728>
- Kim, M., Kim, J., Shin, Y. K., & Kim, K. Y. (2020). Gentisic acid stimulates keratinocyte proliferation through ERK1/2 phosphorylation. *International Journal of Medical Sciences*, 17(5), 626–631. <https://doi.org/10.7150%2Fijms.36484>
- Kocazorbaz, E. K. (2021). Green synthesis, optimization, and characterization of silver nanoparticles from *Euphorbia rigida* leaf extract and investigation of their antimicrobial activities. *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi*, 8(2), 512–522. <https://doi.org/10.35193/bseufbd.843005>
- Kommu, S., Eswaraiah, M. C., Akhila, B., Pushkara, U., & Ramadevi, T. (2023). *In vitro* antiinflammatory activity of *Tamilnadia uliginosa* fruit extract. *Research Journal of Pharmacy and Technology*, 16(4), 2022–2024.
- Konnikova, L., Kotecki, M., Kruger, M. M., & Cochran, B. H. (2003). Knockdown of STAT3 expression by RNAi induces apoptosis in astrocytoma cells. *BMC Cancer*, 3, 1–9. <https://doi.org/10.1186%2F1471-2407-3-23>
- Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89(3), 217–233. <http://dx.doi.org/10.1016/j.fbp.2010.04.008>
- Krishnaveni, M. (2015). Docking, simulation studies of desulphosinigrin–cyclin dependent kinase 2, an anticancer drug target. *International Journal of Pharmaceutical Sciences Review and Research*, 30(2), 115–118.
- Krupanidhi, A. M., Dabadi, P., Sameera, H. R., Anusha, M. M., Deepika, B. V., Srinivas, G., & Soundarya, R. (2022). Anti-oxidant activities of considerable medicinal plants: A review article. *Systematic Reviews in Pharmacy*, 13 (7), 457–463.
- Kuang, X., Li, W., Kanno, Y., Yamashita, N., Nemoto, K., Asada, Y., & Koike, K. (2016). ent-Atisane diterpenoids from *Euphorbia fischeriana* inhibit mammosphere formation in MCF-7 cells. *Journal of Natural Medicines*, 70, 120–126. <https://doi.org/10.1007/s11418-015-0940-6>
- Kumar, A. P. N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T. K. S., & Oz, F. (2023). Major phytochemicals: Recent advances in health benefits and extraction method. *Molecules*, 28(2), 887. <https://doi.org/10.3390%2Fmolecules28020887>
- Kumar, A., & Saikia, D. (2016). *Euphorbia antiquorum* Linn.: A comprehensive review of ethnobotany, phytochemistry and pharmacology. *Journal of Analytical and*

- 
- Pharmaceutical Research*, 2(4), 00024. <https://doi.org/10.15406/japlr.2016.02.00024>
- Kumar, A., Vishakha, D. A., Jeet, K., & Kumar, S. (2022). A review on silver nanoparticles focusing on applications in biomedical sector. *International Journal of Pharmaceutical Science and Developmental Research*, 8, 57-63. <https://doi.org/10.17352/ijpsdr.000043>
- 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, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African Journal of Biochemistry Research*, 4(7), 191-195.
- Kumar, S. S., Melchias, G., Ravikumar, P., Chandrasekar, R., & Kumaravel, P. (2014). Bioinspired synthesis of silver nanoparticles using *Euphorbia hirta* leaf extracts and their antibacterial activity. *Asian Journal of Pharmaceutical Research*, 4(1), 39-43.
- 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
- Kuo, Y. H., Chang, C. I., Li, S. Y., Chou, C. J., Chen, C. F., Kuo, Y. H., & Lee, K. H. (1997). Cytotoxic constituents from the stems of *Diospyros maritima*. *Planta Medica*, 63(4), 363-365. <https://doi.org/10.1055/s-2006-957703>
- Kuruppuarachchi, S. U., Jayawardena, U. A., & Gunathilake, V. K. (2023). Use of the *Allium cepa* model to assess the cytogenotoxicity of *Luffariella herdmanni* marine sponge extract. *Alternatives to Laboratory Animals*, 51(3), 175-187. **Error! Hyperlink reference not valid.**
- Kwan, Y. P., Saito, T., Ibrahim, D., Al-Hassan, F. M. S., Ein Oon, C., Chen, Y., Jothy, S. L., Kanwar, J. R., & Sasidharan, S. (2016). Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells. *Pharmaceutical Biology*, 54(7), 1223-1236. <https://doi.org/10.3109/13880209.2015.1064451>
- Laganà, P., Anastasi, G., Marano, F., Piccione, S., Singla, R. K., Dubey, A. K., Delia, S., Coniglio, M. A., Facciola, A., Di Pietro, A., Haddad, M. A., Al-Hiary, M., & Caruso, G. (2019). Phenolic substances in foods: Health effects as anti-inflammatory and antimicrobial agents. *Journal of AOAC International*, 102(5), 1378-1387. <http://dx.doi.org/10.1093/jaoac/102.5.1378>
- Lahlou, F. A., Hmimid, F., Loutfi, M., & Bourhim, N. (2014). Antioxidant activity and quantification of phenolic compounds of *Euphorbia echinus*. *International Journal of Pharmacy and Pharmaceutical Science*, 6, 357-360.
- Lahmadi, S., Belhamra, M., Karoune, S., Imad, K., Bensouici, C., Kechabar, M. S. A., Halis, Y., & Ksouri, R. (2020). Phenolic constituents and antioxidant activity of *Euphorbia retusa* Forssk. *Natural Product Research*, 34(24), 3545-3547. <https://doi.org/10.1080/14786419.2019.1582040>
- Lai, W. L., Lee, S. C., Chang, K. F., Huang, X. F., Li, C. Y., Lee, C. J., ... & Tsai, N. M. (2021). *Juniperus communis* extract induces cell cycle arrest and apoptosis of
-

- colorectal adenocarcinoma in vitro and in vivo. *Brazilian Journal of Medical and Biological Research*, 54, e10891. <https://doi.org/10.1515/chem-2021-0195>
- Lai, X. Z., Yang, Y. B., & Shan, X. L. (2004). The investigation of euphorbiaceous medicinal plants in Southern China. *Economic Botany*, 58(1), S307-S320.
- Lakshmi, T., Ezhilarasan, D., Vijayaragavan, R., Bhullar, S. K., & Rajendran, R. (2017). *Acacia catechu* ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. *Journal of Advanced Pharmaceutical Technology & Research*, 8(4), 143-149. [http://dx.doi.org/10.4103/japtr.JAPTR\\_73\\_17](http://dx.doi.org/10.4103/japtr.JAPTR_73_17)
- Lan, Y. H., Yen, C. H., & Leu, Y. L. (2020). Chemical constituents from the aerial parts of *Euphorbia formosana* Hayata and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, 88, 103967. [https://ui.adsabs.harvard.edu/link\\_gateway/2020BioSE..8803967L/doi:10.1016/j.bse.2019.103967](https://ui.adsabs.harvard.edu/link_gateway/2020BioSE..8803967L/doi:10.1016/j.bse.2019.103967)
- Laverty, G., Gorman, S. P., & Gilmore, B. F. (2014). Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens*, 3(3), 596-632. <https://doi.org/10.3390/pathogens3030596>
- Le, D. T. K., Bui, H. X., Nguyen, T. T. A., Pham, T. N. K., & Duong, H. T. (2018). Chemical constituents of *Euphorbia tirucalli* L. *VNUHCM Journal of Natural Sciences*, 2(5), 76-82. <http://dx.doi.org/10.32508/stdjns.v2i5.781>
- Leme, D. M., & Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research/Reviews in Mutation Research*, 682(1), 71-81. <https://doi.org/10.1016/j.mrrev.2009.06.002>
- Lepoivre, M., Chenais, B., Yapo, A., Lemaire, G., Thelander, L., & Tenu, J. P. (1990). Alterations of ribonucleotide reductase activity following induction of the nitrite generating pathway in adenocarcinoma. *Journal of Biological Chemistry*, 265(24), 14143-14149.
- Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orčić, D., & Mimica-Dukić, N. (2018). Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *Journal of Functional Foods*, 40, 68-75. <http://dx.doi.org/10.1016/j.jff.2017.10.047>
- Levan, A. (1938). The effect of colchicine on root mitoses in *Allium*. *Hereditas*, 24, 471-486.
- Li, W., Tang, Y. Q., Chen, S. X., Tang, G. H., Gan, L. S., Li, C., Rao, Y., Huang, Z. S., & Yin, S. (2019). Euphorhelipanes A and B, triglyceride-lowering *Euphorbia* diterpenoids with a bicyclo [4.3. 0] nonane core from *Euphorbia helioscopia*. *Journal of Natural Products*, 82(2), 412-416. <https://doi.org/10.1021/acs.jnatprod.8b00780>
- Li, X. Q., Bai, Y. L., Zhang, D. L., Jiao, H. S., & He, R. X. (2018). Euphornin reduces proliferation of human cervical adenocarcinoma HeLa cells through induction of apoptosis and G2/M cell cycle arrest. *Oncotargets and Therapy*, 4395-4405. <https://doi.org/10.2147%2FOTT.S166018>
- Li, X., Niu, Z., Sun, C., Zhuo, S., Yang, H., Yang, X., ... & Zhu, J. (2022). Regulation of p53 signaling in breast cancer by the E3 ubiquitin ligase RNF187. *Cell Death & Disease*, 13(2), 149. <https://doi.org/10.1038/s41419-022-04604-3>

- Li, Y. Y., Yang, Y., Sun, M., Lu, Q. Y., Pu, X. X., Ran, X., ... & Hao, X. J. (2022). Jatrophone polyesters from the leaves of *Euphorbia peplus* with anti-inflammatory activity. *Phytochemistry Letters*, 49, 114-119. <http://dx.doi.org/10.1016/j.phytol.2022.03.017>
- Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*, 21(10), 1374. <https://doi.org/10.3390/molecules21101374>
- Lin, J., Tang, H., Jin, X., Jia, G., & Hsieh, J. T. (2002). p53 regulates Stat3 phosphorylation and DNA binding activity in human prostate cancer cells expressing constitutively active Stat3. *Oncogene*, 21(19), 3082-3088. <https://doi.org/10.1038/sj.onc.1205426>
- Lingegowda, D. C., Kumar, J. K., Prasad, A. D., Zarei, M., & Gopal, S. (2012). FTIR spectroscopic studies on *Cleome gynandra*-Comparative analysis of functional group before and after extraction. *Romanian Journal of Biophysics*, 22(3-4), 137-143.
- Liu, J., Yu, W., Sun, L., Lu, W., Li, H., Liu, C., & Wei, H. (2021). Anti-inflammatory effect of red ginseng oil in lipopolysaccharide-stimulated RAW264.7 cells: Regulation of COX-2/NF- $\kappa$ B signaling pathway. *Frontiers in Pharmacology* 12: 628074.
- 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. <http://dx.doi.org/10.12659/MSMBR.893327>
- Liu, W. X., Zhang, Y. J., Zhou, M., & Zhao, P. (2021). A new ingol diterpenoid from the seeds of *Euphorbia marginata* Pursh. *Natural Product Research*, 37(1), 63-67. <https://doi.org/10.1080/14786419.2021.1948846>
- Liu, W., Cui, X., Zhong, Y., Ma, R., Liu, B., & Xia, Y. (2023). Phenolic metabolites as therapeutic in inflammation and neoplasms: Molecular pathways explaining their efficacy. *Pharmacological Research*, 193, 106812. <https://doi.org/10.1016/j.phrs.2023.106812>
- Liu, X. X., Ma, H., He, W., Sun, Y., & Lan, W. (2018). Phytochemical constituents isolated from *Euphorbia rapulum*. *Chemistry of Natural Compounds*, 54 (5), 1-3. <http://dx.doi.org/10.1007/s10600-018-2510-0>
- Liu, Y. J., Zhan, J., Liu, X. L., Wang, Y., Ji, J., & He, Q. Q. (2014). Dietary flavonoids intake and risk of type 2 diabetes: A meta-analysis of prospective cohort studies. *Clinical Nutrition*, 33(1), 59-63. <https://doi.org/10.1016/j.clnu.2013.03.011>
- Loganayaki, N., Siddhuraju, P., & Manian, S. (2013). Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *Journal of Food Science and Technology*, 50 (4), 687-695. <https://doi.org/10.1007/s13197-011-0389-x>
- Lončarić, M., Strelec, I., Moslavac, T., Šubarić, D., Pavić, V., & Molnar, M. (2021). Lipxygenase inhibition by plant extracts. *Biomolecules*, 11(2), 152-169. <https://doi.org/10.3390/biom11020152>
- Lotfí, N., Yousefi, Z., Golabi, M., Khalilian, P., Ghezelbash, B., Montazeri, M., Shams, M. H., Baghbadorani, P. Z., & Eskandari, N. (2023). The potential anti-cancer effects of

- quercetin on blood, prostate and lung cancers: An update. *Frontiers in Immunology*, 14, 1077531. <https://doi.org/10.3389/fimmu.2023.1077531>
- 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., Czezelewski, 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), 4287. <https://doi.org/10.3390/cancers13174287>
- Lutoti, S., Kaggwa, B., Kamba, P. F., Mukonzo, J., Sesaaizi, C. D., & Katuura, E. (2023). Ethnobotanical survey of medicinal plants used in breast cancer treatment by traditional health practitioners in central Uganda. *Journal of Multidisciplinary Healthcare*, 635-651. <https://doi.org/10.2147/JMDH.S387256>
- Ma, J. H., Qin, L., & Li, X. (2020). Role of STAT3 signaling pathway in breast cancer. *Cell Communication and Signaling*, 18, 1-13.
- Ma, X., Okyere, S. K., Hu, L., Wen, J., Ren, Z., Deng, J., & Hu, Y. (2022). Anti-inflammatory activity and mechanism of cryptochlorogenic acid from *Ageratina adenophora*. *Nutrients*, 14(3), 439. <https://doi.org/10.3390/n14030439>
- Magnani, C., Isaac, V. L. B., Correa, M. A., & Salgado, H. R. N. (2014). Caffeic acid: A review of its potential use in medications and cosmetics. *Analytical Methods*, 6(10), 3203-3210. <https://doi.org/10.1039/C3AY41807C>
- Magozwi, D. K., Dinala, M., Mokwana, N., Siwe-Noundou, X., Krause, R. W., Sonopo, M., ... & Tembu, V. J. (2021). Flavonoids from the genus *Euphorbia*: Isolation, structure, pharmacological activities and structure-activity relationships. *Pharmaceuticals*, 14(5), 428. <https://doi.org/10.3390/ph14050428>
- Magozwi, D. K., Peter, X., Langat, M. K., Mhlanga, R., Vukea, N., de la Mare, J. A., ... & Tembu, V. J. (2021). *In vitro* cytotoxic effects of chemical constituents of *Euphorbia grandicornis* Blanc against breast cancer cells. *Scientific African*, 14, e01002. <https://doi.org/10.1016/j.sciaf.2021.e01002>
- Mahdavi, B., Zare, H., Qorbani, M., Atabati, H., Kakhki, M. R. V., Raoofi, A., & Ebrahimi, V. (2022). *Euphorbia granulata* Forssk: Evaluation of antioxidant activity, cytotoxicity, and apoptosis induction in breast cancer cells. *South African Journal of Botany*, 150, 576-582. <https://doi.org/10.1016/j.sajb.2022.08.015>
- Mahendran, G., & Kumari, B. R. (2016). Biological activities of silver nanoparticles from *Nothapodytes nimmoniana* (Graham) Mabb. fruit extracts. *Food Science and Human Wellness*, 5(4), 207-218. <https://doi.org/10.1016/j.fshw.2016.10.001>
- Mahmod, M. R., Junayed, A., Bhowmick, C., Sompaa, S., Sultana, T., Akter, T., ... & Sikder, M. A. (2021). Antibacterial activity of silver nanoparticles synthesized from leaf and flower extracts of *Galinsoga formosa*. *Journal of Advanced Biotechnology and Experimental Therapeutics*, 4(2), 178-186. <https://doi.org/10.5455/jabet.2021.d118>
- Mahomoodally, M. F., Dall'Acqua, S., Sinan, K. I., Sut, S., Ferrarese, I., Etienne, O. K., Sadeer, N. B., Ak, G., & Zengin, G. (2020). Phenolic compounds analysis of three *Euphorbia* species by LC-DAD-MSn and their biological properties. *Journal of*

- Pharmaceutical and Biomedical Analysis*, 189, 113477. <https://doi.org/10.1016/j.jpba.2020.113477>
- Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Tiliansky, M. E. & Kalinina, N. O. (2014). "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. *Acta Naturae*, 6(1), 35-44. <http://dx.doi.org/10.32607/20758251-2014-6-1-35-44>
- Maleki, S. J., Crespo, J. F., & Cabanillas, B. (2019). Anti-inflammatory effects of flavonoids. *Food Chemistry*, 299, 125124-125135. <https://doi.org/10.1016/j.foodchem.2019.125124>
- Mali, P. Y., & Panchal, S. S. (2013). A review on phyto-pharmacological potentials of *Euphorbia thymifolia* L. *Ancient Science of Life*, 32(3), 165-172. <http://dx.doi.org/10.4103/0257-7941.123001>
- Mali, P. Y., & Panchal, S. S. (2017). *Euphorbia neriifolia* L.: Review on botany, ethnomedicinal uses, phytochemistry and biological activities. *Asian Pacific Journal of Tropical Medicine*, 10(5), 430-438. <https://doi.org/10.1016/j.apjtm.2017.05.003>
- Mans, D. R., Friperon, P., Pawirodihardjo, J., & Djotaroeno, M. (2020). Antioxidant activity of Surinamese medicinal plants with adaptogenic properties and correlation with total phenolic contents. *Journal of Antioxidant Activity*, 2(1), 11-28. <http://dx.doi.org/10.14302/issn.2471-2140.jaa-20-3478>
- Maoulainine, L. B. M., Jelassi, A., Hassen, I., Ould, O. M. S., & Boukhari, A. (2012). Antioxidant properties of methanolic and ethanolic extracts of *Euphorbia helioscopia* (L.) aerial parts. *International Food Research Journal*, 19(3), 1125-1130.
- Marei, H. E., Althani, A., Afifi, N., Hasan, A., Caceci, T., Pozzoli, G., ... & Cenciarelli, C. (2021). p53 signaling in cancer progression and therapy. *Cancer Cell International*, 21(1), 703. <https://doi.org/10.1186/s12935-021-02396-8>
- Marques, R. V., Sestito, S. E., Bourgaud, F., Miguel, S., Cailotto, F., Reboul, P., Jouzeau, J. Y., Rahuel-Clermont, S., Boschi-Muller, S., Simonsen, H. T., & Moulin, D. (2022). Anti-inflammatory activity of bryophytes extracts in LPS-stimulated RAW264.7 murine macrophages. *Molecules*, 27(6), 1940-1947. <https://doi.org/10.3390/molecules27061940>
- Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2), 48-78. <https://doi.org/10.3390/oxygen2020006>
- Marvalim, C., Datta, A., & Lee, S. C. (2023). Role of p53 in breast cancer progression: An insight into p53 targeted therapy. *Theranostics*, 13(4): 1421-1442, <https://doi.org/10.7150/2Fthno.81847>
- Mazumder, K., Aktar, A., Roy, P., Biswas, B., Hossain, M. E., Sarkar, K. K., Bachar, S. C., Ahmed, F., Monjur-Al-Hossain, A. S. M., & Fukase, K. (2022). A review on mechanistic insight of plant derived anticancer bioactive phytochemicals and their structure activity relationship. *Molecules*, 27(9), 3036. <https://doi.org/10.3390/molecules27093036>

- Mechchate, H., Es-Safi, I., Mohamed Al Kamaly, O., & Bousta, D. (2021). Insight into gentisic acid antidiabetic potential using *in vitro* and *in silico* approaches. *Molecules*, 26(7), 1932. <https://doi.org/10.3390%2Fmolecules26071932>
- Meda, R. N. T., Kam, S. E., Kagambega, W., Zongo, E., Ouedraogo, C., Segda, A., Koama, B. K., Somda, F. T., Zongo, E., & Ouedraogo, G. A. (2023). A review on bioactive compounds isolated from *Euphorbia hirta* L. *American Journal of Plant Sciences*, 14(6), 710-726. <https://doi.org/10.4236/ajps.2023.146048>
- Mekam, P. N., Martini, S., Nguetack, J., Tagliazucchi, D., & Stefani, E. (2019). Phenolic compound profile of water and ethanol extracts of *Euphorbia hirta* L. leaves showing antioxidant and antifungal properties. *South African Journal of Botany*, 127, 319-332. <http://dx.doi.org/10.1016/j.sajb.2019.11.001>
- Mendes, V., Vilaça, R., de Freitas, V., Ferreira, P. M., Mateus, N., & Costa, V. (2015). Effect of myricetin, pyrogallol, and phloroglucinol on yeast resistance to oxidative stress. *Oxidative Medicine and Cellular Longevity*, 2015. <https://doi.org/10.1155/2015/782504>
- Mendez-Encinas, M. A., Valencia, D., Ortega-García, J., Carvajal-Millan, E., Díaz-Ríos, J. C., Mendez-Pfeiffer, P., ... & Velazquez, C. (2023). Anti-inflammatory potential of seasonal *Sonoran propolis* extracts and some of their main constituents. *Molecules*, 28(11), 4496-4511. <https://doi.org/10.3390/ molecules28114496>
- Mesas, C., Martínez, R., Ortiz, R., Galisteo, M., López-Jurado, M., Cabeza, L., Perazzoli, G., Melguizo, C., Porres, J. M., & Prados, J. (2021). Antitumor effect of the ethanolic extract from seeds of *Euphorbia lathyris* in colorectal cancer. *Nutrients*, 13(2), 566. <https://doi.org/10.3390%2Fnu13020566>
- Metin, M., & Bürün, B. (2023). Antimicrobial and antioxidant activities of extracts of *Euphorbia rigida* Bieb. *Journal of Animal Plant Science*, 33 (3) 2023. <http://dx.doi.org/10.36899/JAPS.2023.3.0653>
- Michalak, M., Zagórska-Dziok, M., Klimek-Szczykutowicz, M., & Szopa, A. (2023). Phenolic profile and comparison of the antioxidant, anti-ageing, anti-inflammatory, and protective activities of *Borago officinalis* extracts on skin cells. *Molecules*, 28(2), 868-876. <https://doi.org/10.3390/molecules28020868>
- Miller, A. S., & Wilmott, R. W. (2019). The Pulmonary Mycoses. *Kendig's Disorders of the Respiratory Tract in Children*. Elsevier. 507-527. <https://doi.org/10.1016/B978-0-323-44887-1.00031-6>
- Min, X. Y., Chen, Y. N., Li, D. M., Mu, Q. Y., Zhang, Z. T., & Fang, X. (2021). Chemical constituents of *Euphorbia peplus*. *Biochemical Systematics and Ecology*, 98, 104307. <http://dx.doi.org/10.1016/j.bse.2021.104307>
- Mizushima, Y., & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, 20(3), 169-173.
- Mohamad Hanafiah, R., Abd Ghafar, S. A., Lim, V., Musa, S. N. A., Yakop, F., & Hairil Anuar, A. H. (2023). Green synthesis, characterisation and antibacterial activities of *Strobilanthes crispus*-mediated silver nanoparticles (SC-AGNPS) against selected

- bacteria. *Artificial Cells, Nanomedicine, and Biotechnology*, 51(1), 549-559. <https://doi.org/10.1080/21691401.2023.2268167>
- Monika, P. M., Sharma, K., & Yadav, M. (2016). Antioxidant effect of some medicinal plants: A review. *Inventi Rapid: Planta Activa*, 1(1), 1-8.
- Muchanyereyi, N. M., Muchenje, T., Nyoni, S., Shumba, M., & Mupa, M. (2017). Green synthesis of silver nanoparticles using *Euphorbia confinalis* stem extract, characterization and evaluation of antimicrobial activity. *Journal of Nanomaterials & Molecular Nanotechnology*, 6 (3), 1-6. <http://dx.doi.org/10.4172/2324-8777.1000220>
- Muhammad, A. A., Ogunmefun, O. T., Odesanya, B. O., & Okiki, P. A. (2017). *In vitro* anti-inflammatory activities of milkweed (*Euphorbia heterophylla* Linn.). *Der Pharma Chem*, 9(23), 82-88. <http://www.derpharmachemica.com/archive.html>
- Mukherjee, K., Bhagat, N., Kumari, M., Choudhury, A. R., Sarkar, B., & Ghosh, B. D. (2023). Insight study on synthesis and antibacterial mechanism of silver nanoparticles prepared from indigenous plant source of Jharkhand. *Journal of Genetic Engineering and Biotechnology*, 21(1): 30. <https://doi.org/10.1186/s43141-023-00463-3>
- Munro, B., Vuong, Q. V., Chalmers, A. C., Goldsmith, C. D., Bowyer, M. C., & Scarlett, C. J. (2015). Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. *Antioxidants*, 4(4), 647-661. <https://doi.org/10.3390/2Fantiox4040647>
- Mussin, J., & Giusiano, G. (2022). Biogenic silver nanoparticles as antifungal agents. *Frontiers in Chemistry*, 10, 1023542. <https://doi.org/10.3389/2Ffchem.2022.1023542>
- Mustafa, I., Faisal, M. N., Hussain, G., Muzaffar, H., Imran, M., Ijaz, M. U., Sohail, M. U., Ifthikhar, A., Shaukat, A., & Anwar, H. (2021). Efficacy of *Euphorbia helioscopia* in context to a possible connection between antioxidant and antidiabetic activities: A comparative study of different extracts. *BMC Complementary Medicine and Therapies*, 21, 1-12. **Error! Hyperlink reference not valid.**
- Mustapha, T., Misni, N., Ithnin, N. R., Daskum, A. M., & Unyah, N. Z. (2022). A review on plants and microorganisms mediated synthesis of silver nanoparticles, role of plants metabolites and applications. *International Journal of Environmental Research and Public Health*, 19(2), 674. <https://doi.org/10.3390/ijerph19020674>
- Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future Journal of Pharmaceutical Sciences*, 7, 1-13. <https://doi.org/10.1186/2Fs43094-020-00161-8>
- Muzamil, A., Tahir, H. M., Ali, S., Liaqat, I., Ali, A., & Summer, M. (2021). Inflammatory process and role of cytokines in inflammation: An overview. *Punjab University Journal Zoology*, 36, 237-252. <https://dx.doi.org/10.17582/journal.pujz/2021.36.2.237.252>
- Naidu, S. (2017). *Euphorbia venkatarajui* sp. nov. (Euphorbiaceae) from Eastern Ghats of Andhra Pradesh, India. *Nordic Journal of Botany*, 35(3), 359-364. <https://doi.org/10.1111/njb.01488>



- Nair, A., & Manohar, S. M. (2021). A flow cytometric journey into cell cycle analysis. *Bioanalysis*, 13(21), 1627-1644. <http://dx.doi.org/10.4155/bio-2021-0071>
- Naz, R., Ayub, H., Nawaz, S., Islam, Z. U., Yasmin, T., Bano, A., ... & Roberts, T. H. (2017). Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complementary and Alternative Medicine*, 17, 1-13. <https://doi.org/10.1186/s12906-017-1815-z>
- Ndam, L. M., Mih, A. M., Tening, A. S., Fongod, A. G. N., Temenu, N. A., & Fujii, Y. (2016). Phytochemical analysis, antimicrobial and antioxidant activities of *Euphorbia golondrina* LC Wheeler (Euphorbiaceae Juss.): An unexplored medicinal herb reported from Cameroon. *Springerplus*, 5, 1-15. <https://doi.org/10.1186%2Fs40064-016-1928-8>
- Neri, F., Scala, A., Grimato, S., Santoro, M., Spadaro, S., Barreca, F., ... & Fazio, E. (2016). Biocompatible silver nanoparticles embedded in a PEG-PLA polymeric matrix for stimulated laser light drug release. *Journal of Nanoparticle Research*, 18, 1-14.
- Ng, Y. P., Or, T. C. T., & Ip, N. Y. (2015). Plant alkaloids as drug leads for Alzheimer's disease. *Neurochemistry International*, 89, 260-270. <https://doi.org/10.1016/j.neuint.2015.07.018>
- Nicuță, D., Grosu, L., Patriciu, O. I., Voicu, R. E., & Alexa, I. C. (2025). The *Allium cepa* model: A review of its application as a cytogenetic tool for evaluating the biosafety potential of plant extracts. *Methods and Protocols*, 8(4), 88. **Error! Hyperlink reference not valid.**
- Nirmala, C., Sridevi, M., Aishwarya, A., Eswarkarthik, V., & Sindhu, B. (2023). Phytochemical prospecting, green synthesis of silver nanoparticles from *Euphorbia helioscopia* and its antibacterial activity. *International Journal of Nano Dimension*, 14(2), 157-166. <https://doi.org/10.22034/ijnd.2023.1974092.2192>
- Nitha, B., De, S., Adhikari, S. K., Devasagayam, T. P. A., & Janardhanan, K. K. (2010). Evaluation of free radical scavenging activity of morel mushroom, *Morchella esculenta* mycelia: A potential source of therapeutically useful antioxidants. *Pharmaceutical Biology*, 48(4), 453-460. <https://doi.org/10.3109/13880200903170789>
- Njeru, S. N., Matasyoh, J., Mwaniki, C. G., Mwendia, C. M., & Kobia, K. (2013). A review of some phytochemicals commonly found in medicinal plants. *International Journal of Medicinal Plants*, 105, 135-140.
- Njoya, Z. L. F., Mbiantcha, M., Nguemnang, S. F. D., Marthe, V. M. M., Nana, W. Y., Kouam, Y. K. M., ... & Ateufack, G. (2022). Anti-*Helicobacter pylori*, anti-inflammatory, and antioxidant activities of trunk bark of *Alstonia boonei* (Apocynaceae). *BioMed Research International*, 2022, 1-15. <https://doi.org/10.1155/2022/9022135>
- Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: A review. *Plants*, 11(15), 2011. <https://doi.org/10.3390/plants11152011>

- Nugroho, A., Heryani, H., & Istikowati, W. T. (2020). Quantitative determination of quercitrin and myricitrin in three different parts of *Euphorbia hirta* as bioflavonoid source for functional food. In *IOP Conference Series: Earth and Environmental Science* (Vol. 443, No. 1, p. 012042). IOP Publishing. <http://dx.doi.org/10.1088/1755-1315/443/1/012042>
- Nunes, C. D. R., Barreto Arantes, M., Menezes de Faria Pereira, S., Leandro da Cruz, L., de Souza Passos, M., Pereira de Moraes, L., Vieira, I. J. C., & Barros de Oliveira, D. (2020). Plants as sources of anti-inflammatory agents. *Molecules*, 25(16), 3726-3738. <https://doi.org/10.3390/molecules25163726>
- Oeckinghaus, A., Hayden, M. S., & Ghosh, S. (2011). Crosstalk in NF- $\kappa$ B signaling pathways. *Nature Immunology*, 12(8), 695-708. <https://doi.org/10.1038/ni.2065>
- Okokon, J. E., Dar, A., & Choudhary, M. I. (2012). Immunomodulatory, cytotoxic and antileishmanial activity of *Setaria megaphylla*. *International Journal of Phytomedicine*, 4(2), 155-161.
- Olajuyigbe, O. O., Onibudo, T. E., Cooposamy, R. M., Ashafa, A. O. T., & Afolayan, A. J. (2018). Bioactive compounds and *in vitro* antimicrobial activities of ethanol stem bark extract of *Trilepisium madagascariense* DC. *International Journal of Pharmacology*, 14(7), 901-912. <https://doi.org/10.3923/ijp.2018.901.912>
- Olaniyan, O. T., Kunle-Alabi, O. T., & Raji, Y. (2018). Protective effects of methanol extract of *Plukenetia conophora* seeds and 4H-Pyran-4-One 2, 3-Dihydro-3, 5-Dihydroxy-6-Methyl on the reproductive function of male Wistar rats treated with cadmium chloride. *JBRA Assisted Reproduction*, 22(4), 289-300. <https://doi.org/10.5935/1518-0557.20180048>
- Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Buján, J., García-Honduvilla, N., & Coca, S. (2020). Signal transduction pathways in breast cancer: The important role of PI3K/Akt/mTOR. *Journal of Oncology*, 2020, 1-7. <https://doi.org/10.1155/2020/9258396>
- Osemeobo, G. J. (1991). Effects of common property resource utilization on wildlife conservation in Nigeria. *GeoJournal*, 23, 241-248.
- Osman, N. I., Sidik, N. J., Awal, A., Adam, N. A. M., & Rezali, N. I. (2016). *In vitro* xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. *Journal of Intercultural Ethnopharmacology*, 5(4), 343-349. <https://doi.org/10.5455%2Fjice.20160731025522>
- Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., & Legault, J. (2012). Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. *Food Chemistry*, 132(2), 943-947.
- Owolarafe, T. A., Salawu, K., Ihegboro, G. O., Ononamadu, C. J., Alhassan, A. J., & Wudil, A. M. (2020). Investigation of cytotoxicity potential of different extracts of *Ziziphus mauritiana* (Lam) leaf *Allium cepa* model. *Toxicology Reports*, 7, 816-821. <https://doi.org/10.1016/j.toxrep.2020.06.010>
- Owoseni, M. C., Labulo, A. H., Bako, G., Okunade, O., & Hassan, I. (2023). Antimicrobial potency of green synthesized silver nanoparticles from stem extract of *Euphorbia*

- poissonii* on urinary tract pathogens. *Chemistry Africa*, 6(1), 311-321. <http://dx.doi.org/10.1007/s42250-022-00500-7>
- Oyebode, O. A., Erukainure, O. L., Ibeji, C. U., Koorbanally, N. A., & Islam, M. S. (2019). Phytochemical constituents, antioxidant and antidiabetic activities of different extracts of the leaves, stem and root barks of *Alstonia boonei*: An *in vitro* and *in silico* study. *Botany Letters*, 166(4), 444 - 456. <http://dx.doi.org/10.1080/23818107.2019.1624980>
- Oyedepo, O. O., & Femurewa, A. J. (1995). Anti-protease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Olex sub-scorpoides* and *Tetrapleura tetraptera*. *International Journal of Pharmacognosy*, 33, 65-69
- Özbilgin, S., & Çitogl, G. S. (2012). Uses of some *Euphorbia* species in traditional medicine in Turkey and their biological activities. *Turkish Journal of Pharmaceutical Sciences*, 9(2), 241-256.
- Özbilgin, S., Akkol, E. K., Süntar, İ., Tekin, M., & İşcan, G. S. (2019). Wound-healing activity of some species of *Euphorbia* L. *Records of Natural Products*, 13, 104-113. <http://doi.org/10.25135/rnp.81.18.03.255>.
- Özcan, A., & Özcan, A. S. (2004). Comparison of supercritical fluid and soxhlet extractions for the quantification of hydrocarbons from *Euphorbia macroclada*. *Talanta*, 64(2), 491-495. <https://doi.org/10.1016/j.talanta.2004.03.016>
- Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: Substances useful in human healthcare. *Archivum Immunologiae et Therapiae Experimentalis*, 55, 315-327. <https://doi.org/10.1007/s00005-007-0039-1>
- Pahlevani, A. H. (2017). *Diversity of the genus Euphorbia (Euphorbiaceae) in SW Asia* (Doctoral dissertation). Bayreuth University, Germany.
- Pal, S. M., Avneet, G., & Siddhraj, S. S. (2018). Gallic acid: Pharmacological promising lead molecule: A review. *International Journal of Pharmacognosy and Phytochemical Research*, 10(4), 132-138. <https://doi.org/10.25258/phyto.10.4.2>
- Palit, P., Mandal, S. C., & Bhunia, B. (2016). Total steroid and terpenoid enriched fraction from *Euphorbia neriifolia* Linn. offers protection against nociceptive-pain, inflammation, and *in vitro* arthritis model: An insight of mechanistic study. *International Immunopharmacology*, 41, 106-115. <https://doi.org/10.1016/j.intimp.2016.10.024>
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5, e47. <https://doi.org/10.1017%2Fjns.2016.41>
- Pande, J., & Chanda, S. (2020). Mini review: Screening of antioxidant properties of some medicinal plants. In *Proceedings of the National Conference on Innovations in Biological Sciences (NCIBS)*.1-16.
- Pandey, B. P., Adhikari, K., Pradhan, S. P., Shin, H. J., Lee, E. K., & Jung, H. J. (2020). *In-vitro* antioxidant, anti-cancer, and anti-inflammatory activities of selected medicinal plants from western Nepal. *Future Journal of Pharmaceutical Sciences*, 6, 1-12. <http://dx.doi.org/10.1186/s43094-020-00107-0>

- Pankotai-Bodó, G., Oláh-Németh, O., Sükösd, F., & Pankotai, T. (2023). Routine molecular applications and recent advances in breast cancer diagnostics. *Journal of Biotechnology*, 380, 20-28. <https://doi.org/10.1016/j.jbiotec.2023.12.005>
- Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R., & Lewandowski, W. (2021). Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials*, 14(8), 1984. <https://doi.org/10.3390/ma14081984>
- Parys, W., Dołowy, M., & Pyka-Pająk, A. (2022). Significance of chromatographic techniques in pharmaceutical analysis. *Processes*, 10(1), 172-184. <https://doi.org/10.3390/pr10010172>
- Patay, É. B., Bencsik, T., & Papp, N. (2016). Phytochemical overview and medicinal importance of *Coffea* species from the past until now. *Asian Pacific Journal of Tropical Medicine*, 9(12), 1127-1135. <https://doi.org/10.1016/j.apjtm.2016.11.008>
- 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>
- Patil, S. V., Borase, H. P., Patil, C. D., & Salunke, B. K. (2012). Biosynthesis of silver nanoparticles using latex from few euphorbian plants and their antimicrobial potential. *Applied Biochemistry and Biotechnology*, 167, 776-790. <https://doi.org/10.1007/s12010-012-9710-z>
- Patil, S., Imran, M., Jaqueline, R. S. M., & Aeri, V. (2023). Standardization of *Euphorbia tithymaloides* (L.) Poit. (Root) by conventional and DNA barcoding methods. *ACS Omega*, 4 (32), 29324-29335. <https://doi.org/10.1021/acsomega.3c02543>
- Pavithra, K., & Vadivukkarasi, S. (2015). Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. *Food Science and Human Wellness*, 81(1), 42-46. <http://dx.doi.org/10.1016/j.fshw.2015.02.001>
- Peng, Q., Li, G., Ma, Y., Huang, J., Wei, X., & Wang, J. (2012). Chemical constituents of *Euphorbia kansui*. *Biochemical Systematics and Ecology*, 43, 64-66. <http://dx.doi.org/10.1016/j.bse.2012.02.021>
- Periferakis, A., Periferakis, K., Badarau, I. A., Petran, E. M., Popa, D. C., Caruntu, A., Costache, R. S., Scheau, C., Caruntu, C., & Costache, D. O. (2022). Kaempferol: Antimicrobial properties, sources, clinical, and traditional applications. *International Journal of Molecular Sciences*, 23(23), 15054. <https://doi.org/10.3390/2Fijms232315054>
- Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, 6(11), 1-5. <https://doi.org/10.4103/2F0973-7847.95849>
- Pham, T. H., Park, H. M., Kim, J., Hong, J. T., & Yoon, D. Y. (2020). STAT3 and p53: Dual target for cancer therapy. *Biomedicines*, 8(12), 637. <https://doi.org/10.3390/2Fbiomedicines8120637>
- Ping, K. Y., Darah, I., Yusuf, U. K., Yeng, C., & Sasidharan, S. (2012). Genotoxicity of *Euphorbia hirta*: An *Allium cepa* assay. *Molecules*, 17(7), 7782-7791. <https://doi.org/10.3390/2Fmolecules17077782>

- Pitt, J. J. (2009). Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *The Clinical Biochemist Reviews*, 30(1), 19-34.
- Prabu, G. R., Gnanamani, A., & Sadulla, S. (2006). Guaijaverin—a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. *Journal of Applied Microbiology*, 101(2), 487-495. <https://doi.org/10.1111/j.1365-2672.2006.02912.x>
- Prado-Audelo, D., Luisa, M., Cortés, H., Caballero-Florán, I. H., González-Torres, M., Escutia-Guadarrama, L., Giraldo-Gomez, D. M., Magaña, J. J., & Leyva-Gómez, G. (2021). Therapeutic applications of terpenes on inflammatory diseases. *Frontiers in Pharmacology*, 12, 704197. <https://doi.org/10.3389/fphar.2021.704197>
- Prakash, D., Gupta, C., & Sharma, G. (2012). Importance of phytochemicals in nutraceuticals. *Journal of Chinese Medicine Research and Development*, 1(3), 70-78.
- Prasad, K., & Bisht, G. (2011). *Euphorbia thymifolia* Linn. *Current Research in Chemistry*, 3(2), 98-105. <https://doi.org/10.3923/crc.2011.98.105>
- Prashant, Y., Kritika, S., & Anurag, M. (2015). A review on phytochemical, medicinal and pharmacological profile of *Euphorbia antiquorum*. *International Journal of Pharmacy and Pharmaceutical Research*, 4 (1), 56-67.
- Preethamol, S. N., & Thoppil, J. E. (2020). Phenolic content, flavonoid content and antioxidant potential of whole plant extract of *Ophiorrhiza pectinata* Arn. *Indian Journal of Pharmaceutical Sciences*, 82(4), 12-18. <http://dx.doi.org/10.36468/pharmaceutical-sciences.699>
- Preethamol, S. N., & Thoppil, J. E. (2022). Antioxidant potential and chemical composition of *Ophiorrhiza jacobii*: A promising medicinal plant endemic to Western Ghats. *Indian Journal of Pharmaceutical Sciences*, 84(2), 407-414. <http://dx.doi.org/10.36468/pharmaceutical-sciences.934>
- Primikyri, A., Chatziathanasiadou, M. V., Karali, E., Kostaras, E., Mantzaris, M. D., Hatzimichael, E., Shin, J. S., Chi, S. W., Briasoulis, E., Kolettas, E., Gerothanassis, I. P., & Tzakos, A. G. (2014). Direct binding of Bcl-2 family proteins by quercetin triggers its pro-apoptotic activity. *ACS Chemical Biology*, 9(12), 2737-2741. <https://doi.org/10.1021/cb500259e>
- Pucci, B., Kasten, M., & Giordano, A. (2000). Cell cycle and apoptosis. *Neoplasia*, 2(4), 291-299. <https://doi.org/10.1038%2Fsj.neo.7900101>
- Qiao, Y., Bi, J., Chen, Q., Wu, X., Jin, X., Gou, M., Yang, X., & Purcaro, G. (2022). Rapid and sensitive quantitation of DDMP (2, 3-Dihydro-3, 5-Dihydroxy-6-Methyl-4H-Pyran-4-One) in baked red jujubes by HS-SPME-GC-MS/MS. *Food Control*, 135 (41), 108820. <http://dx.doi.org/10.1016/j.foodcont.2022.108820>
- Qin, J. J., Yan, L., Zhang, J., & Zhang, W. D. (2019). STAT3 as a potential therapeutic target in triple negative breast cancer: A systematic review. *Journal of Experimental & Clinical Cancer Research*, 38(1), 195. <https://doi.org/10.1186/s13046-019-1206-z>
- Qin, Y. M. (2018). Chemical constituents from *Euphorbia helioscopia*. *Chinese Traditional and Herbal Drugs*, 49 (7), 1520-1524. <http://dx.doi.org/10.7501/j.issn.0253-2670.2018.07.006>

- Qiu, L., Liu, M., & Pan, K. (2013). A triple staining method for accurate cell cycle analysis using multiparameter flow cytometry. *Molecules*, 18(12), 15412-15421. <https://doi.org/10.3390/molecules181215412>
- Radi, M. H., El-Shiekh, R. A., El-Halawany, A. M., Al-Abd, A. M., & Abdel-Sattar, E. (2023). *In vitro* cytotoxic study of *Euphorbia grantii* Oliv. aerial parts against MCF-7 and MCF-7ADR breast cancer cell lines: A bioactivity-guided isolation. *ACS Omega*, 8(20), 18299-18305. <https://doi.org/10.1021%2Facsomega.3c02091>
- Radi, M. H., El-Shiekh, R. A., Hegab, A. M., Henry, S. R., Avula, B., Katragunta, K., ... & Abdel-Sattar, E. (2023). LC-QToF chemical profiling of *Euphorbia grantii* Oliv. and its potential to inhibit LPS-induced lung inflammation in rats via the NF- $\kappa$ B, CY450P2E1, and P38 MAPK14 pathways. *Inflammopharmacology*, 32(1), 461-494.
- Rahman, M. M., Islam, M. B., Biswas, M., & Khurshid Alam, A. H. M. (2015). *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Research Notes*, 8(1), 1-9. <https://bmcresearchnotes.biomedcentral.com/articles/10.1186/s13104-015-1618-6>
- Rahman, M. S., Rana, S., & Islam, A. A. (2019). Antithrombotic and anti-inflammatory activities of leaf methanolic extract of *Euphorbia hirta* Linn. *International Journal of Complementary & Alternative Medicine*, 12(4), 154-162. <http://dx.doi.org/10.15406/ijcam.2019.12.00466>
- Rahmani, A. H., Almatroudi, A., Allemailem, K. S., Alwanian, W. M., Alharbi, B. F., Alrumaihi, F., Khan, A. A., & Almatroodi, S. A. (2023). Myricetin: A significant emphasis on its anticancer potential via the modulation of inflammation and signal transduction pathways. *International Journal of Molecular Sciences*, 24(11), 9665. <https://doi.org/10.3390/ijms24119665>
- Raja, P., Dhatchanamoorthy, N., Soosairaj, S., & Jansirani, P. (2020). New distribution record of two endemic plant species, *Euphorbia kadapensis* Sarojin. & RRV Raju (Euphorbiaceae) and *Lepidagathis keralensis* Madhus. & NP Singh (Acanthaceae), for Karnataka, India. *Journal of Threatened Taxa*, 12(14), 17045-17048. <http://dx.doi.org/10.11609/jott.4978.12.14.17045-17048>
- Rajendran, P., Rengarajan, T., Nandakumar, N., Palaniswami, R., Nishigaki, Y., & Nishigaki, I. (2014). Kaempferol, a potential cytostatic and cure for inflammatory disorders. *European Journal of Medicinal Chemistry*, 86, 103-112. <https://doi.org/10.1016/j.ejmech.2014.08.011>
- Rajkuberan, C., Prabukumar, S., Sathishkumar, G., Wilson, A., Ravin dran, K., & Sivaramakrishnan, S. (2017). Facile synthesis of silver nanoparticles using *Euphorbia antiquorum* L. latex extract and evaluation of their biomedical perspectives as anticancer agents. *Journal of Saudi Chemical Society*, 21(8), 911-919.
- Ramachandran, R., Parthasarathy, R., & Dhayalan, S. (2022). Silver nanoparticles synthesized by *Euphorbia hirta* exhibited antibacterial activity and induced apoptosis through downregulation of PI3K $\gamma$  mediated PI3K/Akt/mTOR/p70S6K in human lung adenocarcinoma A549 cells. *Environmental Toxicology*, 37(12), 2865-2876. <https://doi.org/10.1002/tox.23643>

- Ramli, N. R., Yusoff, H. M., Maulidiani, M., Asari, A., & Wahab, N. H. A. (2023). Stability of green synthesis of silver nanoparticles by using *Euphorbia milii* (euphorbiaceae) leaves extract with different solvents and polarities. *Malaysian Journal of Analytical Sciences*, 27(4), 766-776.
- Rank, J., & Nielsen, M. H. (1994). Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 312(1), 17-24.
- Rasheed, T., Bilal, M., Li, C., Nabeel, F., Khalid, M., & Iqbal, H. M. (2018). Catalytic potential of bio-synthesized silver nanoparticles using *Convolvulus arvensis* extract for the degradation of environmental pollutants. *Journal of Photochemistry and Photobiology B: Biology*, 181, 44-52. <https://doi.org/10.1016/j.jphotobiol.2018.02.024>
- Rauf, A., Raza, M., Humayun Khan, M., Hemeg, H. A., Al-Awthan, Y. S., Bahattab, O., Bawazeer, S., Na, S., Basoglu, F., Saleem, M., Khan, M., Seyyedamirhossein, H., Mubarak, M. S., & Erdogan Orhan, I. (2022). *In vitro* and *in silico* studies on clinically important enzymes inhibitory activities of flavonoids isolated from *Euphorbia pulcherrima*. *Annals of Medicine*, 54(1), 495-506. <https://doi.org/10.1080/07853890.2022.2033826>
- Rautela, I., Joshi, P., Thapliyal, P., Pant, M., Dheer, P., Bisht, S., Sinha, V. B., Sundriyal, S., & Sharma, M. D. (2020). Comparative GC-MS analysis of *Euphorbia hirta* and *Euphorbia milli* for therapeutic potential utilities. *Plant Archives*, 20(2), 3515-3522.
- Raven, P. H. (2021). Plants make our existence possible. *Plants, People, Planet*, 3(1), 2-6. <http://dx.doi.org/10.1002/ppp3.10173>
- Ravi, L., & Krishnan, K. (2017). Research article cytotoxic potential of N-hexadecanoic acid extracted from *Kigelia pinnata* leaves. *Asian Journal of Cell Biology*, 12, 20-27. <http://dx.doi.org/10.3923/ajcb.2017.20.27>
- Ravipati, A. S., Zhang, L., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Bartlett, J., Smith, P. T., Shanmugam, K., Münch, G., Wu, M. J., Satyanarayanan, M., & Vysetti, B. (2012). Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. *BMC Complementary and Alternative Medicine*, 12(1), 1-14. <https://doi.org/10.1186/1472-6882-12-173>
- Raza, M. A., Kanwal, Z., Rauf, A., Sabri, A. N., Riaz, S., & Naseem, S. (2016). Size-and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. *Nanomaterials*, 6(4), 74. <https://doi.org/10.3390/nano6040074>
- Revathi, S., Hakkim, F. L., Kumar, N. R., Bakshi, H. A., Sangilimuthu, A. Y., Tambuwala, M. M., Changez, M., Nasef, M. M., Krishnan, M., & Kayalvizhi, N. (2019). *In vivo* anti-cancer potential of pyrogallol in murine model of colon cancer. *Asian Pacific Journal of Cancer Prevention: APJCP*, 20(9), 2645-2651. <https://doi.org/10.31557%2FAPJCP.2019.20.9.2645>
- Ribeiro, V. P., Arruda, C., Abd El-Salam, M., & Bastos, J. K. (2018). Brazilian medicinal plants with corroborated anti-inflammatory activities: A review. *Pharmaceutical Biology*, 56(1), 253-268. <https://doi.org/10.1080/13880209.2018.1454480>
- 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.
- Rodríguez-Negrete, E. V., Morales-González, Á., Madrigal-Santillán, E. O., Sánchez-Reyes, K., Álvarez-González, I., Madrigal-Bujaidar, E., Valadez-Vega, C., Chamorro-Cevallos, G., García-Melo, L. F., & Morales-González, J. A. (2024). Phytochemicals and their usefulness in the maintenance of health. *Plants*, 13(4), 523. <https://doi.org/10.3390/plants13040523>
- Rohilla, P., & Gupta, J. P. (2023). A review on herbal medicine in India: Current market trends and opportunities. *International Journal of Research in Ayurveda and Pharmacy*, 14 (4), 141-145. <http://dx.doi.org/10.7897/2277-4343.1404131>
- Rojas-Jiménez, S., Valladares-Cisneros, M. G., Salinas-Sánchez, D. O., Pérez-Ramos, J., Sánchez-Pérez, L., Pérez-Gutiérrez, S., & Campos-Xolalpa, N. (2024). Anti-inflammatory and cytotoxic compounds isolated from plants of *Euphorbia* genus. *Molecules*, 29(5), 1083. <https://doi.org/10.3390/molecules29051083>
- Romulo, A. (2020). The principle of some *in vitro* antioxidant activity methods. In *IOP Conference Series: Earth and Environmental Science*, Vol. 426, No. 1, p. 012177, IOP Publishing. <http://dx.doi.org/10.1088/1755-1315/426/1/012177>
- Rosa, R. L., Campos, P. M., Cruz, L. S., Novak, R. S., Kanunfre, C. C., Esmerino, L. A., Schebelski, D. J., de Paula, J. D. F. P., & Beltrame, F. L. (2022). Biomonitored study of *Euphorbia umbellata* (Pax) Bruyns extracts to be used to prepare silver nanoparticles with antimicrobial and cytotoxic potential. *Research, Society and Development*, 11(12), e367111234804-e367111234804. <http://dx.doi.org/10.30574/gscbps.2019.8.2.0137>
- Ruddaraju, L. K., Pammi, S. V. N., Sankar Guntuk, U. G., Padavala, V. S., & Kolapalli, V. R. M. (2020). A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nanotechnological combinations. *Asian Journal of Pharmaceutical Science*, 15(1): 42–59. <https://doi.org/10.1016/j.ajps.2019.03.002>
- Sabeen, M., Mahmood, Q., Bhatti, Z. A., Irshad, M., Bilal, M., Hayat, M. T., Irshad, U., Akbar, T. A., Arslan, M., & Shahid, N. (2020). *Allium cepa* assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. *Saudi Journal of Biological Sciences*, 27(5), 1368-1374. <https://doi.org/10.1016%2Fj.sjbs.2019.12.011>
- Sadeghi, M., Khomartash, M. S., Gorgani-Firuzjaee, S., Vahidi, M., Khiavi, F. M., & Taslimi, P. (2022).  $\alpha$ -glucosidase inhibitory, antioxidant activity, and GC/MS analysis of *Descurainia sophia* methanolic extract: *In vitro*, *in vivo*, and *in silico* studies. *Arabian Journal of Chemistry*, 15(9), 104055. <https://doi.org/10.1016/j.arabjc.2022.104055>
- Saeb, A., Alshammari, A. S., Al-Brahim, H., & Al-Rubeaan, K. A. (2014). Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. *The Scientific World Journal*, 2014, 1-9. <https://doi.org/10.1155%2F2014%2F704708>
- Sajitha, M. K., & Thoppil, J. E. (2018). Screening of cytotoxicity, metabolic inhibition and possible apoptotic cell death induced by *Gomphostemma heyneanum* Wall. ex



- Benth. var. heyneanum using *Allium cepa* root tips. *International Journal of Pharmacy and Biological Sciences*, 8(2), 56-64.
- Salar, R. K., Sharma, P., & Purewal, S. S. (2015). *In vitro* antioxidant and free radical scavenging activities of stem extract of *Euphorbia trigona* Miller. *Cellmed*, 5(2), e14 (1-6). <http://dx.doi.org/10.5667/tang.2015.0004>
- Saleem, A., Saleem, M., & Akhtar, M. F. (2020). Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. *South African Journal of Botany*, 128, 246-256. <https://doi.org/10.1016/j.sajb.2019.11.023>
- Saleem, H., Zengin, G., Locatelli, M., Mollica, A., Ahmad, I., Mahomoodally, F. M., Zainal Abidin, S. A., & Ahemad, N. (2019). *In vitro* biological propensities and chemical profiling of *Euphorbia milii* Des Moul (Euphorbiaceae): A novel source for bioactive agents. *Industrial Crops and Products*, 130, 9-15. <https://doi.org/10.1016/j.indcrop.2018.12.062>
- Salehi, B., Iriti, M., Vitalini, S., Antolak, H., Pawlikowska, E., Kręgiel, D., Sharif-Rad, J., Oyeleye, S. I., Ademiluyi, A. O., Czopek, K., Staniak, M., Custodio, L., Coy-Barrera, E., Segura-Carretero, A., Cádiz-Gurrea, M. de la L., Capasso, R., Cho, W. C., & Seca, A. M. (2019). *Euphorbia*-derived natural products with potential for use in health maintenance. *Biomolecules*, 9(8), 337. <https://doi.org/10.3390/biom9080337>
- Salman, Z. O., Alwash, B. M., & Kadhim, E. J. (2024). Isolation and identification of flavonoid compounds from *Euphorbia milii* plant cultivated in Iraq and evaluation of its genetic effects on two types of cancer cell line. *Baghdad Science Journal*. 1-15. <http://dx.doi.org/10.21123/bsj.2024.9276>
- Salter, M., Duffy, C., Garthwaite, J., & Strijbos, P. J. (1996). Ex vivo measurement of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity *in vivo*. *Journal of Neurochemistry*, 66(4), 1683-1690.
- Sameena, V. P., & Thoppil, J. E. (2022). Green synthesis of silver nanoparticles from *Euphorbia* and its biological activities. *Nanotechnology for Environmental Engineering*, 7(2), 377-392. <https://doi.org/10.1007/s41204-022-00232-6>
- Sameena, V. P., & Thoppil, J. E. (2023). *In vitro* antibacterial efficacy of methanolic plant extract and biosynthesized silver nanoparticles from *Euphorbia* species against two selected foodborne pathogens. *Nanotechnology for Environmental Engineering*, 8, 1-13.
- Sangiovanni, E., & Dell'Agli, M. (2021). Special issue: Anti-inflammatory activity of plant polyphenols 2.0. *Biomedicines*, 10 (1), 37. <https://doi.org/10.3390/biomedicines10010037>
- Santhosh, A. S., Sandeep, S., & Swamy, N. K. (2019). Green synthesis of nano silver from *Euphorbia geniculata* leaf extract: Investigations on catalytic degradation of methyl orange dye and optical sensing of Hg<sup>2+</sup>. *Surfaces and Interfaces*, 14, 50-54. <https://doi.org/10.1016/j.surfin.2018.11.004>
- Sari, A. N., Putra, W. E., Rifa'i, M., Sustiprijatno, S., Susanto, H., Salma, W. O., Faisal, M., Hidayatullah, A., Heikal, M. F., & Firdaus, S. D. R. A. (2023). Profiling the coulomb energy of chimanine D and desulphosinigrin as potential anti-diabetic drug

- against alpha-glucosidase. In *AIP Conference Proceedings* (Vol. 2634, No. 1). AIP Publishing. [https://ui.adsabs.harvard.edu/link\\_gateway/2023AIPC.2634b0012S/doi:10.1063/5.0111214](https://ui.adsabs.harvard.edu/link_gateway/2023AIPC.2634b0012S/doi:10.1063/5.0111214)
- Sasikumar, J. M., Erba, O., & Egigu, M. C. (2020). *In vitro* antioxidant activity and polyphenolic content of commonly used spices from Ethiopia. *Heliyon*, 6(9), e05027. <https://doi.org/10.1016/j.heliyon.2020.e05027>
- Sathish, S. S., Janakiraman, N., & Johnson, M. (2012). Phytochemical analysis of *Vitex altissima* L. using UV-Vis, FTIR and GC-MS. *International Journal of Pharmaceutical Sciences and Drug Research*, 4(1), 56-62.
- Sawale, J. A., Patel, J. R., & Kori, M. L. (2019). Antioxidant properties of cycloartenol isolated from *Euphorbia neriifolia* leaves. *Indian Journal of Natural Products*, 33(1), 60-64.
- Saxena, P. N., Chauhan, L. K. S., & Gupta, S. K. (2005). Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: Spectroscopic basis of chromosome damage. *Toxicology*, 216(2-3), 244-252. <https://doi.org/10.1016/j.tox.2005.08.008>
- Schottenfeld, D., & Fraumeni, Jr, J. F. (2006). *Cancer epidemiology and prevention*. Oxford University Press.
- Selvam, M. S., Tresina, P. S., Benjamin, J., & Mohan, V. R. (2022). Assessment of phytochemical, FT-IR and GC-MS fingerprint profiling of marine angiosperms *Enhalus acoroides* (Lf) Royle and *Syringodium isoetifolium* (Asch) Dandy, Gulf of Mannar Biosphere Reserve, Tamil Nadu. *Asian Journal of Biological and Life Sciences*, 11(2), 468-475. <http://dx.doi.org/10.5530/ajbls.2022.11.64>
- Selvam, P., Vijayakumar, T., Wadhvani, A., & Muthulakshmi, L. (2019). Bioreduction of silver nanoparticles from aerial parts of *Euphorbia hirta* L.(EH-ET) and its potent anticancer activities against neuroblastoma cell lines. *Indian Journal of Biochemistry and Biophysics (IJBB)*, 56(2), 132-136. <http://op.niscpr.res.in/index.php/IJBB/article/view/27662>
- Serhan, C. N., & Levy, B. D. (2018). Resolvins in inflammation: Emergence of the pro-resolving superfamily of mediators. *The Journal of Clinical Investigation*, 128(7), 2657-2669. <https://doi.org/10.1172/jci97943>
- Shah, M., Khan, F., Ullah, S., Mohanta, T. K., Khan, A., Zainab, R., Rafiq, N., Ara, H., Alam, T., Rehman, N. U., & Al-Harrasi, A. (2023). GC-MS profiling and biomedical applications of essential oil of *Euphorbia larica* Boiss.: A new report. *Antioxidants*, 12(3), 662. <https://doi.org/10.3390/antiox12030662>
- Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608. <http://dx.doi.org/10.22271/chemi.2020.v8.i2i.8834>
- Shaikh, R. U., Pund, M. M., & Gacche, R. N. (2016). Evaluation of the anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in vitro* as well as *in vivo*. *Journal of Traditional and Complementary Medicine*, 6(4), 355-361. <https://doi.org/10.1016/j.jtcme.2015.07.001>
- Shaker, K. H., Al Shehri, B. M., Oteef, M. D. Y., & Mahmoud, M. F. (2015). Antioxidant compounds from *Euphorbia schimperiana* Scheele in Aseer region, Saudi

- 
- Arabia. *International Journal of Pharmaceutical Sciences Review and Research*, 32(1), 117-122.
- Shanmugam, S., Kumar, T. S., & Panneer Selvam, K. (2019). Laboratory handbook on Biochemistry. PHI Learning Pvt. Ltd. Delhi.
- Sharifi-Rad, J., Quispe, C., Castillo, C. M. S., Caroca, R., Lazo-Vélez, M. A., Antonyak, H., Polishchuk, A., Lysiuk, R., Oliinyk, P., De Masi, L., Bontempo, P., Martorell, M., Daştan, S. D., Rigano, D., Wink, M., & Cho, W. C. (2022). Ellagic acid: A review on its natural sources, chemical stability, and therapeutic potential. *Oxidative Medicine and Cellular Longevity*, 22,1-2. <https://doi.org/10.1155/2022/3848084>
- Sharma, A. K., & Sharma, A. (1990). *Chromosome Techniques: Theory and Practice*. Aditya Books, New Delhi.
- 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 Systems Letters*, 10(1), 2.
- Sheikh, N., Kumar, Y., Misra, A. K., & Pfoze, L. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. *Journal of Medicinal Plants*. 1(6), 62-69. <http://www.plantsjournal.com/>
- Shen, S. C., Lee, W. R., Lin, H. Y., Huang, H. C., Ko, C. H., Yang, L. L., & Chen, Y. C. (2002). *In vitro* and *in vivo* inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E2 production. *European Journal of Pharmacology*, 446(1-3), 187-194. [https://doi.org/10.1016/s0014-2999\(02\)01792-2](https://doi.org/10.1016/s0014-2999(02)01792-2)
- Shi, Q. W., Su, X. H., & Kiyota, H. (2008). Chemical and pharmacological research of the plants in genus *Euphorbia*. *Chemical Reviews*, 108 (10), 4295-4327. <https://doi.org/10.1021/cr078350s>
- Shi, Q., Sun, Y. W., & Meng, D. (2017). Phytochemical and cytotoxic studies on the roots of *Euphorbia fischeriana*. *Bioorganic & Medicinal Chemistry Letters*, 27(2), 266-270. <http://dx.doi.org/10.1016/j.bmcl.2016.11.063>
- Shi, X. H., Du, X. L., & Kong, L. Y. (2006). Studies on chemical constituents in roots of *Euphorbia soongarica*. *Zhongguo Zhong yao Za Zhi Zhongguo Zhongyao Zazhi-China Journal of Chinese Materia Medica*, 31(18), 1503-1506.
- Shilpa, K. J., Gopi, K. P., Muzhapravan, A., & Prabhakaran, K. Evaluation of cytotoxicity potential of (Gaertn.) Desr. male flower *Garcinia cambogia* extract using model *Allium cepa*. (2023). *Advance Pharmaceutical Journal*, 8 (5), 136-141. <https://doi.org/10.31024/apj.2023.8.5.2>
- Shlini, P., Clare, H. M., & Immaculate, V. T. M. (2020). Isolation and purification of rutin from *Euphorbia pulcherrima*. *International Journal of Pharmaceutical Sciences Review and Research*, 64(1), 45-49. <http://dx.doi.org/10.47583/ijpsrr.2020.v64i01.008>
- Shomali, N., Kamrani, A., Heris, J. A., Shahabi, P., Nasiri, H., Sadeghvand, S., Ghahremanzadeh, K., & Akbari, M. (2023). Dysregulation of P53 in breast cancer: Causative factors and treatment strategies. *Pathology-Research and Practice*, 247, 154539. <https://doi.org/10.1016/j.prp.2023.154539>
-

- Shrihastini, V., Muthuramalingam, P., Adarshan, S., Sujitha, M., Chen, J. T., Shin, H., & Ramesh, M. (2021). Plant derived bioactive compounds, their anti-cancer effects and *in silico* approaches as an alternative target treatment strategy for breast cancer: An updated overview. *Cancers*, 13(24), 6222-6232. <https://doi.org/10.3390/cancers13246222>
- Siddiqui, A. A., & Ali, M. (1997). *Practical pharmaceutical chemistry*. CBS Publishers & Distributors, New Delhi, (pp. 126-131).
- Simon, S., Sibuyi, N. R. S., Fadaka, A. O., Meyer, M., Madiehe, A. M., & du Preez, M. G. (2021). The antimicrobial activity of biogenic silver nanoparticles synthesized from extracts of Red and Green European pear cultivars. *Artificial Cells, Nanomedicine, and Biotechnology*, 49(1), 613-624. <https://doi.org/10.1080/21691401.2021.1980884>
- Sinan, K. I., Etienne, O. K., Stefanucci, A., Mollica, A., Mahomoodally, M. F., Jugreet, S., Rocchetti, G., Lucini, L., Aktumsek, A., Montesano, D., Ak, G., & Zengin, G. (2021). Chemodiversity and biological activity of essential oils from three species from the *Euphorbia* genus. *Flavour and Fragrance Journal*, 36(1), 148-158. <http://dx.doi.org/10.1002/ffj.3624>
- Singh, S. (2023). A review on some medicinal plant species with the most traditional medicinal usage in India. *International Journal of Biological Innovations*, 5(01), 52-62. <http://dx.doi.org/10.46505/IJBI.2023.5103>
- Sinha, D., Mukherjee, S., & Chowdhury, S. (2022). Methods of extraction of phytochemicals. In *Isolation, characterization, and therapeutic applications of natural bioactive compounds* (pp. 250-279). IGI Global. <http://dx.doi.org/10.4018/978-1-6684-7337-5.ch010>
- Sinha, D., Sarkar, N., Biswas, J., & Bishayee, A. (2016). Resveratrol for breast cancer prevention and therapy: Preclinical evidence and molecular mechanisms. In *Seminars in cancer biology*. Academic Press, 40, 209-232. <https://doi.org/10.1016/j.semcancer.2015.11.001>
- Sisodiya, D., & Shrivastava, P. (2018). Repellent and antifeedant activities of *Euphorbia thymifolia* (Linn.) and *Manilkara hexandra* (Roxb.) against *Rhyzopertha dominica* (Fab.). *International Journal of Research and Analytical Reviews*, 5(3), 257-260.
- Skłodowski, K., Chmielewska-Deptuła, S. J., Piktel, E., Wolak, P., Wollny, T., & Bucki, R. (2023). Metallic nanosystems in the development of antimicrobial strategies with high antimicrobial activity and high biocompatibility. *International Journal of Molecular Sciences*, 24(3), 2104. <https://doi.org/10.3390/ijms24032104>
- Smeriglio, A., Denaro, M., Trombetta, D., Ragusa, S., & Circosta, C. (2021). New insights on *Euphorbia dendroides* L. (Euphorbiaceae): Polyphenol profile and biological properties of hydroalcoholic extracts from aerial parts. *Plants*, 10(8), 1621. <https://doi.org/10.3390/plants10081621>
- Smith, A. R., & Carter, S. (1988). *Flora of tropical east Africa -Euphorbiac v2 (1988)* (Vol. 137). CRC Press.
- Sodde, V. K., Lobo, R., Kumar, N., Maheshwari, R., & Shreedhara, C. S. (2015). Cytotoxic activity of *Macrosolen parasiticus* (L.) Danser on the growth of breast cancer cell

- 
- line (MCF-7). *Pharmacognosy Magazine*, 11(Suppl 1), S156. <https://doi.org/10.4103%2F0973-1296.157719>
- Sophiya, P., Kiran, B. K., Lohith, N. S., Ali, F., Sathisha, A. D., & Dharmappa, K. K. (2021). GC-MS analysis, adme toxicity and *in silico* studies of some isolated compounds from *Melastoma malabathricum* leaves against SPLA2 inhibition. *Applied Biological Research*, 23 (1), 26-36. <http://dx.doi.org/10.5958/0974-4517.2021.00004.5>
- Sosa, A. A., Bagi, S. H., & Hameed, I. H. (2016). Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*, 8(5), 109-126. <http://dx.doi.org/10.5897/JPP2015.0371>
- Sudha, T. S., & Padmini, R. (2023). Evaluation of bioactive compounds in *Euphorbia hirta* Linn. leaves extract using gas chromatographic and mass spectroscopic techniques. *Journal of Pharmaceutical Negative Results*, 14 (2), 1988-1995. <https://doi.org/10.47750/pnr.2023.14.S02.238%20%20>
- Sudhan, T., Varalakshmi, S., Abirami, T., & Karthikeyan, J. (2021). Phytochemical screening, antibacterial and antioxidant activities of *Euphorbia hirta* crude extract. *International Journal of Zoological Investigations*, 7(2), 689-698. <https://doi.org/10.33745/ijzi.2021.v07i02.052>
- Suksungworn, R., & Duangsrirai, S. (2021). Phytochemical contents and antioxidant activity of medicinal plants from the Rubiaceae family in Thailand. *Plant Science Today*, 8(1), 24-31. <http://dx.doi.org/10.14719/pst.2021.8.1.882>
- Sun, Y., Gao, L. L., Tang, M. Y., Feng, B. M., Pei, Y. H., & Yasukawa, K. (2018). Triterpenoids from *Euphorbia maculata* and their anti-inflammatory effects. *Molecules*, 23(9), 2112. <https://doi.org/10.3390%2Fmolecules23092112>
- Sundaraprabha, V., & Rayan, S. (2018). Antimicrobial and antioxidant activity of ethanolic extract of *Euphorbia prostrata* AIT leaves. *International Journal of Innovative Research in Technology*, 5(1), 575-578. <http://dx.doi.org/10.13140/ RG.2.2.17293.87522>
- 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. *CA: A Cancer Journal for Clinicians*, 71(3), 209-249. <https://doi.org/10.3322/caac.21660>
- Swamy, K. M., Pokharen, N., Dahal, S., & Anuradha, M. (2011). Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*. *Journal of Medicinal Plants Research* 5(24), 5785-5788.
- Tadesse, S., Ganesan, K., Nair, S. K. P., Letha, N., & Gani, S. B. (2016). Preliminary phytochemical screening of *Euphorbia cotinifolia* L. (Family: Euphorbiaceae) by using different solvent extracts. *World Journal of Pharmacy and Pharmaceutical Science*, 5(3), 1176-1183.
- Taib, T. M., AlSamara'ai, R. H., & Al-Sofi, A. A. W. S. M. (2022). Chemical classification of some species *Euphorbia* in central and northern Iraq. *Egyptian Academic Journal of*
-

- Biological Sciences, H. Botany*, 13(2), 245-251. <https://dx.doi.org/10.21608/eajbsh.2022.281341>
- Tang, S. M., Deng, X. T., Zhou, J., Li, Q. P., Ge, X. X., & Miao, L. (2020). Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects. *Biomedicine & Pharmacotherapy*, 121, 109604. <https://doi.org/10.1016/j.biopha.2019.109604>
- Tang, S., & Zheng, J. (2018). Antibacterial activity of silver nanoparticles: Structural effects. *Advanced Healthcare Materials*, 7(13), 1701503. <https://doi.org/10.1002/adhm.201701503>
- Tantary, S., Masood, A., Bhat, A. H., Dar, K. B., Zargar, M. A., & Ganie, S. A. (2017). *In vitro* antioxidant and RBC membrane stabilization activity of *Euphorbia wallichii*. *Free Radicals and Antioxidants*, 7(1), 13-22. <http://dx.doi.org/10.5530/fra.2017.1.3>
- Techaoei, S., Jirayuthcharoenkul, C., Jarmkom, K., Dumrongphuttidecha, T., & Khobjai, W. (2020). Chemical evaluation and antibacterial activity of novel bioactive compounds from endophytic fungi in *Nelumbo nucifera*. *Saudi Journal of Biological Sciences*, 27(11), 2883-2889. <https://doi.org/10.1016%2Fj.sjbs.2020.08.037>
- Teoh, Y. P., & Mashitah, M. D. (2016). Extraction of 4h-pyran-4-one, 2, 3-dihydro-6-methyl-, an alternative antifungal agent, from *Schizophyllum commune*: Optimization and kinetic study. *Borneo Science*, 37(1), 1-22. <http://dx.doi.org/10.51200/bsj.v37i1.4969>
- Thomas, O. E., Alabi, O. S., & Osharode, P. E. (2023). Characterization, antimicrobial and catalytic activities of silver nanoparticles biosynthesized using aqueous extract of *Euphorbia graminea*. *Acta Pharmaceutica Scientia*, 61(2), 125. <http://dx.doi.org/10.23893/1307-2080.APS6112>
- Ting, C. Y., Hsu, C. T., Hsu, H. T., Su, J. S., Chen, T. Y., Tarn, W. Y., Kuo, Y. H., Whang-Peng, J., Liu, L. F., & Hwang, J. (2003). Isodiospyrin as a novel human DNA topoisomerase I inhibitor. *Biochemical Pharmacology*, 66(10), 1981-1991. <https://doi.org/10.1016/j.bcp.2003.07.003>
- Toro-Vazquez, J. F., Morales-Rueda, J. A., Dibildox-Alvarado, E., Charó-Alonso, M., Alonzo-Macias, M., & González-Chávez, M. M. (2007). Thermal and textural properties of organogels developed by candelilla wax in safflower oil. *Journal of the American Oil Chemists' Society*, 84, 989-1000. <https://doi.org/10.1007/s11746-007-1139-0>
- Toudert, N., Zakkad, F., Dadda, N., Djilani, A., Dicko, A., & Djilani, S. E. (2021). Phytochemical analysis of bioactive extracts and seed oil of three *Euphorbia* species from Algerian flora by LC-MS and GC-MS. *Indonesian Journal of Chemistry*, 21(3), 546-553. <http://dx.doi.org/10.22146/ijc.56679>
- Tran, V., Kim, R., Maertens, M., Hartung, T., & Maertens, A. (2021). Similarities and differences in gene expression networks between the breast cancer cell line Michigan cancer foundation-7 and invasive human breast cancer tissues. *Frontiers in Artificial Intelligence*, 4, 674370. <https://doi.org/10.3389%2Ffrai.2021.674370>

- Truong, D. H., Ta, N. T. A., Pham, T. V., Huynh, T. D., Do, Q. T. G., Dinh, N. C. G., Dang, C. D., Nguyen, T. K. C., & Bui, A. V. (2021). Effects of solvent—solvent fractionation on the total terpenoid content and *in vitro* antiinflammatory activity of *Serevenia buxifolia* bark extract. *Food Science & Nutrition*, 9(3), 1720-1735. <https://doi.org/10.1002/fsn3.2149>
- Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2024). A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *Journal of Chemistry*, 2024(1), 5594386. <https://doi.org/10.1155/2024/5594386>
- Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, 5(3), 93. <https://doi.org/10.3390/2Fmedicines5030093>
- Türkoğlu, Ş. (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 626(1-2), 4-14. <https://doi.org/10.1016/j.mrgentox.2006.07.006>
- Ul-Haq, I., Ullah, N., Bibi, G., Kanwal, S., Ahmad, M. S., & Mirza, B. (2012). Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iranian Journal of Pharmaceutical Research: IJPR*, 11(1), 241-249.
- Urnuksaikhon, E., Bold, B. E., Gunbileg, A., Sukhbaatar, N., & Mishig-Ochir, T. (2021). Antibacterial activity and characteristics of silver nanoparticles biosynthesized from *Carduus crispus*. *Scientific Reports*, 11(1), 21047.
- Uto, T., Qin, G. W., Morinaga, O., & Shoyama, Y. (2012). 17-Hydroxy-jolkinolide B, a diterpenoid from *Euphorbia fischeriana*, inhibits inflammatory mediators but activates heme oxygenase-1 expression in lipopolysaccharide-stimulated murine macrophages. *International Immunopharmacology*, 12(1), 101-109. <https://doi.org/10.1016/j.intimp.2011.10.020>
- Vajrabhaya, L. O., & Korsuwannawong, S. (2018). Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. *Journal of Analytical Science and Technology*, 9(1), 1-6.
- 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. **Error! Hyperlink reference not valid.**
- Van der Auwera, A., Peeters, L., Foubert, K., Piazza, S., Vanden Berghe, W., Hermans, N., & Pieters, L. (2023). *In vitro* biotransformation and anti-inflammatory activity of constituents and metabolites of *Filipendula ulmaria*. *Pharmaceutics*, 15(4), 1291. <https://doi.org/10.3390/pharmaceutics15041291>
- Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B., & Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC Advances*, 11(5), 2804-2837. <https://doi.org/10.1039/D0RA09941D>

- Venkatachalam, U., & Muthukrishnan, S. (2012). Free radical scavenging activity of ethanolic extract of *Desmodium gangeticum*. *Journal of Acute Medicine*, 2(2), 36-42. <http://dx.doi.org/10.1016/j.jacme.2012.04.002>
- Vuong, Q. V., Goldsmith, C. D., Dang, T. T., Nguyen, V. T., Bhuyan, D. J., Sadeqzadeh, E., Scarlett, C. J., & Bowyer, M. C. (2014). Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant capacity from *Euphorbia tirucalli* using response surface methodology. *Antioxidants*, 3(3), 604-617. <https://doi.org/10.3390%2Fantiox3030604>
- Wahab, S., Khan, T., Adil, M., & Khan, A. (2021). Mechanistic aspects of plant-based silver nanoparticles against multi-drug resistant bacteria. *Heliyon*, 7(2021), 1-12. <https://doi.org/10.1016/j.heliyon.2021.e07448>
- Wal, A., Wal, P., Gupta, N., Vishnoi, G., & Srivastava, R. S. (2013). Medicinal value of *Euphorbia tirucalli*. *International Journal of Pharmaceutical and Biological Archieve*, 4, 31-40.
- Walker, M. C., & Gierse, J. K. (2010). *In vitro* assays for cyclooxygenase activity and inhibitor characterization. In S. A. Samir, J. Roderick, & P. Michael (Eds.), *Cyclooxygenase* (pp. 131-144). New York, NY: Humana Press.
- Wan Mat Khalir, W. K. A., Shameli, K., Jazayeri, S. D., Othman, N. A., Che Jusoh, N. W., & Hassan, N. M. (2020). Biosynthesized silver nanoparticles by aqueous stem extract of *Entada spiralis* and screening of their biomedical activity. *Frontiers in Chemistry*, 8, 620. <https://doi.org/10.3389/fchem.2020.00620>
- Wang, D., Sun, Q., Wu, J., Wang, W., Yao, G., Li, T., ... & Song, S. (2017). A new prenylated flavonoid induces G0/G1 arrest and apoptosis through p38/jnk mapk pathways in human hepatocellular carcinoma cells. *Scientific Reports*, 7(1), 5736.
- Wang, G., Wang, Y., Yao, L., Gu, W., Zhao, S., Shen, Z., Lin, Z., Liu, W., & Yan, T. (2022). Pharmacological activity of quercetin: An updated review. *Evidence-Based Complementary and Alternative Medicine*, 2022, 1-12. <http://dx.doi.org/10.1155/2022/3997190>
- Wang, J., Fang, X., Ge, L., Cao, F., Zhao, L., Wang, Z., & Xiao, W. (2018). Antitumor, antioxidant and anti-inflammatory activities of kaempferol and its corresponding glycosides and the enzymatic preparation of kaempferol. *PLoS One*, 13(5), e0197563. <https://doi.org/10.1371%2Fjournal.pone.0197563>
- Wang, M., Sun, J., Jiang, Z., Xie, W., & Zhang, X. (2015). Hepatoprotective effect of kaempferol against alcoholic liver injury in mice. *The American Journal of Chinese Medicine*, 43(2), 241-254. <https://doi.org/10.1142/s0192415x15500160>
- Wang, X., Zhang, C., & Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. *Frontiers in Oncology*, 13, 1224125. <https://doi.org/10.3389%2Ffonc.2023.1224125>
- Wang, Y., Song, Z., Guo, Y., Xie, H., Zhang, Z., Sun, D., ... & Chen, L. (2021). Diterpenoids from the seeds of *Euphorbia lathyris* and their anti-inflammatory activity. *Bioorganic Chemistry*, 112, 104944. <https://doi.org/10.1016/j.bioorg.2021.104944>
- Wani, A. K., Akhtar, N., Mir, T. U. G., Singh, R., Jha, P. K., Mallik, S. K., Sinha, S., Tripathi, S. K., Jain, A., Jha, A., Devkota, H. P., & Prakash, A. (2023). Targeting



- apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. *Biomolecules*, 13(2), 194. <https://doi.org/10.3390%2Fbiom13020194>
- Wellington, E. O., Peters, D. E., & Onyeike, E. N. (2019). Chemical composition of phytochemicals and essential oil of *Euphorbia heterophylla*. *International Journal of Advanced Research and Publications*, 3(11), 78-85.
- Wesley, J. J., Smith, A. A., & Balakrishnan, N. (2022). Phytochemical investigation *in vitro* antioxidant activity & anti diabetic activity of *Euphorbia Nivulia*. *Journal of Pharmaceutical Negative Results*, 13 (1), 1539-1547. <https://doi.org/10.47750/pnr.2022.13.S01.183>
- Wianowska, D., & Olszowy-Tomczyk, M. (2023). A concise profile of gallic acid - From its natural sources through biological properties and chemical methods of determination. *Molecules*, 28(3), 1186. <https://doi.org/10.3390/molecules28031186>
- Wongprayoon, P., Leelasart, S., Jantham, J., Pootaeng-On, Y., Oekchuae, S., Limpachayaporn, P., Rayanil, K. O., & Charoensuksai, P. (2022). A triterpenoid friedelan-3 $\beta$ -ol isolated from *Euphorbia lactea* exhibited cytotoxic activity against HN22 cells by inducing an S-phase cell cycle arrest. *Journal of Applied Pharmaceutical Science*, 12(10), 031-048. <http://dx.doi.org/10.7324/JAPS.2022.121004>
- World Health Organization. (2007). *The world health report 2007: A safer future: Global public health security in the 21st century*. World Health Organization.
- Wu, D., Liu, J., Pang, X., Wang, S., Zhao, J., Zhang, X., & Feng, L. (2014). Palmitic acid exerts pro-inflammatory effects on vascular smooth muscle cells by inducing the expression of C-reactive protein, inducible nitric oxide synthase and tumor necrosis factor- $\alpha$ . *International Journal of Molecular Medicine*, 34(6), 1706-1712. <https://doi.org/10.3892/ijmm.2014.1942>
- Wu, Y., Qu, W., Geng, D., Liang, J. Y., & Luo, Y. L. (2012). Phenols and flavonoids from the aerial part of *Euphorbia hirta*. *Chinese Journal of Natural Medicines*, 10(1), 40-42. [https://doi.org/10.1016/s1875-5364\(12\)60009-0](https://doi.org/10.1016/s1875-5364(12)60009-0)
- Xiao-Yang, W. A. N. G., Li-Ping, L. I. U., Ting-Guo, K. A. N. G., & Hong-Bing, W. A. N. G. (2012). Chemical constituents of *Euphorbia fischeriana*. *Chinese Journal of Natural Medicines*, 10(4), 299-302. [https://doi.org/10.1016/S1875-5364\(12\)60061-2](https://doi.org/10.1016/S1875-5364(12)60061-2)
- Xu, C., Jia, H. Y., Zuo, B., Liao, Z. X., Ji, L. J., Sun, H. F., & Wang, Q. (2016). Chemical constituents of the aerial parts of *Euphorbia nematocypha*. *Natural Product Communications*, 11(2), 177-180. <http://dx.doi.org/10.1177/1934578X1601100210>
- Yan, X. L., Huang, J. L., Tang, Y. Q., Tang, G. H., & Yin, S. (2021). Euphopanes A–C, three new diterpenoids from *Euphorbia pekinensis*. *Natural Product Research*, 36(1), 114-121. <https://doi.org/10.1080/14786419.2020.1765342>
- Yang, C., Wang, B., Wang, J., Xia, S., & Wu, Y. (2019). Effect of pyrogalllic acid (1, 2, 3-benzenetriol) polyphenol-protein covalent conjugation reaction degree on structure and antioxidant properties of pumpkin (*Cucurbita* sp.) seed protein isolate. *LWT-Food Science and Technology*, 109, 443-449. <http://dx.doi.org/10.1016/j.lwt.2019.04.034>
- Yang, M., Sun, J., Lu, Z., Chen, G., Guan, S., Liu, X., Jiang, B., Ye, M., & Guo, D. A. (2009). Phytochemical analysis of traditional Chinese medicine using liquid

- chromatography coupled with mass spectrometry. *Journal of Chromatography A*, 1216(11), 2045-2062. <https://doi.org/10.1016/j.chroma.2008.08.097>
- Yang, Z. N., Su, B. J., Wang, Y. Q., Liao, H. B., Chen, Z. F., & Liang, D. (2020). Isolation, absolute configuration, and biological activities of chebulic acid and brevifolincarboxylic acid derivatives from *Euphorbia hirta*. *Journal of Natural Products*, 83(4), 985-995. <https://doi.org/10.1021/acs.jnatprod.9b00877>
- Yashaswee, S., & Trigun, S. K. (2020). Cytotoxicity and induction of apoptosis in melanoma (MDA-MB-435S) cells by emodin. *Journal of Scientific Research*, 64(2), 158-166.
- Yashin, A., Yashin, Y., Xia, X., & Nemzer, B. (2017). Antioxidant activity of spices and their impact on human health: A review. *Antioxidants*, 6(3), 70. <https://doi.org/10.3390/antiox6030070>
- Yasuda, M. T., Sakakibara, H., & Shimoi, K. (2017). Estrogen-and stress-induced DNA damage in breast cancer and chemoprevention with dietary flavonoid. *Genes and Environment*, 39 (10), 1-9. <https://doi.org/10.1186%2Fs41021-016-0071-7>
- Ye, Y., Liu, G. H., Dawa, D., Ding, L. S., Cao, Z. X., & Zhou, Y. (2020). Cytotoxic diterpenoids from the roots of *Euphorbia stracheyi*. *Phytochemistry Letters*, 36, 183-187. <http://dx.doi.org/10.1016/j.phytol.2020.03.002>
- Yedjou, C. G., Tchounwou, H. M., & Tchounwou, P. B. (2016). DNA damage, cell cycle arrest, and apoptosis induction caused by lead in human leukemia cells. *International Journal of Environmental Research and Public Health*, 13(1), 56. <https://doi.org/10.3390%2Fijerph13010056>
- Yener, İ., Ölmez, Ö. T., Ertas, A., Yilmaz, M. A., Firat, M., Kandemir, S. İ., Öztürk., M., Kolak, U., & Temel, H. (2018). A detailed study on chemical and biological profile of nine *Euphorbia* species from Turkey with chemometric approach: Remarkable cytotoxicity of *E. fistulosa* and promising tannic acid content of *E. eriophora*. *Industrial Crops and Products*, 123, 442-453. <http://dx.doi.org/10.1016/j.indcrop.2018.07.007>
- Yff, B. T., Lindsey, K. L., Taylor, M. B., Erasmus, D. G., & Jäger, A. K. (2002). The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. *Journal of Ethnopharmacology*, 79(1), 101-107. [https://doi.org/10.1016/s0378-8741\(01\)00380-4](https://doi.org/10.1016/s0378-8741(01)00380-4)
- Yi, X., Zuo, J., Tan, C., Xian, S., Luo, C., Chen, S., Yu, L., & Luo, Y. (2016). Kaempferol, a flavonoid compound from *Gynura medica* induced apoptosis and growth inhibition in MCF-7 breast cancer cell. *African Journal of Traditional, Complementary and Alternative Medicines*, 13(4), 210-215. <https://doi.org/10.21010%2Fajtcam.v13i4.27>
- 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%2FIJN.S246764>
- Yousuf, M., Shamsi, A., Khan, P., Shahbaaz, M., Al Ajmi, M. F., Hussain, A., Hassan, G. M., Islam, A., Rizwanul Haque, Q. M., & Hassan, M. I. (2020). Ellagic acid controls cell proliferation and induces apoptosis in breast cancer cells via inhibition of cyclin-dependent kinase 6. *International Journal of Molecular Sciences*, 21(10), 3526. <https://doi.org/10.3390%2Fijms21103526>

- Yu, C. C., Hsieh, C. R., Hsiao, G., Chen, P. Y., Chang, M. L., Yin, H. W., Lee, T. H., & Lee, C. K. (2012). Regulated expressions of MMP-2,-9 by diterpenoids from *Euphorbia formosana* Hayata. *Molecules*, 17(2), 2082-2090. <https://doi.org/10.3390%2Fmolecules17022082>
- Yu, X., Peng, W., Wang, Y., Xu, W., Chen, W., Huang, L., ... & Xu, Y. (2023). Palmitic acid inhibits the growth and metastasis of gastric cancer by blocking the STAT3 signaling pathway. *Cancers*, 15(2), 388. <https://doi.org/10.3390/cancers15020388>
- Yu, X., Zhao, M., Liu, F., Zeng, S., & Hu, J. (2013). Identification of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose-histidine Maillard reaction products. *Food Research International*, 51(1), 397-403. <http://dx.doi.org/10.1016/j.foodres.2012.12.044>
- Zafaryab, M., Fakhri, K. U., Khan, M. A., Hajela, K., & Rizvi, M. M. A. (2019). *In vitro* assessment of cytotoxic and apoptotic potential of palmitic acid for breast cancer treatment. *International Journal of Life Science Research*, 7(1), 166-174. <http://dx.doi.org/10.13140/RG.2.2.33419.13606>
- Zaghlol, A. A., Kandil, Z. A., Yousif, M. F., EL-Dine, R. S., & Elkady, W. M. (2024). Unveiling the anti-cancer potential of *Euphorbia greenwayi*: Cytotoxicity, cell migration, and identification of its chemical constituents. *Future Journal of Pharmaceutical Sciences*, 10(1), 24.
- Zaheer, J., Najam-Us-Saqib, Q., Anwar, T., Khan, F. S., Akram, M., Munir, N., Rebezov, M., Shariati, M. A., & Thiruvengadam, M. (2021). Phytochemical profile of rock jasmine (*Androsace foliosa* Duby ex Decne) by using HPLC and GC-MS analyses. *Arabian Journal for Science and Engineering*, 46, 5385-5392. <http://dx.doi.org/10.1007/s13369-020-05241-8>
- Zahoor, M., Nazir, N., Iftikhar, M., Naz, S., Zekker, I., Burlakovs, J., Uddin, F., Kamran, A.W., Kallistova, A., Pimenov, N., & Ali Khan, F. (2021). A review on silver nanoparticles: Classification, various methods of synthesis, and their potential roles in biomedical applications and water treatment. *Water*, 13(16), 2216. <https://doi.org/10.3390/w13162216>
- Zare, S., Ghaedi, M., Heiling, S., Asadollahi, M., Baldwin, I. T., & Jassbi, A. R. (2015). Phytochemical investigation on *Euphorbia macrostegia* (Persian wood spurge). *Iranian Journal of Pharmaceutical Research: IJPR*, 14(1), 243-251.
- Zeghad, F., Djilani, S. E., Djilani, A., & Dicko, A. (2016). Antimicrobial and antioxidant activities of three *Euphorbia* species. *Turkish Journal of Pharmaceutical Science*, 13(1), 22-37. <http://dx.doi.org/10.5505/tjps.2016.29491>
- Zhang, C. Y., Wu, Y. L., Zhang, P., Chen, Z. Z., Li, H., & Chen, L. X. (2019). Anti-inflammatory lathyrane diterpenoids from *Euphorbia lathyris*. *Journal of Natural Products*, 82(4), 756-764. <https://doi.org/10.1021/acs.jnatprod.8b00600>
- Zhang, L., Wang, C., Meng, Q., Tian, Q., Niu, Y., & Niu, W. (2017). Phytochemicals of *Euphorbia lathyris* L. and their antioxidant activities. *Molecules*, 22(8), 1335. <https://doi.org/10.3390/molecules22081335>
- Zhang, X. F., Liu, Z. G., Shen, W., & Gurunathan, S. (2016). Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic

- approaches. *International Journal of Molecular Sciences*, 17(9), 1534-1568. <https://doi.org/10.3390%2Fijms17091534>
- Zhang, Y., Fang, J., Yang, J., Gao, X., Dong, L., Zheng, X., ... & Wang, Y. (2022). *Streptococcus mutans*-associated bacteria in dental plaque of severe early childhood caries. *Journal of Oral Microbiology*, 14(1), 2046309. <https://doi.org/10.1080%2F20002297.2022.2046309>
- Zhao, H., Sun, L., Kong, C., Mei, W., Dai, H., Xu, F., & Huang, S. (2022). Phytochemical and pharmacological review of diterpenoids from the genus *Euphorbia* Linn. (2012–2021). *Journal of Ethnopharmacology*, 298, 115574. <https://doi.org/10.1016/j.jep.2022.115574>
- Zhao, T., Sun, Q., Marques, M., & Witcher, M. (2015). Anticancer properties of *Phyllanthus emblica* (Indian gooseberry). *Oxidative Medicine and Cellular Longevity*, 2015. <https://doi.org/10.1155%2F2015%2F950890>
- Zhao, W., Entschladen, F., Liu, H., Niggemann, B., Fang, Q., Zaenker, K. S., & Han, R. (2003). Boswellic acid acetate induces differentiation and apoptosis in highly metastatic melanoma and fibrosarcoma cells. *Cancer Detection and Prevention*, 27(1), 67-75. [https://doi.org/10.1016/s0361-090x\(02\)00170-8](https://doi.org/10.1016/s0361-090x(02)00170-8)
- Zhao, X. L., Yu, L., Zhang, S. D., Ping, K., Ni, H. Y., Qin, X. Y., Zhao, C. J., Wang, W., Efferth, T., & Fu, Y. J. (2020). Cryptochlorogenic acid attenuates LPS-induced inflammatory response and oxidative stress via upregulation of the Nrf2/HO-1 signaling pathway in RAW 264.7 macrophages. *International Immunopharmacology*, 83, 106436. <https://doi.org/10.1016/j.intimp.2020.106436>
- Zhou, C., Ma, J., Su, M., Shao, D., Zhao, J., Zhao, T., ... & Jiao, P. (2018). Down-regulation of STAT3 induces the apoptosis and G1 cell cycle arrest in esophageal carcinoma ECA109 cells. *Cancer Cell International*, 18, 1-12. <https://doi.org/10.1186/s12935-018-0549-4>
- Zhou, L., Zhao, X., Li, M., Lu, Y., Ai, C., Jiang, C., ... & Shi, J. (2021). Antifungal activity of silver nanoparticles synthesized by iturin against *Candida albicans* *in vitro* and *in vivo*. *Applied Microbiology and Biotechnology*, 105(9), 3759-3770.
- Zhou, Y., Hong, Y., & Huang, H. (2016). Triptolide attenuates inflammatory response in membranous glomerulo-nephritis rat via downregulation of NF- $\kappa$ B signaling pathway. *Kidney and Blood Pressure Research*, 41(6), 901-910. <https://doi.org/10.1159/000452591>
- Zhu, N., & Hou, J. (2021). Molecular mechanism of the anti-inflammatory effects of *Sophorae flavescens* Aiton identified by network pharmacology. *Scientific Reports*, 11(1), 1005. <https://doi.org/10.1038/s41598-020-80297-y>
- Zhu, S., Jiao, W., Xu, Y., Hou, L., Li, H., Shao, J., Zhang, X., Wang, R., & Kong, D. (2021). Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway. *Life Sciences*, 286, 120046. <https://doi.org/10.1016/j.lfs.2021.120046>



## APPENDICES

### Wagner's reagent

### Appendix 1

Iodine : 1.27 g

KI : 2 g

Dissolve the above chemicals in 5 ml  $\text{H}_2\text{SO}_4$  and make upto 100 ml.

### Griess reagent

### Appendix 2

Naphthyl ethylenediamine HCl : 0.1% in distilled water

Sulfanilamide : 1% in 5%  $\text{H}_3\text{PO}_4$

Both solutions are mixed in 1 : 1 ratio

### Phosphate buffer saline

### Appendix 3

NaCl : 8 g

KCl : 0.2 g

$\text{Na}_2\text{HPO}_4$  : 1.44 g

$\text{KH}_2\text{PO}_4$  : 0.24 g

Distilled water : 1 litre

The above ingredients are mixed in 800 ml of distilled water and pH was adjusted to 7.4 with HCl. The total volume was then made up to 1 litre with distilled water. The solution was sterilized by autoclaving (20 min,  $121^\circ\text{C}$ ) and stored at room temperature.

### Tris-HCl buffer

### Appendix 4

Tris base : 121.1 g

HCl : 70 ml

The tris base is dissolved in 800 ml distilled water and 70 ml concentrated HCl is added. Then the volume is adjusted to 1 litre with distilled water.

**HEPES buffer****Appendix 5**

|                                 |   |         |
|---------------------------------|---|---------|
| NaCl                            | : | 6.72 g  |
| CaCl <sub>2</sub>               | : | 133 mg  |
| MgCl <sub>2</sub>               | : | 114 mg  |
| K <sub>2</sub> HPO <sub>4</sub> | : | 418 mg  |
| HEPES                           | : | 4.77 g  |
| H <sub>2</sub> O                | : | 1 litre |

Adjust the pH to 7.4 with HCl or NaOH. HEPES buffer can be stored refrigerated for several weeks.

**Blocking buffer****Appendix 6**

|          |   |        |
|----------|---|--------|
| Gelatin  | : | 0.2%   |
| TWEEN 20 | : | 0.005% |

The ingredients are freshly prepared in PBS

**Modified Carnoy's fluid****Appendix 7**

|             |   |       |
|-------------|---|-------|
| Acetic acid | : | 10 ml |
| Ethanol     | : | 30 ml |

**Acetocarmine stain****Appendix 8**

|                  |   |        |
|------------------|---|--------|
| Carmin           | : | 2 g    |
| 45% Acetic acid: | : | 100 ml |

The solution is heated to dissolve carmine and is filtered to remove undissolved stain.

**DMEM (Dulbecco's modified Eagle's medium)****Appendix 9**

|                    |   |         |
|--------------------|---|---------|
| Sodium bicarbonate | : | 1.85 g  |
| HEPES              | : | 2.95 g  |
| DMEM powder        | : | 0.67 g  |
| Distilled water    | : | 1 litre |

Vaccum sterilized and stored at 4°C.