# PHARMACOGNOSTIC PROFILING, PHYTOCHEMICAL CHARACTERIZATION AND BIOACTIVITY SCREENING OF SELECTED SPECIES OF *MEMECYLON* L. (MELASTOMATACEAE)

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

This is to certify that the thesis entitled **" Pharmacognostic profiling, phytochemical characterization and bioactivity screening of selected species of *Memecylon* L. (Melastomataceae) "** 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 **Ramya Sree P. R.** during the period of study (2017-2021) at the Cell and Molecular Biology Division, Department of Botany, University of Calicut under my supervision and guidance and that it has not formed the basis for award of any degree or diploma. Also certified that the contents in the thesis is subjected to plagiarism check using the software URKUND, and that no text or data is reproduced from other works.

**Dr JOHN E. THOPPIL**
Supervising Teacher

# DECLARATION

I, Ramya Sree P. R., hereby declare that the thesis entitled **"Pharmacognostic profiling, phytochemical characterization and bioactivity screening of selected species of *Memecylon* L. (Melastomataceae) "** 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, Professor, Cell and Molecular Biology Division, 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.

Date: **RAMYA SREE P. R.**

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***Ramya Sree P. R.***

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# CONTENTS

# ABBREVIATIONS

| | | |
|---|---|---|
| °C | : | Degree Celsius |
| µg | : | microgram |
| µg/mL | : | microgram/milliliter |
| µL | : | microliter |
| µm | : | micrometer |
| $AlCl_3$ | : | Aluminium chloride |
| AO | : | Acridine Orange |
| ATP | : | Adenosine triphosphate |
| CAT | : | Catalase |
| cDNA | : | Complementary DNA |
| COX | : | Cyclooxygenase |
| DMEM | : | Dulbecco's Modified Eagle Medium |
| DMSO | : | Dimethylsulphoxide |
| DPPH | : | 2,2-diphenyl-1-picrylhydrazyl |
| EDTA | : | Ethylene Diamine Tetra Acetic acid |
| EDX | : | Energy dispersive X- ray spectroscopy |
| EtBr | : | Ethidium Bromide |
| eV | : | Electron volt |
| $FeCl_3$ | : | Ferric chloride |
| g | : | gram |
| GC | : | Gas chromatography |
| GC-MS | : | Gas Chromatography-Mass Spectrometry |
| GSHPx | : | Glutathione peroxidase |
| $H_2O_2$ | : | Hydrogen peroxide |
| $H_2SO_4$ | : | Sulphuric acid |
| HCl | : | Hydrochloric acid |
| HEPES | : | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HMF | : | Hydroxymethylfurfural |
| HPLC | : | High-performance liquid chromatography |
| HPTLC | : | High-performance thin layer chromatography |
| HR-LC/MS | : | High Resolution Liquid Chromatography/Mass spectrometry |

| | | |
|---|---|---|
| $IC_{50}$ | : | Inhibitory Concentration 50% |
| ICP-MS | : | Inductively coupled plasma mass spectrometry |
| IPP | : | Isopentenyl pyrophosphate |
| KOH | : | Potassium hydroxide |
| L | : | Liter |
| LC | : | Liquid chromatography |
| $LD_{50}$ | : | Least Dose 50% |
| LDL | : | Low density lipoproteins |
| M | : | Molar |
| m/z | : | Mass to charge ratio |
| MCF-7 | : | Human Breast Adenocarcinoma (Michigan Cancer Foundation - 7) |
| mg | : | milligram |
| min | : | minutes |
| mL | : | milliliter |
| mM | : | millimolar |
| MS Q-TOF | : | Mass spectrometry quadrupole time of flight |
| MTT | : | 3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide |
| N | : | Normal |
| $Na_2CO_3$ | : | Sodium carbonate |
| $NaNO_2$ | : | Sodium nitrate |
| NaOH | : | Sodium hydroxide |
| NBT | : | Nitro blue tetrazolium |
| nm | : | nanometer |
| PBS | : | Phosphate Buffered Saline |
| PCR | : | Polymerase chain reaction |
| PRXs | : | Peroxiredoxins |
| ROS | : | Reactive oxygen species |
| rpm | : | Revolutions per minute |
| RT- PCR | : | Reverse transcription polymerase chain reaction |
| SE | : | Standard error |
| SEM | : | Scanning electron microscope |
| SOD | : | Superoxide dismutase |
| sq km | : | Square kilometer |
| TBA | : | Thiobarbituric acid |

| | | |
|---|---|---|
| TCA | : | Trichloroacetic acid |
| TE | : | Tris EDTA |
| TRXs | : | Thioredoxins |
| UV | : | Ultraviolet |
| V | : | Volt |
| w/v | : | weight per volume |
| WHO | : | World Health Organization |

# CHAPTER 1 INTRODUCTION

India has a rich tradition in art, food, medicine, literature *etc*. The excellent contributions made in these fields had incredibly enlightened our tradition. The great art and works of literature have been symbolically substantiating our tradition into the forefront of World heritage. Ramayana, Mahabharata and Vedas are milestone works in Indian history. They gave insight into the values and dharmas that one should follow in life. The enormous diversity of food items is really fascinating and it became a trademark of our country. The medical field is another relevant field. We have a rich traditional medicinal system like Ayurveda, Unani and Siddha that are always used by the common people to cure various diseases. This system gave a detailed treatment manual for various human ailments. The main reason behind the usage of these medicinal systems is the curing effects with no side effects. The common people have a strong belief in these medicinal systems because of their wide acceptability. From ancient times, plants and humans are mutually connected. The plants are used for curing human ailments and worshipping are marked in various religious manuscripts such as Bible, Rig Vedas, Ramayana *etc*. The early medicines of Pharaohs (3000 BC), the Greek (460-370 BC; Hippocratis) and the Romans (37 BC; Dioscorides, a Greek physician who wrote Materia Medica -78 AD) are plant-based and they described nearly 600 medicinal plants. In the Medieval period, Arab physicians (Rhazes 865-925 and Avicenna 980-1037) relied mainly on plants for therapy (Subhose et al., 2005).

India has a rich tradition of indigenous medicinal systems. Ayurveda, Siddha and Unani are the common medicinal practices in India. Plants are the basic resources of these practices. The Ayurvedic system of medicine was originated from the Indus valley civilization and still widely practiced in

modern times (Gupta et al., 2014). Plant-based medicines are the common strategy of Ayurvedic treatment. Ayurvedic traditional texts such as *Charaka Samhitha*, *Susrutha Samhitha* and *Ashtanga Samgraha* describes the use of thousands of plants in the Ayurvedic preparations. The Indian subcontinent has a vast geographical distribution and enormous biological diversity. Out of the 10,000 plants used for medicinal purposes in the Indian subcontinent, only 1200 to 1500 have been incorporated into the official Ayurvedic pharmacopoeia (Manohar, 2012). The forest areas are the rich repositories of medicinal and aromatic plants. In India, 90% of medicinal plants are in forest habitats and the remaining 10% in grasslands, freshwater bodies and agricultural lands (Chakraborty et al., 2012).

Siddha medicine is one of the most ancient medical systems of India. It uses safe herbal and herbo-mineral treatment for various diseases like psoriasis, eczema, alopecia, diabetic ulcer, warts, leprosy *etc.* (Thas, 2008). Unani system of medicines was originated from ancient Greece. It is a herbal medicinal system, which includes dietary practices and alternative therapies. The plant kingdom is the cardinal pluck of all indigenous medicinal systems in the World. According to WHO, 80% of populations of developing countries rely on medicinal plants for their primary health care needs. The increasing demands of herbal medicine are satisfied by the exploration of medicinal plants from the forest area. Natural forests are the reservoirs of the majority of medicinal plants and around 20,000 medicinal plants have been recorded. The traditional practitioners use only 7000-7500 plants for curing different diseases. The proportion of use of plants in the different Indian systems of medicine is as follows: Ayurveda 2000, Siddha 1300, Unani 1000, Homeopathy 800, Tibetan 500, Modern 200 and Folk 4500. In India, around 25,000 effective plant-based formulations are used in traditional and folk medicinal practices (Pandey et al., 2008).

# Chapter 1
# Introduction

In India, the Western Ghats region is covered by 34 global biodiversity hotspots and having an area of 159,000 sq km with 4500-15,000 plant species. Almost 1800 of these are endemic to the region and 500 plants have been identified to have potential medicinal value (Sekhar et al., 2015). The proper authentification of plant material is an important step in pharmacological approaches of herbal medicines. There are thousands of plants that have potential medicinal values. The clarifications of the botanical identity of the selected plant materials are very important on their pharmacological application. The misleads in plant authentification can cause adverse effects in pharmacological properties. The medicinal industry ramifies the production of drugs from various plant species. So there is a chance of getting allied species or foreign material on their preparation. This will cause adverse therapeutic effects in testimonials. Pharmacognosy is a leading branch of science that deals with the proper identification of plant materials particularly medicinal plants, which are the possible source of natural drugs. The American Society of Pharmacognosy (ASP) defines it as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from different natural sources" (Perveen & Al-Taweel, 2019). Pharmacognosy has always been a multidisciplinary branch of science that deals with phytomedicine and phytochemistry. It has an important association between medicinal chemistry and pharmacological studies.

Natural products such as plant extracts provide unlimited opportunities for new drug discoveries, mostly because they have a plethora of chemicals. The actions of phytoconstituents on tissues are specific. The phytoconstituents in plants are apportioned into primary and secondary metabolites. Primary metabolites are involved in the normal growth, development and reproduction

of an organism. They are involved in all basic life functions. So they are more or less similar in all living cells. Carbohydrates, vitamins, amino acids and proteins are the primary metabolites in plants. The secondary metabolites are not involved in the life functions of organisms. They are the byproducts of subsidiary pathways that originated as the result of some defensive mechanism of the plants. The medicinal effects of herbals point towards the secondary metabolite profiles of the respective plants. The vast and versatile pharmacological effects of secondary metabolites make them a lead component of natural drugs. Phytochemistry is the basis of the therapeutic uses of herbs. Good knowledge of the chemical composition of plants provides a better understanding of their possible medicinal values. Several secondary metabolites are present in plants, such as alkaloids, terpenes, phenols, coumarins, glycosides *etc*. Secondary metabolites are chemicals that are derived from primary metabolites through specific metabolic pathways. Albrecht Kossel is the one who identify the role of secondary metabolites in organisms (Jones, 1953). Czapek treat them as end-products of nitrogen metabolism. The latest chromatographic separation techniques give a more purified form of secondary metabolites (Bourgaud et al., 2001).

The bioactive secondary metabolites are effective targets of drug discoveries. They perform potential functions like antioxidant, anticancerous, antimicrobial, antidiabetic activities *etc*. The herbal medicinal system has wide acceptability as compared to synthetic medicines. Many plant-derived pharmaceutical drugs are developed from the herbal medicinal system. Vincristine and vinblastine are developed from *Catharanthus roseus*, an Apocynaceae member, which is effectively targeted in cancer chemotherapy for Hodgkin's disease and neuroblastoma. Paclitaxel or taxol is another chemotherapeutic agent derived from *Taxus brevifolia.* Cardiovascular disease can be effectively targeted by the use of digoxin, isolated from the

*Digitalis lanata*. Opiates are another class of chemicals isolated from the opium poppy and are targeted on opioid receptors in the human body that regulate pain and temperature control. Aspirin is the isolated chemical from *Salix alba* (white willow), *Spirea* spp. and *Betula* spp. It is popular in the treatment of pain, inflammation and fever. It works by inhibiting an enzyme known as cyclooxygenase (COX). There are some specific reports on pharmaceutical drugs. However, thousands of plants are still unexplored. The herbal medicinal systems have a wide chance of discovering many natural therapeutic agents (**https**[1]). The growing number of outbreaks of various infectious diseases increases the demand for therapeutic agents. The deleterious side effect of synthetic drugs increases human attention on natural drugs. Natural drugs are safe and their permanent curing effects are attracting mankind. So it opens a gateway for the search for safe natural drugs. Plants are rich reservoirs of phytochemicals. Most of them are rich in alkaloids, phenolics, terpenes, saponins, glycosides *etc*. These secondary metabolites are possessing diverse bioactivities. The synergistic action of phytochemicals contributes to their specific bioactivities. So plants are an immense resource of nature. The plants are viable for modern medicine in four ways; they serve as a direct source of medicine; act as a raw material for the synthesis of complex semi-synthetic chemical compounds; the chemical structure derived from phytochemicals are used for the synthesis of new compounds; plants are used as taxonomic markers for the synthesis of new therapeutics (Raaman, 2006).

The alkaloids represent a group of natural products with a wide spectrum of biological activity. They are alkali-like compounds that react with acids to form salts. Atropine, morphine, quinine and vincristine are some of the popular alkaloids used in the therapeutic field. They are used to treat a wide range of disease conditions that include malaria, asthma, cancer *etc*.

Alkaloids have diverse physiological effects such as antibacterial, anti-inflammatory, analgesic, local anaesthetic, antimitotic, psychotropic, antitumor activity *etc*. In the daily life of human beings, there is always a chance of getting exposed to alkaloids. Alkaloids always form a part of foods and drinks. Coffee seeds (caffeine), cacao seeds (theobromine, caffeine), tea leaves (theophylline), tomato (tomatine) and potato (solanine) is our daily source of alkaloids. Several well-known plant-based alkaloids with potential bioactivities have been already reported. Morphine is one of the well-known alkaloids that had been used as a powerful narcotic agent. The methyl ether derivative of the morphine-codeine complex possesses an excellent analgesic activity. Atropine is another well-known alkaloid used as medication in many clinical applications and it is also used to treat bradycardia. Vincristine and vinblastine are the most potential chemotherapeutic agents in many cancer types. Ephedrine is used to treat bronchial asthma and quinine is an anti-malarial agent. The popular colchicine originated from *Colchicum autumnale* is used to treat acute gout attacks (Kurek, 2019). Thus the extended list of valuable alkaloids reveals their potential role in the pharmaceutical field and creates a gateway for new searches.

Flavonoids are a group of polyphenolic compounds widely distributed in the plant kingdom. It has several biological roles such as antiatherosclerotic, antiviral, antitumor, anti-inflammatory and antimicrobial effects. Flavonoids consist of flavones, flavanones, flavonols, isoflavonoids, catechins and anthocyanins. Kaempferol, catechins, rutin and quercetin are some of the well-known flavonoids (Agrawal, 2011). Flavonoids are good free radical scavengers. Epicatechin and rutin are powerful radical scavengers and the scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase (Hanasaki et al., 1994). In certain *in vitro* studies, flavonoids were found to exhibit an inhibitory effect on LDL oxidation due to

their antioxidant potential (Kerry & Abbey, 1997). Flavonoids like quercetin, naringin, hesperetin and catechins exhibit promising antiviral potential. The immune-responsive mechanism of quercetin is a promising one. The subclasses of flavonoids also have a significant biological role (Panche et al., 2016).

Phenolics are the most abundant group of secondary metabolites, widely distributed in the plant kingdom. The plant polyphenols have marked attention due to their antioxidant activity and remarkable prevention of oxidative stresses associated with various diseases. Currently, more than 8000 plant polyphenols are known. Phenolic compounds are generally found to be a part of the defensive mechanism of plants. They are involved in defence against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to the colours of plant parts. Plant phenolics are mainly phenolic acids and tannins. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables. The esterified caffeic acid and quinic acid forms chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is commonly seen in cereals. Polyphenols can modulate the activity of a wide range of enzyme and cell receptors. In addition to having antioxidant properties, polyphenols have several other specific biological actions in preventing and or treating diseases (Dai & Mumper, 2010).

Among the plant metabolites, terpenoids are the most diverse group of secondary metabolites. Terpenes consist of monoterpenes, sesquiterpenes, diterpenes through the isopentenyl diphosphate (IPP) mediated biosynthetic pathway. Terpenoids have a significant ecological role (Cheng et al., 2007). The antimicrobial effect of sesquiterpene and diterpenes isolated from *Salvia sclarea* was proved against C*andida albicans, Proteus mirabilis* and *Staphylococcus aureus* (Ulubelen et al., 1994). The potential effect of

secondary metabolites opens a gateway for the search for more and more bioactive compounds from nature. From ancient times, nature and human beings are interlinked. Nature satisfies all the needs of mankind. They are in the form of food, medicines, ergotic substances *etc*. The growing outbreaks of infectious diseases increase the demand for natural herbal medicine with less deleterious side effects. The phytochemical analysis gives a picture of the immense resource of bioactive molecules in plants. The identification and isolation of various bioactive molecules from plants will be useful in curing various infectious diseases. As we know, the pharmaceutical research world looks for the immense source of herbal plants in nature. They work out on the identification and purification of bioactive molecules and find out their bioactivity. The phytochemical constituents like saponins, tannins, alkaloids, phenols, flavonoids and terpenoids in the herbals, were found to be responsible for the desired healing effect. In nature, there is a greater area of unexploited resources. So there is always a chance of more and more exploitation in pharmaceutical research. The World is now moving towards the herbal medicine or phytomedicine that repair and strengthens our body systems and help to destroy offending pathogens without toxic side effects.

The proper identification and isolation of phytoconstituents from the plant is important in their specific bioactivity. The selected plant parts (leaves, fruits, bark, flowers, seeds, roots and stem) were extracted upon a suitable solvent using the Soxhlet apparatus. The preliminary phytochemical analysis thus gives an insight into the presence or absence of major phytoconstituents. In these preliminary phytochemical analyses, both quantitative and qualitative analyses are important. The preliminary analysis is a prime step in phytochemical analysis. It is difficult to characterize the metabolites that are at the compound level. The characterization of phytochemicals is done through advanced instrumentation techniques. Chromatographic techniques

are an effective way of phytochemical characterization. Nowadays, combinations of advanced techniques like chromatography and spectrometry are the more reliable ones.

All separation techniques depend upon the physical properties of the compounds. Some time the compounds are similar in their molecular size but differ in any one of the physical characters. Several chromatographic techniques are available for the compound level separation and it may be a single chromatographic technique or a combination of chromatographic techniques. There are several chromatographic techniques like column chromatography, gas chromatography, gas-liquid chromatography, thin-layer chromatography, paper chromatography, liquid chromatography, high-performance liquid chromatography *etc*. Gas chromatography (GC) and liquid chromatography (LC) are more powerful techniques for the qualitative and quantitative determination of compounds. Gas chromatography-mass spectrometry (GC-MS) is a widely used instrument for the qualitative and quantitative evaluation of volatile organic compounds. Here the samples are converted to the gaseous state without decomposition and separate the compounds based on their mass-to-charge ratio. The quantitative determination of non-volatile compounds is performed through liquid chromatography-mass spectrometry (LC-MS). High-performance liquid chromatographic (HPLC) techniques are also available in the quantitative analysis of phytochemicals. The advances in thin layer chromatography *ie*., high-performance thin layer chromatography (HPTLC) can be a valuable tool in separation techniques (Raaman, 2006).

In the last several years, researches on the isolation and separation of compounds from plants have been increased because the biological potential of plant-based compounds is remarkable. They are active against various human ailments. Oxidative stress is one of the most important conditions

behind several disease conditions. Oxidative stress is a shift in the balance between oxidants and antioxidants. Oxidative stress can down-regulate all cellular functions and may lead to several disease conditions. Oxidative stress can generate Reactive Oxygen Species (ROS) inside the body. ROS are the normal byproduct of cellular metabolism in the aerobic organism. ROS have both beneficial and harmful effects. ROS have become harmful when its equilibrium of generation and scavenging gets disturbed. It has a beneficial role as secondary messengers in environmental stress conditions. ROS are highly reactive molecules and can damage cell structures and alter their functions that include carbohydrates, nucleic acids, lipids and proteins. The antioxidant system inside the body can regulate the functioning of ROS, cell proliferation, and organ systems. The main function of antioxidant defence systems is the blocking of initial production of free radicals, scavenging the oxidants, converting the oxidants to less toxic compounds, blocking the production of toxic metabolites or inflammatory mediators and repairing the molecular injury induced by free radicals or enhancing the endogenous antioxidant defence system of the target system. All these defence mechanisms act together to protect the body from oxidative stress (Kabel, 2014). The antioxidant defence system consists of powerful enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), heme oxygenase-1 and redox proteins, such as thioredoxins (TRXs), peroxiredoxins (PRXs) and glutaredoxins are the enzymatic antioxidants. The non-enzymatic antioxidant machinery includes vitamins, bioflavonoids, carotenoids *etc*. The regulation of ROS occur through the reactions of antioxidants, *ie.*, they can neutralize oxidative stress. Otherwise, ROS can disrupt cellular mechanisms and lead to severe pathological conditions and diseases like cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes *etc*. ROS also affects the upregulation of redox-sensitive transcription factors and chromatin

remodelling *via.,* alteration in histone acetylation/deacetylation. Regulation of redox state is critical for cell viability, activation, proliferation and organ function (Birben et al., 2012).

There are several natural antioxidants that perform effective scavenging activity. Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C. *In vivo* studies are also validating the same result (Rice-Evans et al., 1997). The polyphenols present in fruits, vegetables, wine, tea, chocolate *etc.,* shows promising antioxidant activity in *in vitro* studies. Studies reveal that antioxidant responsive elements (AREs) are present in the promoter regions of many of the genes that are inducible by oxidative stress. So dietary polyphenols are good antioxidants and they can stimulate antioxidant transcription and detoxification defence systems through ARE (Masella et al., 2005). Several reports are describing the potential antioxidant efficacy of plants. The chemical constituents of plants have contributed to their potential scavenging activity. Several fruits and vegetables are rich sources of natural antioxidants. It can scavenge the free radicals and boost up overall health. The natural antioxidant sources have great attention as compared to synthetic ones. The plethora of phytochemicals present in natural sources can scavenge the free radicals and neutralize them. This will reduce oxidative stress and pathological conditions inside the body. Mankind always follows safe remedies without deleterious side effects.

Oxidative stress can cause an imbalance in the human body. It may result in the form of serious diseases like cancer, cardiovascular diseases, lungs diseases *etc*. Cancer is one of the leading causes of death in the World. The complicated molecular mechanisms of various cancers block them from complete surveillance. According to WHO, cancer is the second leading cause of death globally. About 9.6 million deaths or one in six deaths, in 2018 is due

to cancer. The most common type of cancer in men are lung, prostate, colorectal, stomach and liver cancer, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women. In studies, ROS plays a complex role in cancer development. They are involved in the transformation, tumor survival, proliferation, invasion and metastasis of cancer cells. ROS can actively take part in cell cycle arrest, apoptosis and necrosis. The influence of ROS in malignancy is dependent on tumor and tissue type, disease stage, treatment strategy, as well as duration, specificity and levels of ROS (Okon & Zou, 2015). The prevalent ROS stress is observed in various cancers. ROS act as pleiotropic signalling molecules in physiologic as well as pathological processes associated with neurodegenerative diseases, carcinogenesis and even cancer heterogeneity. The presence of ROS is notified in non-neoplastic cells associated with tumors like cancer-associated fibroblasts (CAF), endothelial cells, immune cells, adipocytes and pericytes (de Sá Junior et al., 2017).

The emergence of breast cancer in women is higher as compared to other types of cancers. It is affecting nearly 2.1 million women each year. The present study gives special emphasis to *in vitro* studies using breast cancer cell lines. Cell line studies are the preclinical method of screening the effectiveness and toxicity of drugs in laboratory conditions. Cell lines are valuable physiological targets of drug activity and they may be helpful for target validation, efficacy testing and introducing safer remedies into clinical trials. A large proportion of current knowledge on carcinomas is derived from *in vivo* and *in vitro* studies performed using cancer cell lines. There are specific cell lines for each carcinoma. The lung cancer studies were carried out on the cell lines *viz.,* A549, ABC-1, EBC-1, LK-2, LU65, LU99, STC 1 and RERF-LC-MA types. In breast cancer studies, MDAMB134, MCF-7, HCC1428, LY2 *etc.*, are the cell lines used (Watanabe et al., 2010; Dai et al.,

2017). The hCMEC/D3, the human brain endothelial cell line is used as a blood-brain barrier in drug transport studies (Poller et al., 2008). The cell lines are effective *in vitro* models for assessing drug transport and their toxicity levels in the antiproliferative study.

The consumption of nutraceuticals from nature can reduce the incidence of cancer development (Prasad et al., 2017). Several naturally occurring metabolites are being examined for their antiproliferative efficacy leading to the development of new clinical drugs. The anticancer properties of natural medicines have ample demand because of their target-specific activity and they are non-toxic to normal cells. The compounds which have been identified and extracted from plants with anticancerous properties are mainly polyphenols, brassinosteroids and taxols. The polyphenols include flavonoids, curcumins and tannins that show potent anticancerous activity. Resveratrol and gallocatechins are the known polyphenols having potential anticancerous activity and they are said to be natural antioxidant agents. Polyphenols have the apoptosis-inducing ability through the regulation of copper ion mobilization which can interfere with chromatin during DNA fragmentation. Curcumin treated cancer cells show suppression of Tumor Necrosis Factor (TNF) (Greenwell & Rahman, 2015). Several purified flavonoids have potential anticancerous activity. 4'-Methoxy licoflavanone (MLF) and Alpinumisoflavone (AIF) exhibiting cytotoxic effects in HL-60 cells (human leukaemia) are examples of it (Kumar et al., 2013). Brassinosteroids are the other naturally occurring compounds that possess anticancerous property. It can induce growth inhibition and apoptosis through the interaction of the cell cycle. 28-homocastasterone (28-homoCS) and 24-epibrassinolide (24-epiBL) are two brassinosteroids having anticancer effects in various cell lines (Malíková et al., 2008). A vast source of natural drugs new to science is produced every year. Their non-toxic effects on normal cells

and their cytotoxic effects on cancer cells put them in high demand. The increasing demand for plant-derived drugs is the outcome of fruitful research in pharmaceutical science.

The antiproliferative studies have made several advances. It includes the initial screening of cytotoxicity using *in vitro* and *in vivo* systems. *Allium cepa* assay is a basic cytotoxic screening assay used for evaluating the toxic potential of plant extracts. It can be correlated with the chromosome damages induced by the plant extract. Further cell line studies and mammalian *in vivo* studies provide the potential range of plant extract in cancer therapy. The cancer induction and the cell cycle progression are interlinked. The cell cycle consists of the interphase and mitotic phase. G1, S and G2 are the interphase stages. The mitotic phase consists of prophase, metaphase, anaphase, telophase and cytokinesis. Each phase of the cell cycle is tightly regulated, and checkpoints exist to detect potential DNA damages and allow it to be repaired before a cell divides. If the damage cannot be repaired, a cell becomes targeted for apoptosis. So cell cycle analysis is an effective tool in anticancer studies. Flow cytometric analysis of cellular DNA content during the cell cycle will be effective for the identification of abnormal cell populations (Spyratos, 1993). In cancer cells, abnormal cell populations act as marker points. The deregulated cell proliferation and inhibition of cell deaths are the warning force behind cancer development. The cell cycle analysis and apoptotic induction studies give a vivid picture of uncontrolled cell proliferation. The development of clinical strategies for cell cycle and apoptosis can provide a better solution for this malignancy. There are several *in vitro* assays that provide a better understanding of the antiproliferative mechanism of natural herbs. So the *in vitro* assays provide an initial platform for the anticancer drug approaches. In anticancer studies, several *in vitro* assays are practiced. The cell viability assays are the prime ones, which is

based on cellular enzymes and proteins, DNA synthesis, cellular ATP, membrane integrity and impedance. The *in vitro* assays for the detection of apoptosis, cell migration and invasion, angiogenesis, antioxidant and oxidative stress markers and cellular senescence are the other frequently used assays. The techniques to detect gene mutations and chromosomal alterations, techniques for gene and protein expression analysis and assays for monitoring energy metabolism in cancer cells *etc*., are commonly used in cancer drug discovery studies (Ediriweera et al., 2019). In the present study, we explore some of the *in vitro* assays for cell viability, detection of apoptosis, cell migration and cell invasion, chromosomal aberrations, DNA content, protein expression *etc*. The *in vitro* studies are the key factors behind the formulation of *in vivo* strategies for drug discovery.

The present study is also exploring the biosynthesis of silver nanoparticles using plant extracts. The synthesis of nanomaterials has wide application in the field of physics, biology and medicine. Nanomaterials have a particle size of 1-100 nm and have superior bioavailability than larger particles. There are different methods of nanoparticle synthesis *ie.*, chemical, physical, and biological, but chemical and physical methods are involved in the production of toxic byproducts which are hazardous and the methods are very expensive. A stable nanoparticle with controlled size and shape with an inexpensive, safe, reliable and green approach has been recently developed. So there are many reports related to the green synthesis of nanoparticles using several varieties of plant extracts (Khan et al., 2018; Saranyaadevi et al., 2014). Silver has been in use for several decades as nanosilver in various biomedical applications. The antimicrobial potential of silver nanoparticles and it's cytotoxic effects on various test systems and cell lines are being documented (Arunachalam et al., 2015; Rai et al., 2014). The new approaches in nano-medicines, aim to enhance anticancer activities of plant-derived drugs

by controlling the release of the compound and new administration strategies (Greenwell & Rahman, 2015).

Melastomataceae is a dicotyledonous flowering family distributed widely in the tropical and sub-tropical regions with about 300 genera and 8000 species. They are mostly annual or perennial herbs, shrubs or trees. According to the Angiosperm Phylogeny Group (APG), Melastomataceae is placed in the rosids clade (Chase et al., 2016). APG IV (2016) places Melastomataceae in the order Myrtales. The common members of the family are *Melastoma*, *Osbeckia*, *Heterotis*, *Clidemia etc*. The herbal aspect of this family has wide application in folk medicine and some of them have ornamental uses also.

The genus *Memecylon* L., a potent genus of the family Melastomataceae is represented by 289 species. They are distributed in semi-evergreen, evergreen, deciduous and montane forests. Among the 289 species, 40 species are representative of Indian region and in which 21 are found to be endemic (Sivu et al., 2013) most of which are distributed in the Deccan Peninsula exhibiting maximum diversity in the southern states of Kerala and Tamil Nadu. The genus is characterized by simple leaves, small blue, bluish-white, white, purplish, pale pink or rose pink, tetramerous flowers in cymose clusters, with an inferior ovary and 1-2 seeded berry. It can be readily distinguished in the field from the other Melastomataceous genera by its non-acrodromous venation and absence of trichomes. Many *Memecylon* species have potent medicinal properties and are used as an astringent and also for the treatment of eye troubles and skin disorders. *M. umbellatum* Burm. f., shows wound healing activity (Puratchikody & Nagalakshmi, 2007). *M. malabaricum* Clarke is used to cure inflammation and allergic disorders. *M. talbotianum* is used for neurodegenerative diseases, diabetic complications, inflammation, helminthic infections and skin diseases (Prakash et al., 2016). The genus *Memecylon* is one of the least studied groups of plants and hence in

the present study, an attempt will be made to evaluate its phytochemical aspects, various bioactivities and green synthesis efficacy. Some of the *Memecylon* species available in Kerala including *M. grande* Retz., *M. randerianum* S. M. & M. R. Almeida and *M. umbellatum* Burm. f., are selected for the present study. The leaf and fruit of the selected species were used for the study.

The main objectives of the present study are:

- ❖ Pharmacognostic evaluation of selected *Memecylon* species - Powder microscopic analysis, SEM-EDX analysis of fruit samples and ICP-MS analysis
- ❖ Phytochemical characterization - Preliminary qualitative and quantitative analysis, GC/MS and HR-LC/MS
- ❖ Bioactivity studies
- Antioxidant activity - DPPH, hydroxyl, nitric oxide and superoxide free radical scavenging assays.
- Cytotoxicity assay using *Allium cepa* root tip meristem.
- Antiproliferative studies with MCF-7 breast cancer cell lines - Cytotoxicity evaluation using MTT assay on MCF-7 cell line, cytotoxicity evaluation using MTT assay on L929 cell line, comet assay, detection of apoptosis, flow cytometric analysis and gene expression studies.
- Green synthesis of silver nanoparticles and characterization through UV-Vis spectrophotometer and SEM analysis.

# CHAPTER 2 REVIEW OF LITERATURE

An increasing interest in herbal remedies has been observed in several parts of the World. Many of the herbal remedies have been incorporated into orthodox medicinal plant practices. The wide usage of the plant material as natural drugs is due to their efficacy, low side effects, and a broad spectrum of biological activity. So the invention of natural drugs became a promising challenge nowadays. Diseases that have been managed traditionally using medicinal plants include malaria, epilepsy, infantile convulsion, diarrhoea, dysentery, fungal and bacterial infections. Medicinal herbs are considered to be a chemical factory as it contains multitudes of chemical compounds like alkaloids, glycosides, saponins, resins, flavonoids, sesquiterpenes, lactones and essential oils (Singh, 2005).

Melastomataceae is a dicotyledonous flowering family distributed widely in the tropical and sub-tropical regions with about 300 genera and 8000 species. They are mostly annual or perennial herbs, shrubs or trees. According to the Angiosperm Phylogeny Group (APG), Melastomataceae is placed in the rosids clade. APG IV (2016) places Melastomataceae in the order Myrtales. The common members of the family are *Melastoma*, *Osbeckia*, *Heterotis*, *Clidemia etc*. The herbal aspect of this family has wide application in folk medicines and some of them have ornamental uses also. *Memecylon* L., a potent genus of the family Melastomataceae is represented by 289 species. They are distributed in semi-evergreen, evergreen, deciduous and montane forests. Among the 289 species, 40 species are representative of Indian region and in which 21 are found to be endemic (Sivu et al., 2013), most of which are distributed in the Deccan Peninsula exhibiting maximum diversity in the southern states of Kerala and Tamil Nadu. The genus is characterized by simple leaves, small blue, bluish-white, white, purplish, pale

pink or rose pink, tetramerous flowers in cymose clusters. Inferior ovary and 1-2 seeded berry are the salient features with which the plant can be readily distinguished in field from the other Melastomataceous genera together with its non-acrodromous venation and absence of trichomes. Most species of this genus are found in wild habitats and are unexplored. *Memecylon umbellatum* and *M. randerianum* are the two common members of this genus that are widely under consideration. The present study deals with the exploration of *M. grande, M. umbellatum* and *M. randerianum*. All the available pieces of literature related to these species are discussed herein.

## I. TAXONOMIC BACKGROUND OF SELECTED *MEMECYLON* SPECIES

Taxonomically, the identification of *Memecylon* species is quite difficult due to their closely resembling morphological features. The floral morphology of *Memecylon* species is well conserved, although leaf morphology and inflorescence placements are highly varied and can serve as a species-level identification trait. Several species are converged on the basis similar vegetative characters causing taxonomic uncertainty. The shape and size of the leaves, the position and nature of the inflorescence, the length of pedicels, the shape and nature of the calyx cohesiveness and the presence or absence of disc rays are all taxonomic delimiting features (Rao et al., 1980). The species identification of *Memecylon* becomes difficult due to their morphological complexity. The taxonomic position of *Memecylon* is noted below.

| **Bentham and Hooker classification** | **APG IV classification** |
|---|---|
| Kingdom : Plantae | Division : Angiosperms |
| Class : Polypetalae | Clade : Eudicots |

| | |
|---|---|
| Series : Calyciflorae | Clade : Rosids |
| Order : Myrtales | Clade : Malvids |
| Family : Melastomataceae | Order : Myrtales |
| Genus : *Memecylon* | Family : Melastomataceae |

A taxonomic complexity was raised in the case of *M. umbellatum* Burm. f. *M. umbellatum* and *M. edule* were classified as different species. *M. umbellatum* was classified as a synonym of *M. edule* (Neginhal, 2004; Pullaiah & Rao, 2001). *M. umbellatum* and *M. edule* are considered conspecific in several regional floras (Manilal & Sivarajan, 1982; Almeida & Almeida, 1998). The same controversy is also raised in *M. randerianum* species. *M. amplexicaule* var. *malabarica* is assigned to *M. malabaricum* and *M. depressum* by Gamble (1997) in Flora of Presidency of Madras based on their elevation of occurrence. *M. malabaricum* is confined to higher elevations and *M. depressum* to elevations up to 365 m. *M. depressum* is listed as a synonym for *M. amplexicaule* in the Flora of British India (Hooker, 1879). *M. malabaricum* is the approved name for *M. amplexicaule* according to some taxonomists. As a result, the taxonomic position of *M. malabaricum*, *M. amplexicaule* and *M. depressum* is unclear. *M. amplexicaule* var. *malabarica* and *M. malabaricum* are considered as the synonym of *M. randerianum*.

The molecular phylogenetic analysis of *Memecylon* was studied by various researchers. Bharathi et al. (2017a) conducted an isozyme profiling of *Memecylon* species. *M. malabaricum* and *M. wightii* have related similarity indices and are classified into one cluster with 98 percent similarity, while *M. umbellatum*, *M. edule* and *M. talbotianum* are put into another cluster with 79 percent similarity. On the other hand, ITS sequences were used to identify four Indian *Memecylon* species, *M. umbellatum*, *M. malabaricum*, *M. wightii* and *M. talbotianium* (ITS 1, 5.8S and ITS 2). These species genotyping may

be deduced from phylograms derived from their ITS sequences. These species were effectively separated from other *Memecylon* species by the rDNA sequence produced (Bharathi et al., 2016a). The molecular phylogeny of Melastomataceae and Memecylaceae was analyzed by Clausing and Renner (2001). They performed parsimony and maximum likelihood (ML) analyses of cpDNA sequences from the *rbcL* and *ndhF* genes and the *rpl16* intron. They gave detailed information about the phylogenetic relationship and morphological evolution of Melastomataceae and Memecylaceae. The genetic diversity analysis of *Memecylon* species done through the ISSR, RAPD and gene-based barcoding tools reveals the identity of *M. malabaricum* from *M. wightii* and *M. umbellatum* from *M. edule* species. *M. malabaricum* and *M. wightii* are placed together in one clade, whereas *M. umbellatum, M. edule*, and *M. talbotianum* are classified together in another clade (Ramasetty et al., 2016). In some critically endangered *Memecylon* species *ie., M. subcordatum* the ISSR and ITS analyses were carried out to assess genetic diversity and phylogeny. They had suggested utilizing genetic diversity-rich saplings and stem cuttings of the plants to expanse their natural occurrence (gowdu Viswanathan et al., 2018). The infrageneric classification of the African species of *Memecylon* was analyzed by Stone (2014). In 167 samples, the internal and external transcribed spacers of nuclear riboso mal DNA were sequenced and phylogenetically categorized. All over the world, molecular analyses of *Memecylon* species are very frequent. The endemism, species richness and morphological trends in Madagascan *Memecylon* were studied by Stone (2012). In Thailand, the discovery of new species and new species combinations were analyzed by Wijedasa and Hughes (2012).

## II. PHARMACOGNOSY

Pharmacognosy is the study of plants or other natural resources as a possible source of drugs. Several plants are considered as a possible source of medicinal products. Much of the research related to plants has been focused

on the invention of the safe remedy for vulnerable diseases. Most synthetic drugs have many toxic side effects, so it opens a gateway for the search of a natural drug. WHO had proposed some guidelines for herbal medicine preparations that ensure the safety and quality of drugs (Patel et al., 2011). Herbal development occurred through the various stepwise analytical processes. Pharmacognosy is one of the preliminary steps in it. It includes the analysis of the functional purity of the plant sample. The herbal medicines often suffer from quality controversies because of similar species or varieties. Drug discovery from plant sources is a multidisciplinary branch that involves combined approaches of botanical, ethnobotanical, phytochemical and biological areas (Jachak & Saklani, 2007). An outline of drug discovery is given in **Figure 1**. The authenticated plant specimens were primarily exposed to pharmacognostic, anatomical and phytochemical analyses. The functionally active plants were subjected to further bioactivity studies and are finally targeted to drug discovery. The application of pharmacognosy is that it can eliminate the adulterant particles from a powdered drug sample and ensure the functional purity of the sample. So undoubtedly, the plant kingdom holds many plant species with incredible medicinal values, which have yet to be discovered. The plants are being screened for their various pharmacological roles such as cytotoxic, hypotensive, anti-inflammatory and anticancerous activities (Evans, 2009).

Pharmacognostic analysis of herbal medicine involves various methods of evaluation. The light and electron microscopic characterization of plant parts are one of the usual techniques employed in the analysis. Histochemical, physicochemical and phytochemical techniques are often used in drug validation (Alam & Saqib, 2015). In the microscopic analysis of medicinal plants, powder microscopy is a leading technique in quality assessment. The information obtained through the powder microscopic analysis is used as a diagnostic character of medicinal plants. Fluorescence analysis and

physicochemical analysis are often used in the quality assessment of herbal medicine (Sharma, 2013).

A comparative morphological analysis of two *Memecylon* species *ie.*, *M. umbellatum* and *M. angustifolium*, reveals that transverse sections of mature leaves and its powder sample contain druse crystals. Stomatal arrangements, foliar sclereids, and wood anatomy were also recorded (Karunarathne et al., 2017). The different parameters like xylem vessel thickening patterns, foliar sclereids present in the leaf mesophyll, xylem arrangement in the leaf midrib and the pattern of xylem parenchyma arrangement in the wood were used to separate the two species.

The trace elements are essential for maintaining the normal life of humans. Plants are the major source of nutritive elements. The elemental composition analysis of some *Memecylon* species shows that they are rich in macronutrients, micronutrients and nutritional elements. *M. umbellatum* possess a high percentage of moisture and crude fat. *M. malabaricum*, *M. talbotianum* and *M. umbellatum* have the highest levels of macronutrients such as Na, K, Ca, Mg, P, and N. *M. umbellatum* have the high Cu and Mn levels (Asha et al., 2015).

The microscopic evaluation of *M. umbellatum* leaves extract shows non-lignified phloem in midrib region, lignified xylem having well-defined xylem fibres, vessels, parenchyma and phloecentric vascular bundles. Anomocytic stomata were observed on both epidermal surfaces (Killedar et al., 2014b). The pharmacognostic and phytochemical analysis in leaves of *M. edule* revealed the presence of carbohydrates, gums, mucilage, tannins and saponins (Dorababu et al., 2013). These features establish some standards for drug validation. It includes a transverse section of the leaf, powder analysis and physicochemical evaluation like ash values, extractive values, moisture

content and fluorescence analysis. These are important in the identification, purity and quality assurance of medicinal drugs.

## III. MEDICINAL USAGE OF SELECTED PLANTS

*M. umbellatum* is used for curing various diseases. The leaves extract shows wound healing activity and used to treat diabetes (Puratchikody & Nagalakshmi, 2007). The seeds are used in cough and as a sedative. The leaves are used for treating snakebite (Gowda, 2004; Kshirsagar & Singh, 2001). *M. malabaricum* is used to cure inflammation and allergic disorders. The fruit of the plant is used to control sterility in men. The leaves are used for treating psoriasis in some traditional communities (Bharathi et al., 2016a). *M. taboltianum* is used as an antibacterial as well as antioxidant agent (Yashoda et al., 2014).

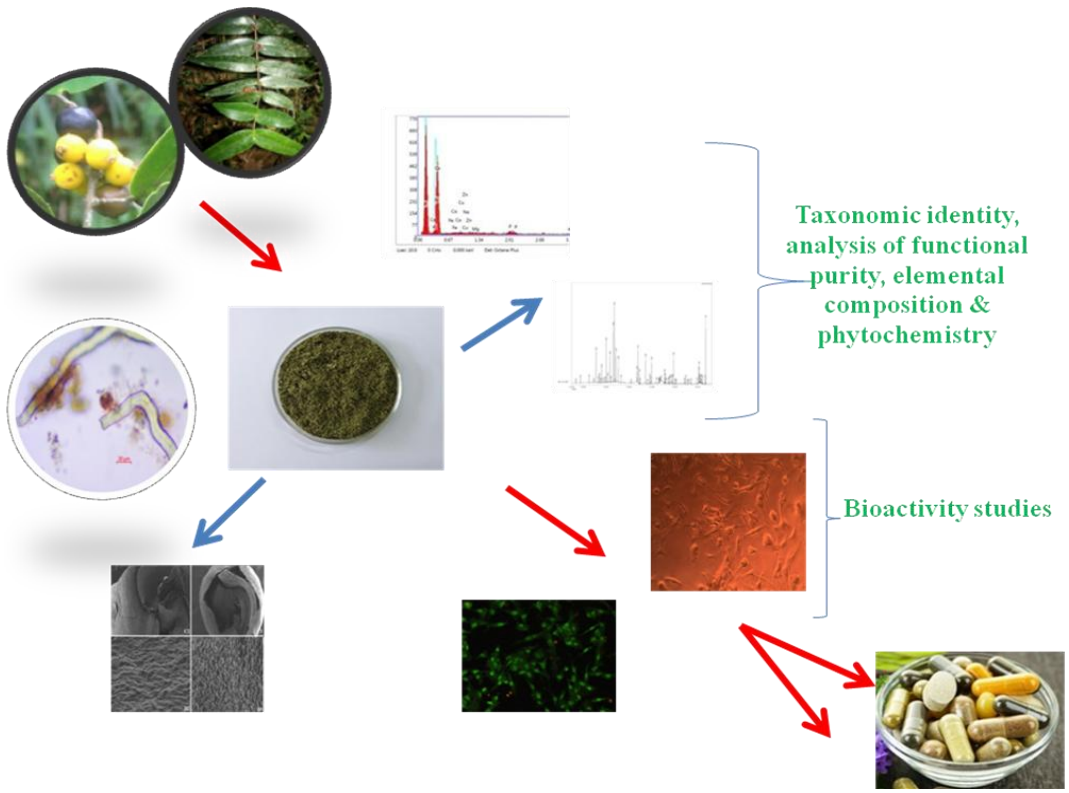


**Figure 1**: **A schematic representation of drug preparation from plant sources**.

## IV. PHYTOCHEMICAL CHARACTERIZATION

Plants are being used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternate source of medicine to maintain health and improve the eminence of life. Many studies are conducted for the assessment of phytochemical aspects of medicinal plants (Maridas, 2010). Medicinal plants are a priceless source of bioactive components. The traditional medicinal systems like Ayurveda, Siddha and Unani always relied on herbal medicine to cure various human ailments. Several drastic discoveries had occurred in the pharmaceutical field, that will enlighten the interest of researchers. Plant-based anticancer agents including vincristine, vinblastine and irinotecan; novel antibacterial agent quinolone from *Evodia rutaecarpa*, which is effective against *Helicobacter pylori*; maloyl glucans derived from *Aloe vera* having wound-healing effects and 4-hydroxypanduratin a potential antimutagenic agent isolated from the rhizome of *Boesenbergia pandurata* are few examples of the drastic discoveries that are enhancing the medicinal field in the past (Ali et al., 2014). Only a very small percentage of plants have been profoundly studied for their potential value as a source of drugs. Many of the plants having medicinal potential remain unassessed. Natural products will continue as extremely important sources of medicinal agents. The discoveries of new drugs from plants require the screening of many thousands of plant extracts and thus require continued access to the vast plant biodiversity of the Earth.

The ecosystem diversity of our country is enormous, ranging from sea level to the highest mountain ranges; hot and arid conditions in the northwest to cold arid conditions in the trans-Himalayan region; tropical wet evergreen forests in Northeast India and the Western Ghats; mangroves of Sundarbans and freshwater to marine ecosystems (Sharma & Singh, 2000). The geographical area covered by the country represents about 2.4% of the

world's total landmass, and it harbours a total of 47,513 plant species (Arisdason & Lakshminarasimhan, 2017). Around the World, the potentiality of herbal medicines is widely discussed (Shad et al., 2014). Authentification and validation of herbs are very essential for their bioactivity assessment. The novel species discoveries are tremendously increasing and the identification becomes a tedious task. So the research in herbal medicine is still waiting for new explorations.

In the tropical region, the genus *Memecylon* L., which belongs to the family Melastomataceae, has about 289 species of shrubs and trees. Several *Memecylon* species have been reported to be in use by tribes for healing various diseases in traditional medical systems such as Ayurveda and Siddha. *M. malabaricum* is used to cure inflammation and allergic disorders. *M. talbotianum* is used for neurodegenerative diseases, diabetic complications, inflammation, helminthic infections and skin diseases (Bharathi et al., 2016b). Only vast taxonomical studies of the genus were carried out by various researchers, but the phytochemical, cytological and pharmacological studies of the genus are still limited (Sivu et al., 2013). The hot and cold extracts of *M. umbellatum* leaves in different solvents namely petroleum ether, chloroform, ethanol and aqueous, were subjected to phytochemical screening. Glycosides and lignin were found to be absent in all the extracts of young and mature leaves. The quantitative analysis of phenols, tannins, steroids, alkaloids, flavonoids, lignins, proteins and carbohydrates reveal that alkaloids and lignin were absent in both young and mature leaves. Whereas phenols, tannins, flavonoids and steroids were recorded and their variation among young and mature leaves was less (Murugesan et al., 2011; Krishnamurthy & Asha, 2011).

*M. malabaricum* and *M. talboltianum* leaves extract were analyzed to identify the phytoconstituents (Yashoda et al., 2014). The preliminary

phytochemical analysis of extracts showed the presence of phytoconstituents *viz.*, saponins, tannins, flavonoids and glycosides in the extracts of both *M. malabaricum* and *M. talboltianum*. But alkaloids and steroids were not detected in both extracts. One of the common *Memecylon* species mostly exploited is *M. umbellatum.* Its methanolic seed extracts possess tannins, phenolic compounds, fats and oils (Harkare et al., 2013; Killedar & More, 2012; Puttaswamy & Achur, 2013). Elavazhagan and Arunachalam (2010) investigated the phytochemical and antibacterial studies of the seed extracts of *Memecylon edule.* The findings imply that this plants ethyl acetate and chloroform extracts have moderate antibacterial activity. It was discovered that the plants secondary metabolites can be utilized to treat wounds and other bacterial diseases. *M. edule* leaves extract possesses antibacterial activity with maximum inhibitory activity against *E. coli*, *Staphylococcus aureus* and minimum for *Klebsiella pneumoniae.* The activity point towards the presence of antimicrobial agents like long-chain fatty acids, steroids, saponins *etc*., in *M. edule* (Palaniselvam et al., 2012). Lowry (1976) discovered anthocyanins in *Memecylon* species such as Mv-3, 5-diglucoside from *M. caeruleum* and Cy-3, 5-diglucoside from *M. amplexicaule*.

The quantitative measurement of phenolic components and flavonoids in methanolic extracts of 32 *Memecylon* species found in the Western Ghats was explored by Sivu et al. (2013). Phenolic content ranged from 89.86 mg/g (*M. gracile* Bedd.) to 05.04 mg/g (*M. depressum* Benth.). *M. grande* has the most flavonoid compounds (39.56 mg/g) while *M. talbotianum* had the least (0.76 mg/g). The phytochemical analysis of leaves and callus extract of *M. umbellatum* revealed the presence of significant secondary metabolites such as phenols, flavonoids, terpenoids, steroids, tannins, saponins, quinones, cardiac glycosides and alkaloids. The tannin content was highest in the callus extract as compared to the leaves extract. So the powerful antibacterial effect

is attributed to the greater amount of tannin compounds present in the callus extracts of *M. umbellatum* (Anbukkarasi et al., 2017).

Phytoconstituents are the key factors behind the biological properties of the plant species. Killedar et al. (2014a) point out the phytochemical nature and antioxidant capacity of the *M. umbellatum* leaves. They found that among the tested solvents methanolic extract shows the highest activity. The antimicrobial activity and phytochemical documentation of *M. umbellatum* inflorescence were done by Killedar and More (2011). The *in vitro* antidiabetic activity of *M. umbellatum* was examined by Rajesh et al. (2014) and found that the phytochemicals present in the plant extracts were responsible for the reduction of glucose level. Antidiabetic potential was assessed using amylase inhibition, non-enzymatic glycosylation of haemoglobin, the glucose diffusion experiment and glucose uptake by yeast cells. The methanolic extracts showed higher antidiabetic activity by the inhibition of glucose uptake as compared to the control.

*M. terminale* phytochemical analysis revealed prominent amount of alkaloids, flavonoids and modest amounts of steroids, tannins and phenols. The presence of carbohydrates, reducing sugars, alkaloids, phenols, flavonoids, cardiac glycosides, steroids, terpenoids and coumarins were found in qualitative analysis of phytoconstituents in *M. randerianum*. In quantitative estimation of phenols, flavonoids and alkaloids indicated the highest percentage of total alkaloid content (Hegde & Hungund, 2020).

The *in vitro* antioxidant and GC/MS spectroscopic analysis of *M. umbellatum* was conducted for elucidating its bioactive compounds by Elangovan et al. (2014). Eight major and minor phytochemical constituents were revealed in the methanolic seed extract of *M. umbellatum.* 1-Butanol, 1H-pyrazole, 2-furancarboxylic acid, pyrrolidine carboxamide, 3-furanmethanol and thiazole are the major compounds identified in the

investigation. The phytoconstituents of stem extract of *M. umbellatum* revealed the presence of many bioactive compounds (Murugesan & Panneerselvam, 2013). Twenty different compounds from chloroform extract, 11 compounds from petroleum ether extract and 10 various compounds from ethanol extract were identified. n-Hexadecanoic acid, octadecanoic acid and oleic acid are the fatty acids present in all three extracts. The compounds like 1-monolinoleoylglycerol trimethylsilyl ether; 1,5, heptadien-4-one, 3,3,6-trimethyl; 1,2-benzenedicarboxylic acid; diisooctyl ester and 9,12-octadecanoic acid (Z,Z) were present only in the petroleum ether extract. A wide spectrum of phytochemical compounds was identified in the various *Memecylon* species (**Table 1**).

Mala and Saravanakumar (2016) studied GC-MS analysis of bioactive compounds present in the methanolic leaves extract of *M. edule*. GC-MS analysis of methanolic leaves extract revealed a total of 28 distinct phytochemicals. All of the chemicals discovered had therapeutic value in the treatment of a variety of human illnesses. Gas chromatography–mass spectrometry analysis of *M. sisparense*, and docking studies along with its nephroprotective activity against cisplatin (CP)-induced nephrotoxicity in mice was studied by Uppu et al. (2018). They observed that out of the 41 compounds identified, 20 were found to be biologically active, such as nephroprotective, anticancer, antioxidant, hepatoprotective, antimicrobial and shows inhibition of uric acid production. The cisplatin-induced nephrotoxicity was reduced by the effect of nephroprotective active compounds like N,N,O-triacetylhydroxylamine, 2(4H)-benzofuranone; 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-; N-{(4-hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide present in the plant extract.

**Table 1: Phytochemical compounds reported from *Memecylon* species.**

| Species | Plant part used | Compounds | References |
|---|---|---|---|
| *M. malabaricum* Clarke | Leaves | Ar-turmerone; 2,6,6-trimethyl bicyclo[3.1.1]heptane; 11,13-dimethyl-12-tetradecen-1-ol acetate; phytol; 9-octadecenoic acid (Z)-dihydroxypropyl ester; 2,6,10-trimethyl dodecane; 3,6-dimethylundecane; heptadecane; hexacosanoic acid; I-(+)-ascorbic acid 2,6-dihexadecanoate; 3-methyl-octadecane; sulphurous acid, heptadecyl 2-pentyl ester; palmitic acid vinyl ester; 4-methyl nonadecane; 4-propyl heptadecane; 2-methyl heptadecane; octadecanoic acid,2,3-bis[(1oxotetradecyl)oxy]propyl ester; hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester; 1,1-dimethoxy-9-octadecene<br>4,9,14,19-tetramethyl-1,6,11,16-tetraoxacycloeicos 3,8,13,19-tetraene (Memecylaene) | Rajalakshmi, 2018<br>Rekha et al., 2014 |
| *M. umbellatum* Burm.<br>*M. edule Roxb.*<br>*M. talbotianum* Brandis<br>*M. malabaricum* Clarke<br>*M. wightii* Thwaites | Leaves | α-tocopherol | Bharathi et al., 2017b |
| *M. umbellatum* Burm. f. | Leaves | β-amyrin | Sridevi et al., 2015 |

| | | | |
|---|---|---|---|
| | | Furfural; 2-cyclopenten-1-one, 2-hydroxy-; 1-benzoyl-3-amino-4-cyano-3-pyrroline; 2(3H) furanone, 3 acetyldihydro; phentermin-propionyl; cis-1, 2-dihydrocatechol; 1, 2-butanediol, 1-phenyl-; hydrouracil, 1-methyl-; methyl 2-furoate; levoglucosenone; 1-deoxy-d-altritol; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; benzoic acid, 2-hydroxy-, methyl ester; 1, 4:3,6-dianhydro-α-d glucopyranose; 2-furancarboxaldehyde, 5-(hydroxymethyl); 2-methoxy-4-vinylphenol; hydroquinone; methyl-α-d-ribofuranoside; 1,2,3-benzenetriol | Mala & Saravanakumar, 2016 |
| | | D-allose; benzene acetic acid, 4-hydroxy-3-methoxy-; 2-cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl- ; n-hexadecanoic acid; umbelactone; β-amyrin; sitosterol | Joshi et al., 2011 |
| | | Oleanolic acid; ursolic acid; sitosterol-β D- glucoside | Agarwal & Rastogi, 1978 |
| | Root | Octocosonoic acid, cerotic acid; ethyl palmitate; palmitic acid; butyric acid | Joshi et al., 2009b |
| *M. edule* Roxb. | Leaves | Rutin | Srinivasan et al., 2015 |
| | | 3,4:5,6-diepoxycyclohex-1-ene; heptanal, 4-methyl-4-nitro-5-oxo; isoxazole, 4-(chloromethyl)- 3,5-dimethyl; bicyclohexan-3-one, 4-methyl- 1-(1-methylethyl)-; 4-vinylphenol; 2-fluoro-1-methoxy-4- methylbenzene; 1-acetoxy-2-(t-butyl)-4- methyl-2,3-pentadiene; N,N'-diacetylethylenediamine; 1,2,3-benzenetriol; N-[2 | Srinivasan et al., 2014 |

| | | | |
|---|---|---|---|
| | | (acetylamino) ethyl] acetamide; 16-heptadecenal; 2-decen-1-ol; decanoic acid; stearic acid; butanoic-3,3-D2 acid, 2-methyl; trimethylsilyl ester of tetracosanoic acid;1,3-methanonaphthalene, decahydro-2,2-dimethyl; 2,2 dimethylglutaric acid; 3,6-bis-dimethyl aminomethyl-2,7-dihydroxy-fluoren-9-one; cyclotetrasiloxane,octamethyl; [2,2']bithiophenyl-5-YL(3-hydroxybenzo[1,2,5]oxadiazol- 5-YL)methanone; 3-(4-dimethylamino- naphthalen-2-(4-nitro-phenyl)-acrylonitrile; 6-fluoro-1,3-bis (fluorodimethylsilyl)-2,2,4,4-tetramethyl-1,3,5-triaza-2,4-disila-6-boracyclohexane; thiophen-2-methylamine, N,N- didecyl-; dimethyl 9-isopropyl-1,6 dimethyltricyclo[5.4.1.0(4,12)] dodeca 3,5,7(120,8,10- pentaene-2,3-dicarboxy | |
| *M. talbotianum* Brandis | Leaves | Gallic acid; cis-ferulic acid; trans-sinapic acid; cis-sinapic acid; 3,7- dihydroxy-4- methoxy flavones; quercetin; mono caffeoylquinic acid; feruloyl sinapoyl glucose; feruloylquinic acid; synapoylhexose formic acid; cyanidin -3-O-malonyl glucoside; 6-C-arabinosyl-8-C-glucosyl-apigenin; 2-O-pentosyl - 8 C-hexosyl luteolin; isorhamnetin-3-O-glycoside-7-O-glycoside; kaempferol 3-O-feruloylhexosyl rhamnoside; quercetin 3-O sinapoyldihexose | Bharathi et al., 2016b |
| *M. caeruleum* Jack.<br>*M. amplexicaule* Roxb. | Flower | Mv-3,5-diglucoside<br>Cy-3,5-diglucoside | Lowry, 1976 |

Srinivasan et al. (2015) discovered an antioxidant chemical quercetin-3-O-α-L-rhamnoside (1→6)β-D-glucose (rutin) obtained from ethyl acetate leaves extract of *M. edule*. The chemical constituents identified in the two accessions of *M. edule* shows nine similar compounds such as levoglucosenone, 4H-pyran-4-one, furfural, hexadecanoic acid *etc*. 1,2,3-benzenetriol shows a higher percentage in both accessions. A total of 44 compounds were identified in which they all belong to various classes of bioactive components (Saravanakumar, 2017).

## V. BIOACTIVITY REPORTS

- **Antioxidant activity**

Reactive oxygen species (ROS) are reactive molecules or free radicals derived from molecular oxygen. Superoxide radicals, hydrogen peroxide, hydroxyl radical and singlet oxygen are major types of ROS. They are mainly formed in cells as a byproduct of the mitochondrial electron transport system and are intermediates of some metal-catalyzed oxidative reactions. The concentrations of ROS in cells are very important. The low incidence of ROS is essential for phosphorylation of proteins, various intracellular signaling and defense against pathogens *etc.* The higher amount of ROS causes several diseases like atherosclerosis, cancer, diabetes, ischemia *etc.* (Rajendran et al., 2014). In cell signalling and homeostasis, reactive oxygen species play an important role. It can set off a series of events that include DNA damage, lipid peroxidation and amino acid oxidation in proteins (**Figure 2**).

Although several synthetic antioxidants are now in use, natural substances derived from plants are of particular interest (Puttaswamy & Achur, 2013). It can scavenge the reactive oxygen species and reduce the rate of cellular damages. ROS production occurs mainly in two ways, enzymatic and non-enzymatic. Enzymatic reactions occur during the electron transport chain, phagocytosis *etc.*, and non-enzymatic reactions occur when oxygen reacts with organic compounds or when cells are exposed to some ionizing

radiations. In enzymatic reaction, NADPH oxidase, xanthine oxidase and peroxidases are responsible for the production of superoxide radicals. Nitric oxide free radical production was mediated by arginine-to-citrulline oxidation by nitric oxide synthase. NADPH oxidase also initiates the production of hydrogen peroxide and hydroxyl radicals (Pizzino et al., 2017). The imbalance between the formation and neutralization of free radicals can damage the cellular metabolism and homeostasis of the human body. The condition of this imbalance is named oxidative stress. One of the best examples is lipid peroxidation, in which excess hydroxyl and peroxynitrite gets accumulated and leads to the formation of malondialdehyde and the diene compounds, which are cytotoxic and mutagenic. These will damage the cell membrane and its lipoprotein composition (Kabel, 2014).

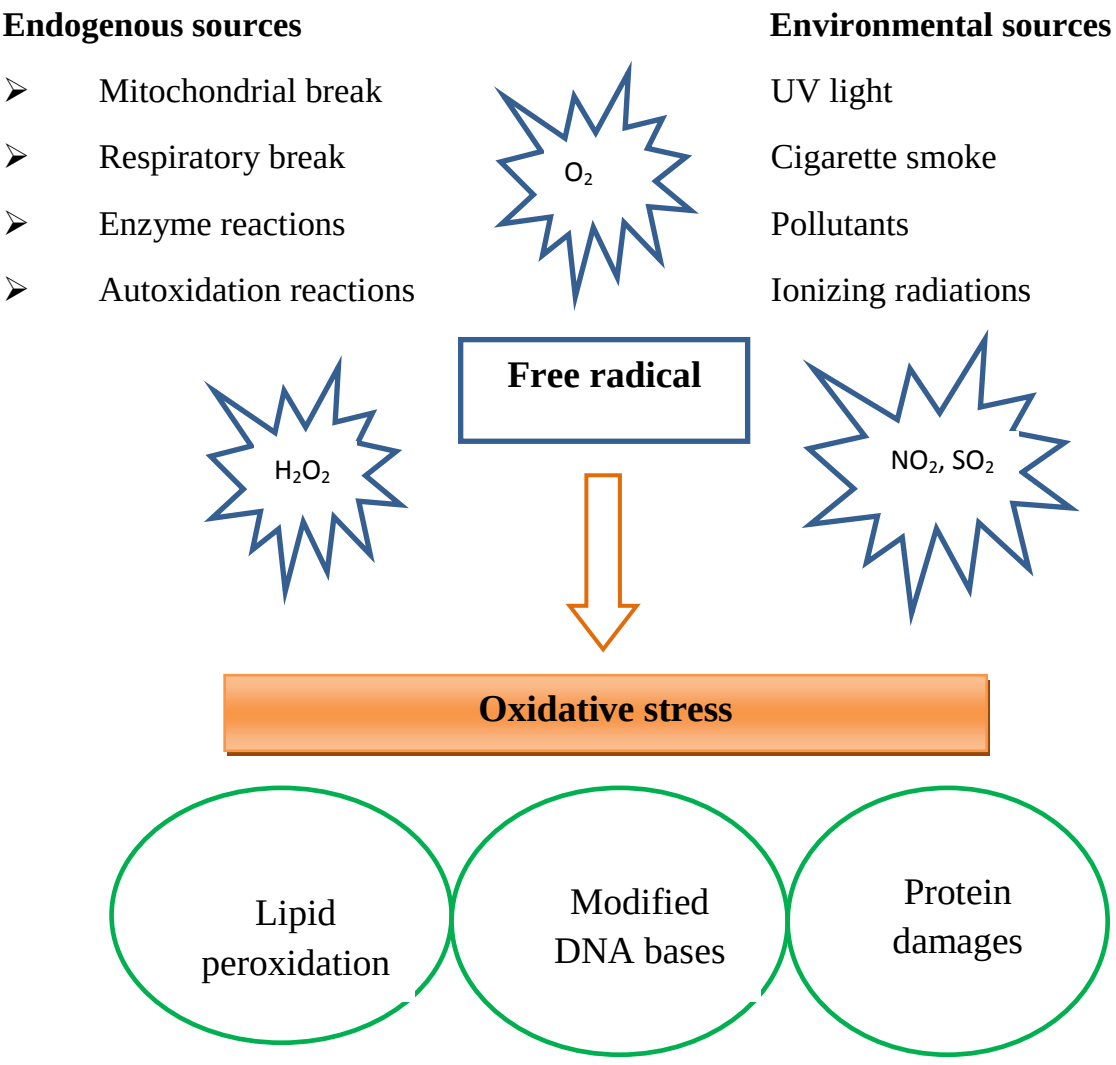

**Figure 2: Action of free radicals on the living system**

Oxidative stress causes various diseases in the human body such as cancer, cardiovascular diseases, ageing, arthritis and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stresses *ie.*, antioxidants, which are the scavengers of free radicals. Antioxidants are either present naturally *in situ* or it has an external source of additives like food/supplements.

The Western Ghats form a rich source of plant diversity, where *Memecylon* species are widely distributed. Sivu et al. (2013) evaluated the antioxidant activity of *Memecylon* species in the Western Ghats region. *In vitro* antioxidant and anti-inflammatory studies of *M. talbotianum* were carried out by Bharathi et al. (2014) in different solvents. The antioxidant and anti-inflammatory effects of the methanol, ethyl acetate and water extracts were promising. Sekhar et al. (2015) investigated the antioxidant activity, lipoxygenase inhibition and DNA protection properties of *M. umbellatum, M. talbotianum* and *M. malabaricum in vitro*. *M. malabaricum* possesses the highest lipoxygenase inhibition and COX-2 activity. Methanolic leaves extract of all plants can prevent DNA nicking by hydroxyl radicals, produced during the Fenton reaction. The antioxidant potential of different solvent extracts of *M. umbellatum* leaves was evaluated by using DPPH, nitric oxide, hydroxyl radical assays *etc*. Among these solvents, the methanolic extract shows the highest antioxidant activity (Sridevi et al., 2014). The antioxidant compound rutin was isolated from leaves extract of *M. edule* (Srinivasan et al., 2015). Hydrogen peroxide scavenging activity, total antioxidant capacity, nitric oxide scavenging activity and reducing power activity of *M. umbellatum* was tested by Rumzhum et al. (2012) and found a pronounced antioxidant activity when compared with ascorbic acid, the standard. The antiangiogenic, antioxidant and proapoptotic chemopreventive properties of

tannins from *M. malabaricum* (Rekha et al., 2015) were analyzed. Tannins are non-nutritive substances from plant sources, which exhibit potent biological activities that lower the risk of chronic diseases. *M. malabaricum* extract shows promising antioxidant activity in 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging ($EC_{50}$ of 2.67 µg/mL), hydroxyl radical scavenging ($EC_{50}$ of 7.73 µg/mL) and nitric oxide radical scavenging assays ($EC_{50}$ of 19 µg/mL) .

The antioxidant activity of *M. edule* was tested by Kumar and Jain (2016) and found that it shows promising results in DPPH, superoxide and reducing power assays. The antioxidant activity of the plant extract is due to the presence of colossal phenolic content. The ursolic acid isolated from *M. edule* aerial parts possesses effective antioxidant activity in various assays like DPPH, nitric oxide, hydroxyl, superoxide radical and ferric reducing antioxidant power assays (Srinivasan et al., 2020).

*Memecylon* is the least explored genus of the Melastomataceae family. There is limited information regarding the bioactive potential of *Memecylon* species. *Memecylon* species have potential effects in relieving many diseases such as diabetes, herpes, gonorrhoea, leucorrhoea and skin diseases (Bharathi et al., 2016a; Puratchikody & Nagalakshmi, 2007). The antihypercholesterolemic activity of *M. edule* extracts in cholesterol-induced Swiss albino mice shows that a significant change occurred in cholesterol level, as well as very-low-density lipoprotein and low-density lipoprotein levels. However the action of the antioxidant system gets enhanced and the high-density lipoprotein level in serum get increased (Kuppusamy et al., 2015). *M. pauciflorum* inhibit glucose-induced fluorescent AGEs, α-amylase, α-glucosidase, ACE and digestive enzymes linked to type II diabetes (Deo et al., 2016). *M. malabaricum* has interesting possibilities as a source of the oral hypoglycemic agent was described by Ramaiah et al. (2013). *M. umbellatum*

extracts show a considerable reduction in urea and creatinine levels when compared to the control group in acute toxicity trials (Puttaswamy et al., 2013). The antidiabetic and antiobesity effect of *M. umbellatum* on high-fat diet-induced obese mice was studied by Sunil et al. (2017). High diet-induced obese mice model was administered with 250 mg/kg body weight of the plant extract. A significant reduction in fasting glucose levels, body weight and triglycerides, as well as amelioration of insulin resistance *etc*., are observed. In gene expression level studies, the down regulation of IL6, PAI1 and ApoB expression is observed.

Pharmacognostic studies, antioxidant, antimicrobial and wound healing activities of *M. umbellatum* and *M. edule* was evaluated by Mohideen (2008). Detailed phytochemical analyses were conducted for deciding the identity and purity of plant material. The histopathological studies are well correlated with the biochemical assays. It was found that a significant pro-healing activity was shown in the wound area treated with plant extracts and confirmed the presence of a significant amount of collagen, hexosamine and uronic acid in the infected area. The pharmacological and phytochemical analysis of *M. malabaricum* was conducted by Abi and Madhusudhanan (2017). It was found that the plant extract possesses effective antimicrobial, antipsoriatic, antihelminthic, antidiabetic and clastogenic activities. It has antibacterial properties against both gram-positive and gram-negative bacteria such as *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antipsoriatic activity was proven through the *in vivo* analysis by mouse tail test and *in vitro* antipsoriatic activity by using HaCaT cells, lipoxygenase inhibition and thymidine phosphorylase inhibition assays. A significant percentage of orthokeratosis in the mouse tail test confirmed the antipsoriatic activity of the plant extract. *M. malabaricum* possess potent antihelminthic

activity against *Pheritima posthuma.* The micronucleus test on *Zakerana keralensis* larvae shows the clastogenic potential of the plant extract. Memecylaene, isolated from the plant *M. malabaricum* exhibited significant anti-inflammatory activity in acute and sub-acute models of inflammation with significant reduction of paw edema and granuloma tissues.

The antimicrobial activity of *M. umbellatum* and *M. edule* shows that they are active against the gram-positive bacteria like *Staphylococcus aureus, S. epidermidis, Micrococcus luteus, Bacillus cereus* and the gram-negative bacteria *like Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The plant extracts exhibit antifungal activity against *Aspergillus niger, A. fumigatus* and *Candida albicans* (Mohideen et al., 2012; Padmavathy et al., 2010b). The anti-inflammatory, analgesic and wound healing activity of *M. edule* was proved by Nualkaew et al. (2009). They found that ethyl acetate extract of *M. edule* stimulates the production of interleukin-10 and thereby support the traditional usage of *M. edule* leaves for pain relief. The ursolic acid isolated from *M. edule* aerial parts shows a profound inhibitory effect on the proliferation of U-937 and HT-60 cell lines. In molecular docking studies, ursolic acid creates an inhibitory effect on the ATPase region of topoisomerase II. So ursolic acid might be used as a good molecular template in the discovery of novel antiproliferative agents (Srinivasan et al., 2020). Antibiotic constituents of endophytic *Bacillus amyloliquefaciens* UD25 were extracted from *M. edule* Roxb (Bhoonobtong et al., 2017). It exhibit growth inhibition of pathogenic bacteria, like *Streptococcus* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis, Bacillus cereus* and *Escherichia coli.* The presence of bioactive endophytic fungi was found in Melastomataceae members, such as *Melastoma malabathricum* and *Memecylon ovatum*. The isolated endophytes have potential bioactivities like antimicrobial, anticancer and antimalarial

efficiencies as reported by Wiyakrutta et al. (2004). There are some reports about the endophytic fungal diversity in *M. umbellatum,* which shows that the diversity is dependent upon the isolation methods, seasons and plant parts. Potential endophytic fungal species documented are species of *Cochliobolus* and *Pestalotiopsis* which showed high antibacterial activity. The winter season supported the high fungal incidence of species of *Cochliobolus, Bipolaris* and *Khuskia* (Suryavamshi & Shivanna, 2020).

The antilarvicidal, antioxidant and antiproliferative activity of ursolic acid and rutin, which are the isolated compounds of *M. edule* leaves exhibit promising results. Ursolic acid shows potent antibacterial and antiproliferative activity. However, rutin possesses the highest antioxidant and antiproliferative activity (Srinivasan, 2014**)**. The larvicidal potential of *M. edule* leaves extract against *Aedes aegypti* (L.) was noted by Sharower and Latif (2018). It is explained that a moderate level of larvicidal activity was shown by the plant extract. The methanolic leaves extract of *M. heyneayanum* to combat pathogenic microorganisms was reported by Manikandan and Ramasubbu (2020). The plant extract has a wide range of antimicrobial potential, which ranges from gram-positive bacteria *viz. Bacillus cereus, Staphylococcus aureus, Micrococcus mucilaginosus* to gram-negative bacteria, *Klebsiella pneumoniae, Pseudomonas aeuruginosa, Escherichia coli* and *Klebsiella terrigena* as well as fungal species *viz., Candida albicans, Candida glabrata* and *Candida* spp.

The anti-inflammatory effect of *M. umbellatum* was investigated in two rat models such as, acute rat paw edoema caused by carrageenan and sub-acute rat granuloma caused by cotton pellets. The weight of adrenal glands was found to be significantly increased in root extract-treated animals. A significant dose-dependent anti-inflammatory activity was shown by the ethanolic plant extracts (Joshi et al., 2009a). Venkategowda et al. (2020)

proved the elevated levels of pro-inflammatory cytokines and IL-6 mRNA levels in *M. umbellatum* treated carrageenan-induced paw edema model in db/db mice. Histopathology of the paw showed significant inflammatory changes such as edema, vascular congestion, leukocyte infiltration, and necrosis. The hepatoprotective activity of *M. umbellatum* root extract on acetaminophen-induced hepatotoxicity in rats showed a significant reduction in the elevated serum enzyme level and it maintains the histological characters of the liver when compared to the control group (Joshi et al., 2008). Kamble and Rao (2017) explained the hepatoprotective role of *M. malabaricum* root extract on paracetamol-induced hepatotoxicity in rat models. The root methanolic extract treated rat models possesses a reduction in SGOT, SGPT, ALKP, TBL, CHL levels and a significant increase in TPTN and ALB levels in a dose-dependent manner.

Four sequential extracts of *M. edule* from hexane, ethyl acetate, ethanol and 50% ethanol was used for the determination of anti-inflammatory and analgesic activity. Ethyl-phenylpropiolate-induced mouse ear edoema was used to test anti-inflammatory activity and analgesic activity was studied by acetic acid-induced writhing test in mice. The ethyl acetate extract of *M. edule* exhibits strong anti-inflammatory and analgesic activity. The presence of terpenoids and flavonoids in *M. edule* extract might be the reason for the activity (Nualkaew et al., 2007). The analgesic activity of *M. umbellatum* root extract was analyzed through a tail-flick, hot plate and acetic acid-induced writhing model assays. The results revealed that a prominent peripheral analgesic activity was shown by animal models than the central effect (Himanshu et al., 2010).

- **Cytotoxicity assay using *A. cepa***

Several plants are used as bioindicators, which are suitable genetic models to monitor the damages induced by environmental pollutants and other lethal mutagens. *Allium cepa*, *Vicia faba* and *Tradescantia* spp. are a few of them. These plant bioindicators assess mitotic index, chromosome aberrations, micronuclei, sister chromatid exchange and mutations in respective test systems. These bioassays are validated, and their protocols are standardized through a program under the International Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme (UNEP) (de Souza et al., 2016). The commonly used plant bioindicators are shown in **Table 2**. *A. cepa* is used as a standard model organism for cytotoxicity or genotoxicity studies. It is used as an initial screening test for acute toxicity at the cellular level. *A. cepa* assay facilitates the detection of chromosomal aberrations and mitotic spindle abnormalities and changes in cell division or mitotic index (Bezerra et al., 2016).

*A. cepa* is commonly used as a test organism for genotoxicity studies because it is cheap, easily available and has advantages over other short-term tests. As previously said, the endpoints of the *A. cepa* assay are the detection of chromosomal aberration and mitotic index. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity, whereas micronucleus analysis is used to verify the mutagenicity of different chemicals (Khanna & Sharma, 2013). There are several advantages of using higher plants like *A. cepa* and *V. faba* as bioindicators 1) Visualization of the chromosomal organization is possible for comparison. 2) They rapidly respond to environmental changes. 3) They allow *in situ* monitoring. 4) They are constantly exposed to pollution. 5) They are easy and inexpensive to grow (de Souza et al., 2016).

The cytotoxic potential of plants is widely discussed by various researchers. They suggest that chromosomal aberrations have resulted from

the action of toxic phytoconstituents present in the sample. The chromosomal aberrations are apportioned into two *ie.*, clastogenic and aneugenic aberrations. In the former, abnormal effects are induced on the genetic material and later interfere with mitotic spindle formation (Bhagyanathan & Thoppil, 2016).

**Table 2: Some common plant bioindicators used in genotoxic/cytotoxic assays**

| Sl. No. | Plant | Common name | Biomarkers | Reference |
|---|---|---|---|---|
| 1 | *Allium cepa* | Onion | Mitotic index, chromosomal aberrations | Rodríguez et al., 2015 |
| 2 | *Vicia faba* | Broad bean | Mitotic index, chromosomal aberrations, micronucleus and sister chromatid exchange | Obidoska et al., 2017 |
| 3 | *Arabidopsis thaliana* | Thale cress | Recombination and point mutation | Menke et al., 2001 |
| 4 | *Tradescantia* spp. | Spiderwort | Micronucleus in pollen grain mother cells and point mutation in staminal hair test | Klumpp et al., 2006; Mišík et al., 2011 |
| 5 | *Lactuca sativa* | Lettuce | Seed germination, root elongation, mitotic index, chromosomal aberrations and micronucleus test | Bagur-González et al., 2011 |
| 6 | *Hordeum vulgare* | Barley | Chromosomal aberrations | Mattiello et al., 2015 |

The abnormality percentage and mitotic index are two important parameters in toxic studies. Based on the toxic level of plant extract, aberration percentage and mitotic index may vary. In several plants, reduced mitotic index percentage and many chromosomal abnormalities like disturbed prophase, C-mitosis, vagrant chromosomes, stickiness, laggards, chromatid bridges and fragment formation in the anaphase are recorded (Karaismailoglu, 2014; Prajitha & Thoppil, 2016). The genotoxic effect of the heavy metal

contaminants in the aquatic system was evaluated by using the *A. cepa* system and the comet assay. The result shows that a significant change was detected in the frequency of chromosome aberrations and in the mitotic index when compared to the negative control system. Comet assay also substantiates the same effect through the significant alteration in the level of DNA breaks (Barbosa et al., 2010).

The genotoxicity evaluation of *M. umbellatum* leaves extract on cyclophosphamide-induced rats was carried out by Shetty et al. (2010). The frequency of chromosomal aberrations and micronuclei formation induced by cyclophosphamide was not found to be statistically significant. The plant extract can prevent the genotoxicity of cyclophosphamide and thus only a slight variation occurs in the mitotic index as compared to the negative control groups. The C-mitotic activity of *M. randerianum* was reported by Ramya Sree and Thoppil (2018). C-mitosis or colchicine mitosis is the interruption of spindle formation during mitosis. This mitotic spindle arrest causes various abnormalities in the cell during mitosis *viz.,* C-metaphase, C-anaphase, polyploidy, vagrance, cytostatic effect *etc*. The highest percentage of the mitotic division was found in the highest concentration (0.1%) at the prolonged exposure period (24 hr), which strongly supports the presence of colchicine like compounds in the plant extracts. The C-mitotic activity of the plant extract of *M. randerianum* led to the exploration of colchicine-like compound, which can be exploited mainly in the field of plant breeding for inducing polyploidy. Limited studies are available on the *Memecylon* genotoxic effects. The understanding of the antigenotoxic potential of plant extract is helpful in the formulation of natural anticancer drugs. We have several examples of natural anticancer agents of plant origin, *viz*., Curcumin from *Curcuma longa*, Epigallocatechin-3-gallate from *Camellia sinensis* and Resveratrol from *Veratrum grandiflorum* are few of them (Wang & Jiang, 2012).

- **Anticancerous activity**

The increasing incidence of various cancers has encouraged researchers to discover novel, more effective drugs from plant sources. Cancer is one of the most treacherous diseases in the world. According to WHO, it is the second leading cause of death globally and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer. The present study gives a special emphasis on breast cancer developments. Breast cancer is the most frequent cancer among women, affecting 2.1 million women each year. In 2018, it is estimated that 627,000 women died from breast cancer - that is approximately 15% of all cancer deaths among women. The breast cancer rates are higher among women in more developed regions and it is increasing alarmingly in nearly every region globally (WHO, 2018). Breast cancer is the second most common cancer worldwide after lung cancer (Siegel et al., 2016). There are several factors associated with breast cancer risk. Age, hormonal - reproductive - menstrual history, alcohol, radiation and hereditary factors are the major ones. Some of the observations related to breast cancer risk is that it is increased in early menarche, late menopause and obesity in postmenopausal women.

From the history of cancer treatments, the accepted way of cancer treatment involves surgery, radiation and drugs. Most of the available cancer chemotherapeutic agents can provide temporary relief from symptoms and cause prolongation of life. An effective anticancer drug should kill or incapacitate cancer cells without causing damage to normal cells. Apoptosis is an effective way of maintaining normal cell growth rather than cancerous growth. So inducing apoptosis in the cancer cell is the most ideal situation. Apoptosis or programmed cell death is essential for maintaining the homeostasis of the body. Apoptosis can restrict the growth of cancer cells. The outstanding feature of apoptosis involves cytoplasmic breakage, cytoplasmic disintegration, cytoplasmic shrinkage, heterochromatinisation, cytoplasmic vacuolation *etc*. A detailed study on apoptosis is essential because apoptosis is the key mechanism behind the management and

prevention of cancer. Synthetic drugs have less durability to prevent cancerous growth and the side effect of these cannot be predicted. In most of the case, the availability of the drug sample become limited. These limitations are pointed to the discovery of a safe natural therapeutic drug for cancer. Many of the research work around the world are aiming to develop drugs for the ultimate prevention of this dangerous disease. New natural templates are developed around the world for this purpose (Taraphadar et al., 2001).

**Table 3: Anticancer drugs isolated from plants**

| Natural compounds | Plant source | Cell lines | Reference |
|---|---|---|---|
| *Catharanthus* alkaloids | *Catharanthus roseus* | Acute lymphocytic leukaemia, Non-small cell lung cancer, Bladder cancer | Lichota & Gwozdzinski, 2018 |
| *Viscum album* extract | *Viscum album* | Human bladder carcinoma (T24, TCCSUP, J82 and UM-UC-3) Squamous cell carcinoma of the tongue cell lines SCC-9 and SCC-25 | Urech et al., 2006 |
| Camptothecin | *Camptotheca acuminata* | Human colon cancer HCT116, Breast cancer MCF-7, Prostate cancer DU145, Leukaemia (CEM) | Goldwasser et al., 1995 |
| Taxanes | *Taxus baccata* | Breast cancer cell SK-BR-3, MCF-7, Human prostate cancer PPC-1 | Jelínek et al., 2015 |
| Artesunate, Artemisinin | *Artemisia annua* | Colon cancer HT 116, Lung cancer A549, Breast cancer MCF-7, Melanoma A375, | Crespo-Ortiz & Wei, 2012 |

| | | | |
|---|---|---|---|
| Salvicine | *Salvia pronitis* | G-361, LOX Colon cancer HT-1376, HeLa and Breast cancer MCF-7 | Deng et al., 2011 |

Numerous anticancer drugs isolated from plant materials are tested on cells (including various cancer cell lines) and experimental animals. In recent years, there has been a dynamic increase in the number of newly discovered natural compounds. Banerjee et al. (2015) documented that in 2006, about 50,000 natural compounds were known, whereas, in 2014, the number of newly discovered molecules increased to approximately 326,000. Among these, there were approximately 170,000 compounds in the toxic chemical class and there are 195,000 of them designated as pharmacologically active compounds. Some of the common anticancer drugs isolated from plants are listed in **Table 3.**

Naidu et al. (2013) investigated the effects of ethyl acetate extract of *M. edule* leaves on human gastric cancer cells via a mitochondrial-dependent pathway. When comparing the cytotoxicity effect on gastric cancer cells (NUGC and MKN-74) to normal gastric cells (GES-1), the results showed that the cytotoxicity was more specific to the malignant cells (NUGC and MKN-74), implying greater specific cytotoxicity to the malignant cells. Puttaswamy and Achur (2013), studied the anticancer activity of *M. umbellatum* leaves extract. The maximum amount of inhibition observed in the methanolic extract was found to be 83% and 81% respectively for DLA and EAC cells. Minimum inhibitory concentration was found to be 42% at 20 µg/mL. The chloroform extract doesn't exhibit significant activity when compared to methanolic extract. The *in vitro* anticancer activity of *M. malabaricum* and *M. umbellatum* on human colon cancer cell line HCT-116 was found to be effective. *M. umbellatum* extract possesses the highest

inhibitory effect as compared with *M. malabaricum* extract. Apoptosis is the preferred mode of cell death executed by the plant extract in HCT-116 and was evaluated by acridine orange/ethidium bromide (AO/EB) staining (Chaudhary et al., 2017). *M. randerianum* antiproliferative efficacy was studied by Hegde and Hungund (2020). It was tested against breast (MCF), oral (KB) and lung (A-549) cancer cell lines, with $IC_{50}$ values of 159.81 ± 7.54 g/mL, 240.21 ± 2.57 g/mL and 124.17 ± 2.10 g/mL respectively. Srinivasan et al. (2020) reported that ursolic acid is an anticancer agent found in the leaves of *M. edule*. It is active against human leukemic monocyte lymphoma (U-937) and human acute promyelocytic leukemia (HT-60) cell lines.

The anticancer properties of natural medicines have an ample demand because of their target specific-activity and non-toxicity to normal cells. The consumption of nutraceuticals from nature can reduce the incidence of cancer development (Prasad et al., 2017). A safe remedy against cancer is the most essential requisite. So it is important to screen apoptotic inducers of plant origin. Apoptosis occurs normally as a homeostatic mechanism during development and ageing in tissues to maintain cell populations. Apoptosis is also occurring as a defensive mechanism in response to external stimuli, noxious chemicals and certain pathological conditions (Norbury & Hickson, 2001). According to Elmore (2008), the apoptotic cell appears as rounded bodies having eosinophilic cytoplasm and chromatin fragments in dense purple colour. Early during the chromatin condensation phase, the electron-dense nuclear material aggregates peripherally under the nuclear membrane and can be seen as dense nuclei. The apoptotic bodies can be distinguished from necrotic cells by passive process and uncontrolled process that usually affects large fields of cells. Apoptosis is reversely controlled, energy-dependent and can affect individual cells or clusters of cells.

Two molecular signaling pathways occur during apoptosis *viz.*, extrinsic and intrinsic (mitochondrial) pathways. The extrinsic pathway initiates an apoptotic mechanism through the transmembrane receptors. They are the death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily. In the intrinsic pathway, the apoptotic mechanism is regulated by the array of non-receptor-mediated stimuli, which are produced in mitochondrial-initiated events.

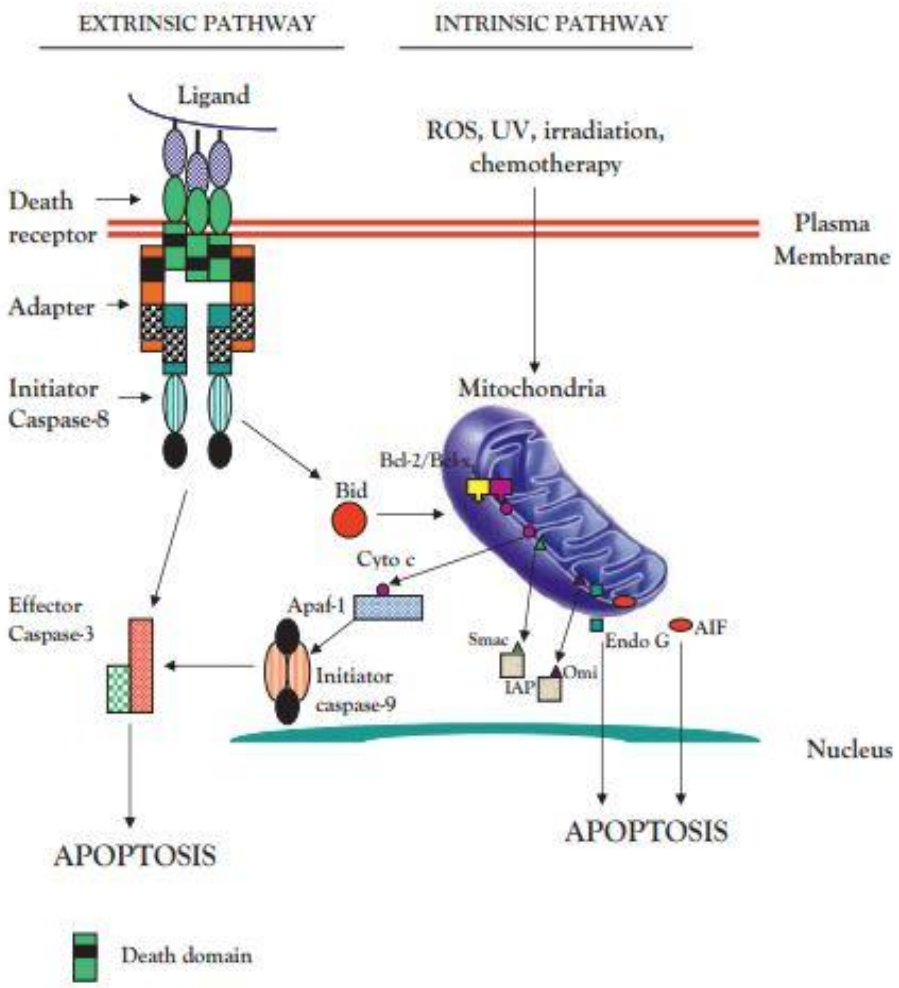


**Figure 3: Representation of extrinsic and intrinsic pathway of apoptosis** (Source: Gupta et al., 2006)

The schematic representation of extrinsic and intrinsic pathways is given in **Figure 3.** In the extrinsic pathway, the death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways. The best-characterized death receptors include CD95 (APO-1/Fas), TNF receptor 1 (TNFRI), TNF-related apoptosis-inducing ligand-receptor 1 (TRAIL-R1) and TRAIL-R2. However, the role of DR3 (TRAMP/Apo-3/WSL-1/LARD) or DR6 has not exactly been identified. The corresponding ligands of the TNF superfamily comprise death receptor ligands such as CD95 ligand (CD95L), TNF-α, lymphotoxin-α, TRAIL and TWEAK, a ligand for DR3 (Fulda & Debatin, 2006). The extrinsic apoptotic pathway activated by the stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily such as CD95 (APO-1/Fas) or TNF-related apoptosis-inducing ligand (TRAIL) receptors results in activation of the initiator caspase-8. This can propagate the apoptosis signal by direct cleavage of downstream effector caspases such as caspase-3 and execute apoptosis (Walczak & Krammer, 2000).

Intrinsic apoptotic pathway is initiated by the release of apoptogenic factors from the mitochondrial intermembrane space to the cytosol, such as cytochrome c, apoptosis-inducing factor (AIF), Smac (second mitochondria-derived activator of caspase), DIABLO (direct inhibitor of apoptosis protein [IAP]-binding protein with low PI) and Omi/HtrA2 or endonuclease G. The release of cytochrome c into the cytosol triggers caspase-3 activation through the formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex. Sma c/DIABLO and Omi/HtrA2 promote caspase activation by neutralizing the inhibitory effects to the IAPs (Saelens et al., 2004).

In most anticancer therapies, apoptosis pathways and signal-transducing molecules have been shown to play a crucial role in killing tumor cells in response to cytotoxic agents. Understanding the molecular events that regulate apoptosis in response to anticancer chemotherapy, and how cancer

cells evade apoptotic death, provides novel opportunities for a more rational approach to the cancer research field.

- **Breast cancer research**

The BRCA (BReast CAncer) gene is a tumor suppressor gene that prevents uncontrolled cell division and helps in DNA break repair. The mutation in the BRCA gene produces BRCA1 and BRCA 2 which predisposes to human breast cancer. BRCA1 and BRCA2 encode very large proteins widely expressed in different tissues during the S and G2 phases, which are localized in the cell nucleus (Venkitaraman, 2002). BRCA 1 and BRCA 2 possess several biological roles and they can act as a caretaker of chromosome structures *ie*., they can control the gross chromosomal rearrangements including translocations, deletions and fusions of the non-homologous chromosomes. BRCA 2 has a main role in double-strand break repair, particularly it can control RAD 51 recombinase, an eukaryotic homolog of bacterial Rec A essential for double-strand break repair. BRCA 1 deficiency can cause DNA damage and thereby block cell proliferation and apoptosis (Deng, 2006). Heterogeneity in breast cancer makes them a fascinating and challenging stream to diagnose and treat. Women with a BRCA 1 or BRCA 2 mutation are candidates for some additional risk factors. Some additional susceptibility genes have been identified, including PTEN, ATM, TP53, CHEK2, CASP8, PBRL and BRIP1 (Bradbury & Olopade, 2007).

PI3K/AKT/mTOR pathway aberrations are the common abnormalities associated with breast cancer risk. Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes involved in cell growth, proliferation, differentiation and intracellular trafficking. It is a heterodimer composed of regulatory (p85) and catalytic (p110) subunits. The stimulation of receptor tyrosine kinases activating the signalling cascade through PI3K activation is followed by

phosphorylation of AKT and mTOR complex 1 (mTORC1). In TNBC (Triple Negative Breast Cancer), oncogenic activation of the PI3K/AKT/mTOR pathway resulted as a function of overexpression of upstream regulators (e.g., epidermal growth factor receptor [EGFR]) and the down regulators. It also includes the mutations of PI3K catalytic subunit α (PIK3CA), loss of function or expression of phosphatase, tensin homolog (PTEN) and the proline-rich inositol polyphosphatase (Costa et al., 2018). PI3K/AKT/mTOR pathway had become an essential tool in cancer therapy. Fillmore et al. (2010) suggest that estrogen can induce the secretion of paracrine acting proteins, which in turn increase percentages of $CD44^{+}/CD24^{-}/ESA^{+}$ populations and corresponding cancer stem-like properties in many breast cancer cell lines.

Lack of HER2 expression, estrogen and progesterone receptors is another reason for breast cancer. Breast tissues are estrogen-responsive and BRCA deficient cells in the breast and ovarian tissues can escape from apoptosis and lose their damage repairing capacity. Estrogens can regulate the expression and function of c-Myc, cyclin D1 and activate cyclin E-Cdk2 complexes, which are the rate-limiting steps in $G_1$-S phase progression. The activation of cyclin E-Cdk2 by estrogen promotes the formation of high molecular weight complexes, lacking the CDK inhibitor p21 (Doisneau-Sixou et al., 2003). In human breast cancer cells, deregulation of several miRNAs can be revealed through the microarray and northern blot analysis *ie., mir*-125 b, *mir*-145, *mir*-21, *mir*-155. The role of miRNA in tumorigenesis and its potential usage as breast cancer biomarkers are the future perspectives for the early diagnosis and treatment of breast cancer.

Nowadays the tumor markers have increasing attention in breast cancer clinical studies. The cancer antigen 15-3 (CA15-3) is a member of the mucin-1 (MUC-1) family of glycoprotein that is over-expressed in tumors. A cohort study in Chinese women proposes that pre-operative prediction of breast cancer markers is validating the cancer survival rate and offering a personalized treatment strategy in cancer subtypes (Li et al., 2020**)**. They

found that tumor markers like CA15-3, CA125 and CEA levels before surgery may have the potential in predicting breast cancer survival. The level of tumor marker is different in different breast cancer subtypes. It is lower in the triple-negative group as compared to the luminal groups (Kos et al., 2013). Several studies have been conducted all over the world to eradicate breast cancer malignancy. The increased number of population studies on breast cancer forms an alarming signal that it continues to spread all over the world (Kalager et al., 2012; Abubakar et al., 2018).

- **Nanoparticle biosynthesis**

Nanoscience is a rousing discipline of science, which has numerous novel and cost-effective yields and applications. Nanomaterials have a particle size of 1-100 nm and have superior bioavailability than larger particles. This property can enhance their usage in single cells, tissues and organ systems. The growing demands for nanoparticles always search for the biosynthesis method, which is profitable and ecofriendly with high utility factors. The biogenic nanoparticle has numerous physical, biological and pharmaceutical applications. Nano-silver is the most studied and utilized nanoparticle. Silver nanoparticles (AgNPs) have become the topic of researchers because of their unique properties. Nanoparticle research is an intense scientific research area due to its potential application in the biomedical, optical and electronics fields. There are different groups of nanoparticles like metal NPs, ceramic NPs, polymeric NPs and fullerenes. The unique surface feature and nanoscale size are the key factors behind the chemical and physical properties of NPs. Due to these characters, they are suitable candidates for various applications *ie.*, catalysis, biomedical application and environmental usages. There are different methods of nanoparticle synthesis *ie.*, chemical, physical and biological methods. The chemical and physical methods are involved in the production of toxic byproducts which are hazardous, moreover the methods are very expensive. A stable nanoparticle with controlled size and shape with an inexpensive, safe,

reliable and green approach has been recently developed. So there are many reports related to the green synthesis of nanoparticles using several plant extracts (Khan et al., 2018; Saranyaadevi et al., 2014).

The two most commonly employed processes of nanoparticle synthesis is the top-down and bottom-up pathway. In a top-down pathway, the materials are broken down into small particles of the nanoscale by many lithographic methods like mechanical milling/ball milling and chemical etching. This approach creates imperfections in the surface structure of the product. In the bottom-up pathway, the NPs are formed by the oxidation and bioreduction procedures. In this method, the smaller particles are aggregated to form nanomaterials. They have lesser defects and are more homogeneous in chemical composition (Thakkar et al., 2010). Several methods of biosynthesis of nanoparticles are attempted by researchers. The bacterium, fungus, yeast and plant-mediated biosynthesis of nanoparticles are widely accessible. The microorganism based nanoparticle synthesis have several limitations, such as the rate of synthesis is slow, difficulty in maintaining microbial cultures, difficulty in the isolation of microbial strains and only a limited number of sizes and shapes are amenable as compared to the conventional process of NPs synthesis. Apart from this, advanced studies had reflected the succeeding role of plants in green synthesis because of the ease of process involved in the synthesis, much stable NPs and cost-effectiveness (Sastry et al, 2003; Durán et al., 2005). The plant-mediated synthesis of the nanoparticles is represented in **Figure 4.**

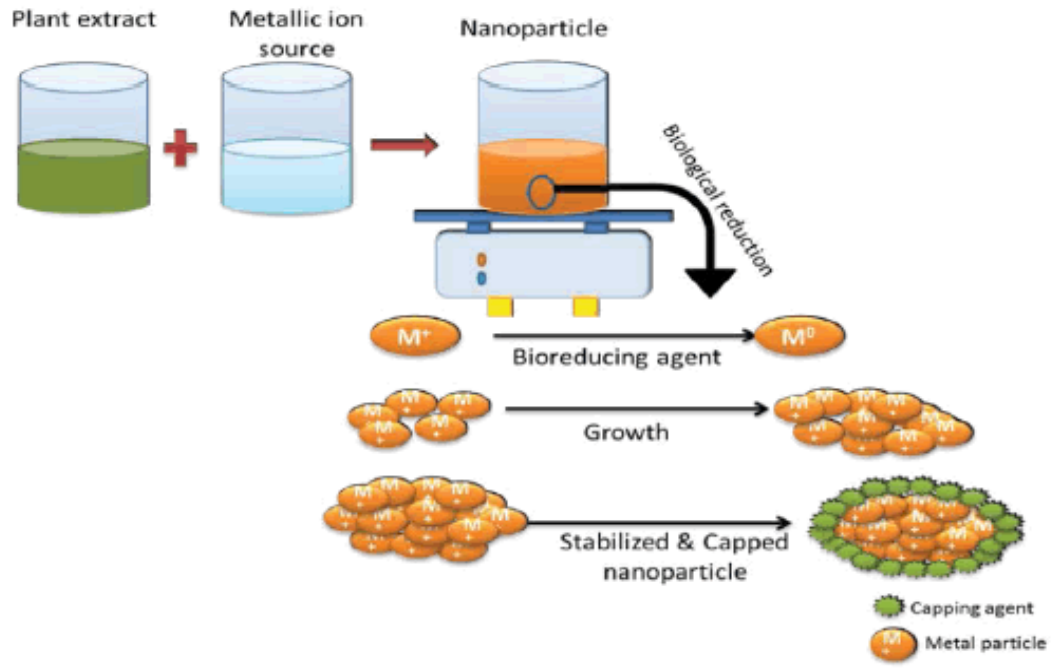


**Figure 4: Schematic representation of biosynthesis of nanoparticles using plant extract.** (Source**:** Qidwai et al., 2018)

The biological procedure involves capping and stabilizing mediators (phytochemicals like phenolics, flavonoids, terpenoids and cofactors) that contribute to higher stability. Elavazhagan and Arunachalam (2011) reported that the biosynthesis of silver nanoparticles using *M. edule* leaves extract is an excellent bioreductant. For the green synthesis of silver and gold nanoparticles, it is a readily available plant source. Limited studies are available on the nano-synthesis from *Memecylon* species. Research works regarding the phytogenic synthesis of nanomaterials using plant extracts are reported in **Table 4**.

**Table 4: Reports of phytogenic synthesis of nanoparticles**

| Plant | Nanoparticle component | Activities & Application | References |
|---|---|---|---|
| *Azadirachta indica* | Silver | Antimicrobial activity | Roy et al., 2017 |

| | | | |
|---|---|---|---|
| *Clitoria ternatea* | Silver | Antibacterial activity against common nosocomial pathogens | Krithiga et al., 2015 |
| *Cocos nucifera* | Silver | Antibacterial activity against human pathogens | Mariselvam et al., 2014 |
| *Acorus calamus* | Gold | Antibacterial and UV blocking applications | Ganesan & Prabu, 2019 |
| *Salvia officinalis* | Silver | Cytotoxicity | Sehnal et al., 2019 |
| *Sida acuta* | Silver | Larvicidal activity | Veerakumar et al., 2013 |
| *Gossypium hirsutum* | Silver | Antibacterial activity against plant pathogens | Vanti et al., 2019 |

The nanoparticle characterization was done through microscopic and spectrometric methods. The two important parameters are the size and shape of the nanoparticles. UV-Vis spectroscopy (UV-Vis) is another relatively facile and low-cost characterization method of nanoparticles. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from reference material. Nanoparticles have optical properties that are sensitive to size, shape, concentration and agglomeration state, which makes UV-Vis spectroscopy an important tool for characterizing nanoparticles. The size and shape of synthesized nanoparticles are determined by Scanning Electron Microscopic analysis (SEM). There is a direct relationship between the size and shape of the nanoparticle and its biological activity (Hamouda et al., 2019). TEM, XRD, NTA, DLS and XPS are some of the common techniques for the characterization of NPs (Mourdikoudis et al., 2018). The biological activities of nanoparticles are very specific and mostly unexploited. So the green nanoscience can be effectively targeted towards therapeutic research.

This literature survey suggests that *Memecylon* species are potential for various bioactivities and it could be attributed to the presence of a certain unique classes of compounds found in them. It strengthens the necessity for conducting more studies on further exploration of the phytochemical components and isolation of the active components. The *in vivo* screening of bioactivities and formulation of natural herbal drugs opens a new gateway in pharmaceutical research.

The materials and methods used in the present study are given in the next chapter.

# CHAPTER 1 MATERIALS AND METHODS

The present work has been designed to study pharmacognostic profiling, phytochemical characterization and bioactivity screening of selected species of *Memecylon* L. The leaves and fruits of selected species are used for the present study. The materials and methods adopted for the entire study are divided into three phases.

**Phase I:** Pharmacognostic profiling of selected species of *Memecylon* through powder microscopy, SEM-EDX and ICP-MS analysis.

**Phase II:** Phytochemical characterization by GC/MS and HR-LC/MS analyses.

**Phase III**: Bioactivity studies of selected species of *Memecylon* - Evaluation of *in vitro* free radical scavenging activity, cytotoxicity screening on *Allium cepa*, antiproliferative efficacy on MCF-7 cell lines and biosynthesis of silver nanoparticles.

## I. MATERIALS

### Plant specimens

*Memecylon grande* Retz., *Memecylon randerianum* S. M. & M. R. Almeida and *Memecylon umbellatum* Burm. f., are collected from different parts of Kerala **(Plate 1)**. The taxonomic authentification of the plant materials was done by Dr A. K. Pradeep, Assistant Professor, Angiosperm Taxonomy Division, Department of Botany, University of Calicut. Voucher specimens are deposited at the Herbarium of Botany Department, University of Calicut (CALI). The voucher number allotted to each plant is given in brackets.

- ***Memecylon grande* Retz. (CALI No. 123777 )**

**Family: Melastomataceae**

**Habitat: Small tree**

**Locality: Kodungallur, Thrissur**

**Flowering and fruiting: April-November**

Small trees. Leaves 8 x 3 cm, ovate-lanceolate, obtusely acuminate, base cuneate, coriaceous, slight brown when dry, lateral nerves and intramarginal nerves faint, petiole 8 mm. Flowers blue, peduncles 1.5 cm, bracts ovate, minute, pedicels 3 mm. Calyx lobes 1 mm, ovate, petals 2.5 mm, obovate, stamens 8, recurved. Ovary embedded in calyx tube, 2 mm. Berry globose, brownish-black.

- ***Memecylon randerianum* S. M. & M. R. Almeida (CALI No. 123776)**

**Family: Melastomataceae**

**Habitat: Large shrub**

**Locality: Puthoor vayal, Wayanad**

**Flowering and fruiting: February-May**

Large shrubs, branchlets terete. Leaves 9-10 x 3-3.5 cm, subsessile, ovate-oblong, greenish-yellow below when dry, nerves 10-12 pairs, scarcely visible. Cymes 1.5-2 cm across, peduncle very short or absent, pedicel 3-4 mm long, slender. Flowers 2-3 mm across, many-together, calyx lobes ovate, white margined, glabrous. Petals 1-2 mm across, obovate, blue. Berry globose, bluish.


**Plate 1: Plant materials- a-** *Memecylon grande,* **a1-** inflorescence, **a2-** fruit, **b-** *Memecylon randerianum* **b1-** inflorescence, **b2-** fruit, **c-** *Memecylon umbellatum*, **c1-** inflorescence, **c2-** fruit.

- ***Memecylon umbellatum* Burm. f. (CALI No. 123775)**

**Family: Melastomataceae**

**Habitat: Small tree**

**Locality: Mezhuveli, Pathanamthitta**

**Flowering and fruiting: February-March**

Small trees. Branchlets terete. Leaves 6-7 x 2-2.5 cm, elliptic-lanceolate, acuminate and shortly cuspidate at the tip, yellow when dry, petiole 5-6 mm long, slender. Peduncle 1-2 together, 2-3 mm long, stout, umbel, 3-6 flowered, pedicel 2-3 mm long. Flowers 6-7 mm across, calyx lobes acute, petals 2-3 mm across, blue. Berry 5-7 mm across, globose, puberulus, yellow.

## II. METHODOLOGY

### PHASE I- PHARMACOGNOSTIC PROFILING

The functional purity of the plant sample is essential for pharmaceutical trials. Pharmacognosy is a field in which the authenticity of the plant specimens is characterized. In this study, powder microscopy, SEM-EDX and ICP-MS analyses were carried out to determine the pharmacognostic status of the selected specimens.

**a) Powder microscopy**

The powder of selected *Memecylon* species was treated with 4% KOH and mounted in glycerine on clean slides and the powder characters were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam ERc 5s digital camera.

**b) SEM analysis**

Scanning electron microscopic (SEM) analysis was performed using the ZEISS Gemini SEM 300 machine. The samples were prepared on a carbon-coated copper grid.

**c) EDX analysis**

SEM-EDX analysis was done by using Octane plus with Gemini 300/EDS. The active area selected for the present study is 30 $mm^2$.

**d) ICP-MS analysis**

Inductively coupled mass spectrometric analysis was performed by using Agilent 7800 ICP-MS with Integrated Sample Introduction System (ISIS 3) and SPS 4 autosampler. The standard torch of 2.5 mm diameter injector was used. The instrument also used a Ni sampler and Ni skimmer cones. 0.2 g of sample material is dissolved in 15 mL con. nitric acid. Add 2-3 drops of hydrogen peroxide to the above solution and boil till the solution become clear. The solution is filtered through the Whatman No. 1 filter paper into a 50 mL standard flask. The solution is made up to the mark by using Milli Q water. This solution is used for the ICP-MS analysis.

## PHASE II- PHYTOCHEMICAL CHARACTERIZATION

The shade dried leaves and fruits of *Memecylon* species are used for extraction. 30 g of powdered samples were subjected to Soxhlet extraction using methanol as a solvent and the extraction requires 10 hrs. The methanolic extract thus obtained was cooled, filtered through a Whatman No. 1 filter paper and concentrated by removing the complete content of the solvent. It was stored in amber coloured glass bottles at 4°C for all further experiments.

**Preliminary qualitative phytochemical analysis**

The methanolic extract was subjected to phytochemical analysis for the presence or absence of secondary phytoconstituents using standard chemical tests (Sofowora, 1993; Trease & Evans, 1989; Harborne, 1973)

**1) Tests for alkaloids**

The crude extracts of *Memecylon* species were treated with dilute HCl and the filtrate was used for various tests.

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

**(b) Hager's test**: Filtrate was treated with Hager's reagent (saturated Picric acid). The formation of a yellow coloured precipitate indicates a positive test.

**2) Test for anthraquinones**

**Borntrager's test**: 3 mL of aqueous extract was shaken with 3 mL of benzene, filtered and 5 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicates the presence of free anthraquinones.

**3) Test for coumarins**

1 mL of each extract was treated with alcoholic NaOH solution. The production of dark yellow colour indicates the presence of coumarins.

**4) Tests for flavonoids**

**(a) Alkaline reagent test**: A few drops of NaOH solution were added to the extracts. The presence of flavonoids is indicated by the production of a bright yellow colour that become colourless on addition of few drops of weak acid.

**(b) Lead acetate test**: Extracts were treated with few drops of lead acetate solution. The formation of a yellow coloured precipitate indicates the presence of flavonoids.

**5) Test for glycosides**

**Keller Kiliani test**: 0.5 g of the extract was treated with 2 mL of glacial acetic acid and a drop of 5% (w/v) $FeCl_3$ was added to it. Glacial acetic acid containing 1% (w/v) $FeCl_3$ gives a brown ring in the presence of 2-deoxysugar in the glycone portion of the phytochemical.

**6) Test for phenolic compounds**

**$FeCl_3$ test**: 0.5 g of the powdered sample is boiled in 20 mL distilled water and then filtered using a filter paper. 5% (w/v) $FeCl_3$ is added to the filtered samples and observed for the presence of brownish-green or blue-black colour.

**7) Test for phlobatannins**

**HCl test**: About 2 mL of aqueous extract was added to 2 mL of 1% HCl and the mixture was boiled. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

**8) Test for resins**

0.5 g of the extract was diluted to 10 mL with water and shaken for 5 min. The formation of turbidity indicates the presence of resins.

**9) Test for saponins**

**Foam test**: 0.5 g of the extract was shaken well with 2 mL of water. The test is based on the production of persistent foam, indicating a positive test for saponins.

**10) Test for steroids**

**Liebermann Burchard test**: Extracts were treated with chloroform and filtered. Filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated $H_2SO_4$ was added. The reagents, acetic anhydride and concentrated $H_2SO_4$ react with the hydroxyl group of phytosterols to produce a dark green colour in the upper layer indicating the presence of steroids.

**11) Test for tannins**

**Breymer's test**: 2 mL extract was treated with 10% alcoholic $FeCl_3$. Blue or greenish colour appeared, which indicates the presence of tannins.

**12) Test for terpenoids**

**Salkowski's test**: 2 mL of extracts were treated with 2 mL chloroform and filtered. Filtrates were treated with few drops of concentrated $H_2SO_4$, shaken and allowed to stand. The appearance of golden yellow colour at the interface indicates the presence of a terpenoid ring.

a) **Preliminary quantitative phytochemical analysis**

**1) Total alkaloid content**

The total alkaloid content was determined by using the protocol developed by Shamsa et al. (2008). The plant sample of 1 mg was dissolved in 2 N HCl and filtered. Add 5 mL phosphate buffer (pH 4.7), 5 mL BCG solution and shake the mixture with 1, 2, 3 and 4 mL of chloroform. The chloroform layer containing alkaloid was separated. Caffeine is used as a standard. The absorbance of the complex in chloroform was measured at 470 nm against the blank prepared as above but without sample. The calibration curve of caffeine is plotted and a regression equation was generated. The total

alkaloid content of the extract was calculated and expressed as mg caffeine equivalents (CE). Samples were analyzed in triplicates.

**2) Total flavonoid content**

Total flavonoid content was measured by the aluminium chloride colorimetric assay (Chang et al., 2002). An aliquot (1 mL) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. 0.30 mL 5% $NaNO_2$ was added to the flask, followed by 0.3 mL 10% $AlCl_3$ in 5 min interval. After five minutes, 2 mL 1 M NaOH was added, followed by distilled water to make the volume up to 10 mL. The solution was mixed, and the absorbance was measured at 510 nm against a blank. The calibration curve of quercetin was plotted and a regression equation was generated. The total flavonoid content of the extract was calculated and expressed as mg quercetin equivalents (QE). Samples were analyzed in triplicates.

**3) Total phenolic content**

The total phenolic content was determined by using the Folin Ciocalteu assay (Singleton & Rossi, 1965). An aliquot (1 mL) of extract or standard solution of Gallic acid (100, 200, 300, 400, and 500 µg/mL) was added to 25 mL of the volumetric flask, containing 9 mL of distilled water. The blank used is distilled water. The mixture was mixed with 1 mL of Folin-Ciocalteu phenol reagent. After 5 minutes, the mixture was mixed with 10 mL of a 7% $Na_2CO_3$ solution. After that, the volume was raised up to the mark of volumetric flask. The absorbance value against the reagent blank was measured at 550 nm with a UV-Visible spectrophotometer after 90 minutes of incubation at room temperature. The calibration curve of gallic acid was plotted and a regression equation was generated. Using the regression equation, the total phenolic content of the extract was calculated and

expressed as mg gallic acid equivalents (GAE). Samples were analyzed in triplicates.

**4) Total terpenoid content**

The total terpenoid content of the plant extract was estimated by the method of Ghori et al. (2012). The reaction mixture contains an aliquot of extract along with few drops of chloroform and $H_2SO_4$. The absorbance was measured at 538 nm against blank. Linalool was used as the standard. The calibration curve of linalool is plotted and a regression equation was generated. The total terpenoid content of the extract was calculated and expressed as mg linalool equivalents (LE). Samples were analyzed in triplicates.

b) **Phytochemical profiling by GC/MS**

The chemical composition of selected species of *Memecylon* was determined by GCMS-QP2010 Ultra. Helium was used as a carrier gas at a flow rate of 1.21 mL/min at a column pressure of 73.3 kPa. During the analysis, 260°C was the injector and detector temperatures. Samples (6 µL) were injected into the column with a split ratio of 10:0. Component separation was achieved following a linear temperature program of 70 - 260°C at 3°C/min and then held at 260°C for 6 min, with a total run time of 66.63 min. The MS parameters used were: electron ionization (EI) voltage 70 eV, peak width 2s, mass range 40 - 850 m/z and detector voltage 1.5 V. The constituents were identified by comparison of their retention indices. The MS fragmentation was analyzed through comparison with pure compounds of known composition and searching the matching MS fragmentation patterns with National Institute of Standards and Technology (NIST) mass spectra libraries. Finally, their quantification was done based on the GC peak areas.

### c) Phytochemical profiling by HR-LC/MS

The non-volatile chemical compositions of selected species of *Memecylon* were performed on an HR-LC/MS Q-TOF (Agilent, USA) equipped with an electrospray ionization source. The column employed for separation is G1316C, 4.6 mm × 250 mm dimensions with a particle size of 5 µm; by applying the following gradient at a flow rate of 0.5 mL/min. The elution was performed using a mobile phase consisting of 95% A (water) and 5% B (acetonitrile); 1 - 3 min linear from 5 to 95% A; isocratic 10% A. The injection volume was 5 µL and the total run time was 30 min. Eluted compounds were detected with MS Q-TOF equipped with an electrospray ion source in positive ion modes using nebulizer gas as nitrogen 13 L/min; gas temperature is 250°C and nozzle voltage is 1000 V.

## PHASE III- BIOACTIVITY STUDIES

### a) FREE RADICAL SCAVENGING ACTIVITY STUDIES

The antioxidant activity of *Memecylon* species was determined based on free radicals produced by various substrates like DPPH, $Fe^{3+}$ - ascorbate - EDTA - $H_2O_2$ system, sodium nitroprusside and potassium ferricyanide.

#### 1) DPPH free radical scavenging assay

The DPPH free radical scavenging activity of *Memecylon* extracts was carried out by using the method of Chang et al. (2001). DPPH is a free radical, which reacts with antioxidant agents and gets reduced to DPPH-H. The pink coloured DPPH turns yellow when scavenged by antioxidants. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts. Ascorbic acid (10 mg/mL DMSO) was used as a reference compound. The different volumes of extracts *viz.* 1.25 µL - 20 µL (12.5 - 200 µg/mL) from a stock concentration of 10 mg/mL were made up to

a final volume of 20 µL with DMSO and 1.48 mL DPPH (0.1 mM) solution was added. Control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3 mL of DPPH was taken as blank.

$$\% \text{ inhibition} = \frac{control-test}{control} X100$$

**2) Hydroxyl free radical scavenging activity**

Hydroxyl free radical scavenging activity was performed by the method of Kunchandy and Rao (1990). Different concentration of samples such as 125 - 2000 µg/mL from a stock concentration of 10 mg/mL was mixed with 500 µL reaction mixture [2-deoxy 2-ribose (2.8 mM), $FeCl_3$ (100 µm), EDTA (100 µm), $H_2O_2$ (1.0 mM), ascorbic acid (100 µm) in $KH_2PO_4$ - KOH buffer (20 mM pH 7.4)] was made up to a final volume of 1 mL. Control without the test compound, but an equivalent amount of distilled water was taken. After 1 hour of incubation at 37°C, add 1 mL of 2.8% TCA, followed by 1 mL of 1% aqueous TBA and incubate for 15 minutes at 90°C to develop the colour. The absorbance was measured at 532 nm against a blank solution after cooling. Here gallic acid (10 mg/mL DMSO) was used as reference.

$$\% \text{ inhibition} = \frac{control-test}{control} X100$$

**3) Nitric oxide free radical scavenging activity**

The determination of nitric oxide free radical scavenging activity of *Memecylon* extracts was performed by using the method of Kumaran and Karunakaran (2006). Different quantities of extracts, ranging from 125 to 2000 µg/mL from a stock solution, were combined with sodium nitroprusside (5 mmolL-1) in phosphate-buffered saline solution (pH 7.4) (**Appendix 2**). It

is incubated at 25°C for 30 min. Control without the test compound, but an equivalent amount of distilled water was taken. 1.5 mL of the incubated solution was withdrawn after 30 minutes and diluted with 1.5 mL of Griess reagent (**Appendix 3**). The absorbance was measured at 546 nm. Here gallic acid (10 mg/mL DMSO) was used as reference.

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

**4) Superoxide free radical scavenging activity**

Superoxide free radical scavenging activity was carried out by using the method of Valentão et al. (2003). Various concentrations of sample (125 - 2000 µg/mL) were prepared from a stock solution of 10 mg/mL, 0.05 mL of riboflavin solution (0.12 mM), 0.2 mL of EDTA solution (0.1 M), and 0.1 mL NBT (Nitro blue tetrazolium) solution (1.5 mM) were mixed in a test tube, and the reaction mixture was diluted up to 2.64 mL with phosphate buffer (0.067 M). Control without the test compound, but an equivalent amount of distilled water was taken. After a 5 min. of incubation in fluorescent light, the solution's absorbance was measured at 560 nm. Measurement was also taken after illumination for 30 min. at 560 nm on UV visible spectrophotometer. Ascorbic acid (10 mg/mL DMSO) was used as a reference.

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

**b) CYTOTOXICITY SCREENING USING *ALLIUM CEPA***

Germinated bulbs having healthy roots of *A. cepa* were collected at the time of peak mitotic activity (9 - 9.30 am) and washed thoroughly with distilled water. The onion bulbs were kept at the rim of the bottle in which the different concentrations of the extracts were taken (12.5, 25, 50,100 µg/mL) and the roots were completely immersed in the solution. Distilled water was taken as negative control (NC) and hydrogen peroxide (CAS No: 7722-84-1,

10 µg/mL), as a positive control (PC). A few root tips were cut from each bottle after treatment for different time durations *viz.*, ½, 2 and 24 hours. Root tips were washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (**Appendix 4**) for one hour. Mitotic squash experiments were conducted with the help of improved techniques (Sharma & Sharma, 1990). Then root tips were subjected to hydrolysis with 1 N HCl for 5 - 10 min and washed thoroughly in distilled water. Acetocarmine (**Appendix 5**) was used to stain the tissues for one hour and destained in 45% acetic acid. Slides were prepared and photomicrographs were taken using a light microscope (Leica DM 2000 LED, Germany). From ten different fields, numbers of mitotic cells, aberrant cells, and total cells were counted. Mitotic index (%) and abnormality percentage (%) were calculated using the following formulae, and values were expressed as mean ± SE.

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

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

## c) ANTIPROLIFERATIVE ACTIVITY OF *MEMECYLON* SPECIES

### 1) Cytotoxicity assay on MCF-7 cell lines

MCF-7 (Human Breast Adenocarcinoma) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India, and maintained in DMEM medium (Dulbecco's Modified Eagles Medium - Sigma Aldrich, USA) (**Appendix 6**). Direct observation using an inverted phase-contrast microscope is carried out to analyze the viability of cells and followed by the MTT assay for cytotoxicity analysis.

i) Sample preparation

1mg of sample was weighed and dissolved in 1 mL DMEM using a cyclomixer. To ensure the sterility of the sample solution it was filtered through 0.22 µm Millipore syringe filter. The growth medium was replaced with freshly prepared 5% DMEM after 24 hours. Which were then five times serially diluted by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µL of 5% DMEM). The sample of 100 µL solution was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% $CO_2$ incubator. Non treated cells were maintained as control.

**ii) Cytotoxicity assay by direct microscopic observation**

After 24 hours the entire cell plate was observed using an inverted phase-contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and photographs were taken. The changes in the cell morphology were noticed. These are considered indicators of apoptosis.

**iii) Cytotoxicity assay by MTT method**

15 g of MTT (Sigma, M-5655) was dissolved in 3 mL PBS and sterilized by filter sterilization. The sample content in wells was removed and 30 µL of reconstituted MTT solution was added to all test and cell control wells, after 24 hours of the incubation period. The plate was gently shaken thoroughly and then incubated at 37°C in a humidified 5% $CO_2$ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µL of MTT solubilization solution (Dimethyl sulphoxide, DMSO - Sigma Aldrich, USA) was added. Later wells were mixed gently to solubilize the formazan crystals. The absorbance values were measured at 540 nm using a microplate reader (Talarico et al., 2004).

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The percentage of viability was calculated using the formula:

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

**2) Cytotoxicity assay on L929 cell lines**

The most effective extract from the cytotoxic assay using MCF-7 *ie.*, *M. umbellatum* fruits (MUF) extract was selected for further studies. L929 (Fibroblast) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's Modified Eagles Medium, DMEM ( Sigma Aldrich, USA). The viability of cells was evaluated by direct observation of cells by an Inverted phase contrast microscope and followed by the MTT assay method.

**i) Sample preparation**

1 mg of *M. umbellatum* fruit extract was weighed and dissolved in 1 mL DMEM using a cyclomixer. The sample solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. After 24 hours the growth medium was removed, freshly prepared compounds in 5% DMEM were five times serially diluted by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µL of 5% DMEM) and each concentration of 100 µL was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% $CO_2$ incubator. Non treated control cells were also maintained.

**ii) Cytotoxicity assay by direct microscopic observation**

After 24 hours the entire cell plate was observed using an inverted phase-contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and photographs were taken. The changes in the cell morphology were noticed.

**iii) Cytotoxicity assay by MTT Method**

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization. After 24 hours

of the incubation period, the sample content in wells was removed and 30 µL of reconstituted MTT solution was added to all test and cell control wells. The plate was gently shaken well and then incubated at 37°C in a humidified 5% $CO_2$ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µL of MTT solubilization solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm.

The percentage of viability was calculated using the formula:

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

**3) Genotoxicity evaluation using comet assay**

The MCF-7 cells were cultured in 6 well plates and treated with $LD_{50}$ concentration of the sample (MUF - 78.48 ± 0.8 µg/mL) and incubated overnight. The cells were trypsinized and washed with fresh media and used for the comet assay. Fully frosted microscope slides were pre-coated with 1 mL of 0.75% normal melting point agarose (NMA Invitrogen, USA) and stored at 4°C. This layer was removed before use and 120 µL of 0.75% NMA was pipetted into the slides, which were then covered with coverslips. Cell suspensions ($1\times10^4$/5-30 µL) were mixed with 10 µL of low melting point agarose (Novex, Invitrogen) and pipetted over the first layer of agarose. NMA (80 µL) was used as a final protective layer. After each step the slides were incubated at 4°C for 10 min to allow agarose to set (Dhawan et al., 2009).

Slides were then placed in a cold lysing solution (**Appendix 7**) and after lysis, slides were placed in electrophoresis buffer (**Appendix 8**) for 20 min to allow unwinding of DNA. Electrophoresis was conducted in the same

buffer by applying an electric current of 0.8V/cm (300 mA) for 20 min using an electrical supply (Power case, Life Technologies). Finally, slides were washed in neutralization buffer (**Appendix 9**) three times for 5 min each, dried and stained with 50 µL ethidium bromide (20 µg/mL). The slides were photographed using an inverted epifluorescent microscope Olympus CKX41 attached with an Opitka Pro5 CCD camera. Comets were scored using Tritek comet scoring software and correlated statistically.

**4) Detection of apoptosis by the double staining method**

The DNA-binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) (Sigma, USA) (**Appendix 10**) were used for the morphological detection of apoptotic and necrotic cells (Zhang et al., 1998). The MCF-7 cell lines are treated with sample (MUF) at a final concentration of 78.48 ± 0.8 µg/mL ($LD_{50}$ concentration) for 24 hours. The cells were washed with cold PBS and then stained with a mixture of AO (100 µg/mL) and EtBr (100 µg/mL) at room temperature for 10 min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in the blue filter of a fluorescent microscope (Olympus CKX41 with Optika Pro5 camera).

**5) Cell cycle analysis by using flow cytometry**

MCF-7 cells were cultured as per standard procedures described earlier and treated with $LD_{50}$ values of compound (MUF - 78.48 ± 0.8 µg/mL) for 24 hours. The cell sample was transferred to a 12 x 75 mm polystyrene tube or 50 mL conical flask. The minimum recommended number of cells for fixation in a tube is 1 x 106 cells. The samples were then centrifuged at 3000 rpm for 5 min. The supernatant was removed without disturbing the pellet. After centrifugation, the cell pellet forms either a visible pellet or a white film on the bottom of the tube. Cells were repeatedly washed with PBS and fixed with

ice-cold 70% ethanol (1 mL) at - 20°C overnight. After the overnight incubation, the samples were centrifuged at 3000 rpm for 5 min at room temperature. The supernatant was removed and 250 µL PBS was added to the pellet.

Then the centrifugation was done again at the same rpm and time. The pellet was taken after discarding the supernatant, 250 µL of cell cycle reagent was added. This was incubated in the dark for 30 min (which is light sensitive). After this, it was analyzed using a flow cytometer. Gating was performed with reference to untreated control cells and samples were analyzed.

**6) Gene expression study using RT- qPCR**

**i) Isolation of total RNA**

Total RNA was isolated using the total RNA isolation kit according to the manufacture instruction (Invitrogen - Product code 10296010). The addition of TRIzol solution causes the disruption of cells and the release of RNA. During chloroform extraction following centrifugation, RNA was exclusively in the aqueous phase whereas proteins remained in the interphase and organic phase. The supernatant was mixed with isopropanol, RNA gets precipitated as a white pellet on the side and the bottom of the tube.

After attaining 70% confluency of cells in 6 well plates (approximately 4 x $10^5$ cells), the cells were treated with $LD_{50}$ concentration of the sample (MUF - 78.48 ± 0.8 µg/mL) and incubated for 24 hours. A set of untreated control cells was also incubated at 37° C for 24 hours in a $CO_2$ incubator. After incubation DMEM media was removed aseptically and 200 µL of TRIzol reagent was added to the culture well plate and incubated for 5 min. The contents were then transferred to a fresh sterile Eppendorf tube. 200 µL of chloroform was added and shaking was done vigorously for 15 seconds and

incubated for 2-3 min at room temperature, followed by centrifugation at 14000 rpm for 15 min at 4°C. The aqueous layer was collected and 500 µL of 100% isopropanol was added. It was incubated for 10 min at room temperature and then centrifuged at 14000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet thus obtained was washed with 200 µL of 75% ethanol (Merck). It was then centrifuged at 14000 rpm for 5 min at 4° C in a cooling centrifuge (Remi CM12). The RNA pellet was dried and suspended in TE buffer (**Appendix 11**).

**ii) cDNA synthesis**

Total RNA was extracted using TRI Reagent (Sigma). The purity and the concentration of total RNA were determined. Template complementary DNA was synthesized using the cDNA preparation kit (Thermo Scientific, Product code - AB1453A, Verso cDNA Synthesis kit). About 4 µL of 5X cDNA synthesis buffer, 2 µL of dNTP mix, 1 µL of anchored oligo dT, 1 µL of RT Enhancer, 1 µL of Verso Enzyme Mix and 5 mL of RNA template (1 ng of total RNA) were added to an RNAse free tube. Then the total reaction volume was made up to 20 µL with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling conditions were employed, 30 min at 42°C and 2 min at 95°C.

**iii) Gene expression analysis using RT-qPCR**

Real-Time qRT-PCR analysis was carried out using SYBR Green Master Mix (Applied Biosystem, Life technologies). All reactions were performed in triplicates and data were analysed according to $\Delta\Delta C_t$ method (using Light Cycler 96 SW 1.1 Software). The primer sequences used were summarized in **Table 10.**

**Table 10:** Primer sequences used for cDNA synthesis

| OLIGO NAME | FORWARD | | REVERSE | |
|---|---|---|---|---|
| | SEQUENCE (5'->3') | Tm | SEQUENCE (5'->3') | Tm |
| Human β-actin | TCACCCACACTGTGCCCATCTACGA(25) | 66.3 | CAGCGGAACCGCTCATTGCCAATGG(25) | 67.9 |
| Human p21 | GAGGCCGGATGAGTTGGGAGGAG(24) | 69.6 | CAGCCGGCGTTTGGAGTGGTAGAA(24) | 66.1 |
| Human p53 | CCCCTCCTGGCCCCTGTCATCTTC(24) | 69.6 | GCAGCGCCTCACAACCTCCGTCAT(24) | 67.8 |

### iv) Agarose gel electrophoresis

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The fragments are separated by charge and size and move through the agarose gel matrix when subjected to an electric field. The electric field is generated by applying a potential across an electrolyte solution (buffer). When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. 1.5% agarose gel was prepared in 1 x TE buffer and melted in the hot water bath at 90°C. Then the melted agarose was cooled down to 45°C. 6 µL of 10 mg/mL of ethidium bromide was added and poured into a gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer (**Appendix 8**) was poured into the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 min. The stained gel was visualized using a gel documentation system (E-Gel Imager, Invitrogen).

## d) GREEN SYNTHESIS OF SILVER NANOPARTICLES

### i) Preparation of plant extract

The methanolic residue of selected species of *Memecylon* (10 mg) was dissolved in 20 mL of deionized water. The extract was filtered using Whatman No. 1 filter paper.

### ii) Green synthesis of silver nanoparticle

About 10 mL of the collected filtrate was treated with 90 mL of silver nitrate (2 mM) and boiled at 80°C for 10 min, resulting in the formation of brownish-yellow coloured solution indicating the synthesis of silver nanoparticles (Gnanadesigan et al., 2011).

### iii) Collection of silver nanoparticle pellets

The solution was centrifuged at 12,000 rpm for 10 min and redispersed in sterile distilled water. The centrifugation and redispersion were repeated three times to ensure the complete separation of silver nanoparticles. The pellet thus obtained was stored at room temperature for further characterization studies.

### iv) Characterization of silver nanoparticle

- UV-Vis spectral analysis

About 1 mL of the solution was analyzed to detect the reduction of $Ag^+$ ions at a wavelength range of 200 - 700 nm.

- SEM analysis

The dried pellet of silver nanoparticles was mounted on an aluminum stub using carbon tape and examined using a scanning electron microscope (Hitachi SU 6600, Japan).

## PHASE I- PHARMACOGNOSTIC PROFILING

Pharmacognosy studies deal with the finding of functional purity of the plant samples. Functional purity and taxonomic authentification are very crucial in pharmaceutical preparations. Powder microscopy, SEM and EDX analysis and ICP-MS analysis gave a vivid picture of the pharmacognostic profile of the selected *Memecylon* species.

### a) Powder microscopy

The powder of *M. grande* leaves is light green coloured, odourless and have a slightly acrid taste. It showed the characters like fragments of the epidermis, long trichosclereids, epidermal cells with tannin contents, paracytic stomata, mesophyll cells with contents, vessels with spiral thickenings, thick-walled fibre bundles and cluster crystals of calcium oxalate (**Plate 2**). The powder of *M. grande* fruits was brown coloured, odourless and slightly astringent (**Plate 3**). The characters found in the powders are epicarp cells, parenchyma cells with starch grains from mesocarp, stone cells from mesocarp, sclereids from endocarp, vessels with spiral and annular thickenings and rosette crystals. The various diagnostic characters of the *M. randerianum* leaf powder are depicted in **Plate 4.** The powder is light green coloured with a characteristic smell and strongly acrid in taste. The leaf powder showed fragments of the epidermis, paracytic stomata, trichosclereids, Mesophyll cells with tannin content, vessels with spiral and reticulate thickenings, fibre bundles and rosette crystals. In the case of *M. randerianum* fruit powder, it is brown coloured, odourless with a characteristic taste (**Plate 5**). It contains epicarp cells, mesocarp parenchyma cells, stone cells, sclereids from endocarp, tracheids, fibre bundles and rosette

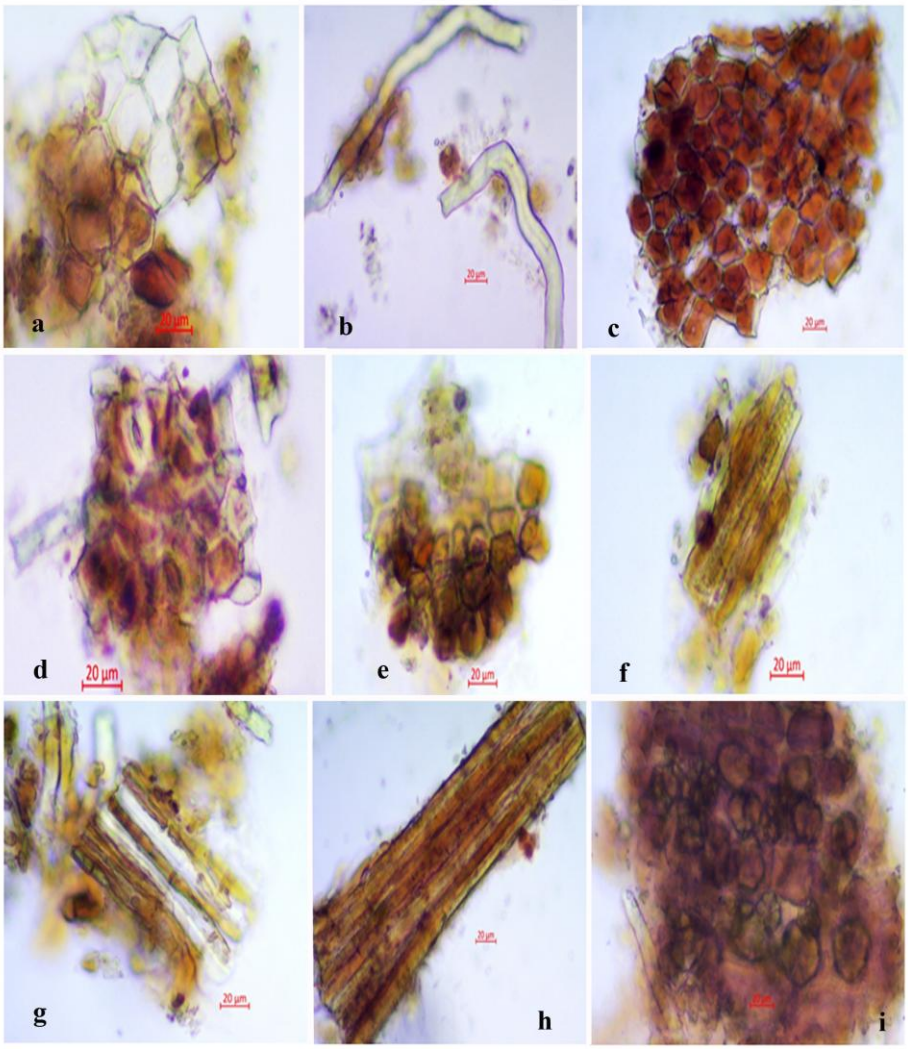



**Plate 2: Powder microscopic analysis of *M. grande* leaves. a** - Epidermal cells, **b** - Trichosclereids, **c** - Epidermal cells with content, **d** - Paracytic stomata, **e** - Mesophyll cells with content, **f** - Spiral vessels, **g** - Elongated parenchyma cells, **h** - Thick walled fiber bundles, **i** - Cluster crystals

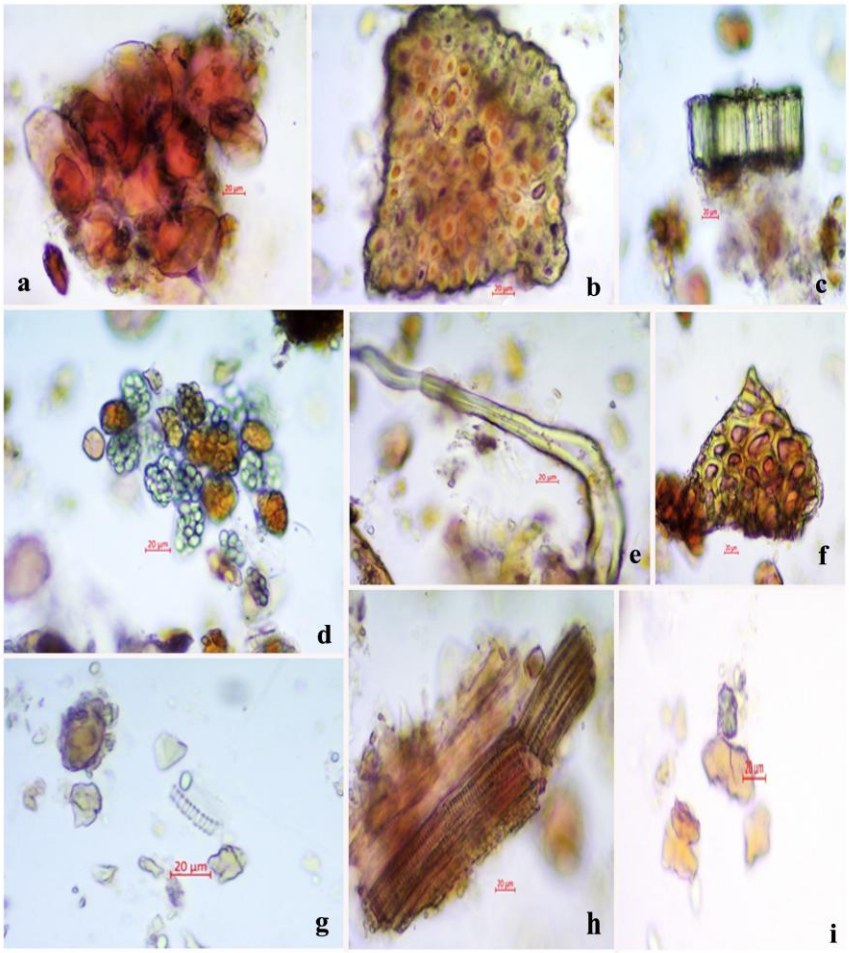



**Plate 3: Powder microscopic analysis of *M. grande* fruits. a -** Epicarp cells, **b -** Testa in surface view, **c -** Transversely cut testa, **d -** mesocarp parenchyma cells with starch grains, **e -** Sclereidal fibers, **f -** Sclereids from endocarp, **g -** Spiral vessels, **h -** Annular vessels, **i -** Rosette crystals

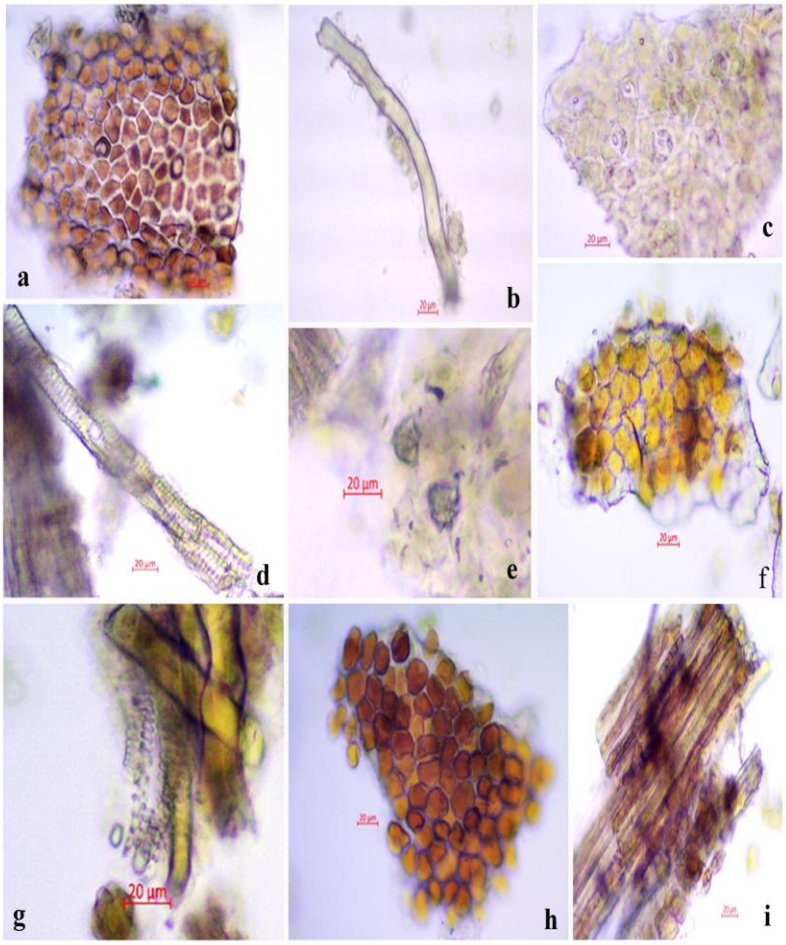



**Plate 4: Powder microscopic analysis of *M. randerianum* leaves. a -** Epidermis of petiole in surface view, **b -** Trichosclereids**, c -** Epidermis with paracytic stomata, **d -** Reticulate vessel, **e -** Rosette crystals, **f -** Mesophyll cells, **g -** Spiral vessels, **h -** Cells with tannin content, **i -** Fiber bundles

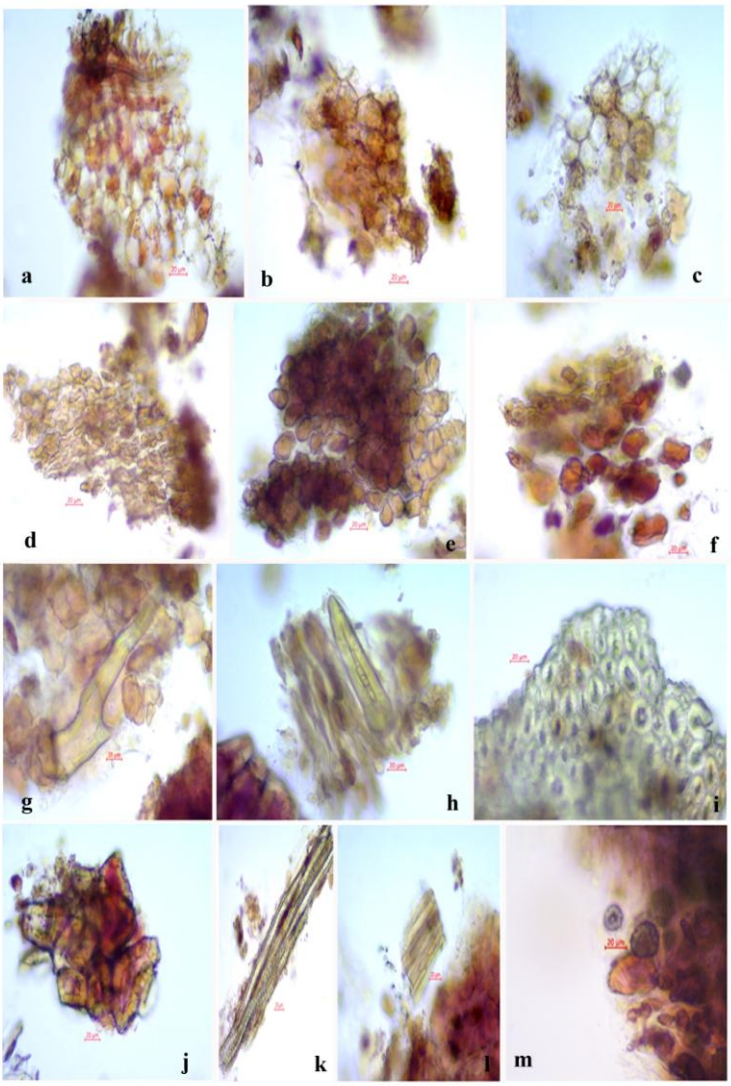



**Plate 5: Powder microscopic analysis of *M. randerianum* fruits. a -** Mesocarp in sectional view, **b -** Epicarp in surface view, **c, d, e, f -** Mesocarp cells, **g, h -** Sclereids, **i, j -** Stone cells, **k -** Tracheids, **l -** Fiber bundles, **m -** Rosette crystals

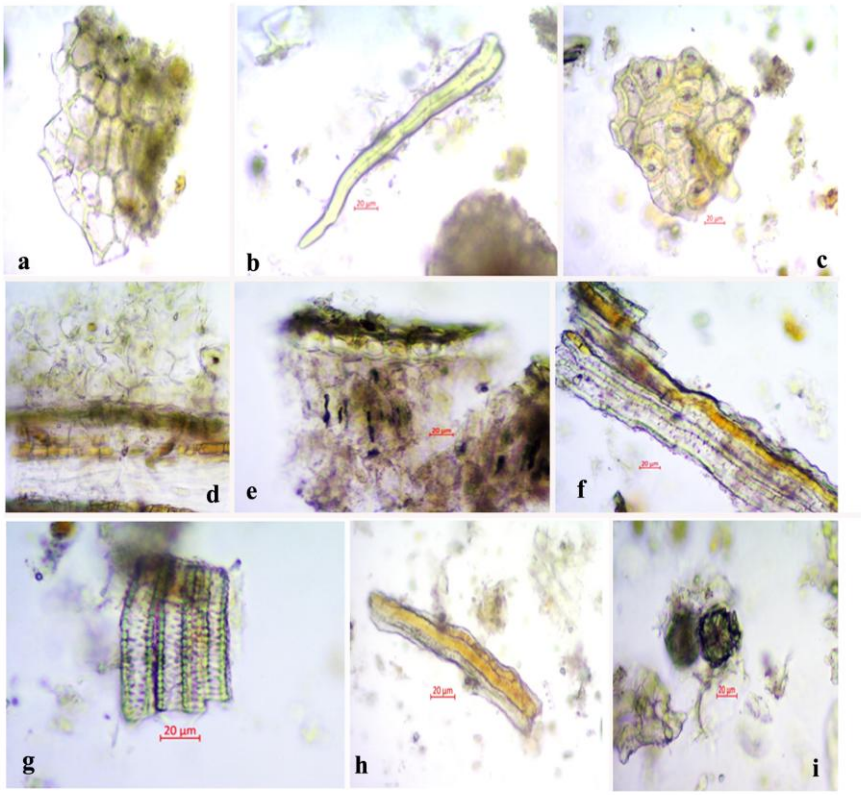



**Plate 6: Powder microscopic analysis of *M. umbellatum* leaves. a -** Epidermal cells, **b -** Trichosclereids, **c -** Epidermal cells with paracytic stomata, **d -** Parenchyma cell **e -** Transversely cut epidermis and mesophyll cells, **f -** Pitted sclereids**, g -** Reticulate vessels, **h -** Tracheidal fiber, **i -** Rosette crystals

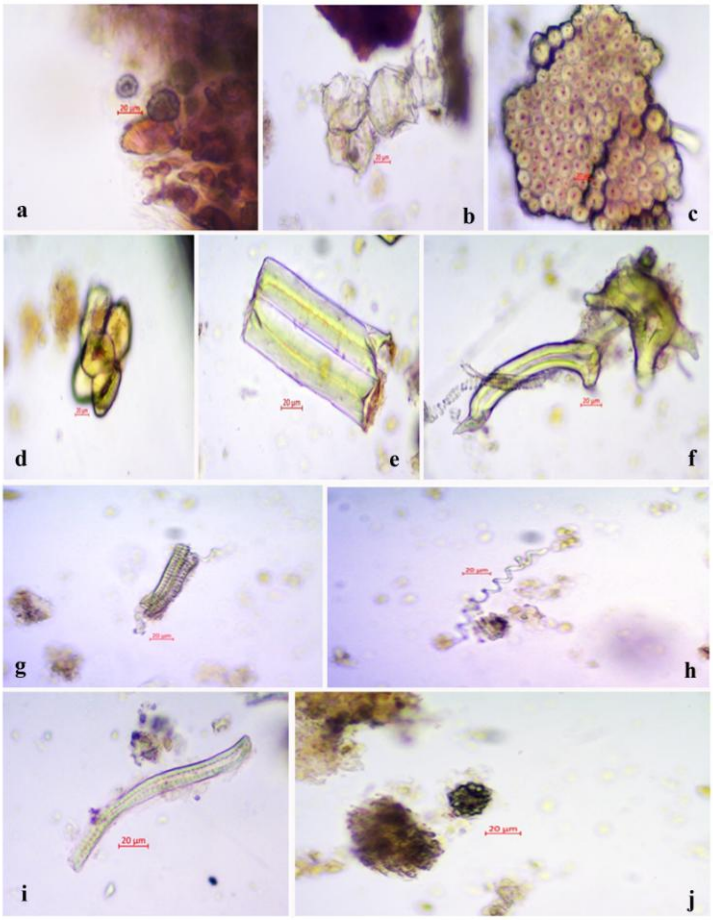



**Plate 7: Powder microscopic analysis of *M. umbellatum* fruits. a -** Epicarp cells, **b -** Pitted parenchyma cells of mesocarp, **c -** Testa in surface view, **d -** Stone cells, **e, f -** Sclereids, **g, h -** Spiral vessels, **i -** Fibrosclereid, **j -** Rosette crystals

crystals. The *M. umbellatum* leaf powder is light green coloured, odourless with a characteristic taste. It showed the presence of fragments of epidermal

cells, epidermal cells with contents, paracytic stomata, trichosclereids, mesophyll cells, parenchyma cells, vessels with reticulate and pitted thickenings, fibro-sclereids and rosette crystals (**Plate 6**). The fruit of the plant is having a brown colour and characterized by epicarp cells, pitted parenchyma cells from mesocarp, stone cells, sclereids, spiral vessels, fibro-sclereids and rosette crystals (**Plate 7**). The powder microscopic analysis confirms that the botanical origin of these plant samples is pure and devoid of foreign particles. So this result can be used as a future reference for the identification of *Memecylon* species.

**b) SEM analysis**

Scanning electron microscopic analysis of fruit endocarp and the entire seed of selected *Memecylon* fruits show a distinct morphological pattern. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships. In the case of *M. grande*, fruits show a colliculate pattern with 6.2 mm endocarp and the seed surface possesses a tuberculate pattern with a width of 5.8 mm (**Plate 8 a1, a2, a3, a4**). Scanning electron microscopic technique reveals that the fruit endocarp of *M. randerianum* has ruminate reticulate type pattern. The width of the endocarp was 4.2 mm. The seed surface of *M. randerianum* is having a reticulate pattern and has 3.5 mm width (**Plate 8 b1, b2, b3, b4**). *M. umbellatum* fruit endocarp possesses a smoothened pattern having 5.6 mm width and its seed surface shows a wrinkled pattern with 3.81 mm width (**Plate 8 c1, c2, c3, c4**).

**c) EDX analysis**

Scanning electron microscope coupled with energy dispersive X-ray microanalysis (EDX) is a technique for analyzing elemental composition at

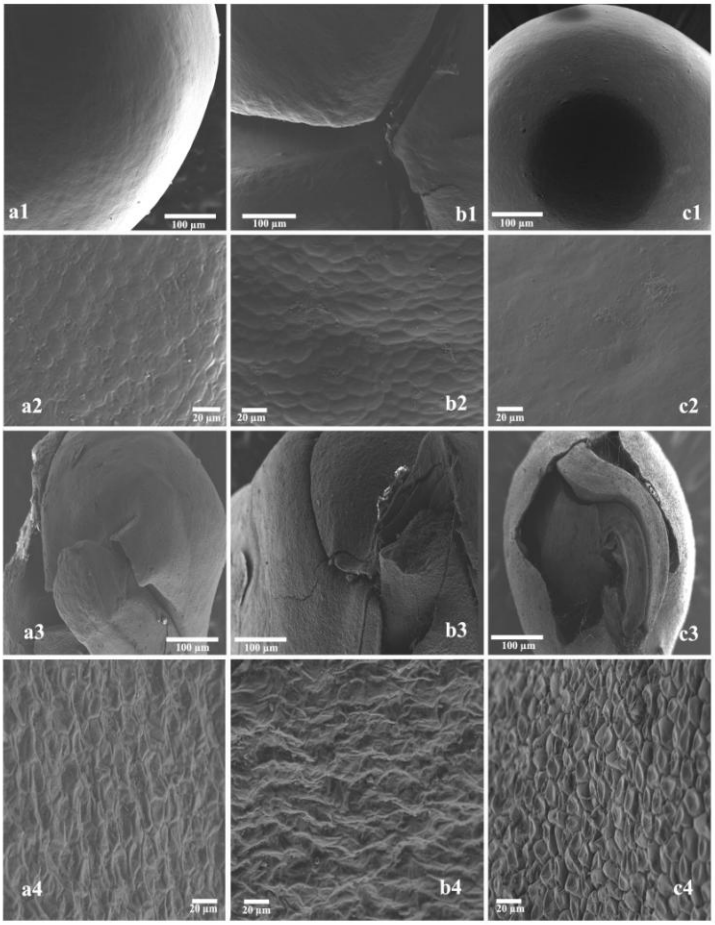
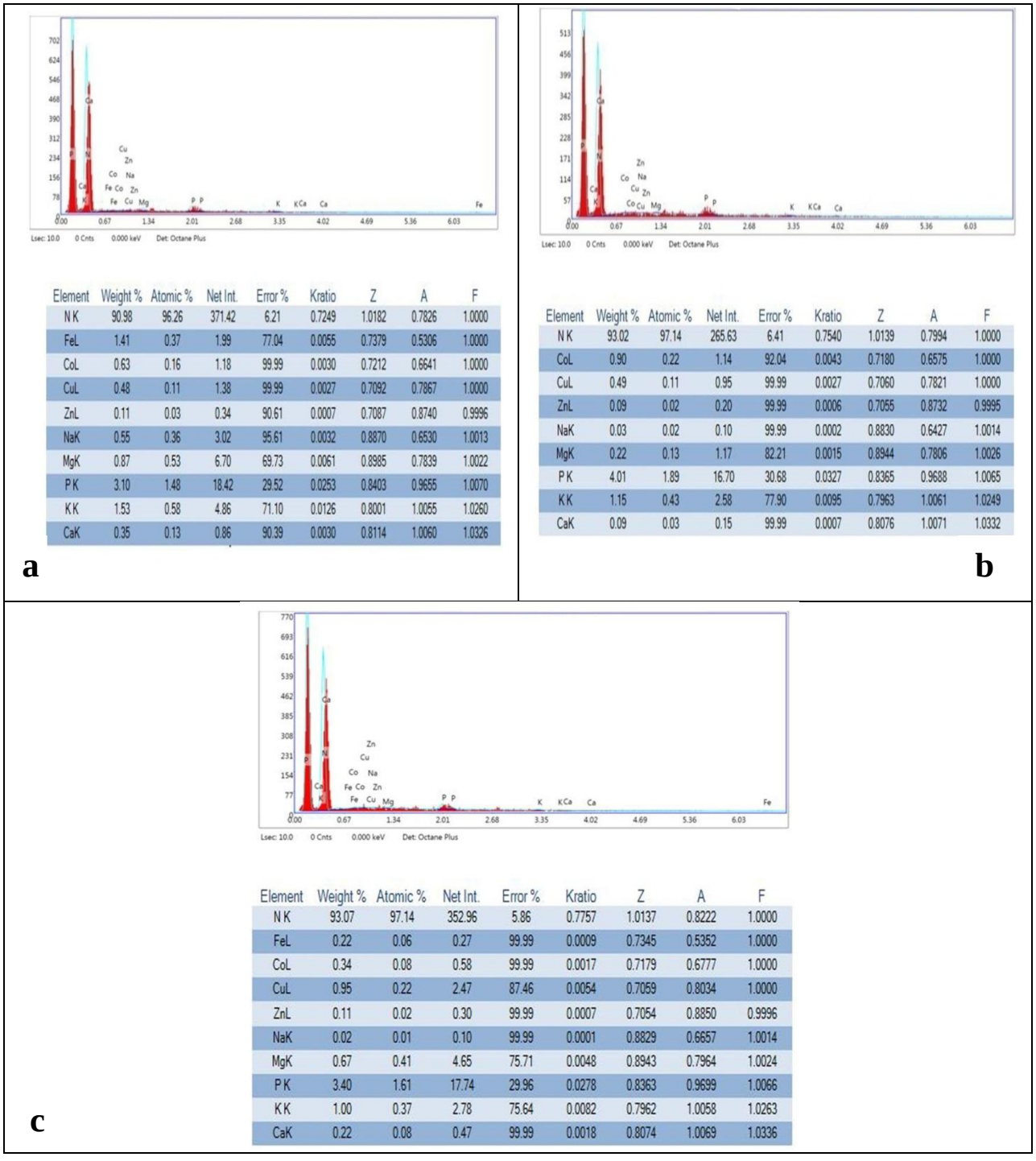



**Plate 8: Scanning electron microscopic analysis of *Memecylon* fruits. a1**- *M. grande* fruit endocarp, **a2-** enlarged view, **a3-** seed surface, **a4-** enlarged view. **b1-** *M. randerianum* fruit endocarp, **b2-** enlarged view, **b3-** seed surface, **b4-** enlarged view. **c1-** *M. umbellatum* fruit endocarp, **c2-** enlarged view, **c3-** seed surface, **c4-** enlarged view.

the microscopic level from an untreated specimen. The elemental composition of *M. grande* fruit shows that nitrogen content with 91% and other elements were found to be like phosphorus 3.10%, potassium 1.53%, iron 1.41%,



**a**

| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 90.98 | 96.26 | 371.42 | 6.21 | 0.7249 | 1.0182 | 0.7826 | 1.0000 |
| FeL | 1.41 | 0.37 | 1.99 | 77.04 | 0.0055 | 0.7379 | 0.5306 | 1.0000 |
| CoL | 0.63 | 0.16 | 1.18 | 99.99 | 0.0030 | 0.7212 | 0.6641 | 1.0000 |
| CuL | 0.48 | 0.11 | 1.38 | 99.99 | 0.0027 | 0.7092 | 0.7867 | 1.0000 |
| ZnL | 0.11 | 0.03 | 0.34 | 90.61 | 0.0007 | 0.7087 | 0.8740 | 0.9996 |
| NaK | 0.55 | 0.36 | 3.02 | 95.61 | 0.0032 | 0.8870 | 0.6530 | 1.0013 |
| MgK | 0.87 | 0.53 | 6.70 | 69.73 | 0.0061 | 0.8985 | 0.7839 | 1.0022 |
| P K | 3.10 | 1.48 | 18.42 | 29.52 | 0.0253 | 0.8403 | 0.9655 | 1.0070 |
| K K | 1.53 | 0.58 | 4.86 | 71.10 | 0.0126 | 0.8001 | 1.0055 | 1.0260 |
| CaK | 0.35 | 0.13 | 0.86 | 90.39 | 0.0030 | 0.8114 | 1.0060 | 1.0326 |

**b**

| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 93.02 | 97.14 | 265.63 | 6.41 | 0.7540 | 1.0139 | 0.7994 | 1.0000 |
| CoL | 0.90 | 0.22 | 1.14 | 92.04 | 0.0043 | 0.7180 | 0.6575 | 1.0000 |
| CuL | 0.49 | 0.11 | 0.95 | 99.99 | 0.0027 | 0.7060 | 0.7821 | 1.0000 |
| ZnL | 0.09 | 0.02 | 0.20 | 99.99 | 0.0006 | 0.7055 | 0.8732 | 0.9995 |
| NaK | 0.03 | 0.02 | 0.10 | 99.99 | 0.0002 | 0.8830 | 0.6427 | 1.0014 |
| MgK | 0.22 | 0.13 | 1.17 | 82.21 | 0.0015 | 0.8944 | 0.7806 | 1.0026 |
| P K | 4.01 | 1.89 | 16.70 | 30.68 | 0.0327 | 0.8365 | 0.9688 | 1.0065 |
| K K | 1.15 | 0.43 | 2.58 | 77.90 | 0.0095 | 0.7963 | 1.0061 | 1.0249 |
| CaK | 0.09 | 0.03 | 0.15 | 99.99 | 0.0007 | 0.8076 | 1.0071 | 1.0332 |

**c**

| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 93.07 | 97.14 | 352.96 | 5.86 | 0.7757 | 1.0137 | 0.8222 | 1.0000 |
| FeL | 0.22 | 0.06 | 0.27 | 99.99 | 0.0009 | 0.7345 | 0.5352 | 1.0000 |
| CoL | 0.34 | 0.08 | 0.58 | 99.99 | 0.0017 | 0.7179 | 0.6777 | 1.0000 |
| CuL | 0.95 | 0.22 | 2.47 | 87.46 | 0.0054 | 0.7059 | 0.8034 | 1.0000 |
| ZnL | 0.11 | 0.02 | 0.30 | 99.99 | 0.0007 | 0.7054 | 0.8850 | 0.9996 |
| NaK | 0.02 | 0.01 | 0.10 | 99.99 | 0.0001 | 0.8829 | 0.6657 | 1.0014 |
| MgK | 0.67 | 0.41 | 4.65 | 75.71 | 0.0048 | 0.8943 | 0.7964 | 1.0024 |
| P K | 3.40 | 1.61 | 17.74 | 29.96 | 0.0278 | 0.8363 | 0.9699 | 1.0066 |
| K K | 1.00 | 0.37 | 2.78 | 75.64 | 0.0082 | 0.7962 | 1.0058 | 1.0263 |
| CaK | 0.22 | 0.08 | 0.47 | 99.99 | 0.0018 | 0.8074 | 1.0069 | 1.0336 |

**Figure 5: SEM-EDX analysis of *Memecylon* fruits. a - *M. grande*, b - *M. randerianum*, c - *M. umbellatum***

magnesium 0.87%, cobalt 0.63%, sodium 0.55%, copper 0.48%, calcium 0.35% and zinc 0.11% (**Figure 5 a**). In the case of *M. randerianum* fruit, nitrogen is the prominent element with 93% of the weight. Phosphorus 4.01%, potassium 1.15%, cobalt 0.90%, copper 0.49%, magnesium 0.22%, zinc 0.09%, calcium 0.09% and sodium 0.03% are the revealed composition of other elements (**Figure 5 b**). The absence of iron content is also noticed in it. *M. umbellatum* fruit also possesses an elevated amount of nitrogen (93%) and all other elements in a trace amount. Phosphorus 3.4%, potassium 1%, copper 0.95%, magnesium 0.67%, cobalt 0.34%, iron 0.22%, calcium 0.22%, zinc 0.11% and sodium 0.02% are the other elements detected (**Figure 5 c**). These findings reveal that *Memecylon* fruits are a reservoir of essential elements and they can be exploited in the pharmaceutical or nutritional field.

**d) ICP-MS analysis**

In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples with regards to their elemental composition, an Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) analysis was carried out. This technique gave the details of elements present in the sample in part per million units and determination of twelve elements were done *ie.,* aluminium (Al), arsenic (As), cobalt (Co), strontium (Sr), selenium (Se), zinc (Zn), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), barium (Ba) and manganese (Mn).

The selected *Memecylon* species shows a promising amount of the majority of these elements (**Table 5**). The highest amount of Al is present in *M. grande* fruit extract, whereas the absence of Al in *M. umbellatum* leaf extract was also noticed. The amount of Cr was found to be the highest in *M. grande* leaf extract (204.32 ppm) and lowest in *M. randerianum* leaf extract (3.65 ppm). A consistent amount of all the elements are present in the *M. grande* leaf extract. As compared to all other extracts, *M. grande* leaf extract

show the highest concentration of Co, Ni, Cu, Ba, As and Mo. The amount of Sr is higher in *M. grande* fruit extract (880.17 ppm) and highest Mn concentration is found in *M. umbellatum* leaf extract (9157.39 ppm).

**Table 5: ICP-MS analysis of selected species of *Memecylon***

| Elements | MGL | MGF | MRL | MRF | MUL | MUF |
|---|---|---|---|---|---|---|
| Al | 199.12 | 83135.86 | 79826.02 | 41909.73 | - | 38739.42 |
| Cr | 204.42 | 10.23 | 3.65 | 5.92 | 16.80 | 4.01 |
| Mn | 201.95 | 1790.17 | 3325.01 | 786.65 | 9157.39 | 272.02 |
| Co | 200.02 | 1.65 | 1.13 | 0.62 | 1.10 | 0.709 |
| Ni | 202.16 | 46.03 | 19.83 | 9.31 | 25.01 | 30.25 |
| Cu | 200.74 | 191.23 | 62.75 | 69.67 | 57.43 | 85.96 |
| Zn | 199.50 | 320.48 | 214.81 | 119.41 | 98.64 | 118.66 |
| As | 200.62 | 1.62 | 0.41 | 1.04 | 0.62 | 0.95 |
| Se | 202.08 | 14.15 | 2.10 | 2.83 | 5.68 | 0.33 |
| Sr | 171.67 | 880.17 | 211.97 | 148.72 | 461.35 | 275.34 |
| Mo | 200.74 | 0.84 | 3.59 | 18.42 | 61.66 | 3.92 |
| Ba | 191.41 | 451.71 | 130.62 | 111.35 | 66.23 | 105.19 |

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits.

## PHASE II- PHYTOCHEMICAL CHARACTERIZATION

The phytochemical analysis comprises four sections. The preliminary qualitative analysis, quantitative determinations of certain phytochemicals, determination of volatile phytoconstituents through the GC/MS analysis and identification of non-volatile constituents through HR-LC/MS analysis.

### a) Preliminary qualitative phytochemical analysis

The preliminary phytochemical analysis is carried out to determine the presence or absence of phytochemicals in the samples. Various standard protocols are available for the determination of phytoconstituents. The chemical reactions produce certain colour change or precipitate and thereby indicate the presence or absence of phytoconstituents. The presence of

alkaloids, flavonoids, phenolics, steroids and tannins were confirmed in all the selected species. The presence of glycosides is revealed in the leaf and fruit samples of *M. umbellatum*. The complete absence of resins and anthraquinones was confirmed in all the selected species. The results of preliminary qualitative phytochemical analyses are summarized in **Table 6**.

**Table 6: Preliminary qualitative phytochemical analysis of selected species of *Memecylon***

| Sl No. | Chemical test | *M. grande* | | *M. randerianum* | | *M. umbellatum* | |
|---|---|---|---|---|---|---|---|
| | | Leaves | Fruits | Leaves | Fruits | Leaves | Fruits |
| 1 | Alkaloids | | | | | | |
| | a) Hager's test | - | + | + | + | + | + |
| | b) Wagner's test | + | - | + | - | + | - |
| 2 | Anthraquinones | | | | | | |
| | Borntrager's test | - | - | - | - | - | - |
| 3 | Coumarins | - | - | + | - | + | - |
| 4 | Flavonoids | | | | | | |
| | a) Alkaline reagent test | + | - | + | + | + | + |
| | b) Lead acetate test | - | + | + | - | + | - |
| 5 | Glycosides | | | | | | |
| | Keller Killiani test | - | - | - | - | + | + |
| 6 | Phenolic compounds | | | | | | |
| | $FeCl_3$ test | + | + | + | + | + | + |
| 7 | Phlobatannins | | | | | | |
| | HCl test | - | - | + | + | + | - |
| 8 | Resins | - | - | - | - | - | - |
| 9 | Saponins | | | | | | |
| | Foam test | + | + | + | + | + | - |
| 10 | Steroids | | | | | | |
| | Liebermann-Burchard test | + | + | + | + | + | + |
| 11 | Tannins | | | | | | |
| | Breymer's test | + | + | + | + | + | + |
| 12 | Terpenoids | | | | | | |
| | Salkowski's test | + | + | + | + | + | + |

## b) Preliminary quantitative phytochemical analysis

The quantitative determinations of alkaloids, flavonoids, phenolics and terpenoids were conducted during this analysis section. Each of the phytoconstituents was determined by using the standard calibration curve method and regression equation. All the plant extracts studied were found to have considerable amounts of potential secondary metabolites. The amount of alkaloids present in the plant extract was quantified by using caffeine as standard. Quercetin, gallic acid and linalool are used as the standard compounds of flavonoids, phenolics and terpenoids respectively. The calibration curve and regression equations of standard compounds are given in **Figures 6, 7, 8, 9.** *M. grande* fruit extract possess the highest amount of alkaloids 52.16 ± 3.23 mg CE/g DW, phenolics 370.28 ± 1.36 mg GAE/g DW and terpenoids 378.21 ± 1.02 mg LE/g DW. The amount of flavonoids was found to be highest in *M. grande* leaf extract with 215.96 ± 1.87 mg QE/g DW. The results of the preliminary quantitative phytochemical analysis are summarized in **Table 7**.

**Table 7: Preliminary quantitative phytochemical analysis of selected species of *Memecylon***

| Plants | Alkaloids (mg CE/g DW) ± SE | Flavonoids (mg QE/g DW) ± SE | Phenolics (mg GAE/g DW) ± SE | Terpenoids (mg LE /g DW) ± SE |
|---|---|---|---|---|
| MGL | 46.48 ± 4.34 | 215.96 ± 1.87 | 179.96 ± 3.18 | 268.39 ± 2.98 |
| MGF | 52.16 ± 3.23 | 91.77 ± 2.65 | 370.28 ± 1.36 | 378.21 ± 1.02 |
| MRL | 47.45 ± 2.99 | 50.42 ± 1.23 | 49.52 ± 4.72 | 127.65 ± 1.42 |
| MRF | 32.17 ± 1.41 | 21.40 ± 2.72 | 276.06 ± 1.12 | 355.03 ± 1.31 |
| MUL | 41.39 ± 1.01 | 59.45 ± 2.06 | 188.88 ± 1.85 | 207.43 ± 1.44 |
| MUF | 36.47 ± 0.66 | 57.57 ± 4.40 | 60.83 ± 5.70 | 127.5 ± 1.50 |

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits. Values are expressed as mean ± standard error (SE).

**c) Phytochemical profiling by GC/MS**

The identification of volatile phytoconstituents in selected *Memecylon* species was done through the GC/MS analysis. A total of 83 compounds were detected in the methanolic extract of selected species. The identified compounds belong to the classes of terpenoids, phenolics, fatty acids, fatty acid esters, steroids *etc*. The identified compounds in selected *Memecylon* species are enlisted in **Table 8** and the gas chromatogram is given in **Figures 10 a & b, 11 a & b, 12 a & b**. The GC/MS analysis of *M. grande* leaf extract reveals the presence of 17 compounds. The major constituents were oleic acid (29.01%) and methyl oleate (23.63%) followed by palmitic acid (14.11%). Palmitic acid is a common saturated fatty acid found in all the selected plant extracts. Cholesterilene (0.64%), campesterol acetate (1.88%) and α-phytosterol (2.19%) are the steroid compounds detected in *M. grande* leaf extract. The mass spectra of individual compounds are shown in **Figures 13 (i-xvii).** The presence of fatty acid esters is in a significant amount. Methyl linoleate, propyl palmitate, 9-hexadecenoic acid, methyl ester, methyl arachidate, methyl 9-cis,11-trans-octadecadienoate and methyl linoleate are detected in GC/MS analysis. A negligible amount of the organic compound oleamide, was also present in the plant extract. In the case of *M. grande* fruit extract, 17 compounds were detected through the analysis. Leaf and fruit extract of *M. grande* shows a similar composition of phytoconstituents. The fatty acid esters are found to be in the highest amount. Methyl elaidate (25.53%) was in the highest amount. The fatty acid methyl esters noticed were methyl octanoate, ethyl 9-hexadecenoate, methyl myristate, methyl pentadecanoate, methyl linoleate, methyl palmitoleate, methyl stearate and methyl arachidate. The presence of trace amounts of cholesterilene (0.64%) and campesterol acetate (1.65%) was also noticed.

**Table 8: Chemical constituents detected in the selected *Memecylon* species by GC/MS analysis**

| Sl. No. | RT (min) | Compounds | Class | Content % | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | MGL | MGF | MRL | MRF | MUL | MUF |
| 1. | 5.551 | α-Angelica lactone | Carbohydrate lactone | - | - | 0.52 | - | - | - |
| 2. | 7.229 | β-Thujone | Monoterpene | - | - | - | 0.45 | - | - |
| 3. | 8.101 | 3-Thujanol | Monoterpene alcohol | - | - | - | 0.49 | - | - |
| 4. | 8.201 | 2-Hydroxy-4-methyl pyrimidine | Organic compound | - | - | - | - | - | 23.11 |
| 5. | 8.644 | 4-methyl 2,5-dihydrofuran -2-one | Organic compound | - | - | - | - | - | 0.82 |
| 6. | 8.883 | Methyl octanoate | Fatty acid methyl ester | - | 2.54 | - | - | - | - |
| 7. | 11.236 | Dehydromevalonic lactone | Carbohydrate lactone | - | - | - | - | - | 1.29 |
| 8. | 12.269 | Chavicol | Phenylpropene | - | - | 1.11 | - | - | - |
| 9. | 12.667 | Methyl 3-methyl-5-oxooxolane-2-carboxylate | Heterocyclic compound | - | - | - | - | - | 21.4 |
| 10. | 12.805 | Hydroxymethylfurfural | Organic compound | - | - | - | - | 1.00 | - |
| 11. | 14.002 | Eugenol | Phenolic compound | - | - | 2.82 | - | - | - |
| 12. | 14.558 | Methyl cinnamate | Fatty acid methyl ester | - | - | 0.56 | - | - | - |
| 13. | 14.583 | Quinic acid | Organic compound | - | - | - | 0.67 | - | - |
| 14. | 14.657 | 2-Heptylacetate | Carboxylic acid ester | - | - | - | - | 0.20 | - |
| 15 | 14.759 | Methyl eugenol | Phenylpropane | - | - | 0.68 | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | 14.760 | 4-Vinylguaiacol | Phenolic compound | - | - | - | - | 0.21 | - |
| 17 | 15.675 | 2-Methoxy-3-allylphenol | Phenylpropanoid | - | - | - | - | 0.16 | - |
| 18 | 16.493 | Levoglucosan | Organic compound | - | - | - | - | 7.40 | - |
| 19 | 17.139 | Hordenine | Alkaloid | - | - | - | - | - | 21.35 |
| 20 | 17.325 | Lauric acid | Fatty acid | - | - | 1.73 | - | - | 0.71 |
| 21 | 18.009 | Methyl pentadecanoate | Fatty acid methyl ester | - | 0.74 | - | - | - | - |
| 22 | 18.492 | γ-Eudesmol | Sesquiterpene | - | - | 0.62 | - | - | - |
| 23 | 18.646 | Methyl 4-hydroxyphenyl acetate | Phenolic compound | - | - | - | - | 0.26 | - |
| 24 | 18.826 | α-Cadinol | Sesquiterpene alcohol | - | - | 1.28 | - | - | - |
| 25 | 18.831 | 9-Hexadecenoic acid, methyl ester | Fatty acid methyl ester | 1.09 | - | - | - | - | - |
| 26 | 18.995 | Maaliol | Sesquiterpene alcohol | - | - | 1.57 | - | - | - |
| 27 | 19.121 | β-Eudesmol | Sesquiterpene alcohol | - | - | 4.58 | - | - | - |
| 28 | 19.487 | Palmitic acid | Fatty acid | 14.11 | 13.46 | 15.51 | 4.90 | 11.95 | 3.28 |
| 29 | 20.044 | Mustakone | Sesquiterpene | - | - | - | - | - | 0.35 |
| 30 | 20.152 | Octadecanoic acid | Fatty acid | - | - | 2.09 | 1.86 | - | 4.37 |
| 31 | 20.409 | Cyperenone | Sesquiterpene ketone | - | - | - | - | - | 2.05 |
| 32 | 20.444 | Ethyl 9-hexadecenoate | Fatty acid ester | - | 0.48 | - | - | - | - |
| 33 | 20.591 | Methyl myristate | Fatty acid methyl ester | 0.90 | 0.84 | - | - | 0.31 | 1.41 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 34 | 20.636 | Propyl palmitate | Fatty acid ester | 1.126 | - | - | - | - | - |
| 35 | 20.749 | Methyl oleate | Fatty acid methyl ester | 23.63 | - | - | - | - | - |
| 36 | 20.756 | Methyl elaidate | Fatty acid methyl ester | - | 25.53 | - | - | - | - |
| 37 | 20.804 | 10,13-Octadecadienoic acid, methyl ester | Fatty acid methyl ester | 1.897 | - | - | - | - | - |
| 38 | 21.107 | Myristic acid | Fatty acid | - | - | - | - | - | 6.91 |
| 39 | 21.113 | Farnesyl acetate | Sesquiterpene | - | - | 3.44 | - | - | - |
| 40 | 21.196 | Oleic acid | Fatty acid | 29.01 | 2.89 | - | - | - | - |
| 41 | 21.558 | Methyl 9-cis,11-trans-octadecadienoate | Fatty acid methyl ester | 1.53 | - | - | - | - | - |
| 42 | 21.748 | Dihydroconiferyl alcohol | Organic compound | - | - | - | - | 0.54 | - |
| 43 | 22.169 | Linoleic acid | Fatty acid | 3.37 | 2.00 | - | 0.76 | - | - |
| 44 | 22.223 | Propyl oleate | Fatty acid ester | 5.17 | - | - | - | - | - |
| 45 | 22.830 | Methyl arachidate | Fatty acid methyl ester | 0.91 | 0.87 | - | - | - | - |
| 46 | 23.283 | Oleamide | Organic compound | 0.75 | 31.27 | - | - | - | - |
| 47 | 25.146 | Neophytadiene | Sesquiterpene | - | - | - | - | 3.63 | - |
| 48 | 25.230 | Hexahydrofarnesyl acetone | Ketone | - | - | - | - | 0.39 | - |
| 49 | 25.473 | Phytol | Diterpene alcohol | - | - | 7.11 | - | 2.70 | - |
| 50 | 25.563 | 3,7,11,15-Tetramethyl-2-hexadecen | Diterpene alcohol | - | - | - | - | 2.70 | - |
| 51 | 25.662 | Agathenic acid | Diterpene | - | - | 14.74 | - | - | - |
| 52 | 25.828 | Bicyclogermacrene | Sesquiterpene | - | - | 3.59 | - | - | - |
| 53 | 26.376 | Virdiflorene | Sesquiterpene | - | - | 0.67 | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 54 | 26.409 | Farnesyl acetone | Terpene ketone | - | - | - | - | 0.18 | - |
| 55 | 26.656 | Methyl palmitate | Fatty acid methyl ester | - | 1.20 | 2.26 | 1.89 | 9.25 | - |
| 56 | 26.771 | 2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin | Organic compound | - | - | - | - | 0.28 | - |
| 57 | 27.001 | Isophytol | Diterpene alcohol | - | - | - | - | 0.41 | - |
| 58 | 27.161 | 4,8,12,16-Tetramethylheptadecan-4-olide | Isoprenoid γ-lactone | - | - | 0.49 | - | 0.26 | - |
| 59 | 27.896 | Phenol, 2,4-bis (1-phenylethyl) - | Phenylpropanoid | - | - | 1.23 | - | - | - |
| 60 | 27.968 | Friedlein | Triterpene | - | - | - | 31.30 | - | - |
| 61 | 28.269 | Methyl heptadecanoate | Fatty acid methyl ester | - | - | - | - | 0.31 | - |
| 62 | 28.391 | Dehydroabietic acid | Diterpene | - | - | 0.96 | - | - | - |
| 63 | 29.322 | Methyl linoleate | Fatty acid methyl ester | 8.88 | 8.01 | 0.66 | - | 0.72 | - |
| 64 | 29.429 | Linoleoyl chloride | Fatty acid derivative | - | - | - | - | 6.94 | 1.47 |
| 65 | 29.518 | Methyl cis-11 octdecenoate | Fatty acid methyl ester | - | - | - | - | 0.31 | - |
| 66 | 29.836 | Methyl stearate | Fatty acid methyl ester | 2.85 | 2.92 | - | 0.70 | 1.26 | - |
| 67 | 30.522 | Cholesterilene | Steroid | 0.64 | 0.64 | - | - | - | - |
| 68 | 31.039 | Ledol | Sesquiterpene | - | - | 1.37 | - | - | - |
| 69 | 31.996 | Campesterol acetate | Steroid | 1.88 | 1.65 | - | - | - | - |
| 70 | 33.450 | Stigmast-5-en-3-ol | Sterol | - | 1.65 | - | 12.80 | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 71 | 35.925 | 4-Campestene-3-one | Steroid | - | - | - | 5.99 | - | - |
| 72 | 36.535 | 4,22-Stigmastadiene-3-one | Steroid | - | - | - | 1.25 | - | - |
| 73 | 37.916 | Isopentacosane | Alkane | - | - | - | - | 0.28 | - |
| 74 | 38.217 | Campesterol | Sterol | - | - | 2.76 | - | 0.88 | - |
| 75 | 39.212 | Squalene | Triterpene | - | - | 8.75 | 7.69 | 9.84 | - |
| 76 | 39.493 | α-Springene | Diterpene | - | - | - | - | 0.33 | - |
| 77 | 40.325 | Tetratetracontane | Alkane | - | - | - | - | 0.47 | - |
| 78 | 40.703 | Lupeol | Triterpene | - | - | 5.38 | - | - | - |
| 79 | 43.069 | Stigmasta- 5,22-dien-3-ol | Sterol | - | - | - | - | 0.74 | - |
| 80 | 43.443 | alpha-phytosterol | Sterol | 2.19 | - | - | - | 17.72 | - |
| 81 | 43.608 | Z,Z-6,28-Heptatriacontadien-2-one | Ketone | - | 1.54 | - | - | - | - |
| 82 | 44.202 | Vitamin E | Organic compound | - | - | - | 0.85 | 5.73 | - |
| 83 | 51.428 | Methyl commate B | Triterpene glycoside | - | - | - | - | 10.60 | - |

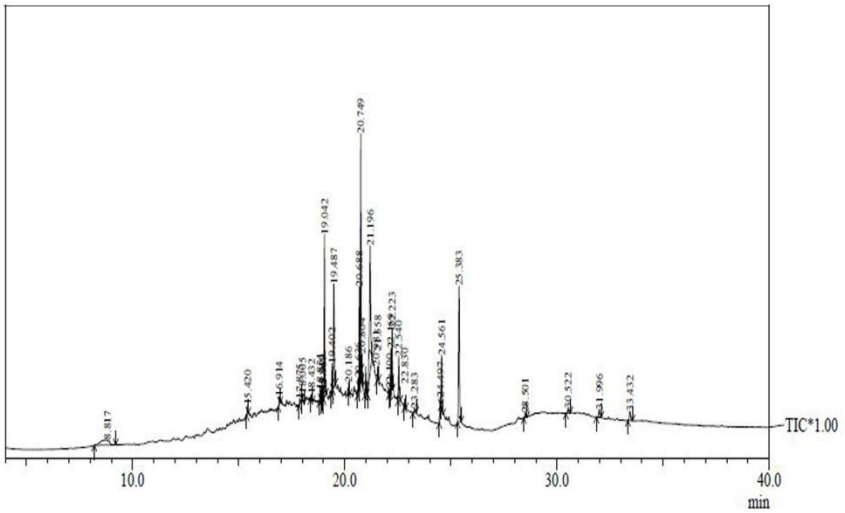



**Figure 10a: Gas chromatogram of methanolic leaves extract of *M. grande***

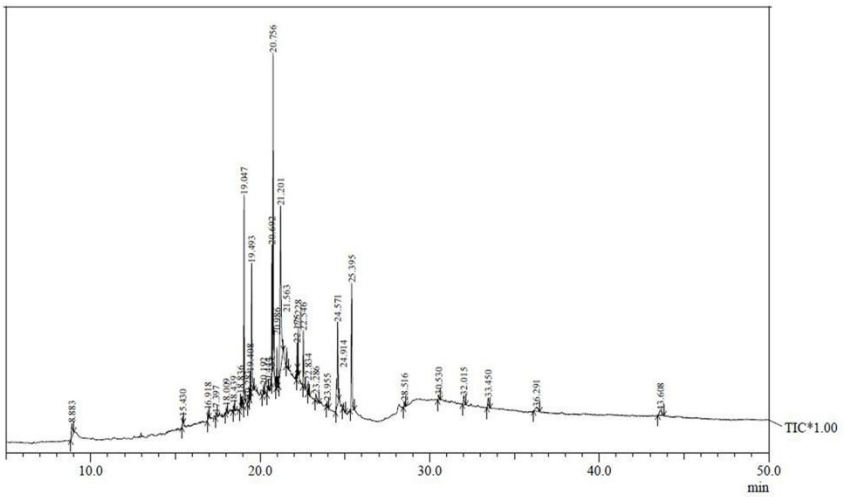



**Figure 10b: Gas chromatogram of methanolic fruit extract of *M. grande***

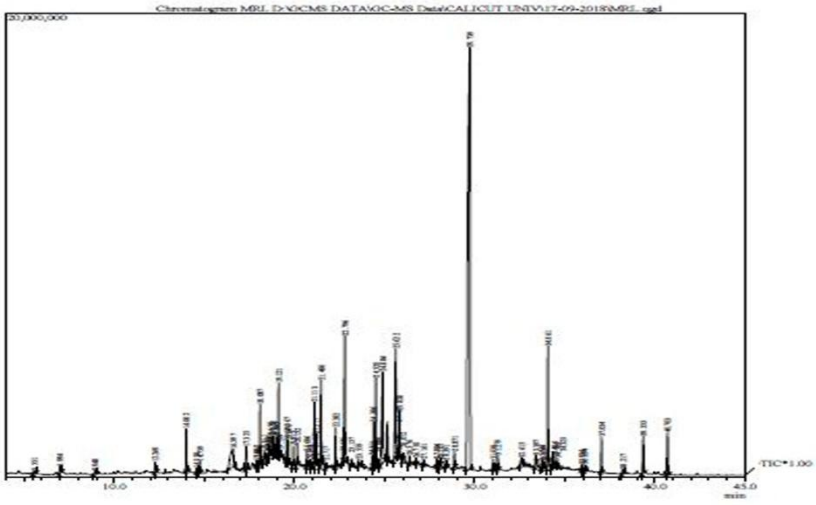



**Figure 11a: Gas chromatogram of methanolic leaves extract of *M. randerianum***

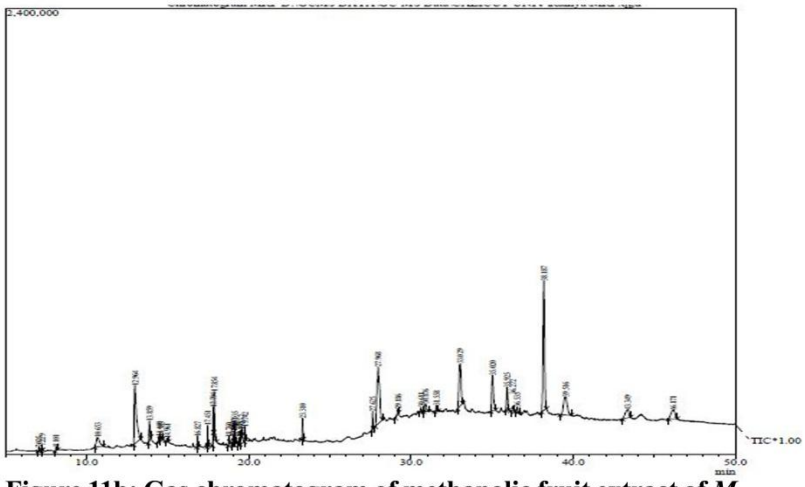



**Figure 11b: Gas chromatogram of methanolic fruit extract of *M. randerianum***

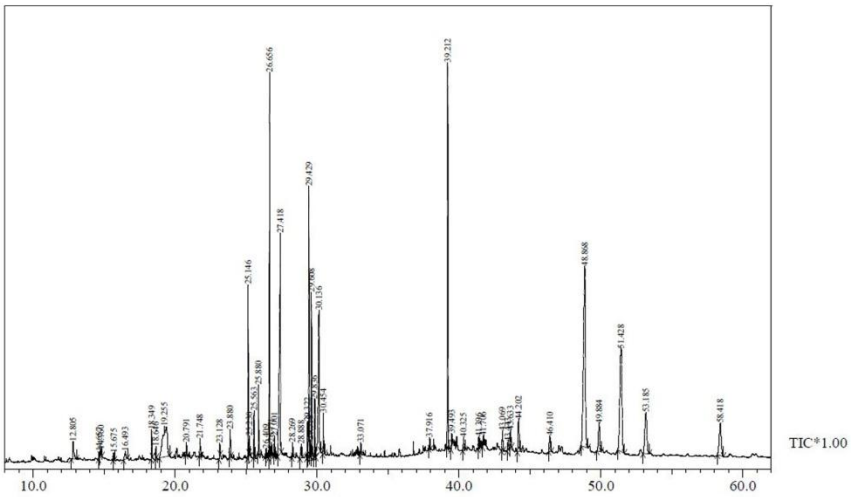



**Figure 12a: Gas chromatogram of methanolic leaves extract of *M. umbellatum***

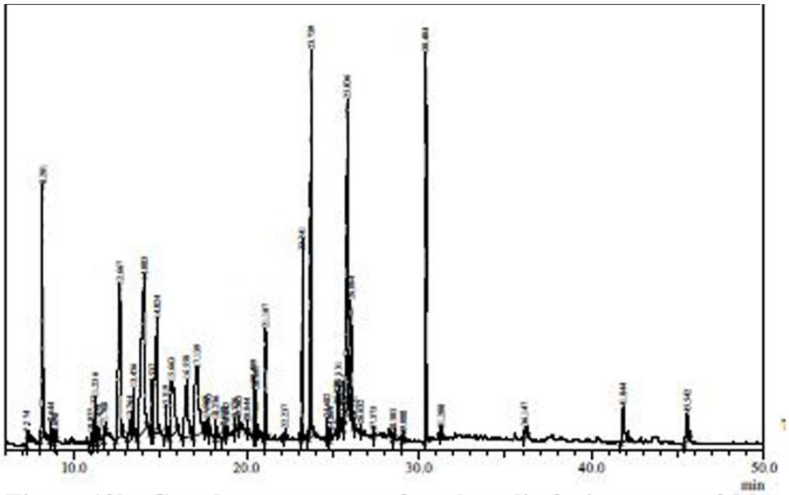



**Figure 12b: Gas chromatogram of methanolic fruit extract of *M. umbellatum***

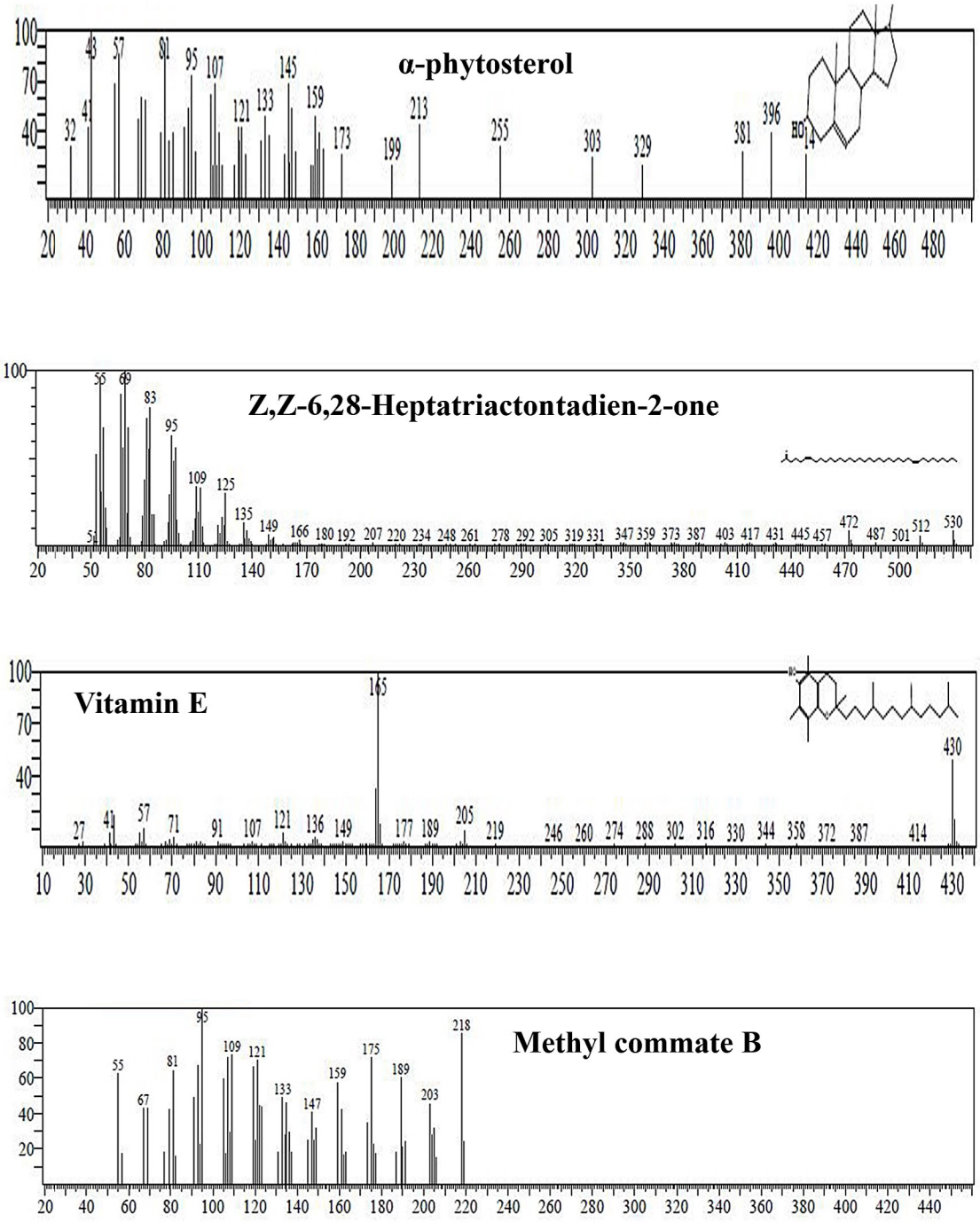



**Figure 13 (xvii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

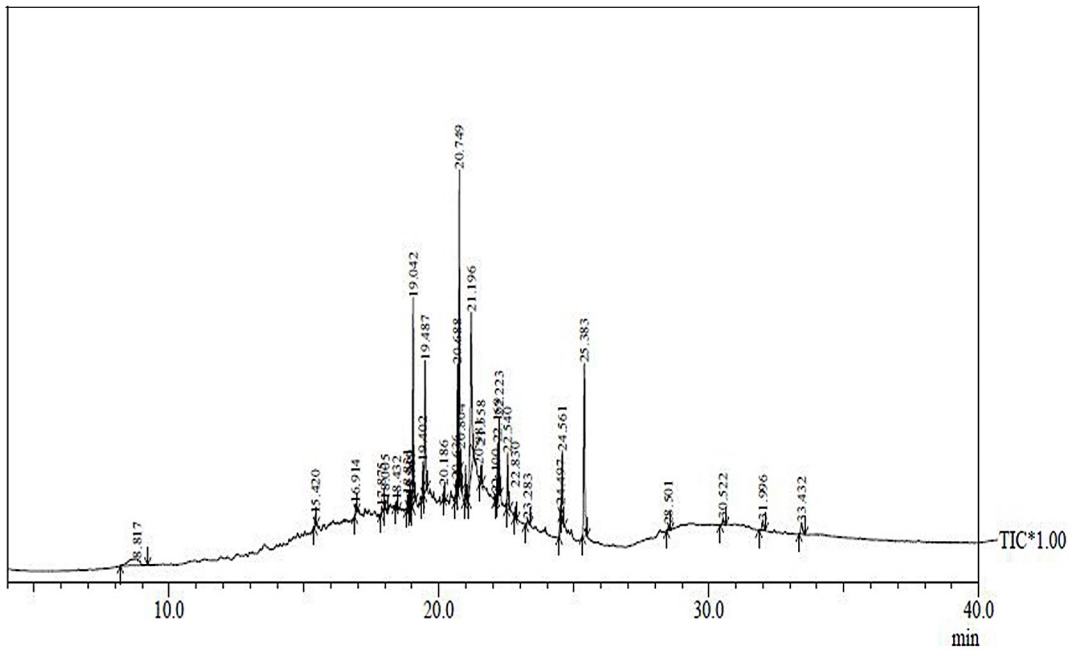



**Figure 10 a: Gas chromatogram of methanolic leaf extract of *M. grande***

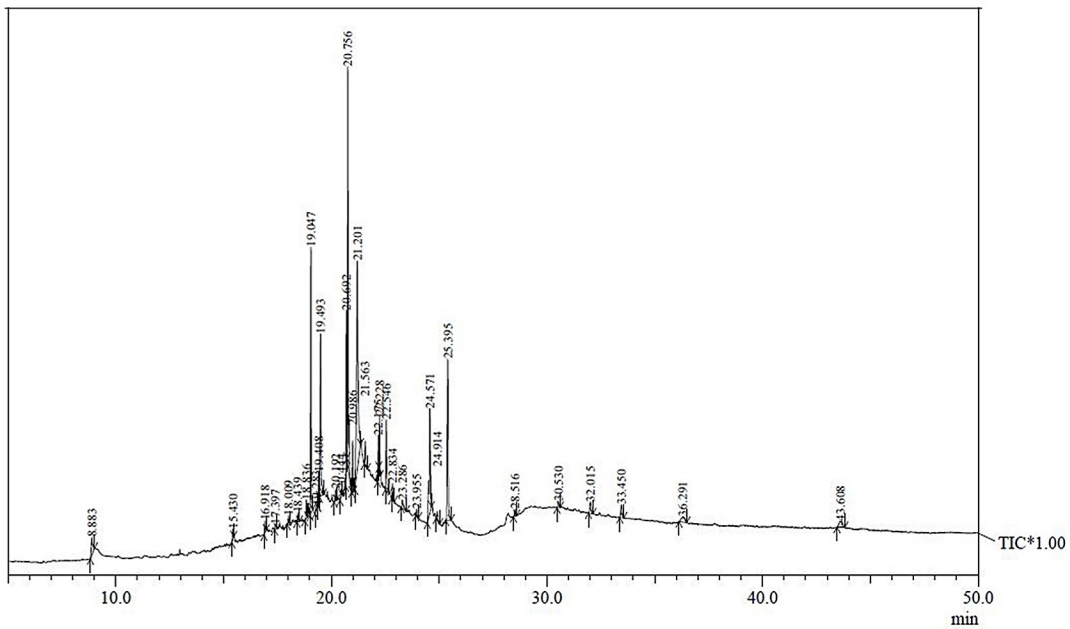



**Figure 10 b: Gas chromatogram of methanolic fruit extract of *M. grande***

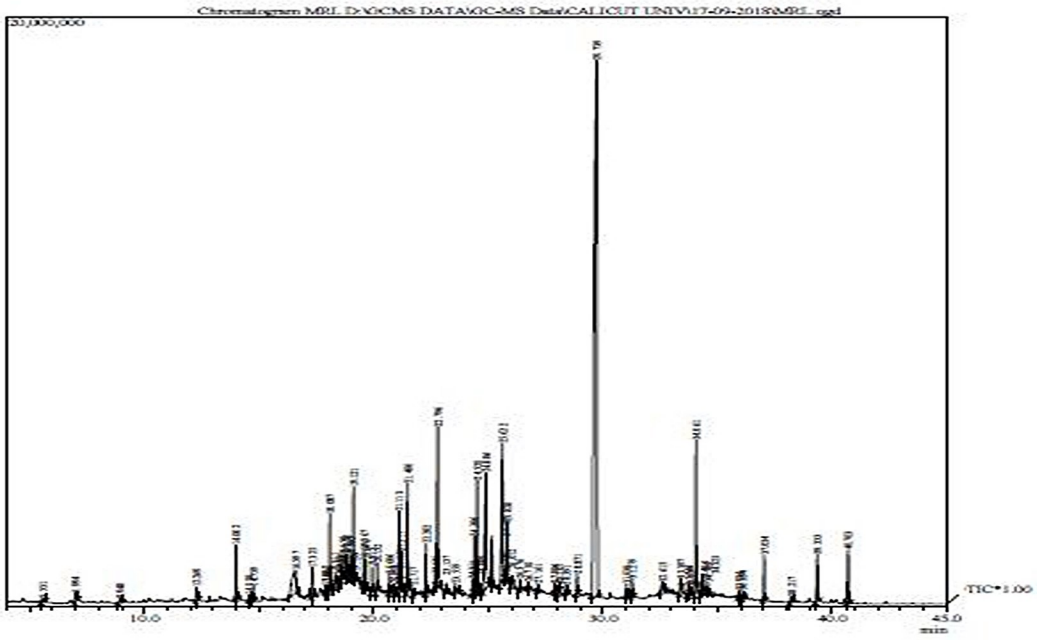



**Figure 11 a: Gas chromatogram of methanolic leaf extract of *M. randerianum***

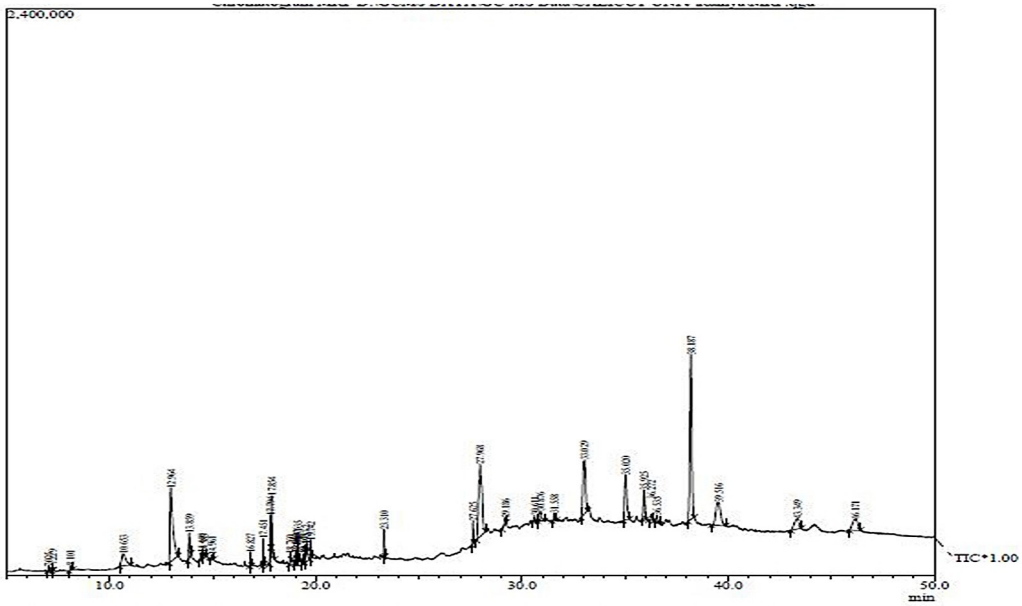



**Figure 11 b: Gas chromatogram of methanolic fruit extract of *M. randerianum***

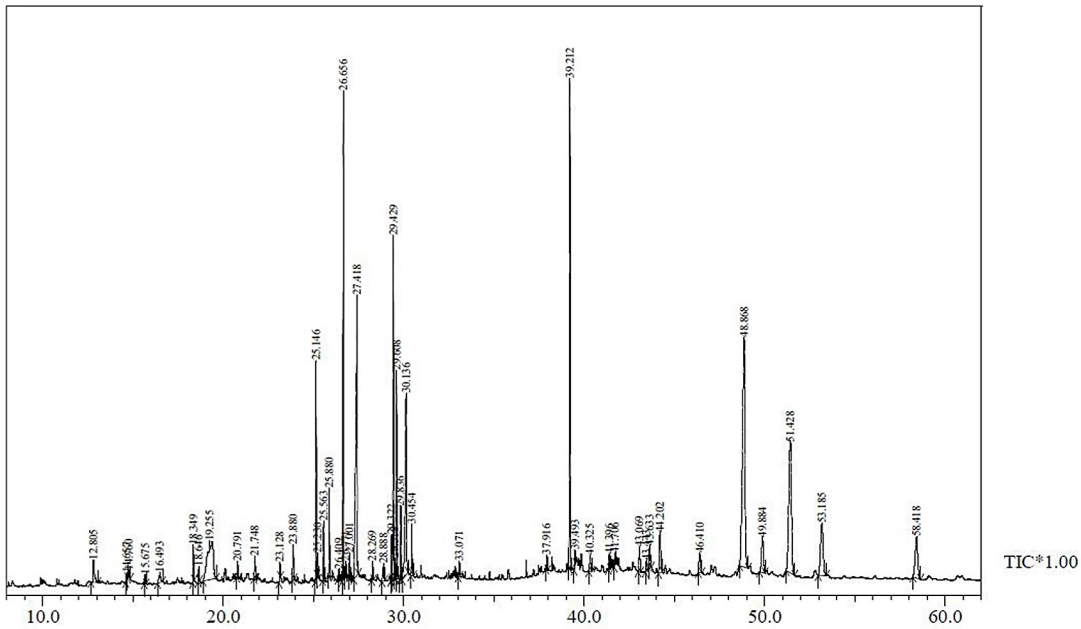



**Figure 12 a: Gas chromatogram of methanolic leaf extract of *M. umbellatum***

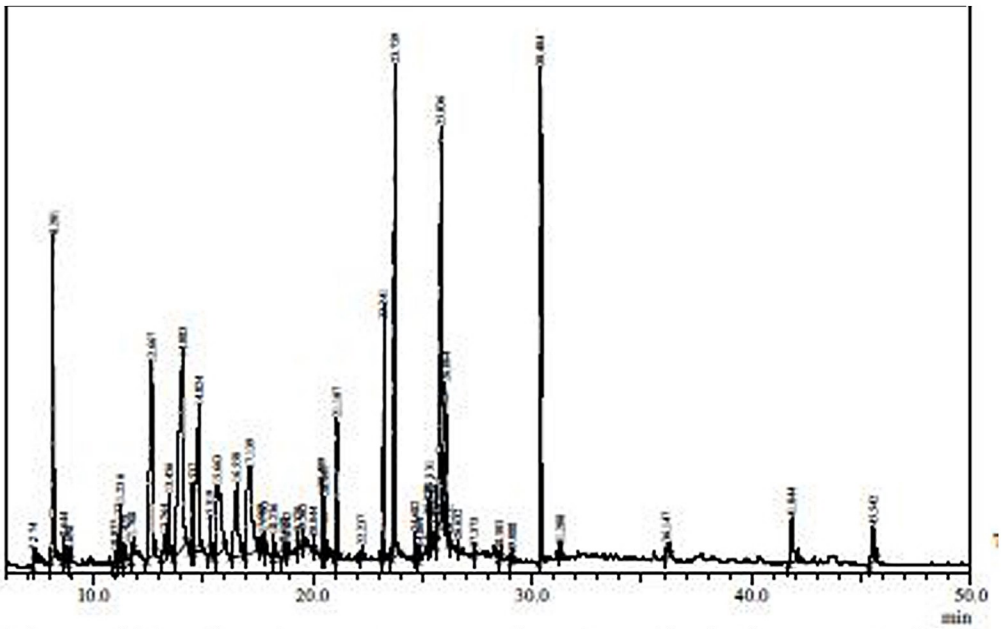



**Figure 12 b: Gas chromatogram of methanolic fruit extract of *M. umbellatum***

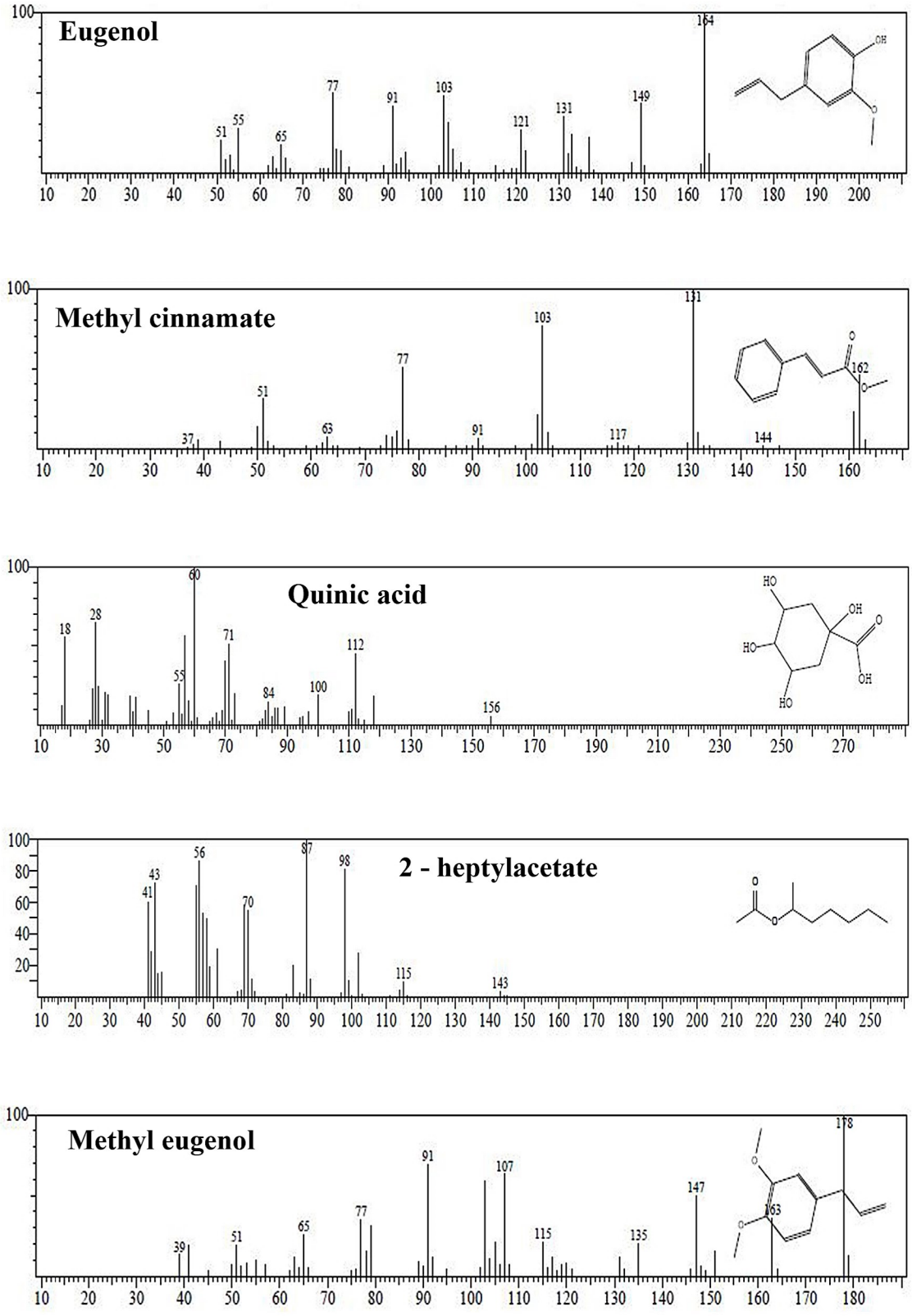



**Figure 13 (iii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

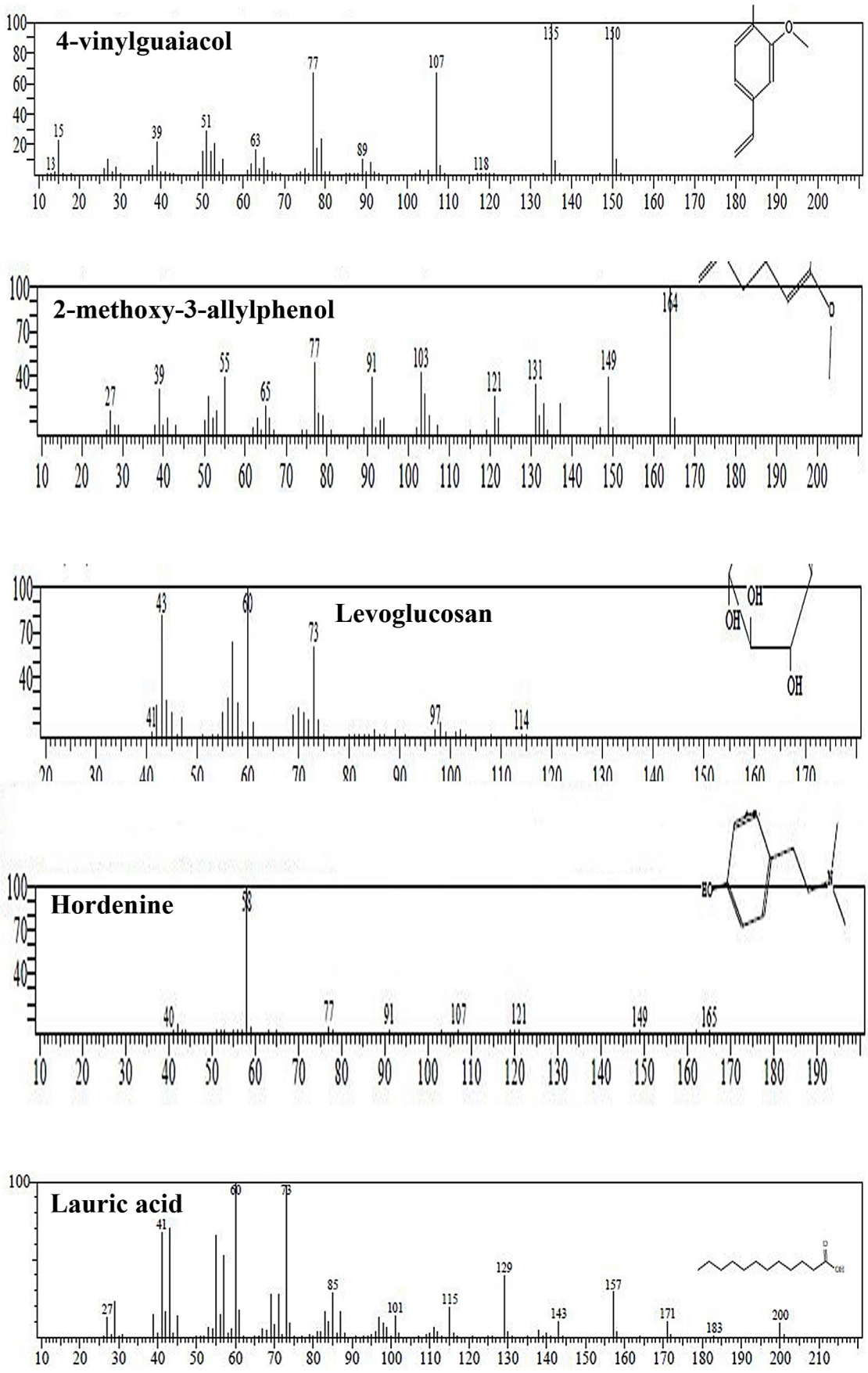



**Figure 13 (iv): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

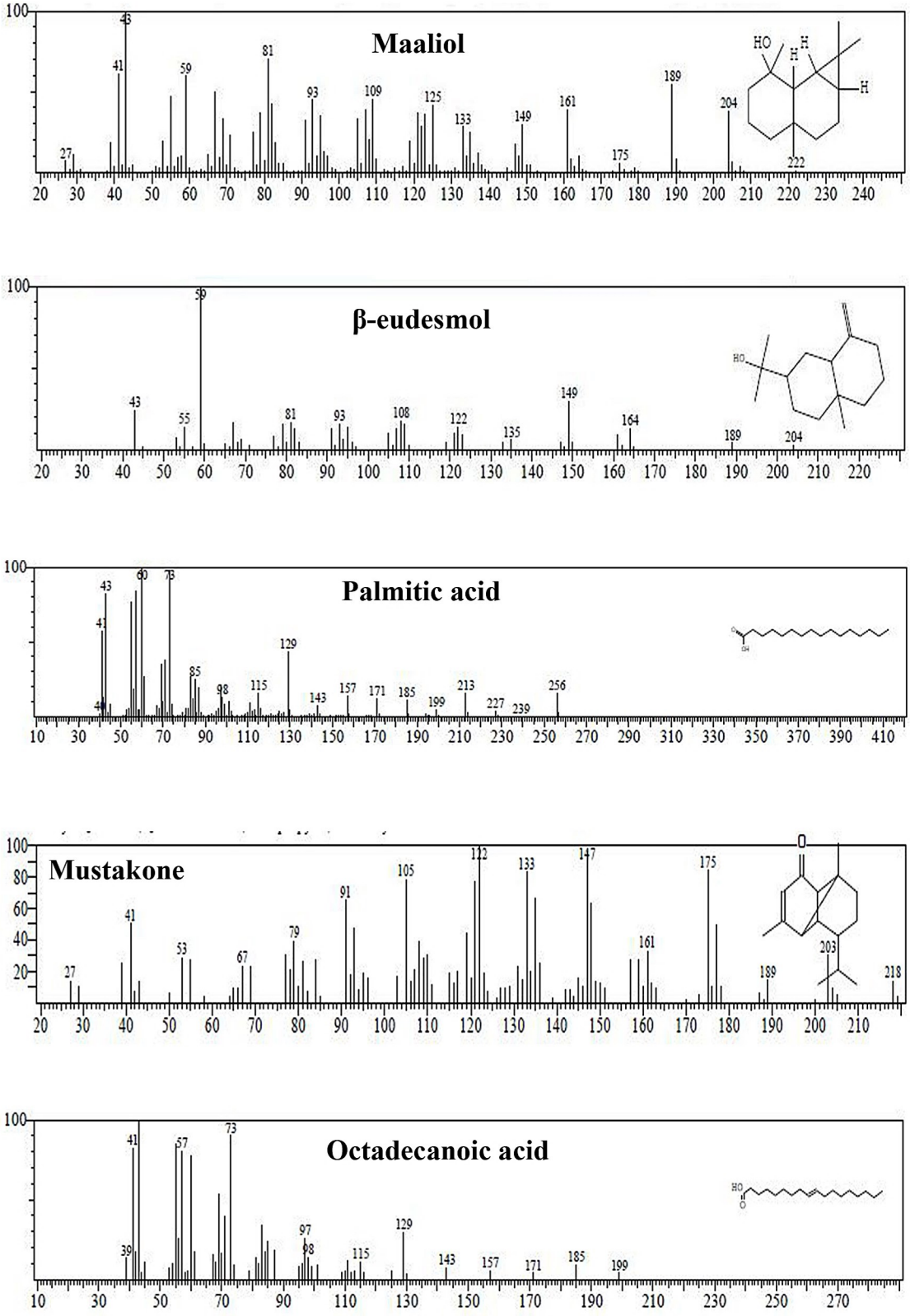



**Figure 13 (vi): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

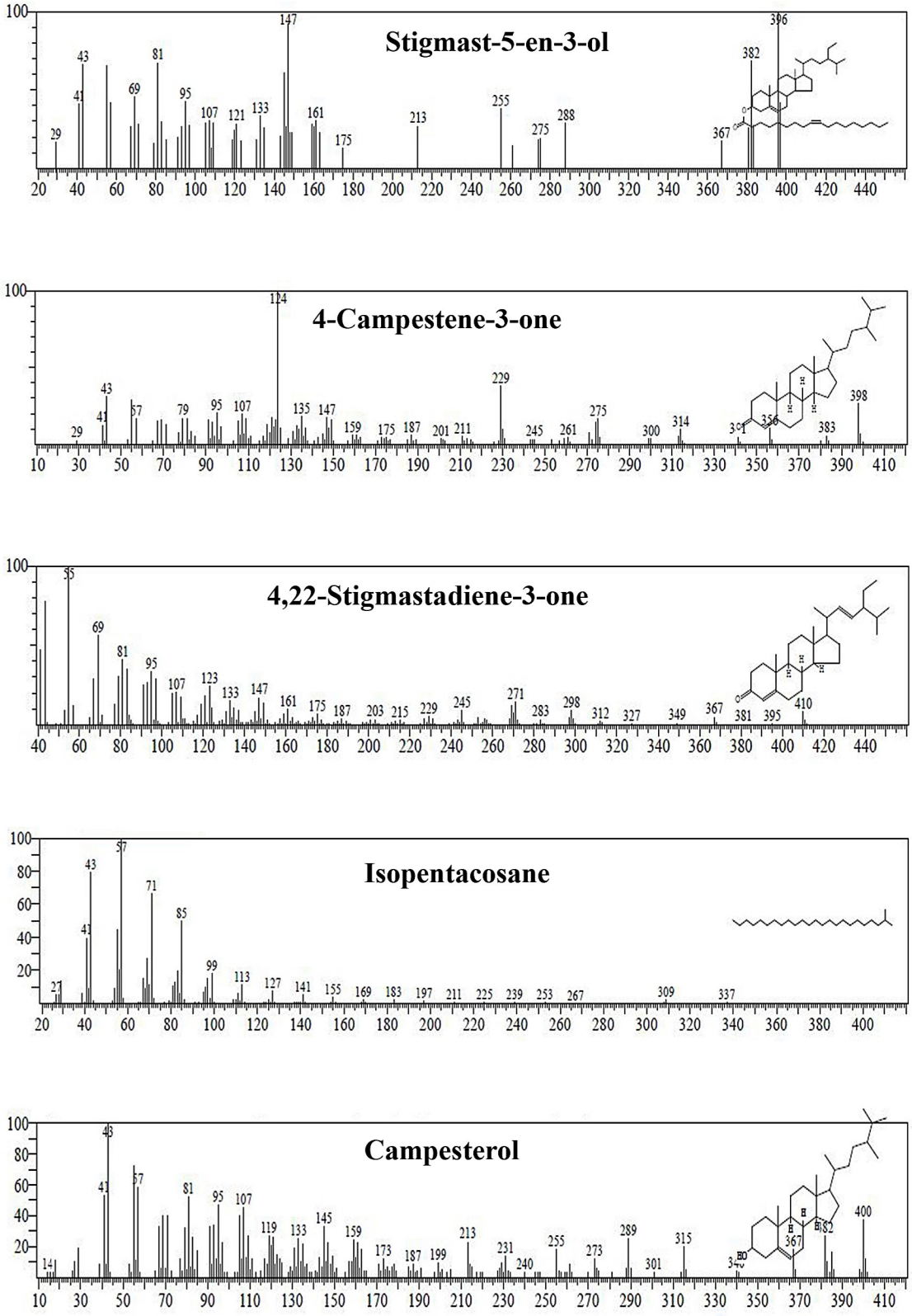



**Figure 13 (xv): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

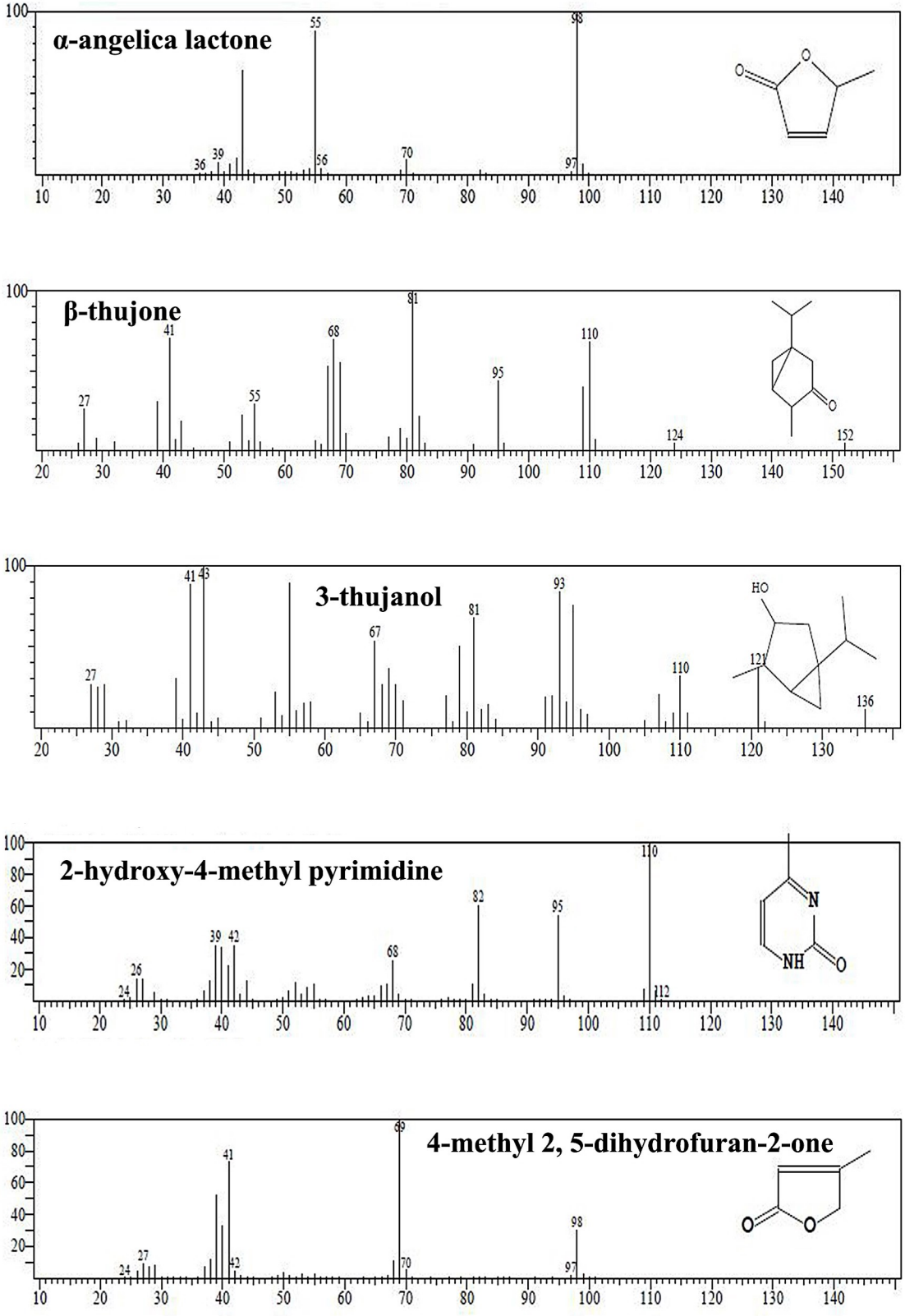



**Figure 13 (i): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

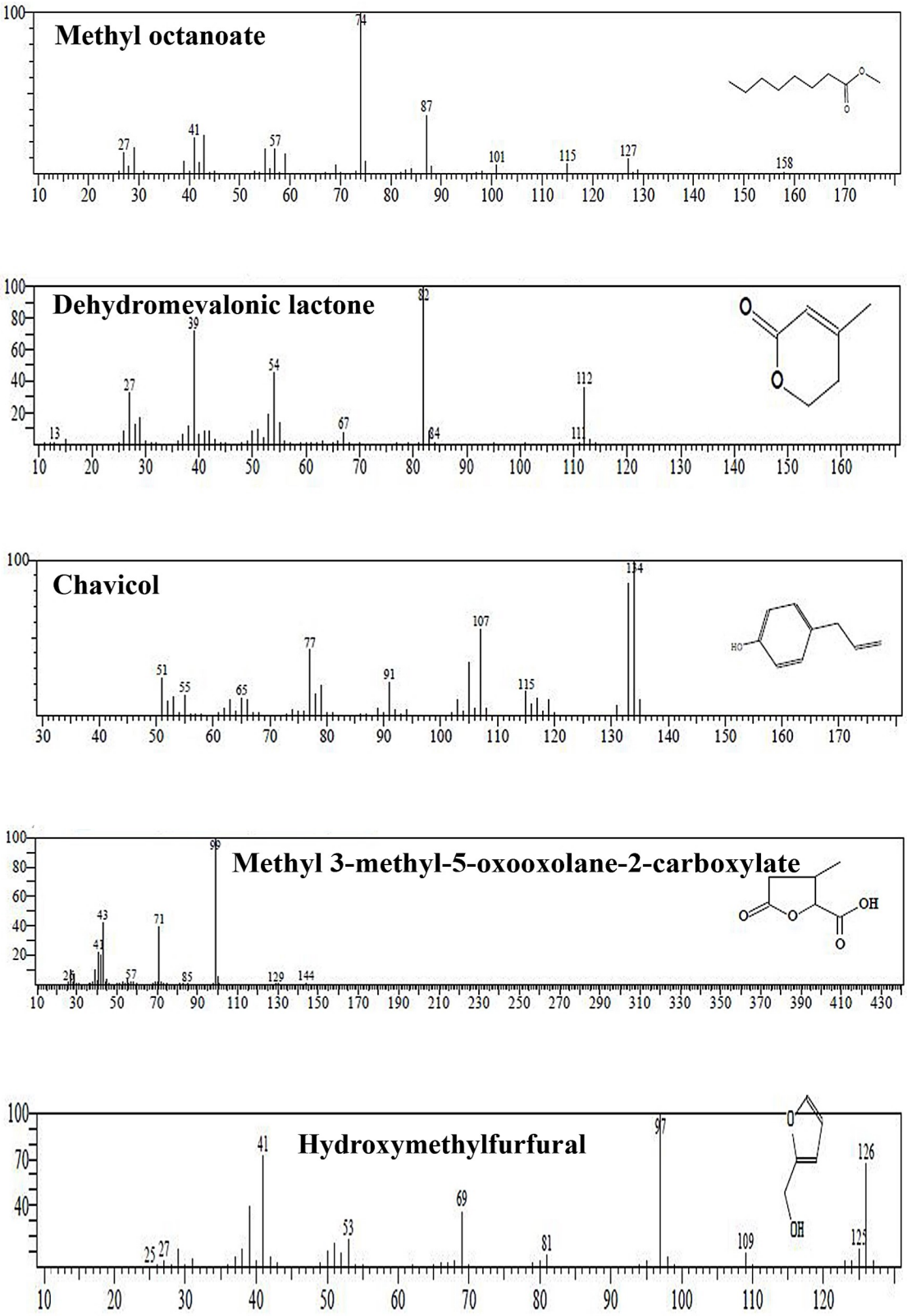



**Figure 13 (ii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

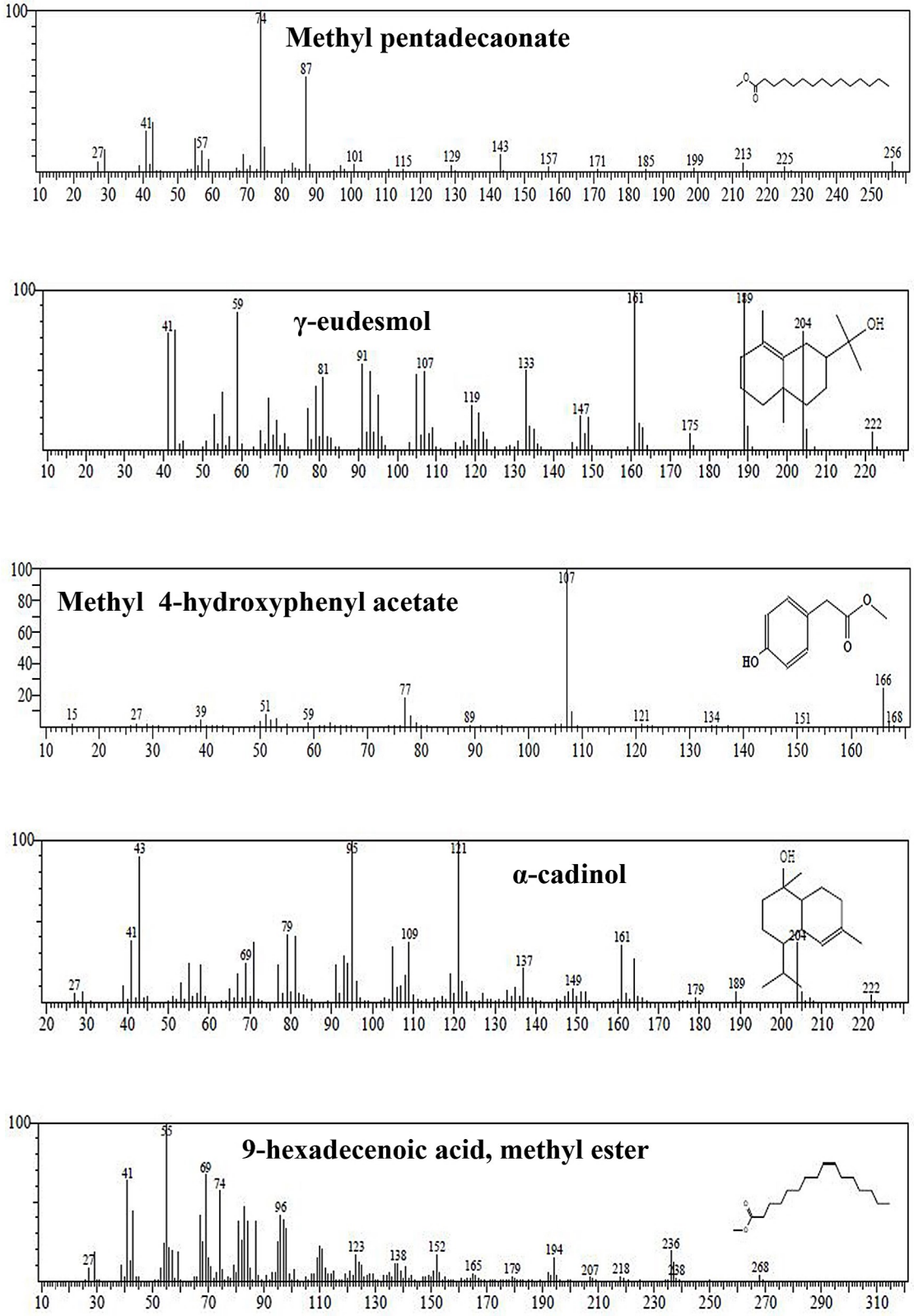



**Figure 13 (v): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

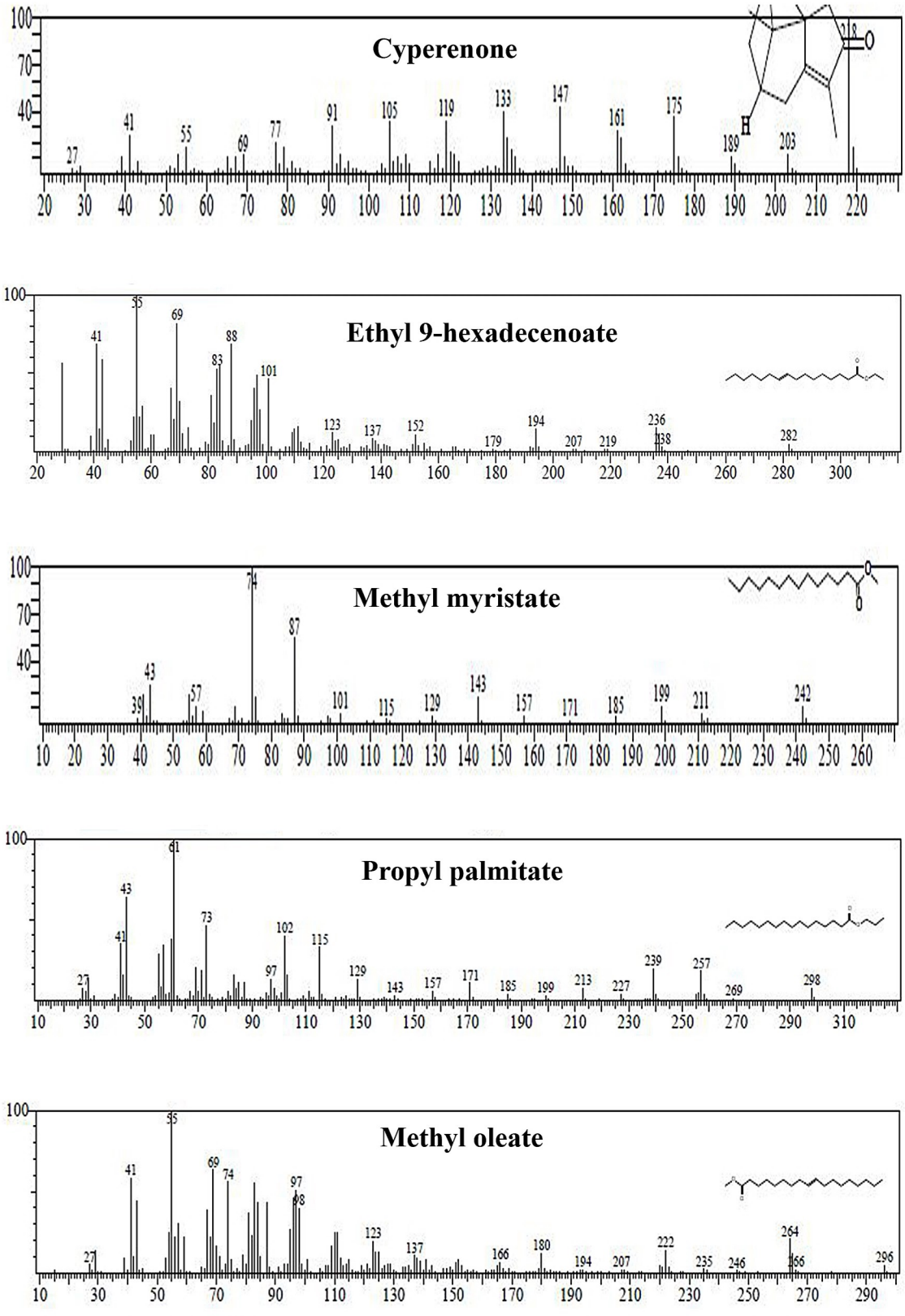



**Figure 13 (vii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

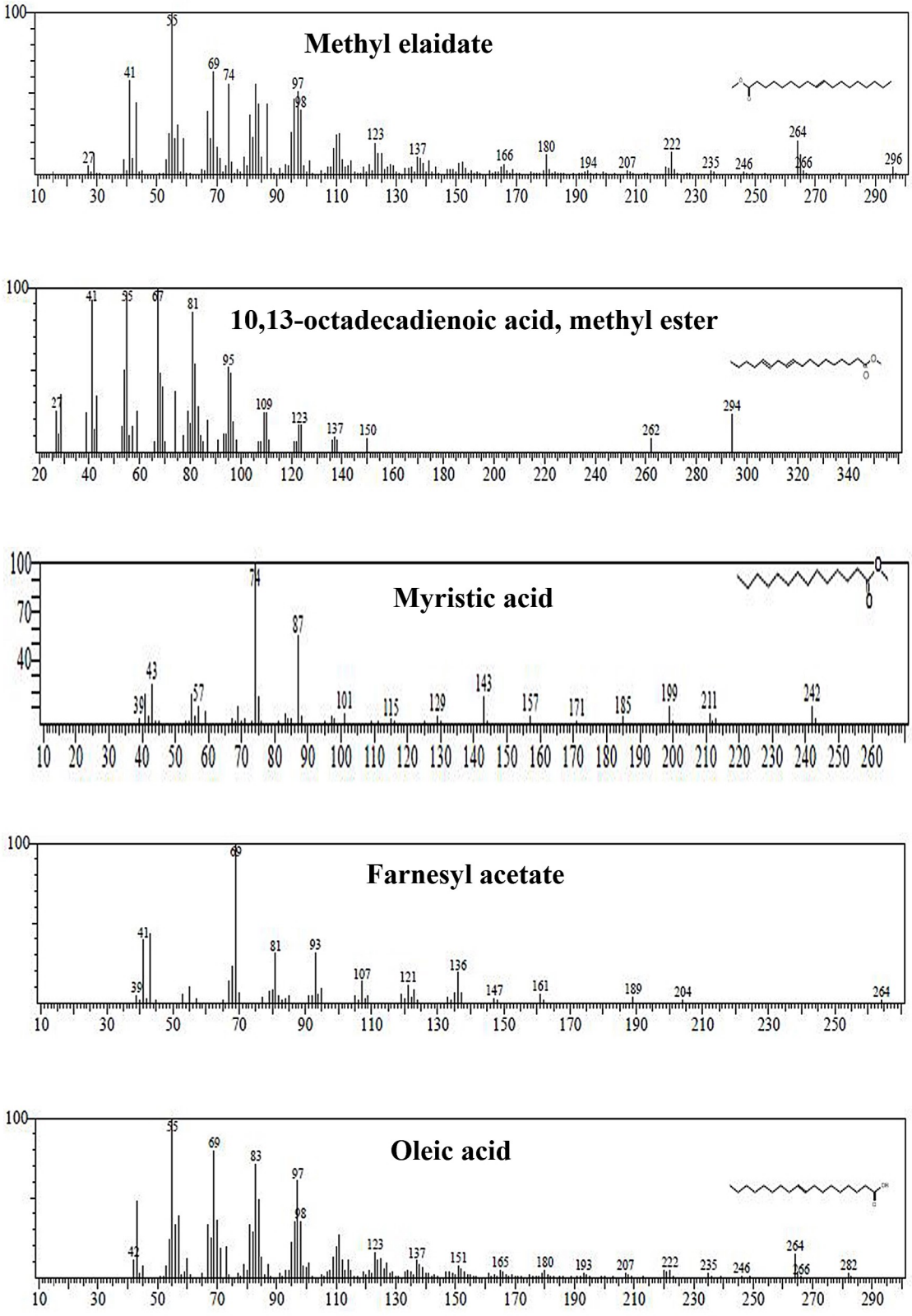



**Figure 13 (viii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

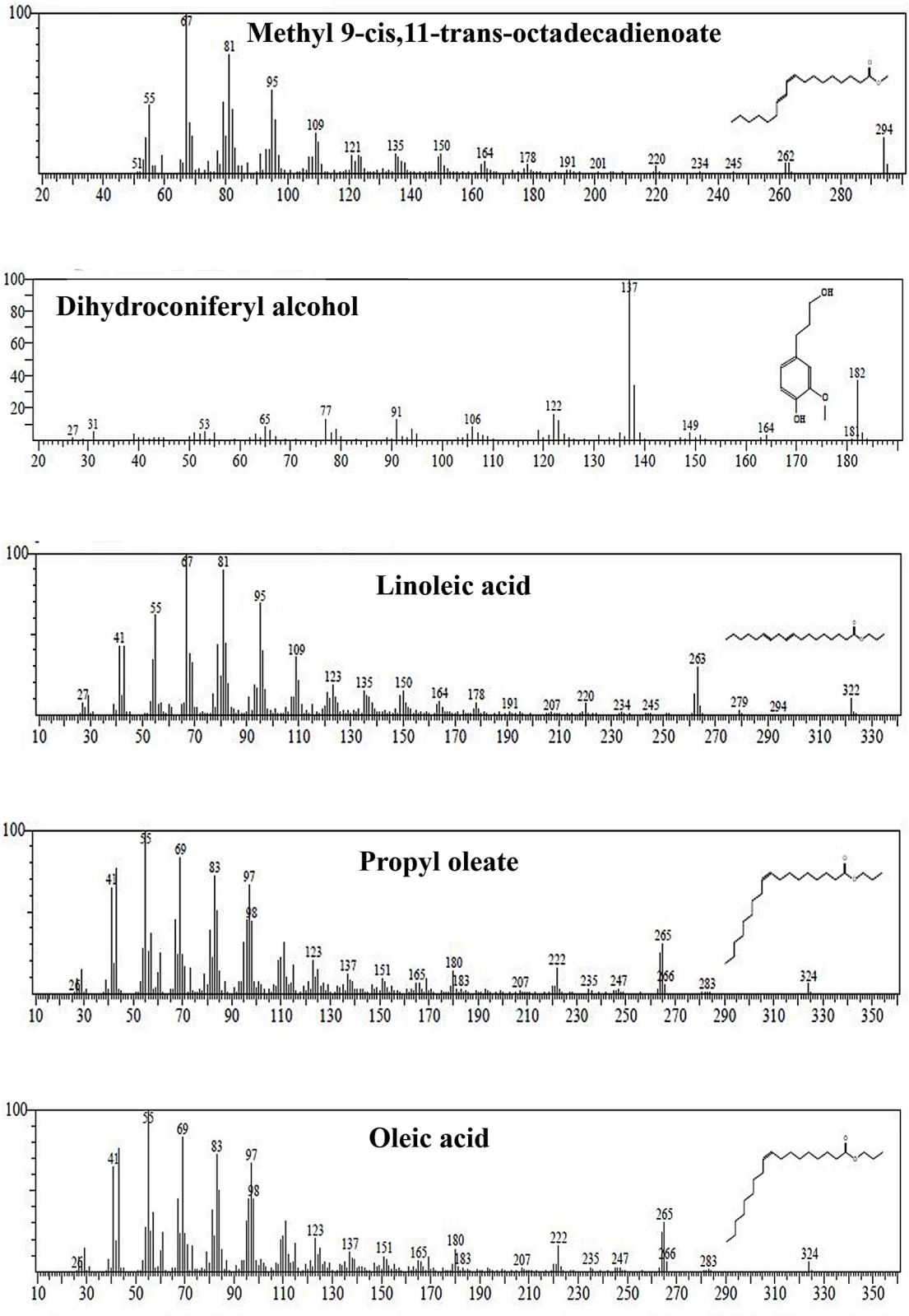



**Figure 13 (ix): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

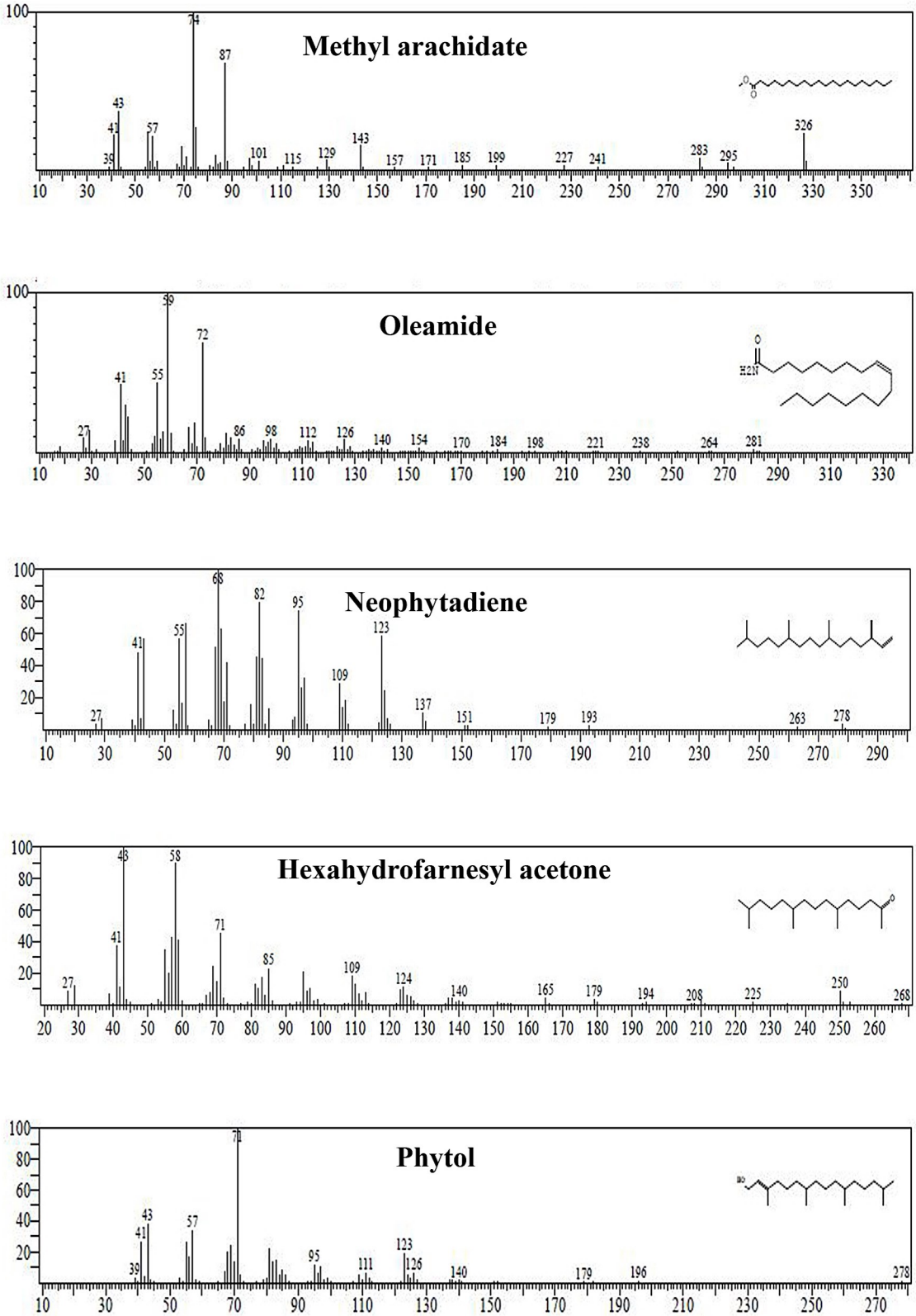



**Figure 13 (x): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

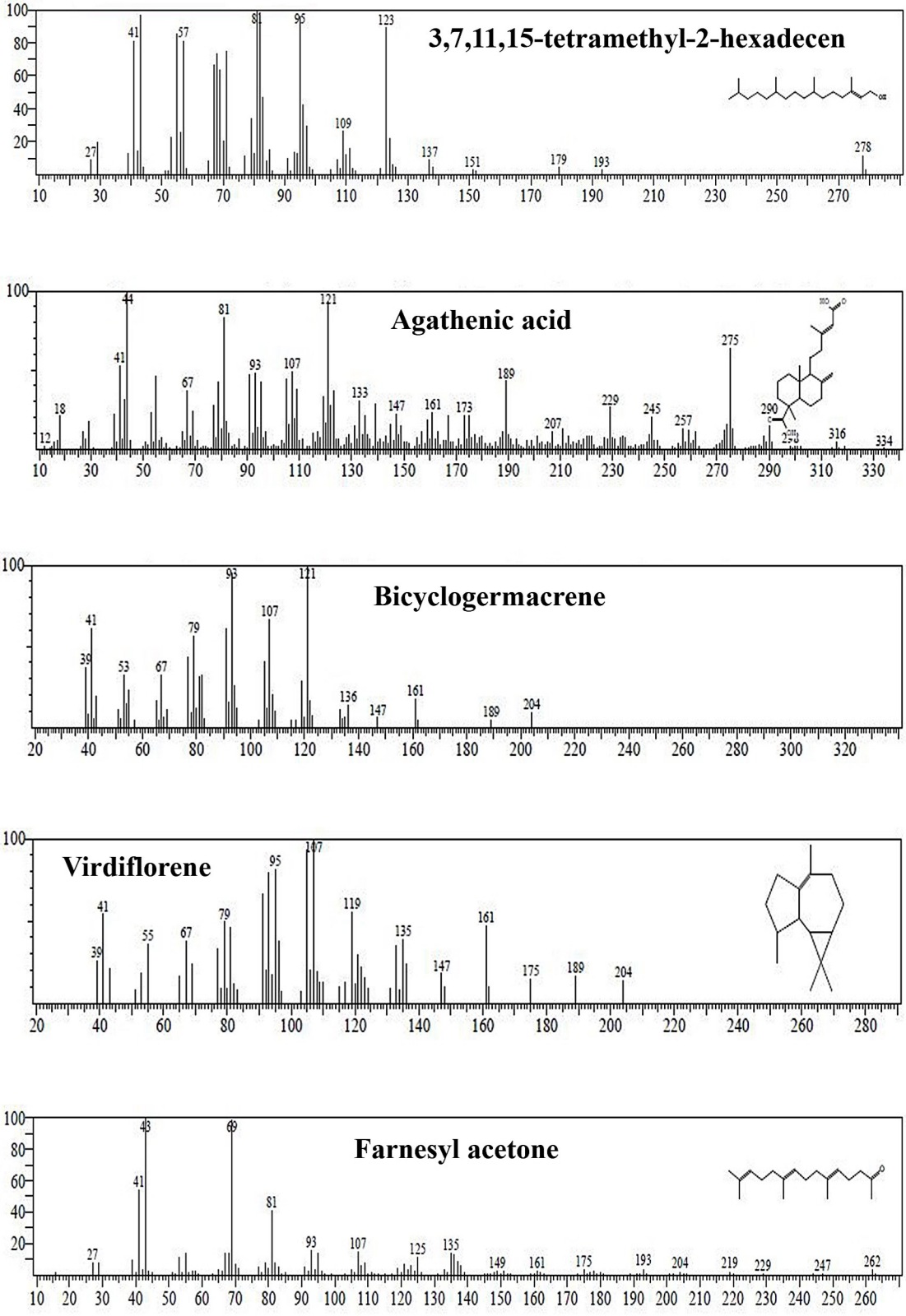



**Figure 13 (xi): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

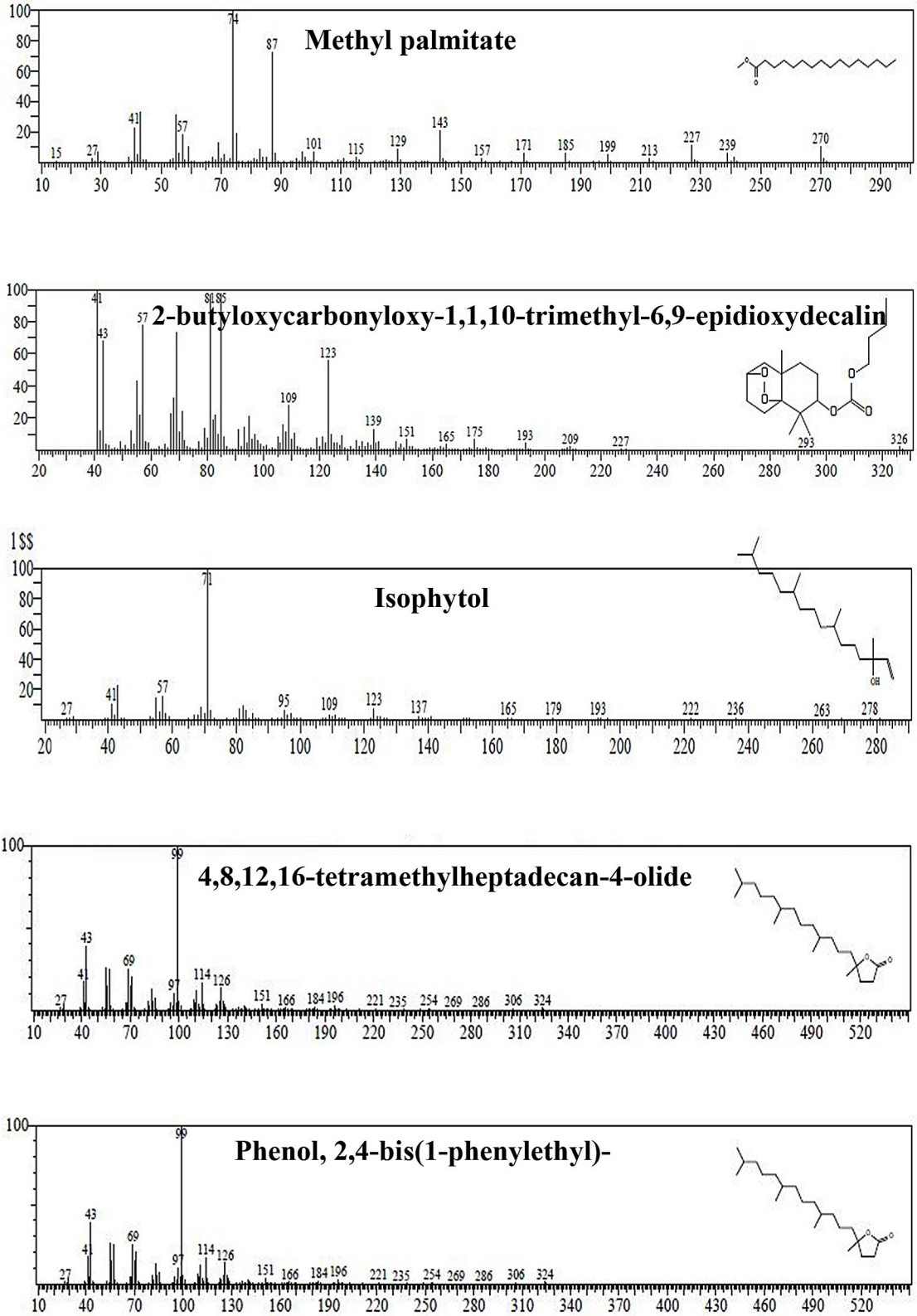



**Figure 13 (xii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

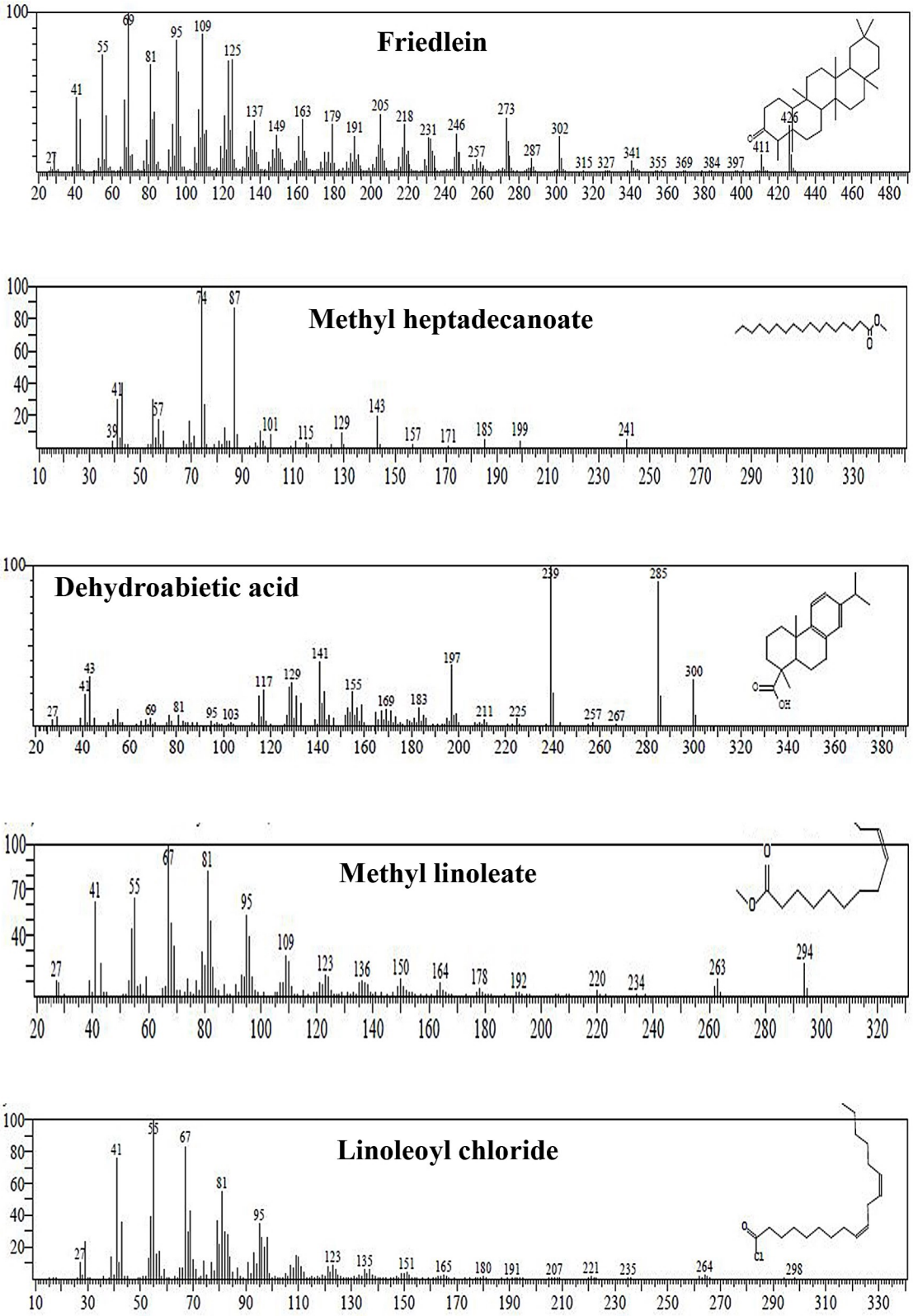



**Figure 13 (xiii): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

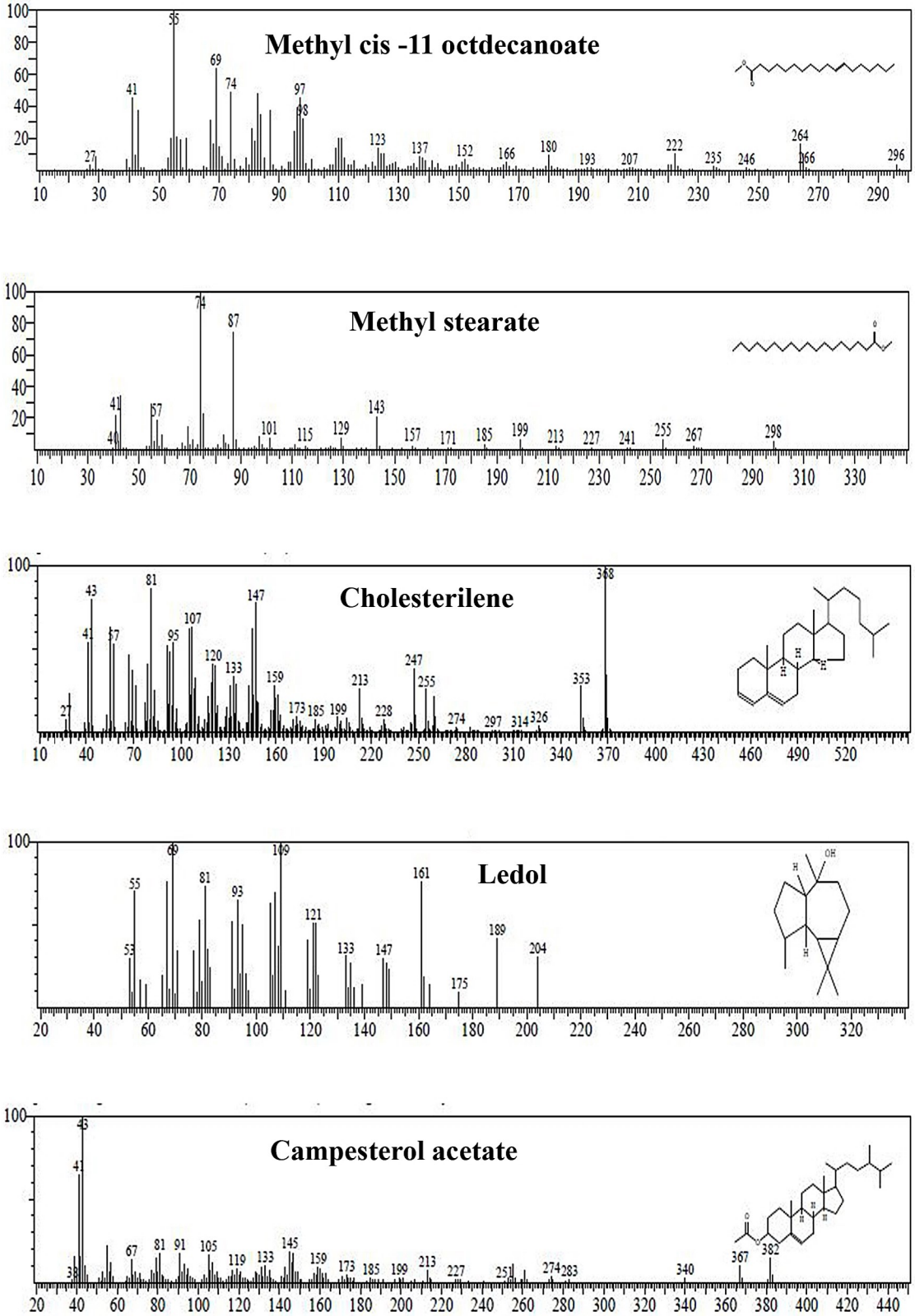



**Figure 13 (xiv): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

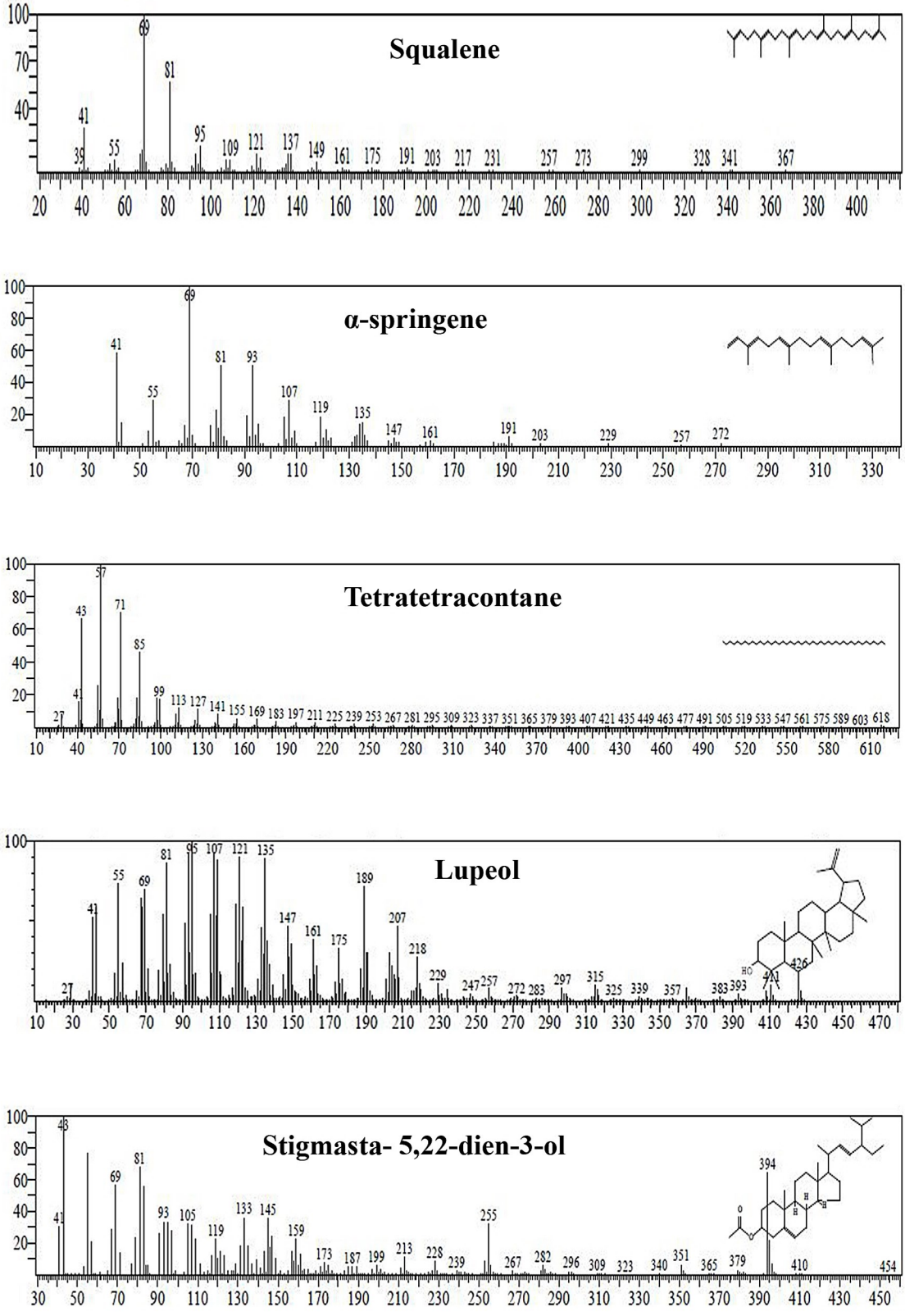



**Figure 13 (xvi): Mass spectra of compounds detected by GC/MS analysis in the methanolic extract of selected species of *Memecylon***

A total of 26 compounds were detected in the methanolic leaf extract of *M. randerianum* by GC-MS analysis. These compounds belong to various classes *viz.,* terpenoids, phenolics, steroids, organic compounds, fatty acids *etc*. Among the revealed 26 compounds, major compounds detected were palmitic acid (15.51%), agathenic acid (14.78%), squalene (8.75 %), phytol (7.11%) and lupeol (5.38%). Among the selected species, the presence of lupeol was noticed only in *M. randerianum* leaf extract. Terpenes were the predominant class of compounds present in the extract. The terpene compounds like agathenic acid (14.745%), bicyclogermacrene (3.59%), γ-eudesmol (0.62%) and terpene alcohol compounds such as phytol (7.11%), maaliol (1.57%), β-eudesmol (4.58%) were revealed through the analysis. Terpenes are classified as monoterpene, diterpene, triterpene, sesquiterpene *etc.* Among the resulted phytoconstituents, diterpenes were found to be the predominant group. Terpenes comprise 52.42% of total phytoconstituents. Octadecanoic acid, dodecanoic acid and palmitic acid are the fatty acids present in the extract. Phenylpropanoid compounds like chavicol, methyl eugenol and phenol, 2, 4-bis (1-phenylethyl) were detected in GC-MS analysis in negligible amounts. The organic compounds detected through the analysis are α-angelica lactone and salicylate glycol. Friedlein (31.30%) and stigmast-5-en-3-ol (12.80%) were the terpenoid group of compounds observed in highest amount in *M. randerianum* fruit extract. The other terpenoid compounds detected in GC/MS analysis are β-thujone, 3- thujanol and squalene. The steroid compounds like stigmast-5-en-3-ol (12.80%), 4-campestene-3-one (5.99%) and 4, 22-stigmastadiene-3-one (1.25%) are also noticed. The organic compounds, quinic acid and vitamin E occurred in trace amounts. The presence of fatty acids and fatty acid methyl esters are also confirmed in a total of 14 compounds.

*M. umbellatum* leaf extract consists of 32 compounds. It includes phenols, terpenoids, steroids and organic compounds. A steroid compound, α-phytosterol (17.72%) occurred in the highest amount. It is followed by a fatty acid, palmitic acid (11.95%) and a triterpene glycoside, methyl commate B (10.60%). Squalene, vitamin E, campesterol, neophytadiene and phytol were the major bioactive compounds identified in *M. umbellatum* leaf extract. A negligible amount of carboxylic acid esters, organic compounds, alkanes and ketones were also present in the extract. Methyl 4-hydroxyphenyl acetate and 4-vinylguaiacol are the phenolic compounds observed in the plant extract. 2-Methoxy-3-allylphenol, a phenylpropanoid was also found. The fruit extract of *M. umbellatum* possesses 12 bioactive phytoconstituents. The presence of an alkaloid, hordenine (21.35%) was found to be prominent. The terpenoids like mustakone (0.35%) cyperenone (2.05%) and a heterocyclic compound, methyl 3-methyl-5-oxooxolane-2-carboxylate (21.4%) were also detected through the analysis. Lauric acid, ocatdecanoic acid and myristic acid are the fatty acid compounds detected in *M. umbellatum* fruit extract. The presence of carbohydrate lactones, fatty acid derivatives and fatty acid methyl esters are also noticed. The diverse array of bioactive phytochemicals present in the *Memecylon* species are revealed through the GC/MS analysis.

### d) Phytochemical profiling by HR-LC/MS

Non-volatile composition of selected *Memecylon* species was identified through the HR-LC/MS analysis. The identified compounds with retention time, molecular mass, molecular formula, m/z ratio and class of compounds are enlisted in **Table 9**. A total of 48 compounds were identified through the analysis. The liquid chromatograms of selected six plant extracts are represented as **Figures 14 a & b, 15 a & b, 16 a & b**. The mass spectra of individual compounds are represented in **Figures 17 (i-xii).**

**Table 9: Chemical constituents identified by HR-LC/MS analysis in the selected species of *Memecylon***

| Sl NO | RT | Compound | Molecular formula | MM | Class | $[M+H]^+$ (m/z) | MGL | MGF | MRL | MRF | MUL | MUF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 1.037 | Elephantopin | $C_{19}H_{20}O_7$ | 360.122 | Sesquiterpene lactone | 365.100 | - | - | - | - | + | - |
| 2. | 1.173 | Tamarixetin | $C_{16}H_{12}O_7$ | 316.057 | Flavonoid | 315.050 | - | + | - | - | - | - |
| 3. | 3.633 | Glu Tyr | $C_{14}H_{18}N_2O_6$ | 310.116 | Biopeptide | 203.112 | - | - | - | + | - | - |
| 4. | 3.697 | Chlorogenic acid | $C_{16}H_{18}O_9$ | 354.092 | Polyphenolic compound | 337.088 | - | - | - | - | - | + |
| 5. | 3.700 | Violastyrene | $C_{17}H_{18}O_3$ | 270.123 | Organic compound | 275.102 | - | - | - | + | - | + |
| 6. | 4.167 | Lupanyl acid | $C_{14}H_{24}N_2O_2$ | 252.180 | Triterpenoid | 235.177 | + | - | - | - | - | - |
| 7. | 4.338 | Amygdalin | $C_{20}H_{27}NO_{11}$ | 457.157 | Glycoside | 480.146 | - | - | - | - | - | + |
| 8. | 4.624 | Tyr Asp Met | $C_{18}H_{25}N_3O_7S$ | 427.142 | Biopeptide | 445.176 | + | - | - | - | - | - |
| 9. | 4.676 | Asp Tyr Met | $C_{18}H_{25}N_3O_7S$ | 427.142 | Biopeptide | 428.495 | + | - | - | - | - | - |
| 10. | 4.827 | Ile Tyr Phe | $C_{24}H_{31}N_3O_5$ | 441.215 | Biopeptide | 445.176 | + | - | - | - | - | - |
| 11. | 5.059 | Indican | $C_{14}H_{17}NO_6$ | 295.105 | Organic compound | 318.094 | - | - | - | - | - | + |
| 12. | 5.251 | Deutzioside | $C_{15}H_{22}$ | 346.125 | Monoterpenoid | 351.104 | - | - | - | - | - | + |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $O_9$ | | | | | | | | | |
| 13. | 5.912 | Cys Thr Arg | $C_{13}H_{26}N_6O_5S$ | 378.167 | Biopeptide | 401.156 | - | - | - | + | - | - |
| 14. | 6.05 | Norstictic acid | $C_{28}H_{24}O_{15}$ | 600.120 | Ester | 599.112 | - | - | - | - | - | + |
| 15. | 6.203 | Bergenin | $C_{14}H_{16}O_9$ | 328.077 | Glycoside | 333.056 | - | - | + | - | - | - |
| 16. | 7.098 | Aesculin | $C_{15}H_{16}O_9$ | 340.077 | Glycoside | 345.055 | + | - | - | - | - | - |
| 17. | 7.383 | Gibberellin A8-catabolite | $C_{19}H_{22}O_7$ | 362.135 | Terpenoid | 345.132 | - | - | - | + | - | - |
| 18. | 7.419 | Arg Asp Cys | $C_{13}H_{24}N_6O_6S$ | 392.148 | Biopeptide | 397.125 | - | - | - | + | - | - |
| 19. | 8.321 | 9,12,13-trihydroxy-10,15-octadecadienoic acid | $C_{18}H_{32}O_5$ | 328.222 | Fatty acid | 333.201 | - | - | + | - | - | - |
| 20. | 8.511 | Gln Gln Val | $C_{15}H_{27}N_5O_6$ | 373.197 | Biopeptide | 356.193 | - | + | - | - | - | - |
| 21. | 8.925 | Rescinnamine | $C35_5H_{42}N_2O_9$ | 634.277 | Alkaloid | 639.280 | - | + | - | + | - | + |
| 22. | 8.976 | Swietenine | $C_{32}H_{40}O_9$ | 568.271 | Tetranortriterpenoid | 573.250 | + | + | - | + | - | - |
| 23. | 9.502 | 9S,10S,11R-trihydroxy-12Z-octadecenoic acid | $C_{18}H_{34}O_5$ | 330.237 | Fatty acid | 329.241 | - | - | - | - | - | + |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24. | 9.614 | Protoveratrine A | $C_{41}H_{63}NO_{14}$ | 793.432 | Alkaloid | 821.377 | - | - | - | - | - | + |
| 25. | 9.825 | C16 Sphinganine | $C_{16}H_{35}NO_2$ | 273.262 | Ceramide | 274.269 | + | - | - | - | - | - |
| 26. | 10.922 | Ganglioside GM1 | $C_{79}H_{141}N_3O_{31}$ | 1627.965 | Lipids | 822.970 | - | + | - | + | - | - |
| 27. | 11.203 | Phytosphingosine | $C_{18}H_{39}NO_3$ | 317.292 | Phospholipid | 318.800 | - | - | - | - | - | + |
| 28. | 11.632 | N-Hexadecyl-L-hydroxyproline | $C_{21}H_{41}NO_3$ | 355.308 | Glycoprotein | 356.315 | - | - | - | - | - | + |
| 29. | 11.857 | Trp Phe Asp | $C_{24}H_{26}N_4O_6$ | 466.186 | Biopeptide | 467.193 | - | - | - | + | - | - |
| 30. | 12.057 | 6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide | $C_{24}H_{32}O_7$ | 432.213 | Terpenoid | 415.210 | - | - | - | - | - | + |
| 31. | 12.098 | Arg Phe Gln | $C_{20}H_{31}N_7O_5$ | 449.241 | Biopeptide | 437.192 | - | + | - | - | - | - |
| 32. | 12.417 | Madecassic acid | $C_{30}H_{48}O_6$ | 504.341 | Triterpenoid | 503.947 | - | + | - | - | - | - |
| 33. | 13.075 | 7,8-Didehydroastaxanthin | $C_{40}H_{50}O_4$ | 594.376 | Carotene | 653.392 | - | + | - | - | - | - |
| 34. | 13.512 | beta-Erythroidine | $C_{16}H_{19}NO_3$ | 273.136 | Alkaloid | 256.133 | - | - | - | + | - | - |
| 35. | 14.599 | 8,13-dihydroxy-9,11- | $C_{18}H_{32}$ | 312.230 | Fatty acid | 295.226 | - | - | - | - | - | + |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | octadecadienoic acid | $O_4$ | | | | | | | | | |
| 36. | 14.785 | Calcifedol | $C_{29}H_{48}O_2$ | 428.364 | Vitamin D analogue | 411.861 | - | + | - | - | - | - |
| 37. | 15.402 | Isorenieratene | $C_{40}H_{48}$ | 528.365 | Carotene | 546.399 | - | - | - | - | - | + |
| 38. | 15.742 | 3-Dehydro-6-deoxoteasterone | $C_{28}H_{48}O_3$ | 432.356 | Steroid | 437.834 | + | - | - | - | - | - |
| 39. | 17.156 | Stigmasta-7,22E,25-trien-3beta-ol | $C_{29}H_{46}O$ | 410.353 | Sterol | 303.350 | - | - | - | - | - | + |
| 40. | 17.583 | Dihydroxylycopene/ OHRhodopin | $C_{40}H_{60}O_2$ | 572.458 | Carotene | 573.705 | - | + | - | - | - | - |
| 41. | 17.851 | Ursolic acid | $C_{30}H_{48}O_3$ | 456.370 | Triterpenoid | 455.363 | - | + | - | - | - | - |
| 42. | 17.949 | Glycerol palmitate | $C_{19}H_{38}O_4$ | 330.277 | Monoglyceride | 313.274 | - | - | - | + | - | - |
| 43. | 18.127 | 6-Deoxocastasterone | $C_{28}H_{50}O_4$ | 450.372 | Steroid | 455.350 | - | - | - | + | - | - |
| 44. | 18.164 | Cosmosiin hexaacetate | $C_{33}H_{32}O_{16}$ | 684.163 | Phenolic compound | 686.573 | - | - | - | + | - | - |
| 45. | 18.356 | Campestanol | $C_{28}H_{50}O$ | 402.387 | Steroid | 425.376 | - | + | - | - | - | - |
| 46. | 18.523 | Khayanthone | $C_{32}H_{42}O_9$ | 570.286 | Limonoid | 593.275 | - | + | - | - | - | - |
| 47. | 26.201 | Embelin | $C_{17}H_{26}O_4$ | 294.184 | Benzoquinone | 293.184 | - | - | - | - | - | + |
| 48. | 27.105 | 14-hydroxy-5Z-tetradecenoic acid | $C_{14}H_{26}O_3$ | 242.184 | Hydroxy fatty acid | 247.163 | - | - | - | - | + | - |

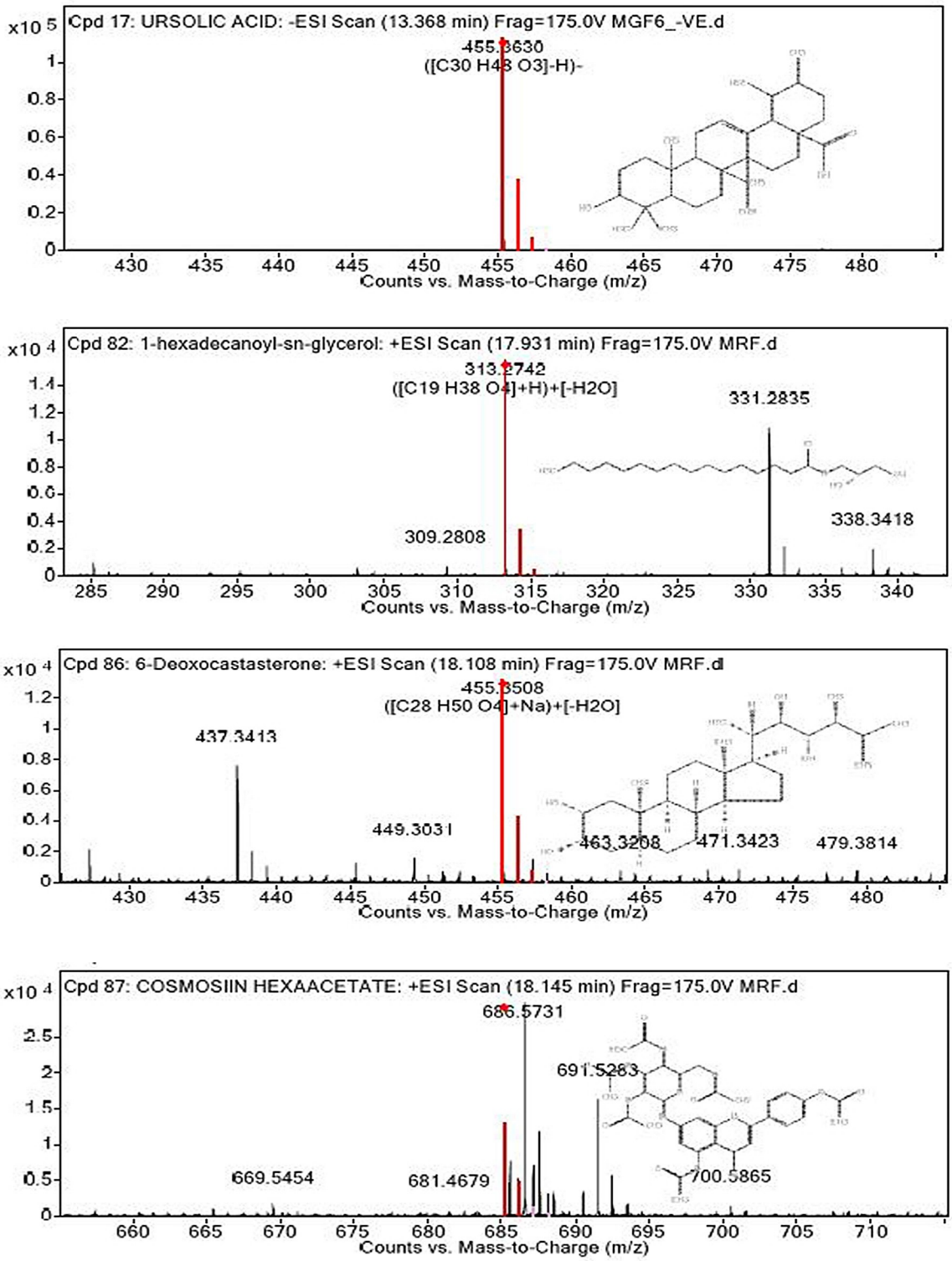



**Figure 17 (xi): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

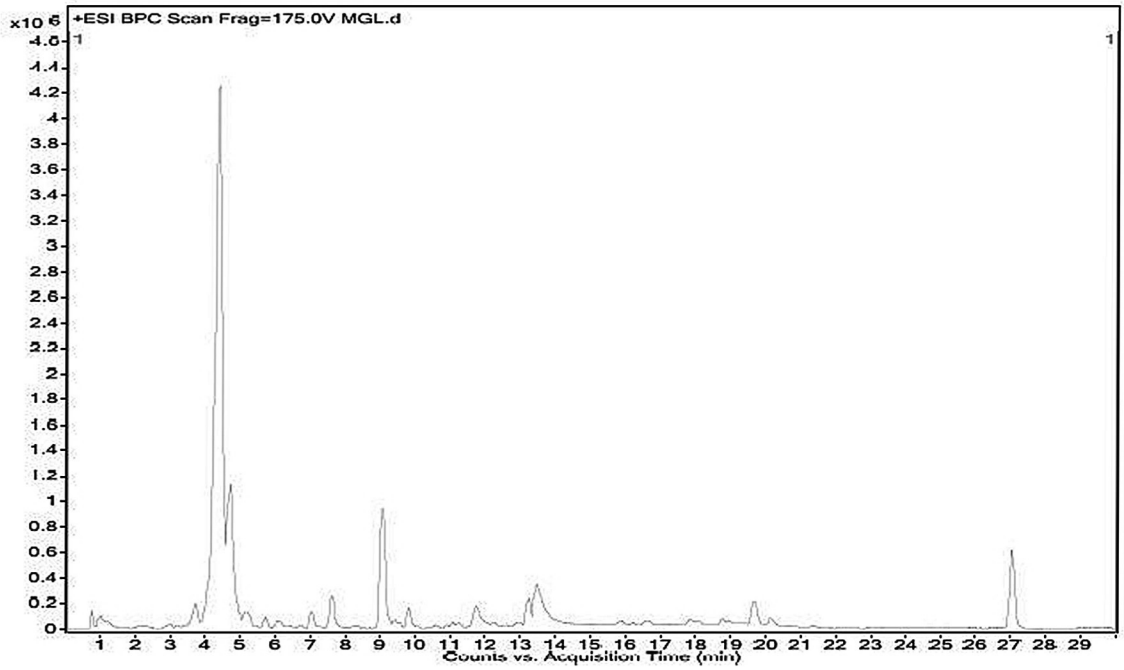



**Figure 14 a: Liquid chromatogram of methanolic leaf extract of *M. grande***

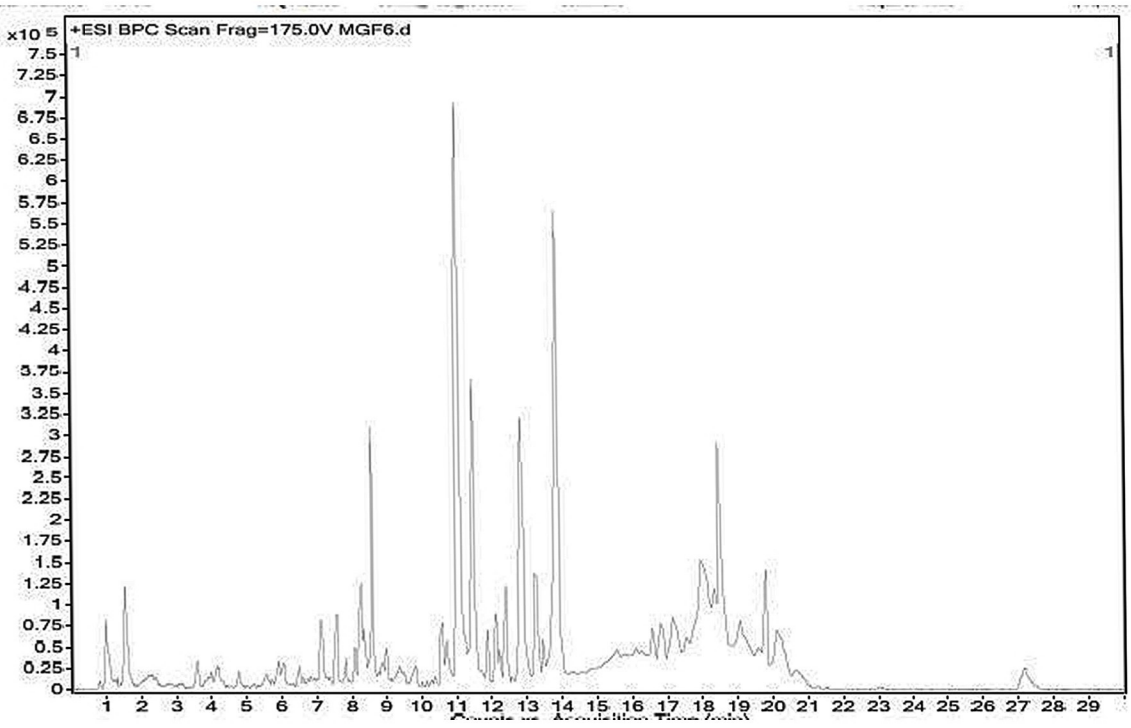



**Figure 14 b: Liquid chromatogram of methanolic fruit extract of *M. grande***

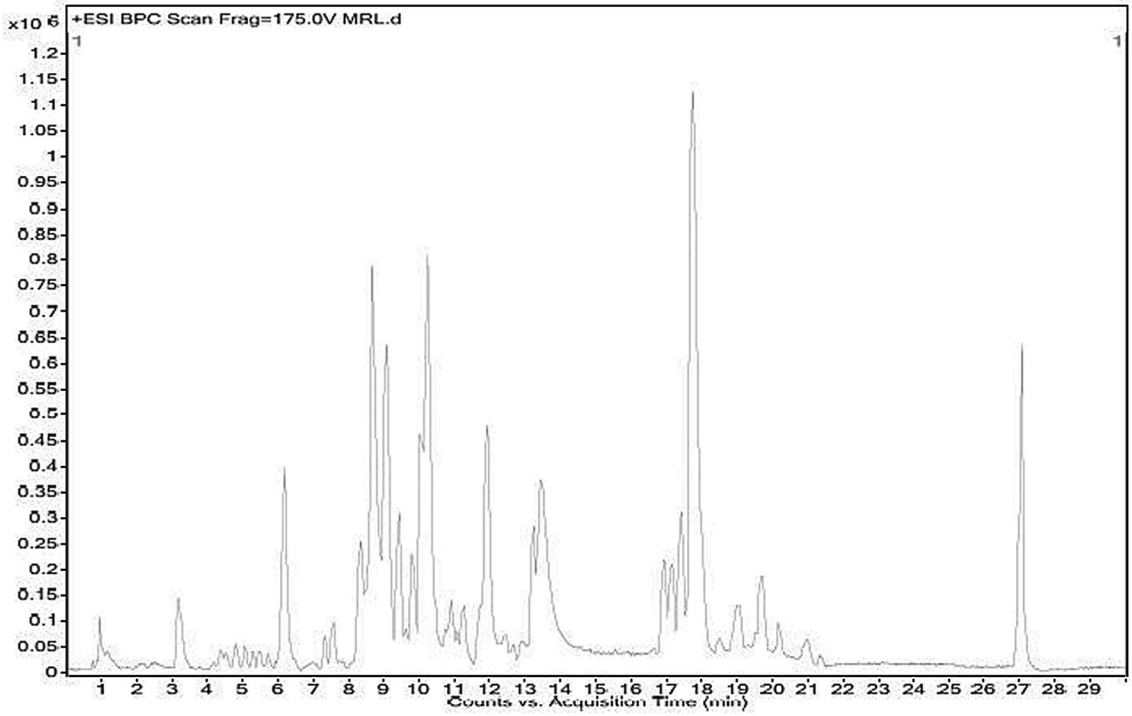



**Figure 15 a: Liquid chromatogram of methanolic leaf extract of *M. randerianum***

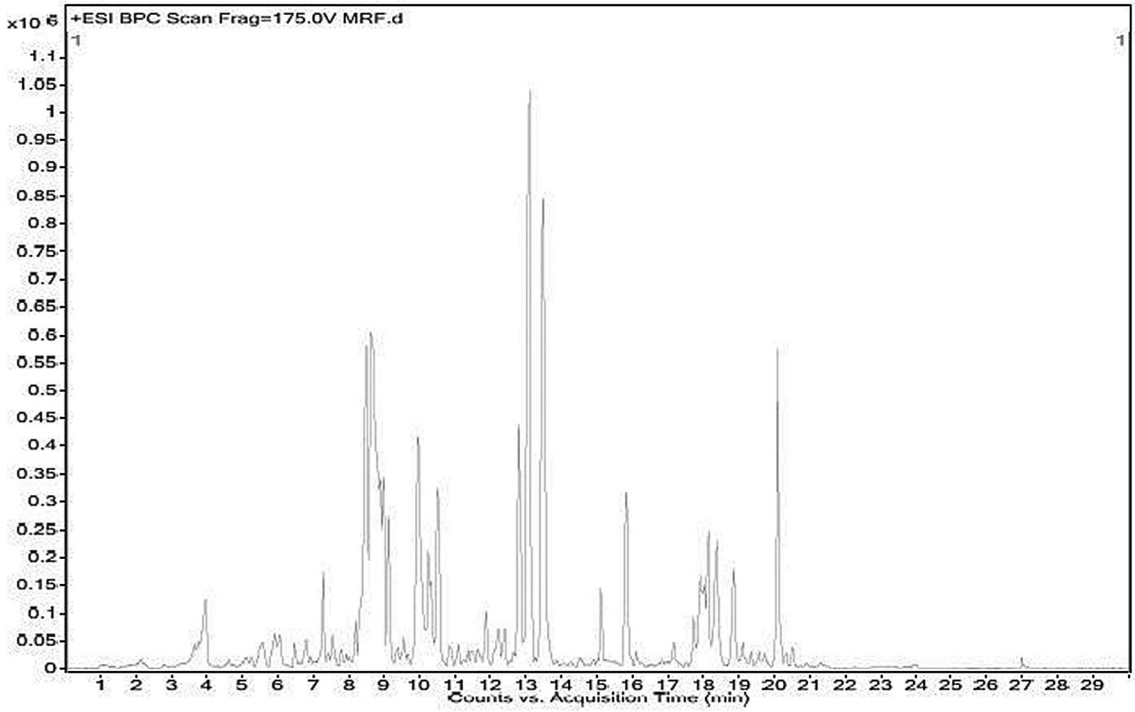



**Figure 15 b: Liquid chromatogram of methanolic fruit extract of *M. randerianum***

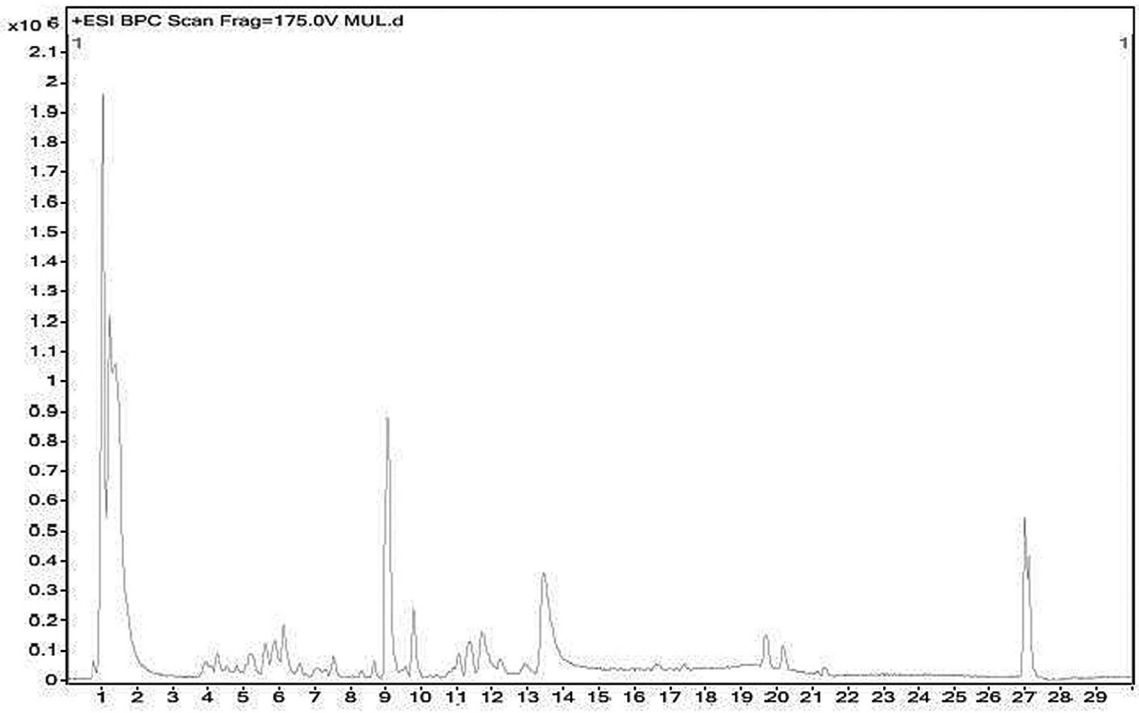



**Figure 16 a: Liquid chromatogram of methanolic leaf extract of *M. umbellatum***

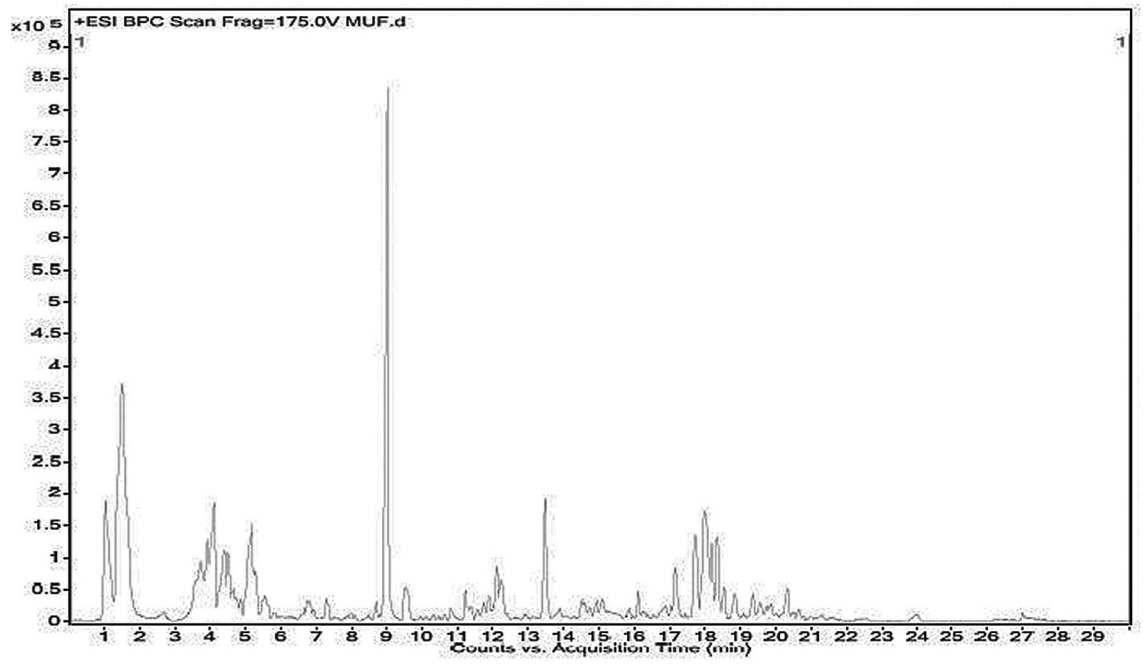



**Figure 16 b: Liquid chromatogram of methanolic fruit extract of *M. umbellatum***

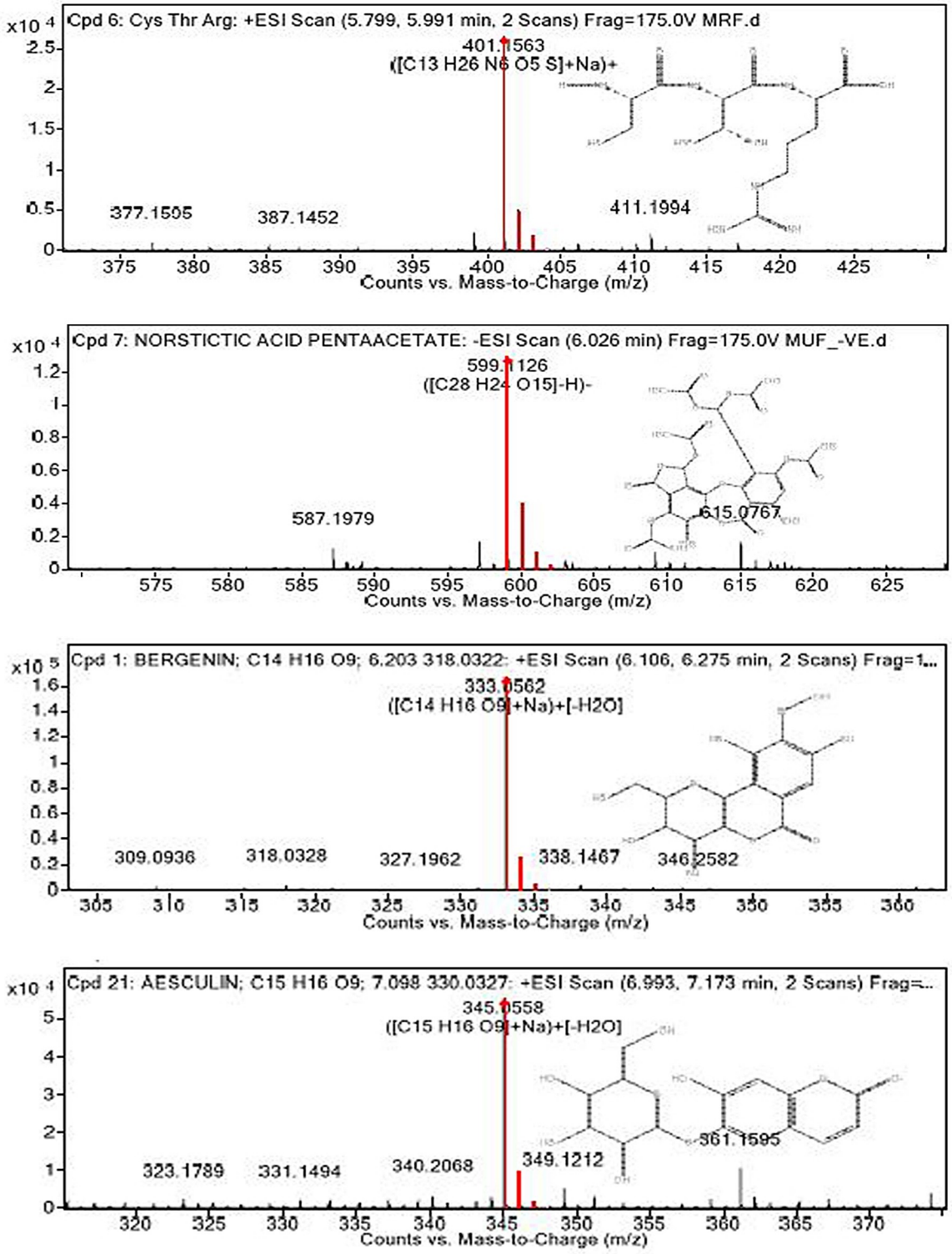



**Figure 17 (iv): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

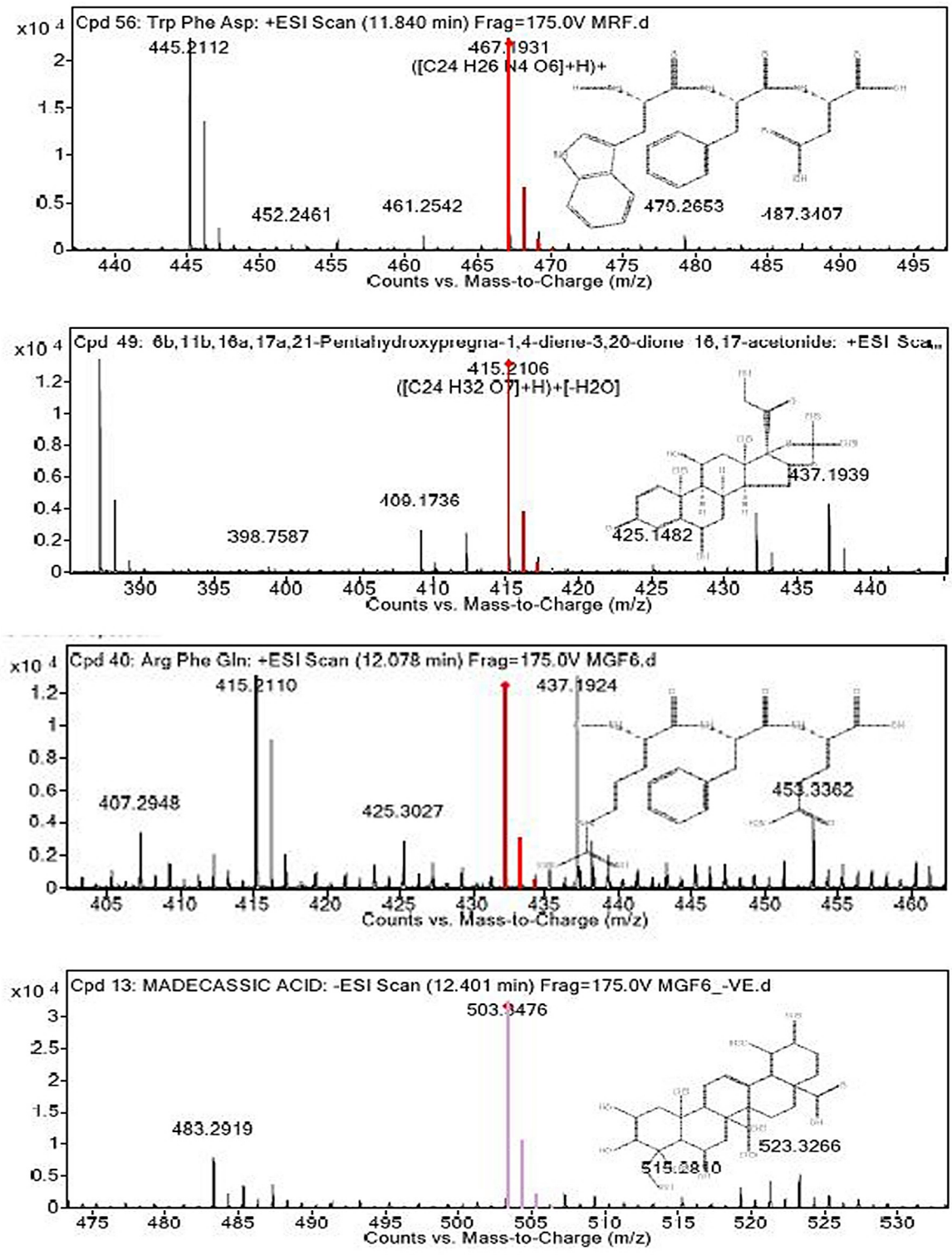



**Figure 17 (viii): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

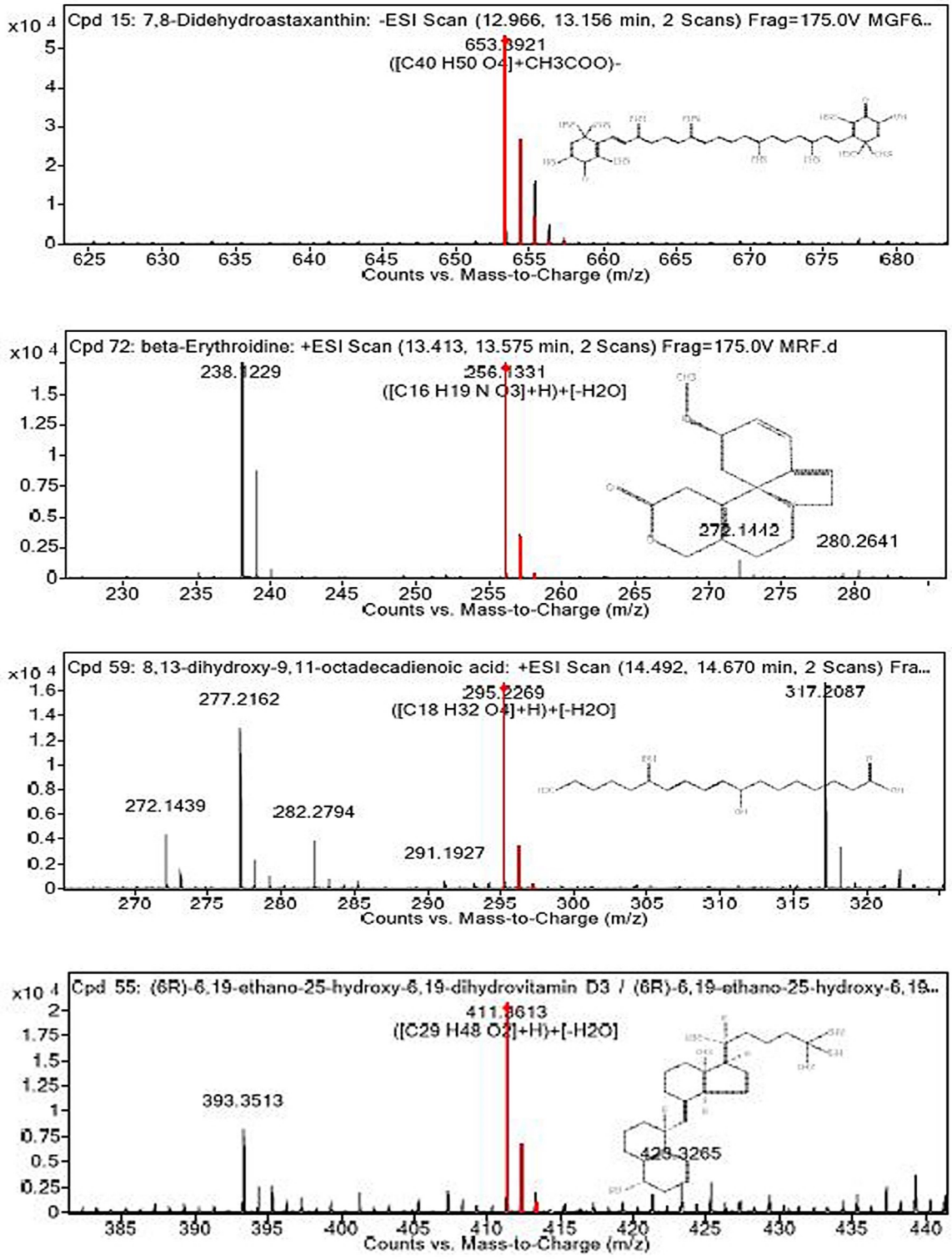



**Figure 17 (ix): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

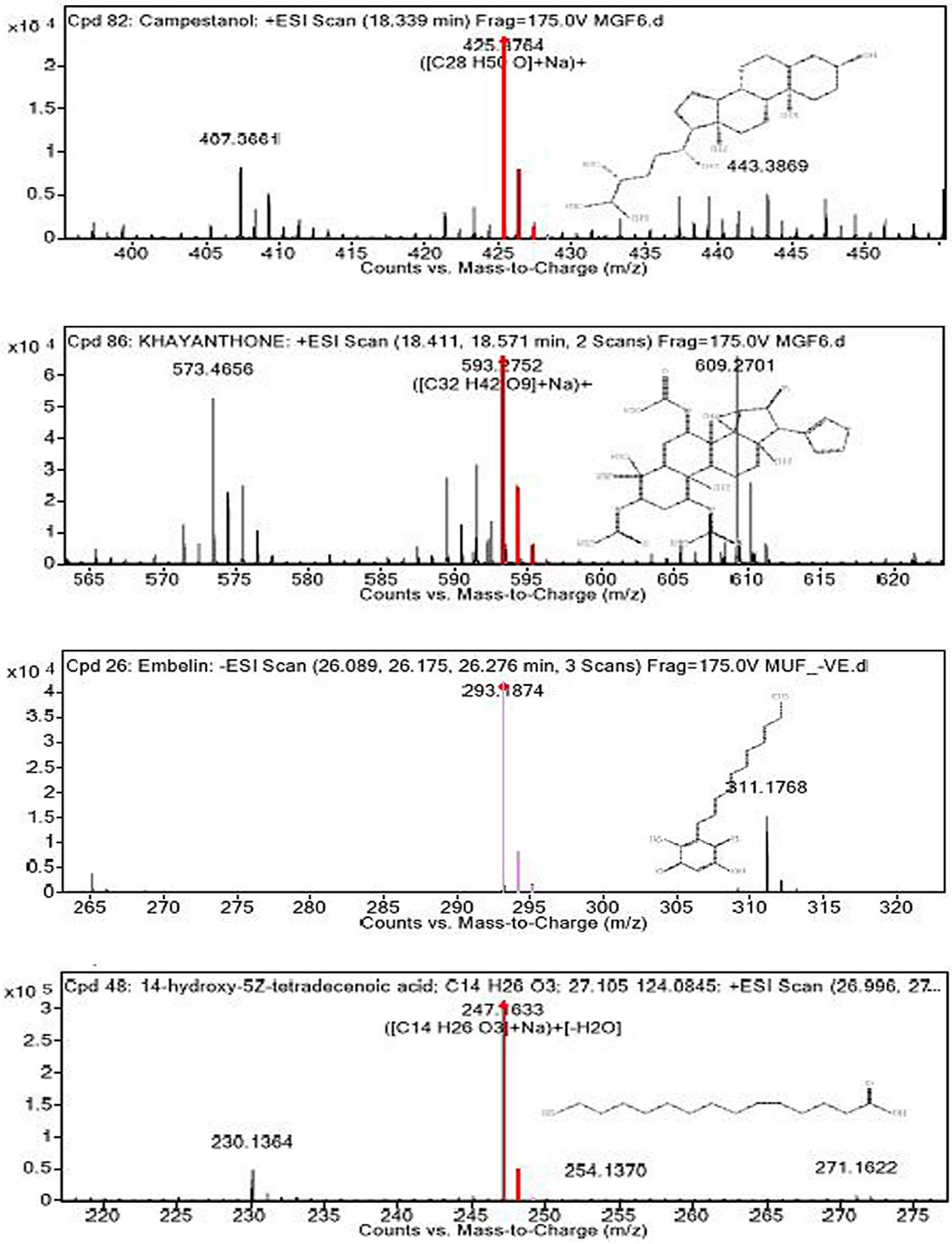



**Figure 17 (xii): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

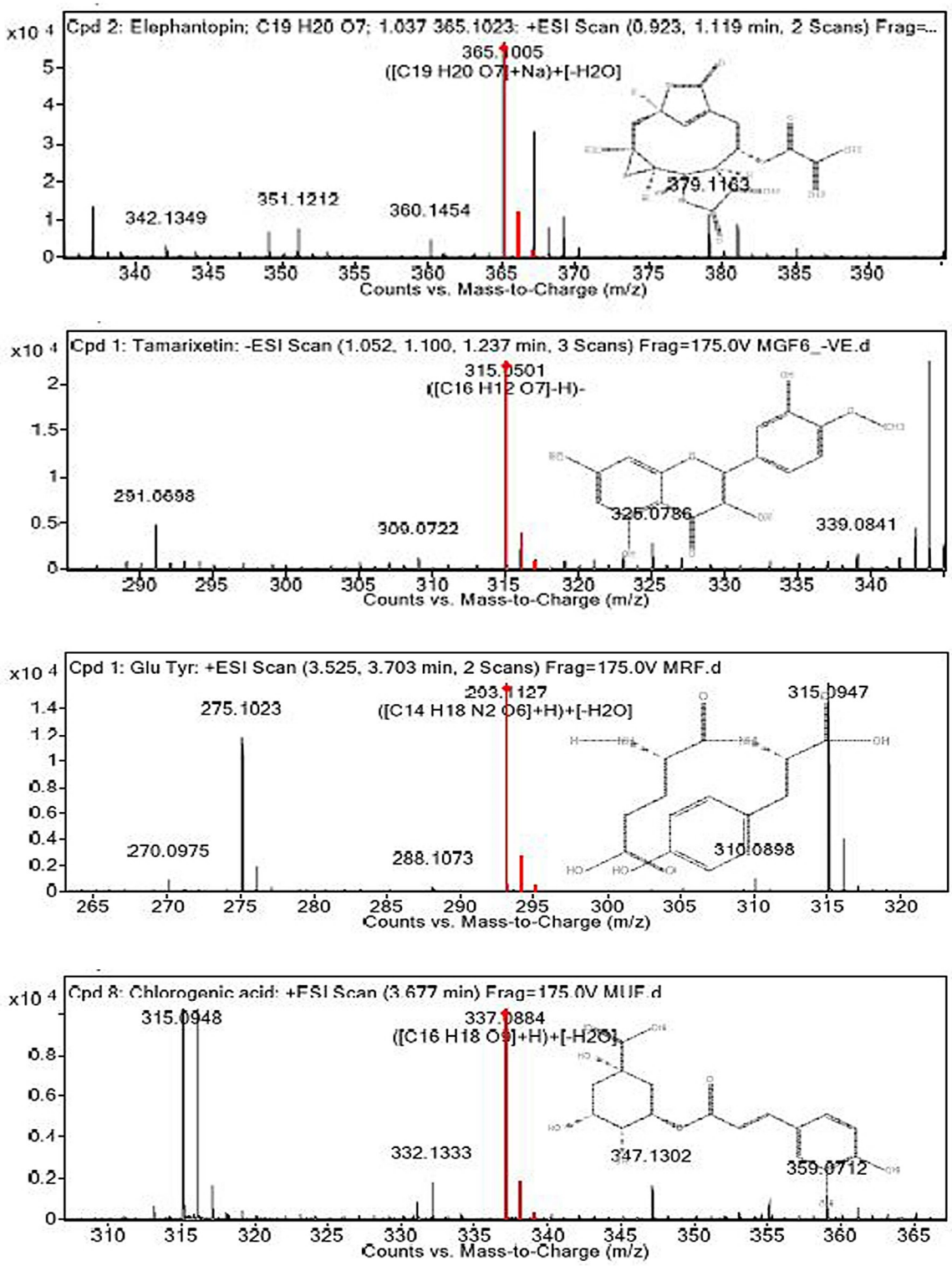



**Figure 17 (i): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

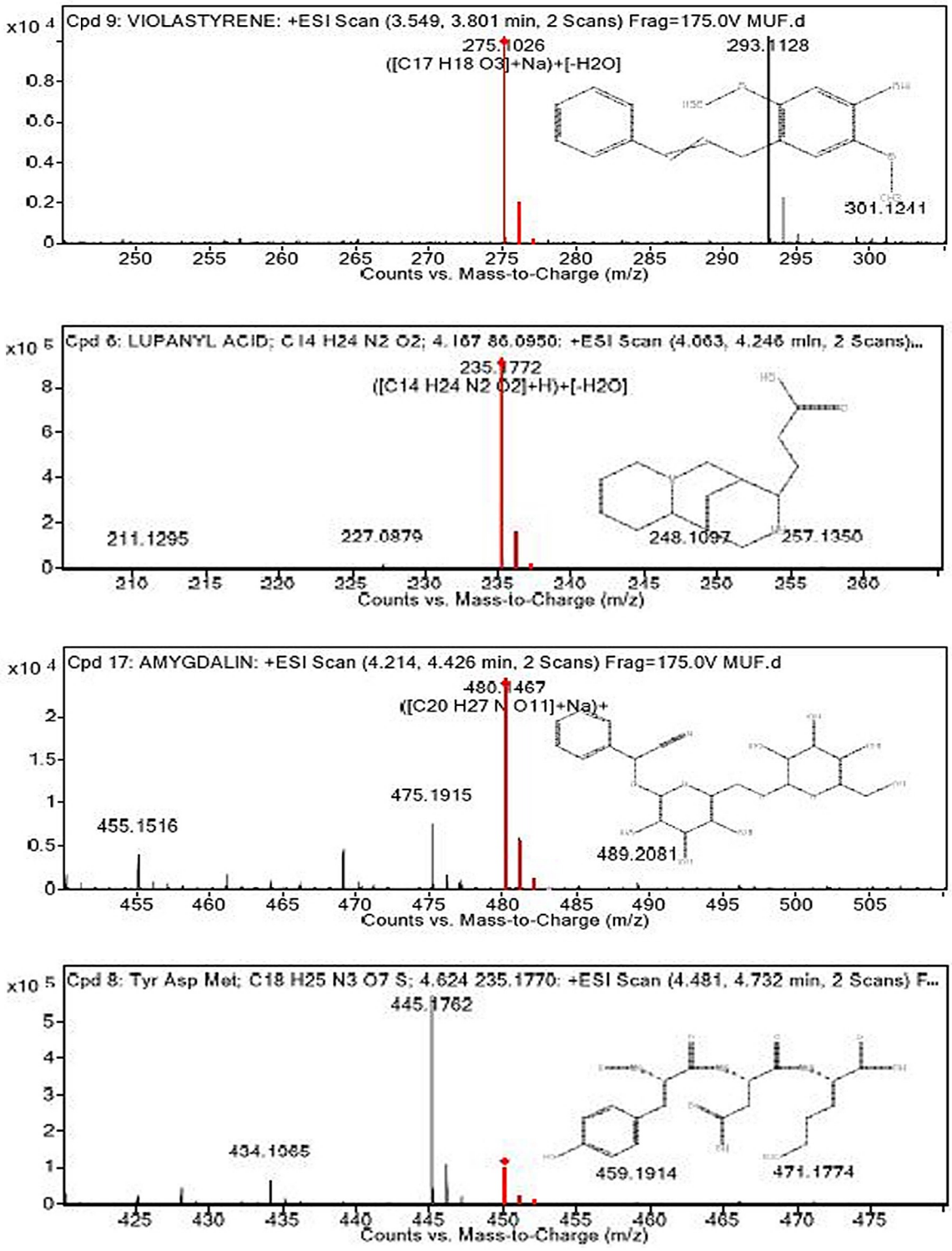



**Figure 17 (ii): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

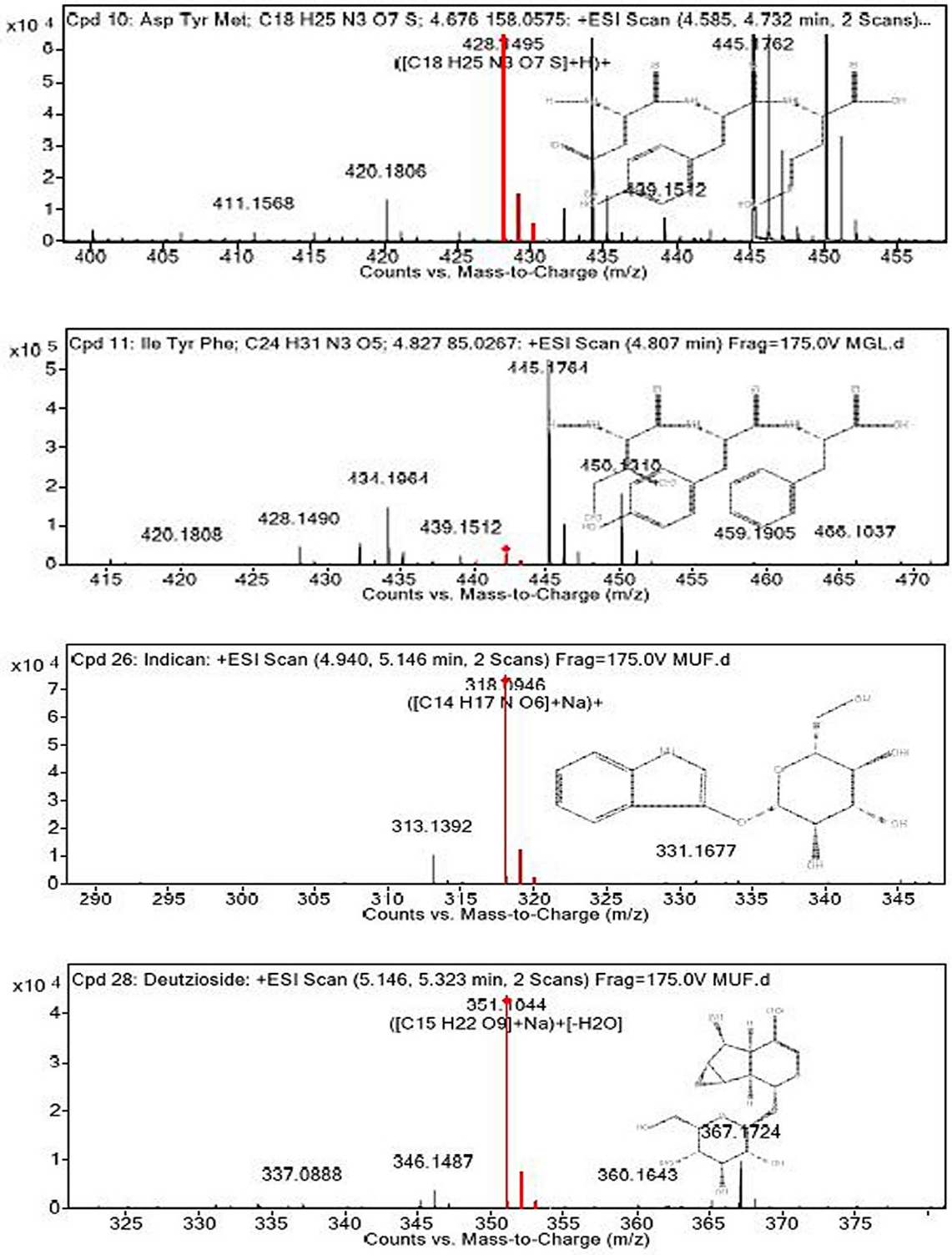



**Figure 17 (iii): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

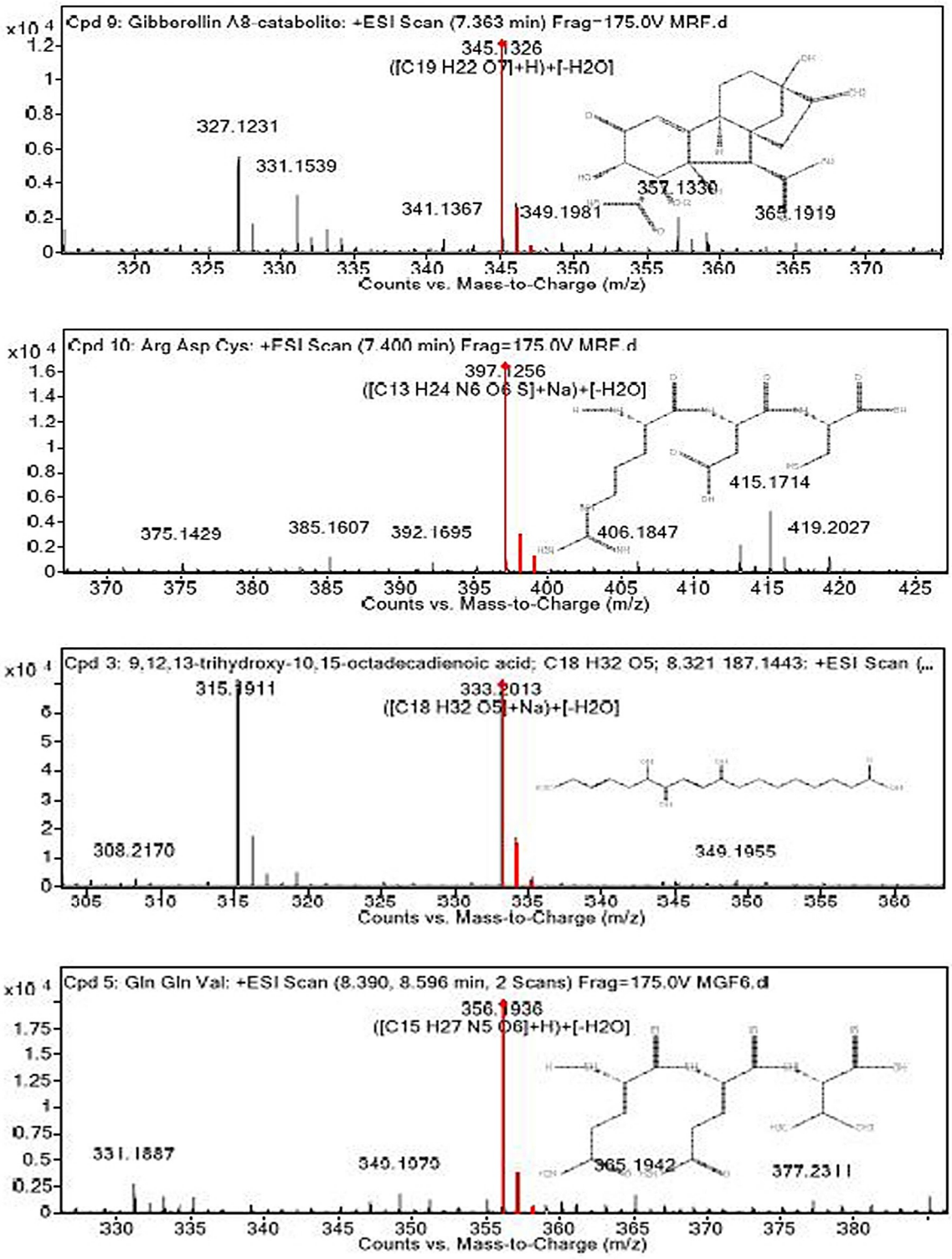



**Figure 17 (v): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

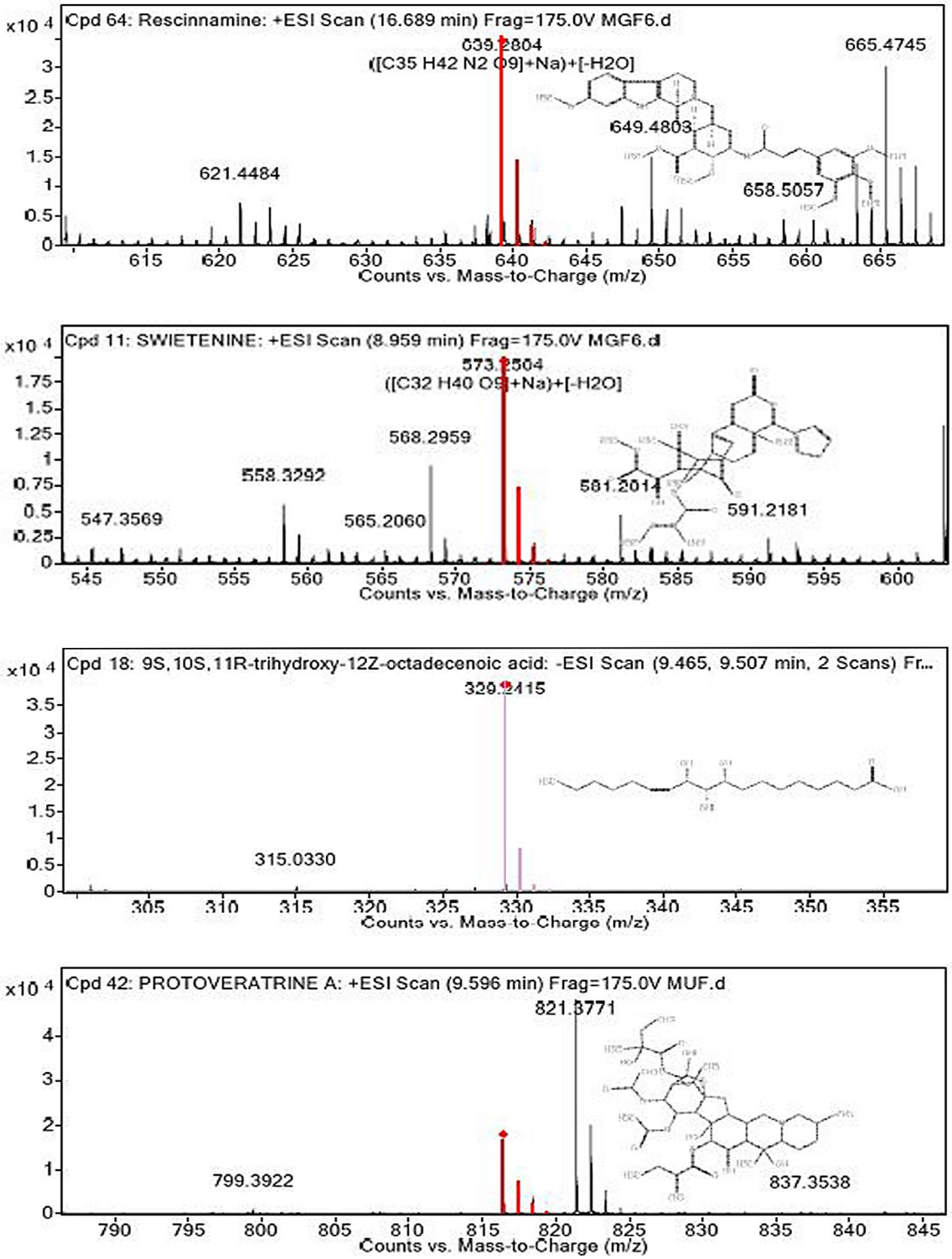



**Figure 17 (vi): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

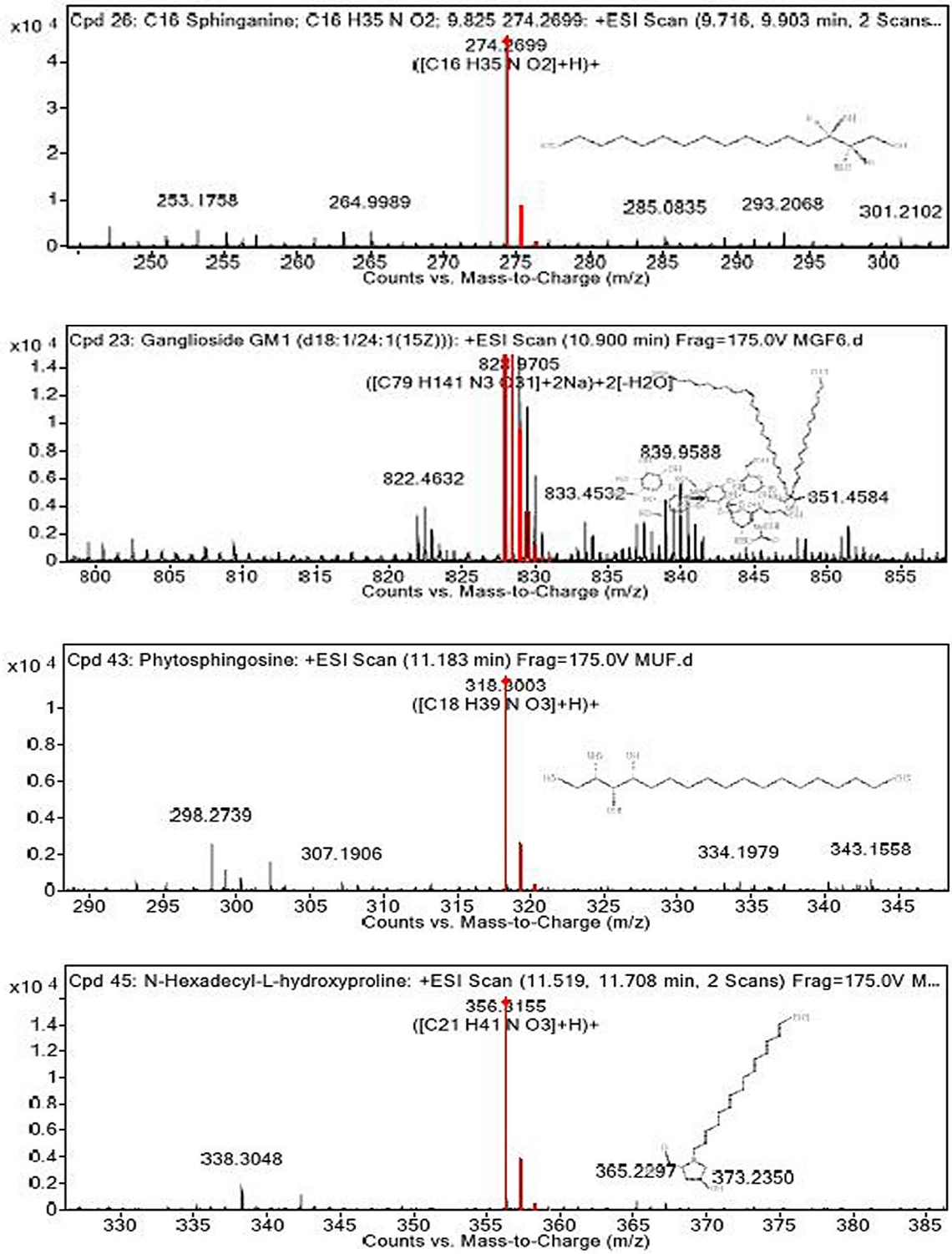



**Figure 17 (vii): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

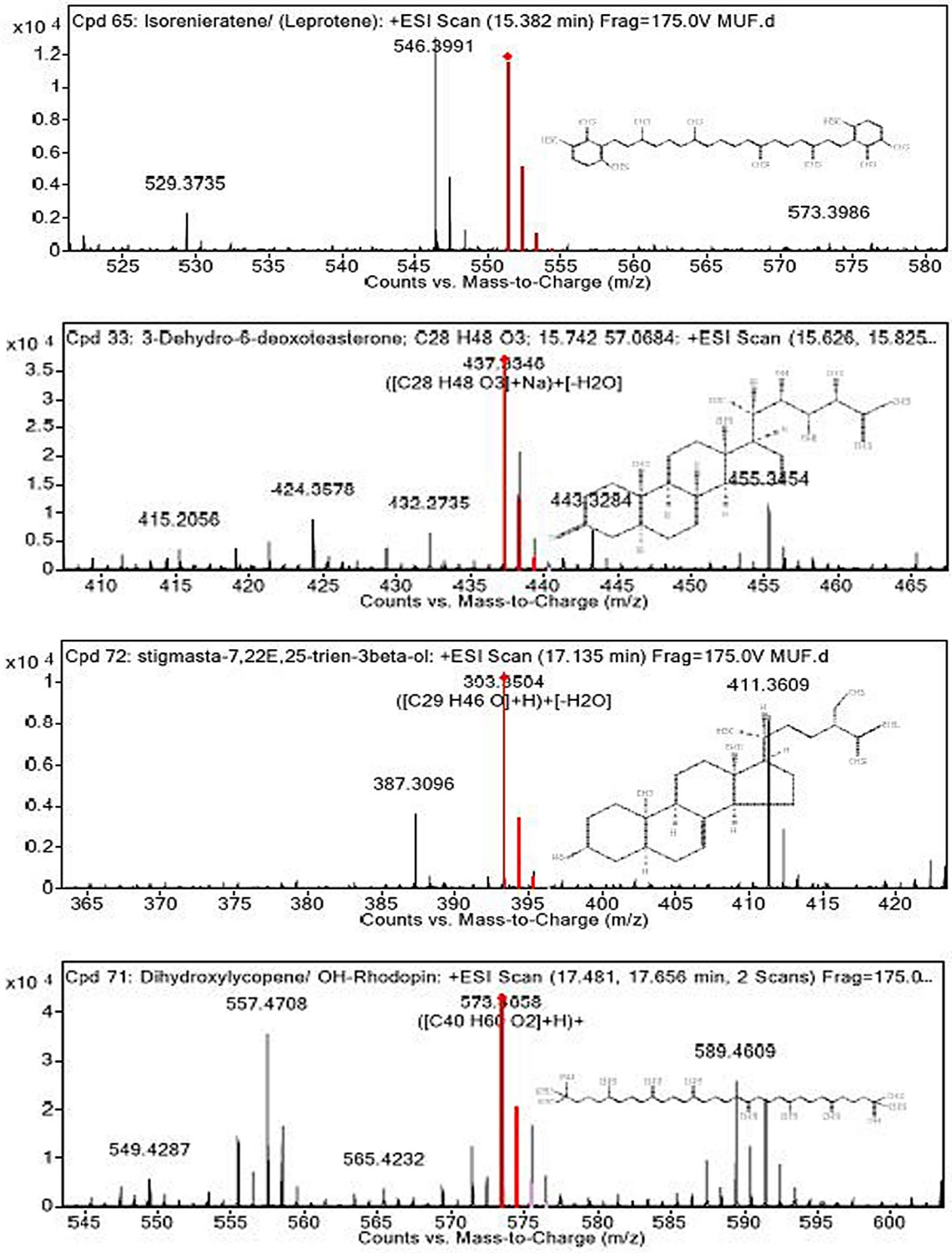



**Figure 17 (x): Mass spectra of compounds detected by HR-LC/MS analysis in the methanolic extract of selected species of *Memecylon***

The identified compounds belong to classes like terpenoids, steroids, fatty acids, biopeptides, hydroxyl benzoquinones, glycosides, alkaloids, esters, carotenes *etc*. A terpenoid compound swietenine was found to be common in *M. grande* leaf, fruit and *M. randerianum* fruit extracts. It possesses a strong signal (peak 5) with $[M+H]^+$ m/z 573.250 and retention time is 8.976 min. Lupanyl acid, aesculin, C16 sphinganine, 3-dehydro-6-deoxoteasterone and biopeptides are the major constituents of *M. grande* leaf extract. The presence of a flavonoid tamarixetin was confirmed in *M. grande* fruit extract with a molecular mass of 316.057 with $[M+H]^+$ m/z 315.050 at a retention time of 1.173 min. Ganglioside GM1 have the strongest signal (peak 2) with $[M+H]^+$ m/z 822.970. Rescinnamine, madecassic acid, campestanol and carotenes are also found to be part of non-volatile constituents of the plant extract. The presence of khayanthone, a limonoid compound was also detected in *M. grande* fruit extract with a retention time of 18.523 min.

The presence of bergenin and 9,12,13-trihydroxy-10,15-octadecadienoic acid are noticed in *M. randerianum* leaf extract. The compound 9,12,13-trihydroxy-10,15-octadecadienoic acid is a fatty acid, which shows a stronger signal in peak 3 with a m/z ratio of 333.201. Violastyrene, gibberellin A8-catabolite, rescinnamine, β-erythroidine, glycerol palmitate, 6-deoxocastasterone and cosmosiin hexaacetate were found in *M. randerianum* fruit extract. Ganglioside GM1, a lipid molecule possess a stronger signal with $[M+H]^+$ m/z 822.970 at 10.9220 min. Rescinnamine is an alkaloid compound detected at 8.925 min with 639.280 $[M+H]^+$ m/z ratio. Among the selected *Memecylon* species, β-erythroidine is the only alkaloid compound that resulted in *M. randerianum* fruit extract through the HR-LC/MS analysis.

*M. umbellatum* leaf extract possess elephantopin, a sesquiterpene lactone as the strongest signal with $[M+H]^+$ m/z ratio of 365.100 at a retention

time of 1.037 min. In addition, 14-hydroxy-5Z-tetradecenoic acid, a hydroxy fatty acid was also noticed in the extract. *M. umbellatum* fruit extract possesses a diverse array of non-volatile chemical constituents. It includes alkaloids, quinones, glycoproteins, sterols, carotene, terpenoids and fatty acids. Protoveratrine A, an alkaloid compound possess a strong signal with 821.377 $[M+H]^+$ m/z. Embelin, a benzoquinone was noticed at 26.201 min. 9S,10S,11R-trihydroxy-12 Z-octadecenoic acid and 8,13-dihydroxy-9,11-octadecadienoic acid are the fatty acids found in *M. umbellatum* fruit extract. A glycoside compound noticed at 4.338 min of analysis, was identified as amygdalin. Stigmasta-7, 22 E, 25-trien-3beta-ol, a sterol was resulted with $[M+H]^+$ m/z ratio 303.350. Chlorogenic acid, violastyrene, indican, deutzioside, norstictic acid, rescinnamine, phytosphingosine, N-hexadecyl-L-hydroxyproline, 6b,11b,16a,17a,21-pentahydroxypregna-1,4-diene-3,20-dione16,17-acetonide and isorenieratene are the revealed non-volatile constituents of the plant extract. While comparing the non-volatile composition of *Memecylon* species, fruit extract shows much more diverse phytoconstituents. The wide spectra of bioactive phytochemicals present in *Memecylon* species are revealed through the HR-LC/MS analysis.

## PHASE III- BIOACTIVITY STUDIES

### a) FREE RADICAL SCAVENGING ACTIVITY STUDIES

Free radical scavenging activity of the selected *Memecylon* species was analyzed through four different assays, such as DPPH, hydroxyl, nitric oxide and superoxide radical scavenging assay.

#### 1) DPPH free radical scavenging assay

In DPPH free radical scavenging assay, a dose-dependent scavenging activity was shown by all the selected plant extracts. The selected concentrations of the plant extracts for the assays were 12.5, 25, 50, 100 and

200 µg/mL. The calibration curve was prepared by using ascorbic acid as the standard (**Figure 18**). The highest scavenging activity was shown by 200 µg/mL concentration of *M. grande* fruit extract. *M. grande* fruit extract exhibit an inhibition percentage of 75.77 ± 0.01. This is followed by *M. umbellatum* fruit extract, which possesses a scavenging effect of 73.97 ± 1.22% in 200 µg/mL concentration (**Figure 19**). *M. umbellatum* leaf extract shows an activity of 70.17 ± 0.50 %. The lowest DPPH scavenging activity was resulted in *M. grande* leaf extract with 64.37 ± 2.05%. *M. randerianum* leaf and fruit extracts exhibit moderate scavenging potential of 68.44 ± 0.08 and 71.92 ± 0.52 % respectively. Ascorbic acid was used as a standard which showed inhibition of 96.15 ± 0.07% at higher concentrations. The $IC_{50}$ value of *M. grande* fruit extract was 83.91 ± 0.14 µg/mL and that of standard compound ascorbic acid is 48.84 ± 1.50 µg/mL (**Table 10**). These results are pointing that among the tested six plant extracts, *M. grande* fruit extract shows significant antioxidant potential.

**2) Hydroxyl free radical scavenging activity**

In hydroxyl radical assays, as the concentration increases, scavenging potential also increases. The standard was gallic acid and the calibration curve was plotted using the mean values obtained (**Figure 20**). Here also *M. grande* fruit extract shows the highest activity *ie.*, 61.69 ± 0.52%. This is followed by *M. umbellatum* fruit extract with 53.46 ± 1.89% (**Figure 21**). *M. umbellatum* leaf extract possesses 46.16 ± 0.15%, similarly, *M. randerianum* fruit extract was with 46.16 ± 0.02% activity. *M. randerianum* leaf extract exhibit 45.01 ± 0.01% activity. The lowest activity was observed in *M. grande* leaf extract with 36.77 ± 0.62%. Gallic acid was used as a standard compound with an inhibitory effect of 62.98 ± 0.16% in 200 µg/mL. $IC_{50}$ value of *M. grande* fruit was 1231± 0.48 µg/mL. $IC_{50}$ value of the standard compound was 1347 ± 0.27 µg/mL (**Table 10**). Here the inhibitory concentration of the standard

compound was higher as compared to the effective plant extract. So these results confirm that *M. grande* fruit extract is a good hydroxyl radical scavenging agent.

**Table 10**: **The effect of methanolic extracts of selected species of *Memecylon* in different antioxidant assays IC $_{50}$ Values (µg/mL)**

| Plants | DPPH radical scavenging assay | Hydroxyl radical scavenging assay | Nitric oxide radical scavenging assay | Superoxide radical scavenging assay |
|---|---|---|---|---|
| Standard | 48.84 ± 1.5 | 1347.51 ± 0.27 | 346.20 ± 0.01 | 238.35 ± 0.03 |
| MGL | 125.90 ± 0.23 | 2773.93 ± 0.07 | 2414.51 ± 0.02 | 1211.01 ± 0.04 |
| MGF | 83.91 ± 0.14 | 1231 ± 0.48 | 696.73 ± 0.06 | 698.99 ± 0.03 |
| MRL | 118.36 ± 0.08 | 2103.31 ± 0.08 | 1348.38 ± 0.02 | 1960.11 ± 0.01 |
| MRF | 104.17 ± 0.13 | 2029.57 ± 0.14 | 1081.61 ± 0.01 | 1311.24 ± 0.02 |
| MUL | 108.08 ± 0.09 | 1973.05 ± 0.02 | 1413.44 ± 0.03 | 1210.93 ± 0.03 |
| MUF | 91.10 ± 0.12 | 1696.73 ± 0.05 | 916.98 ± 0.04 | 1129.34 ± 0.01 |

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits; $IC_{50}$ – concentration effecting 50% inhibition. Values are expressed as mean ± standard error.

### 3) Nitric oxide free radical scavenging activity

Nitric oxide assay also shows notable trends of scavenging potential. *M. grande* fruit extract shows the highest scavenging activity of 76.85 ± 0.08% and the lowest was in *M. grande* leaf extract with 40.86 ± 0.20%. *M. umbellatum* fruit and leaf extracts exhibit a scavenging potential of 75.23 ± 0.01% and 59.61 ± 0.01% respectively. Gallic acid was used as the standard compound, which shows an inhibition of 92.9 ± 0.51% (**Figure 22**). The $IC_{50}$ value of *M. grande* fruit was 696.73 ± 0.06 µg/mL and that of the standard compound was 346.20 ± 0.01 µg/mL (**Table 10**). In the case of *M. randerianum*, fruit extract exhibit 65.74 ± 0.05 % of activity and similarly leaf

extract possess 63.54 ± 0.02% of activity (**Figure 23**). In this assay, all extracts show a moderate antioxidant activity.

**4) Superoxide free radical scavenging activity**

Superoxide assay shows a significant scavenging potential. A dose-dependent activity was shown by the *Memecylon* extracts. *M. grande* fruit extract shows the highest scavenging activity of 72.17 ± 0.02% (**Figure 25**). The lowest activity was shown by *M. randerianum* leaf extract with 47.36 ± 0.01%. *M. grande* leaf extract with 58.6 ± 0.36% of activity and *M. randerianum* fruit extract exhibit 63.03 ± 0.01% activity. In the case of *M. umbellatum* leaf and fruit extract, 61.09 ± 0.01 and 65.14 ± 0.02% of activity was resulted respectively. The standard compound, ascorbic acid shows a potential scavenging activity of 90.68 ± 0.27% (**Figure 24**). The $IC_{50}$ value of *M. grande* fruit was 698 ± 0.03 µg/mL and that of the standard compound was 238.35 ± 0.03 µg/mL (**Table 10**). Here also a moderate antioxidant activity was shown by all the plant extracts.

In all the antioxidant assays, the selected plant species follow similar trends of activity. *M. grande* fruit extract has the highest scavenging potential and lowest was in *M. grande* leaf extract. An exception was noted in superoxide radical assay, where the lowest activity was shown by *M. randerianum* leaf extract. *M. randerianum* and *M. umbellatum* exhibit a moderate range of activity in all the assays. In the four antioxidant assays, *M. grande* fruit extract was found to be more efficient in hydroxyl radical scavenging activity. In DPPH, nitric oxide, and superoxide radical scavenging assays, it shows a moderate scavenging potential. From the analysis, it may also be concluded that the fruit extract of *Memecylon* species was more active than the leaf extract.

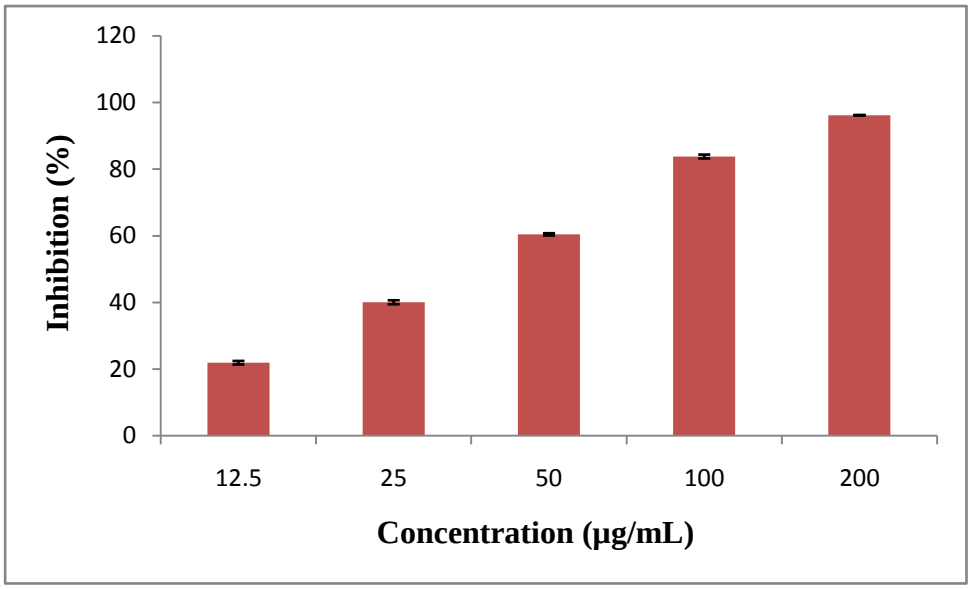



**Figure 18**: ***In vitro*** **DPPH radical scavenging activity of ascorbic acid**

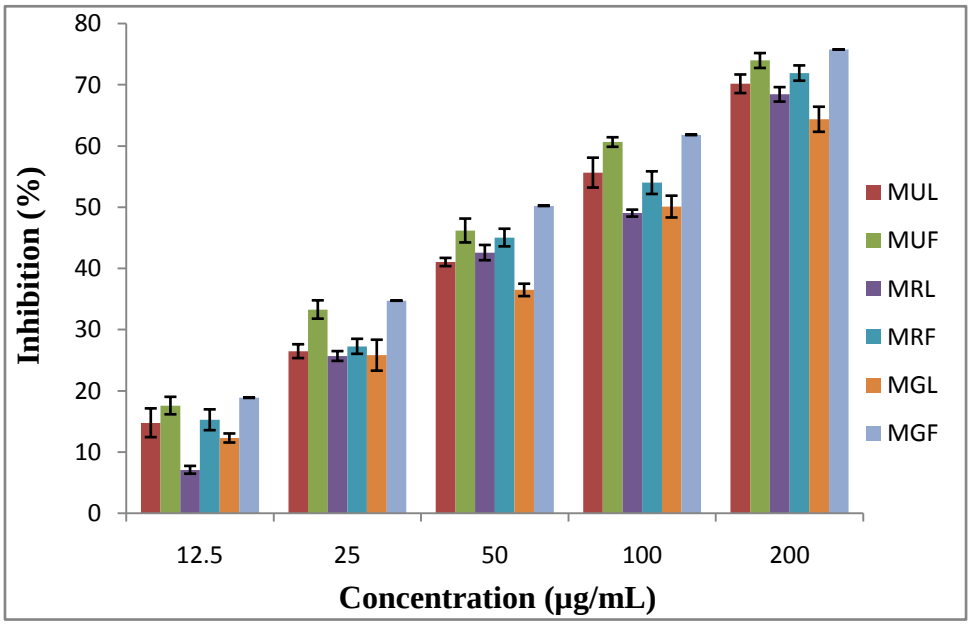



**Figure 19**: ***In vitro*** **DPPH radical scavenging activity of selected *Memecylon* species**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits.

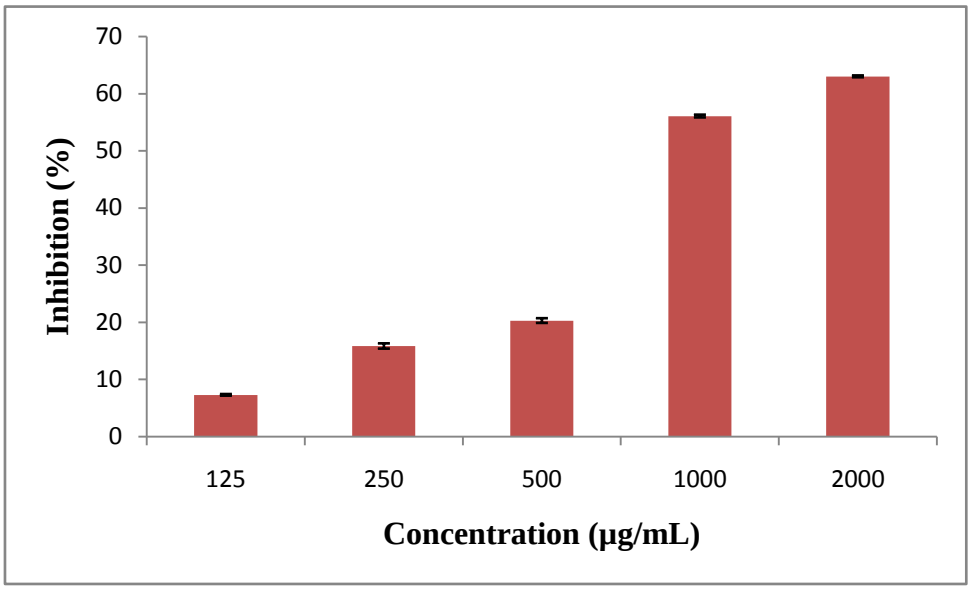



**Figure 20: *In vitro* hydroxyl radical scavenging activity of gallic acid**

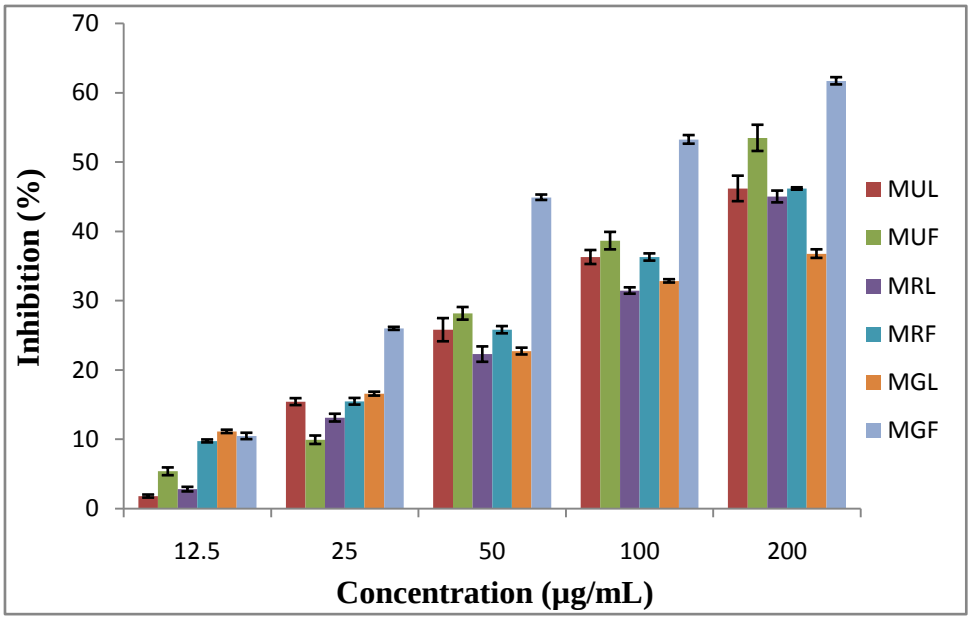



**Figure 21: *In vitro* hydroxyl radical scavenging activity of selected *Memecylon* species**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits.

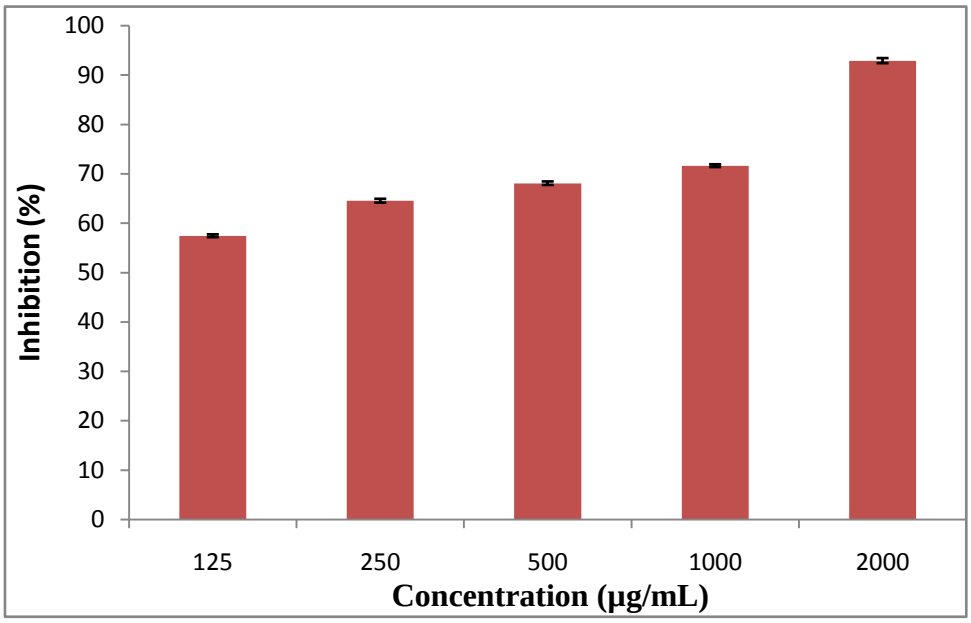



**Figure 22**: ***In vitro*** **nitric oxide radical scavenging activity of gallic acid**

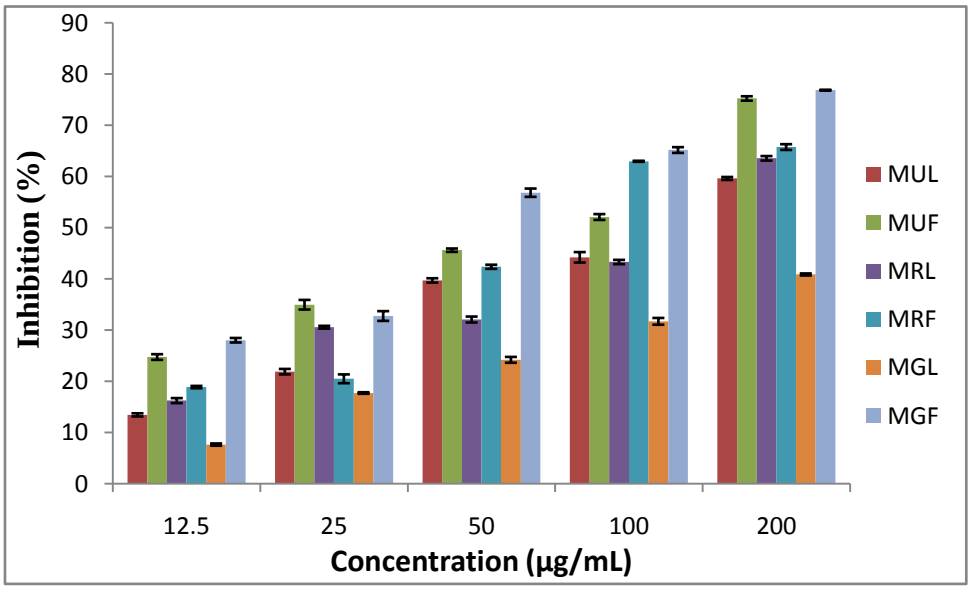



**Figure 23**: ***In vitro*** **nitric oxide radical scavenging activity of selected *Memecylon* species**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits

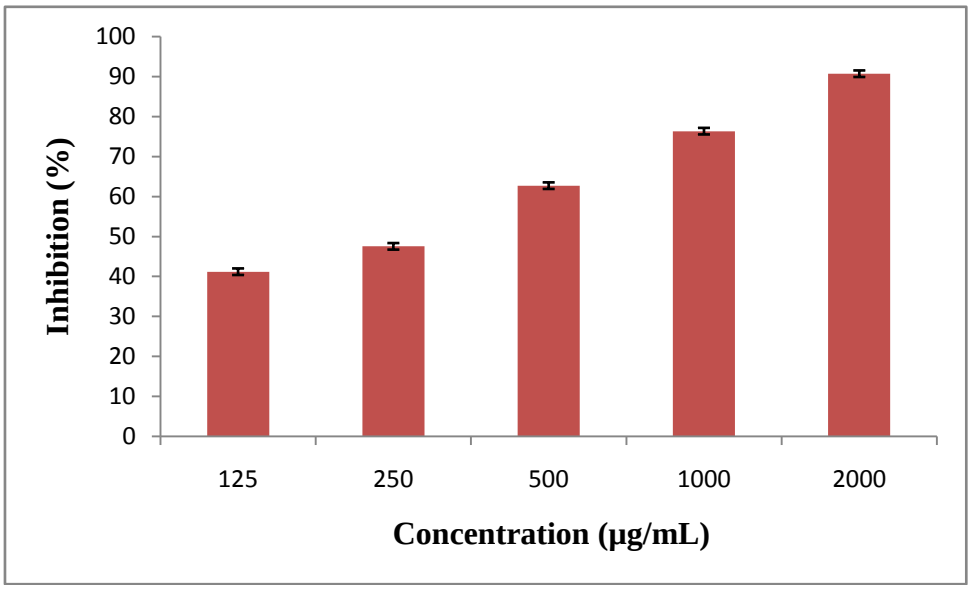



**Figure 24**: ***In vitro*** **superoxide radical scavenging activity of ascorbic acid**

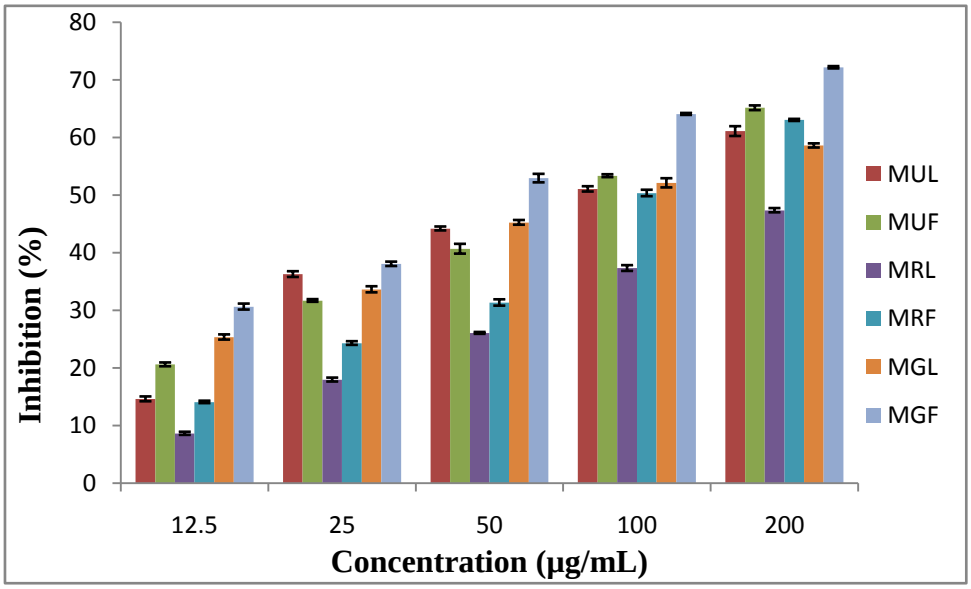



**Figure 25**: ***In vitro*** **superoxide radical scavenging activity of selected *Memecylon* species**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits

## b) CYTOTOXICITY SCREENING USING *ALLIUM CEPA*

The cytotoxicity potential of the selected *Memecylon* species is analyzed by using *A. cepa* root tip meristem. The selected concentrations of methanolic plant extracts are 100, 50, 25, 12.5 µg/mL. Distilled water was taken as negative control (NC) and hydrogen peroxide as positive control (PC). The cytotoxicity screening was carried on different time intervals like ½ hr, 2 hr and 24 hr. The toxic potential of plant extracts were analyzed through the assessment of mitotic index and aberration percentage. Dose dependent mitotic index and abnormality percentage were resulted, and found that time has no role in the cytotoxicity effect of the plant extracts. The abnormal cytotoxic stages of interphase, prophase, metaphase, anaphase, telophase and cytokinesis were also noticed.

The number of dividing cells reduces as the concentration of plant extract increases. The mitotic index of *A. cepa* root tip cell after treatment with *M. grande* leaf extract ranges from 43.66 ± 3.74 to 75.17 ± 3.33%. In negative control, it ranges from 89.1 ± 2.16 to 91.33 ± 2.10% and that of positive control is 40.83 ± 2.71 to 43.48 ± 7.67%. The lowest mitotic index was found in the 24 hr exposure time with 100 µg/mL concentration of the plant extract (**Figure 28 a**). In the ½ hr treatment (**Figure 26 a**) of *M. grande* leaf extract, mitotic index ranges from 51.96 ± 5.83 to 72.28 ± 4.63% in the range of extract concentration from 100-12.5 µg/mL. Similarly 2 hr treatment shows that mitotic index ranges from 60.33 ± 5.72 to 75.17 ± 3.33% (**Figure 27 a**) and that of 24 hr exposure period exhibit the mitotic index range from 43.66 ± 3.84 to 57.61 ± 2.68%. These results show that the exposure time has no role in cytotoxic effect. The abnormality percentage of *A. cepa* root cells treated with *M. grande* leaf extract ranges from 28.85 ± 2.43 to 89.4 ± 2.29%. It was found to be higher as compared to the positive control, which offers the highest abnormality percentage at 24 hr exposure period of 81.83 ± 5.76%

and that of negative control is 19.76 ± 2.49% (**Figure 28 b**). In ½ hr treatment, the abnormality percentage ranges from 89.4 ± 2.29 to 36.75 ± 2.64% (**Figure 26 b**) and in 2 hr treatment it was found to be 85.77 ± 1.85 to 28.85 ± 2.43% (**Figure 27 b**). The prolonged exposure period of 24 hr shows an abnormality percentage in a range of 89.31 ± 2.00 to 51.16 ± 6.35%. These results confirm that the dose dependent cytotoxicity effect offered by the *M. grande* leaf extract and the exposure time has no role in the toxic effect. *M. grande* fruit extract, induces a dose dependent toxic effect in *A. cepa* root tip cells. It exhibits a mitotic index in the range of 48.11 ± 7.39 to 70.63 ± 3.12% and the abnormality percentage from 27.15 ± 4.29 to 90.72 ± 1.45%. So *M. grande* leaf and fruit extracts show a prominent cytotoxicity. The abnormality percentage was found to be higher in *M. grande* fruit extract and it was found to be higher when compared to the positive control (80.1 ± 2.49 to 81.83 ± 5.76%). Here also ½ hr, 2 hr and 24 hr exposure time shows only a dose dependent cytotoxicity.

*M. randerianum* leaf extract offered an increase in mitotic index from 35.57 ± 4.00 to 64.36 ± 8.94% in a concentration range of 100-12.5 µg/mL at various time intervals. The mitotic index range of negative control is 89.1 ± 2.16 to 91.33 ± 2.10% and the highest mitotic index was seen in lowest concentration of 12.5 µg/mL at prolonged exposure time period (24 hr). The mitotic index in ½ hr treatment ranges from 38.73 ± 2.86 to 63.22 ± 8.73%, in 2hr exposure, range from 35.57 ± 4.00 to 61.67 ± 6.22% and in 24 hr exposure period shows 35.80 ± 2.05 to 64.36 ± 8.94% (**Figures 26 a, 27 a, 28 a**). As compared to the positive control (80.53 ± 3.11 to 81.83 ± 5.76%), *M. randerianum* leaf extract shows a higher abnormality percentage (35.66 ± 5.58 to 82.39 ± 2.74) in 2 hr exposure period. *M. randerianum* fruit extract shows a lower mitotic index range as compared to the leaf extract. It ranges from 46.33 ± 7.42 to 81.06 ± 5.24%. The abnormality percentage for *M.*

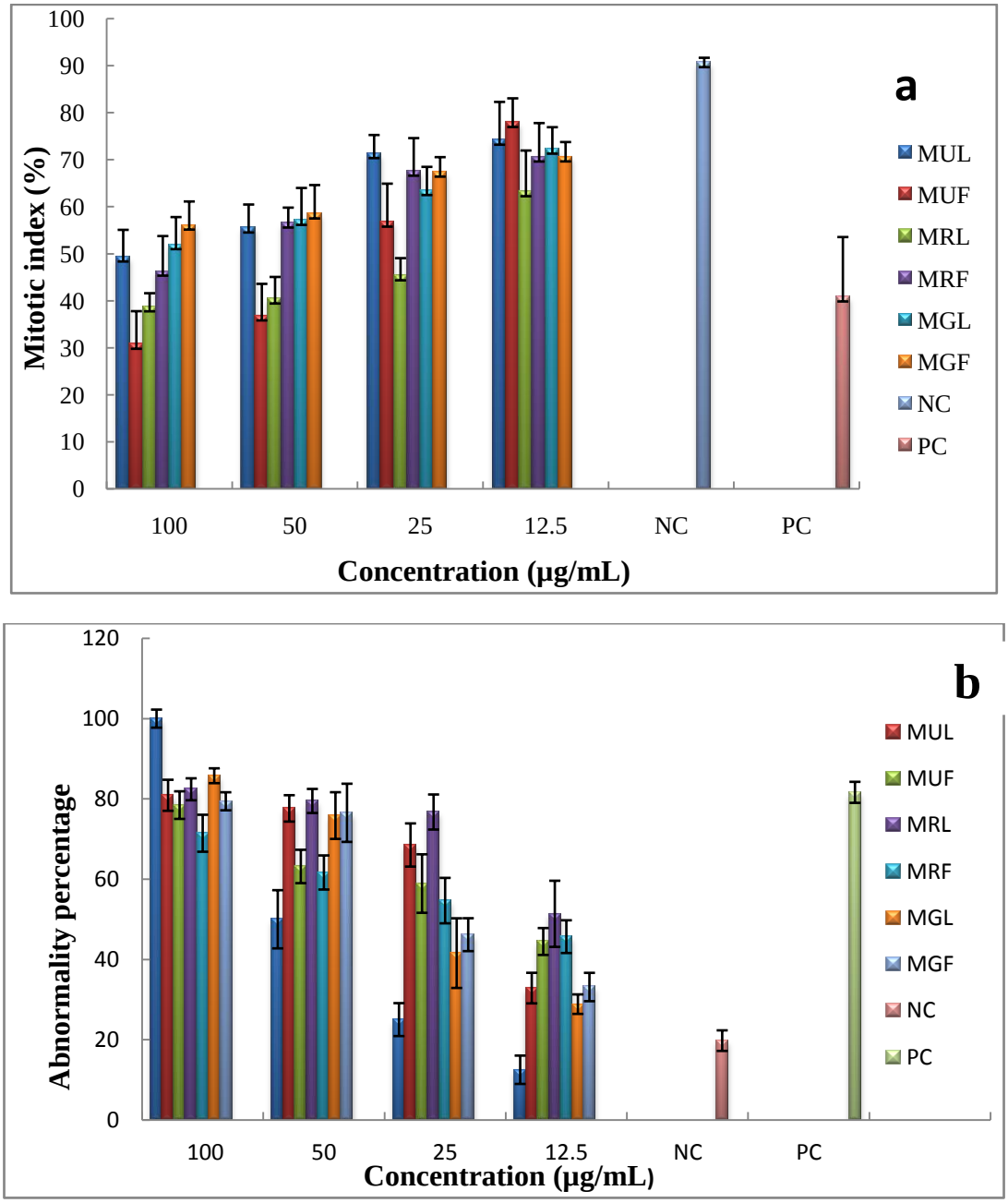



**Figure 26**: **The effects of different concentrations of methanolic extract of selected species of *Memecylon* on *Allium cepa* meristematic root tips after ½ hour treatment. a - Mitotic index, b - Abnormality percentage**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits; NC: Negative control; PC: Positive control. Values are represented as mean ± standard error.

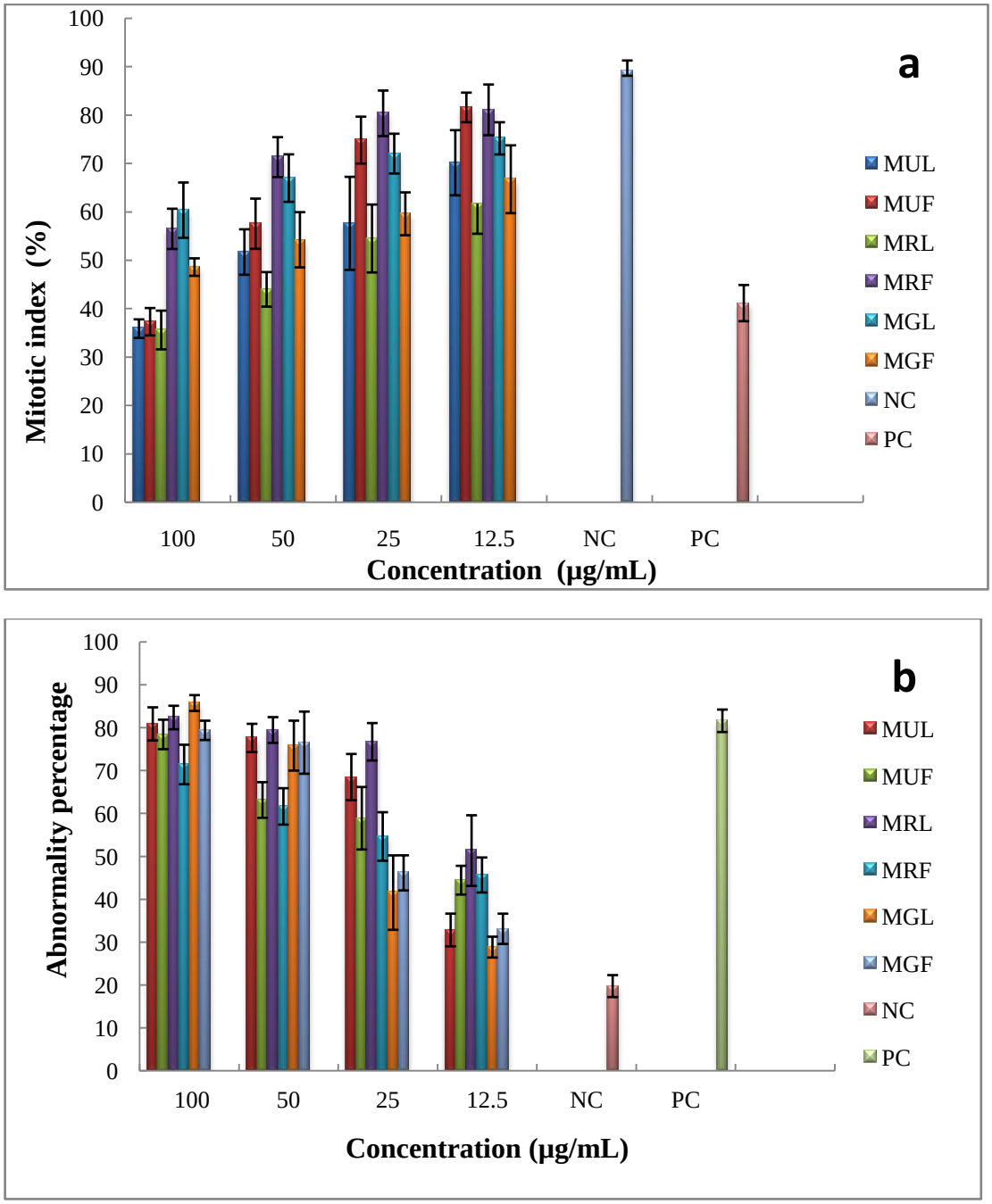
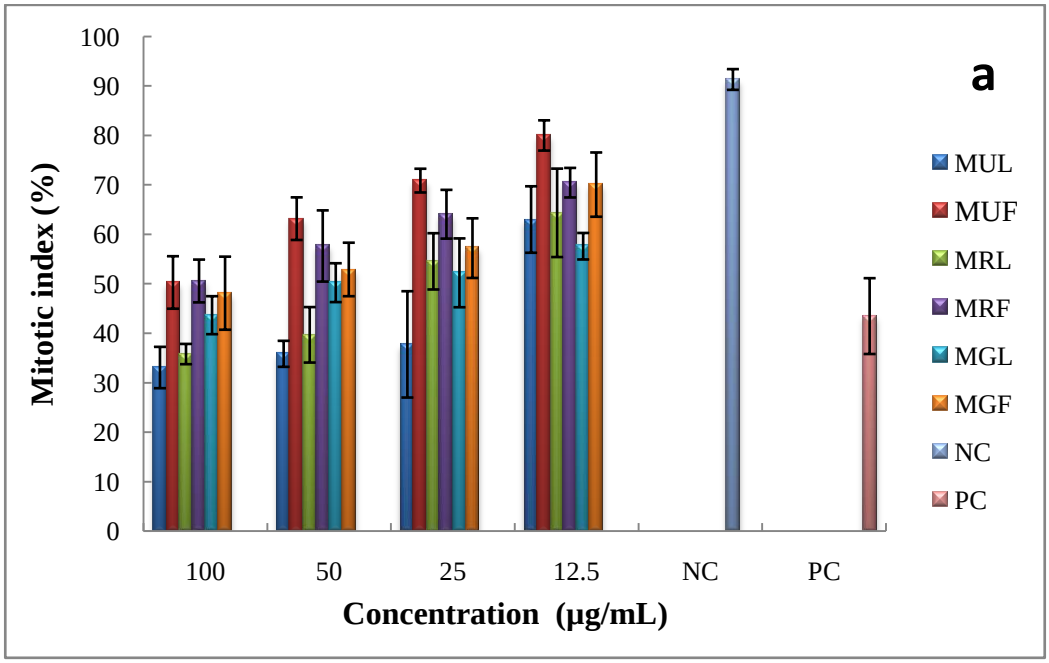



**Figure 27**: **The effects of different concentrations of methanolic extract of selected species of *Memecylon* on *Allium cepa* meristematic root tips after 2 hour treatment. a - Mitotic index, b - Abnormality percentage**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits; NC: Negative control; PC: Positive control. Values are represented as mean ± standard error.



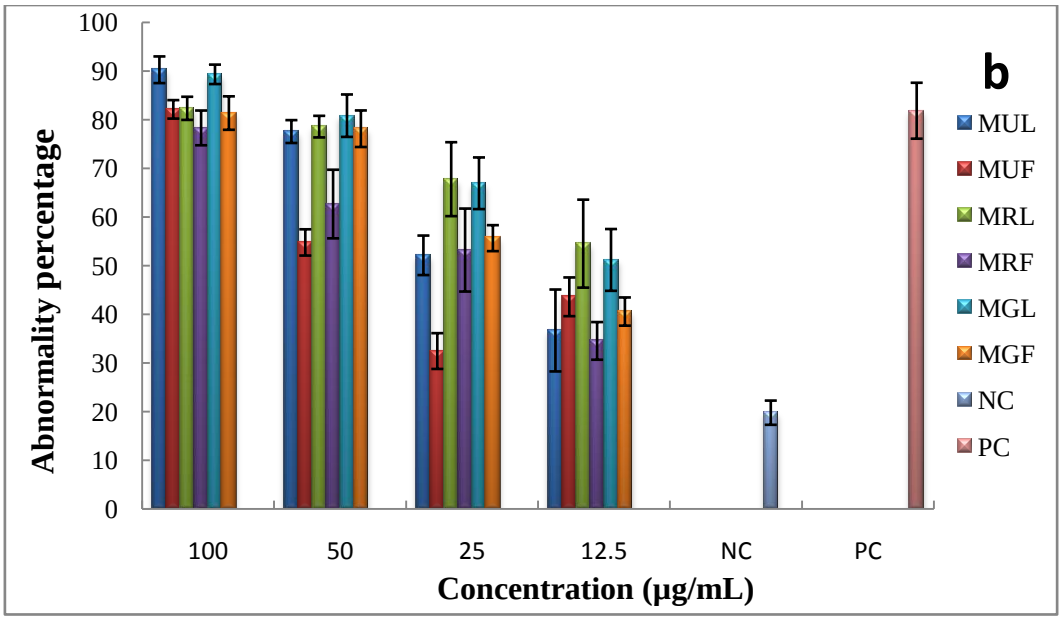




**Figure 28**: **The effects of different concentrations of methanolic extract of selected species of *Memecylon* on *Allium cepa* meristematic root tips after 24 hour treatment. a - Mitotic index, b - Abnormality percentage**

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits; NC: Negative control; PC: Positive control. Values are represented as mean ± standard error.

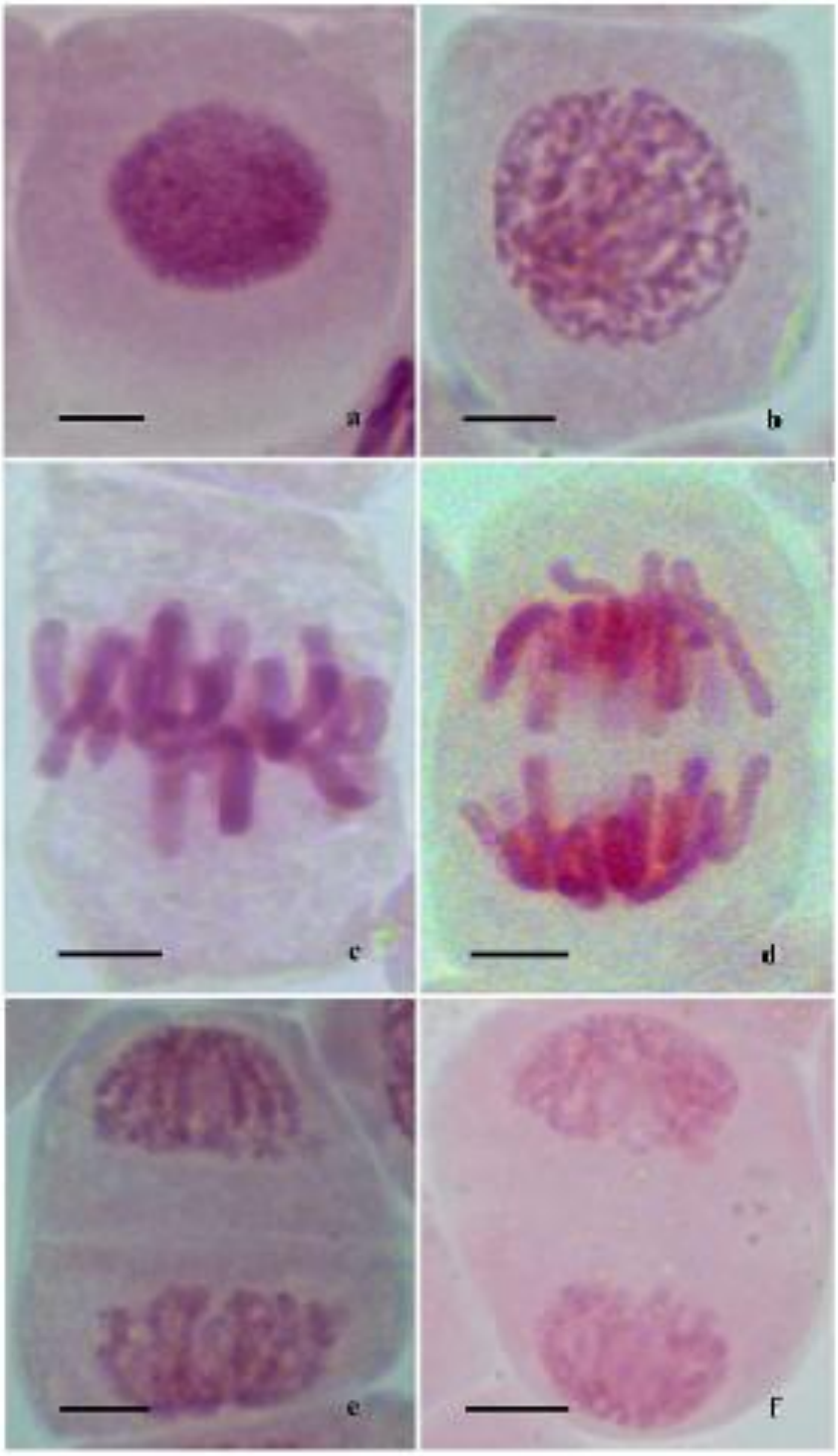



**Plate 9: Normal mitotic stages in *Allium cepa*** **a** - interphase, **b** - prophase, **c** - metaphase, **d** - anaphase, **e** - telophase, **f** - cytokinesis, Bar - 10 µm

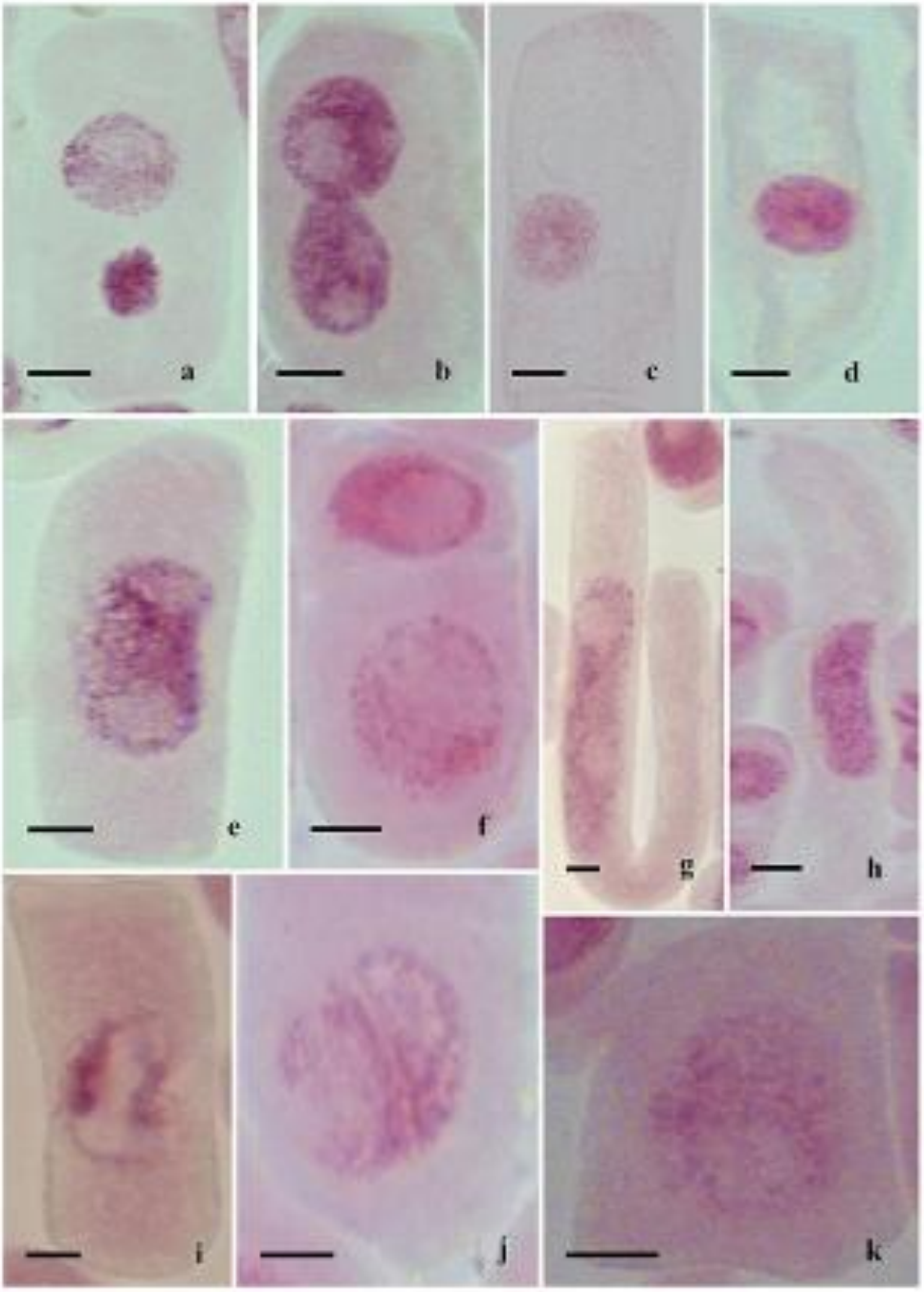



**Plate 10: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at interphase a** - Binucleate cell showing non-synchronous chromatin condensation, **b** - Binucleate cell showing single and double nuclear lesions, **c** - Cytoplasmic vacuolation and single nuclear lesion, **d** - Cytoplasmic vacuolation, **e** - Double nuclear lesion, **f** - Macro and micro cell formation, **g** - Double nuclear lesions in a strap cell, **h** - Strap cell, **i** - Nuclear disintegration, **j** - Nuclear breakage, **k** - Pulverized chromatin leading to ghost cell formation, Bar - 10 µm

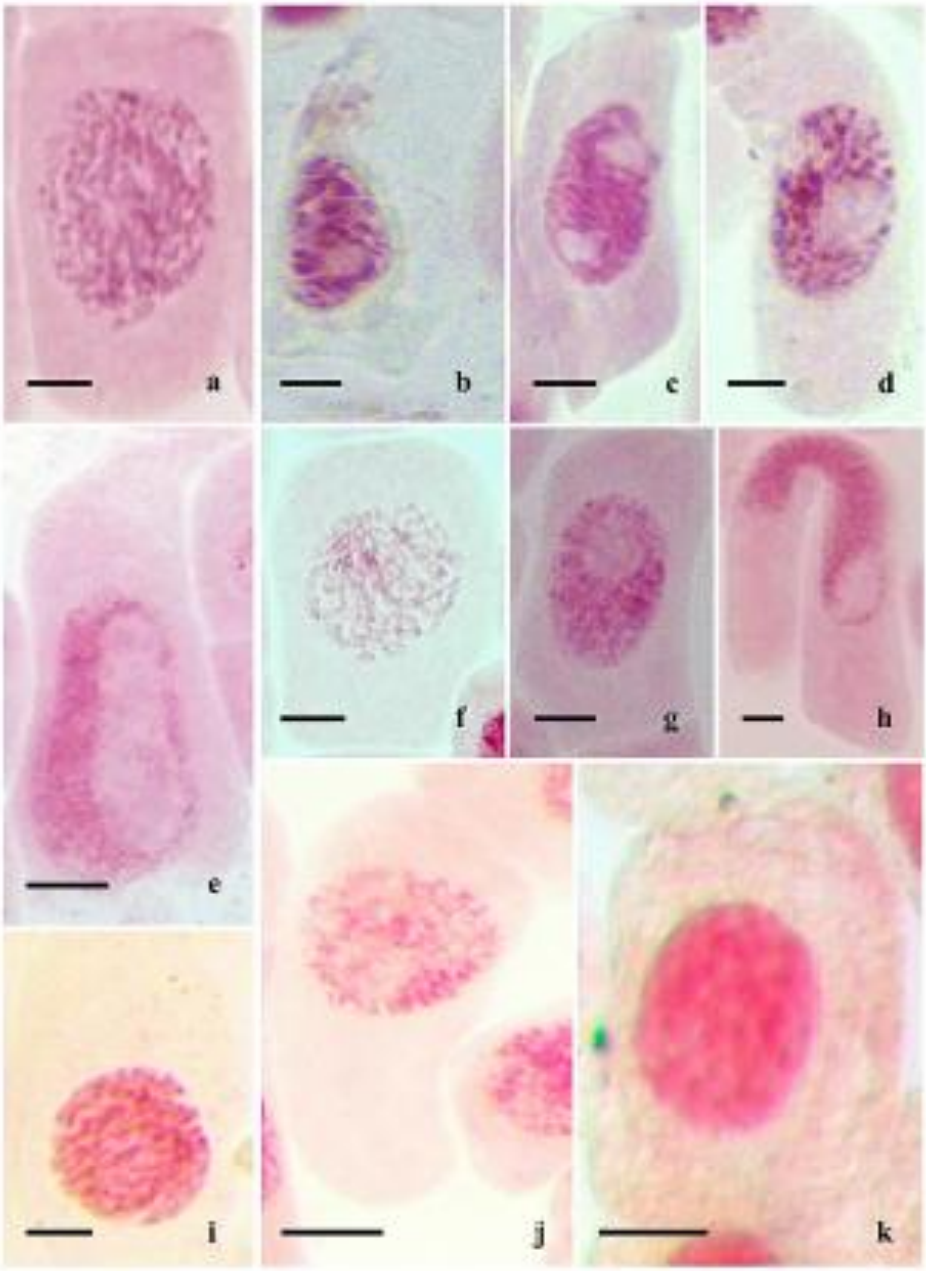



**Plate 11: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at prophase a** - Chromatin erosion, **b** - Chromatin granule and fragmented chromatin, **c** - Double nuclear lesion, **d** - Nuclear lesion and erosion at early prophase, **e** - Large nuclear lesion, **f** - Pulverized chromatin, **g** - Sticky prophase showing single lesion, **h** - Strap cell showing lesion, **i** - Nuclear breakage, **j** - Pulverized chromatin, **k** - Coagulated prophase, Bar - 10 µm

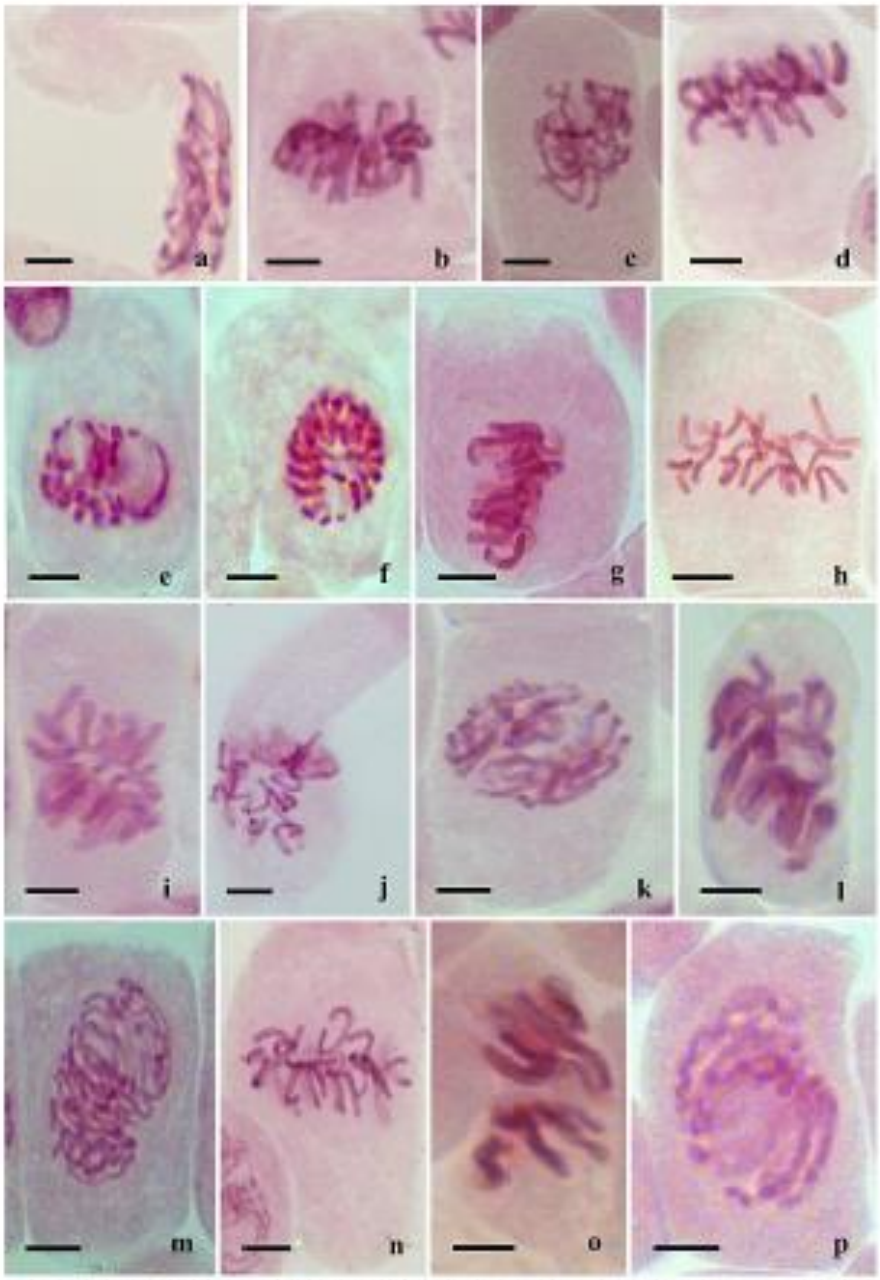



**Plate 12: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at metaphase a** - Chained early metaphase, **b** - Chromosome clumbing, **c** - Chromosome fragments, **d** - Displaced metaphase, **e** - Chromosome gaps, **f** - Ball metaphase, **g** - Diagonal early metaphase, **h** - Partial C- metaphase, **i** - Chromosome rosette, **j** - Displaced metaphase, **k** - Disturbed early metaphase, **l** - Somatic pairing, **m** - Early diagonal metaphase in a hyperploid cell, **n** - Chromosome vagrants, **o** - Extreme stickiness and misorientation, **p** - Lesion and chromosome gaps at early metaphase, Bar - 10 µm

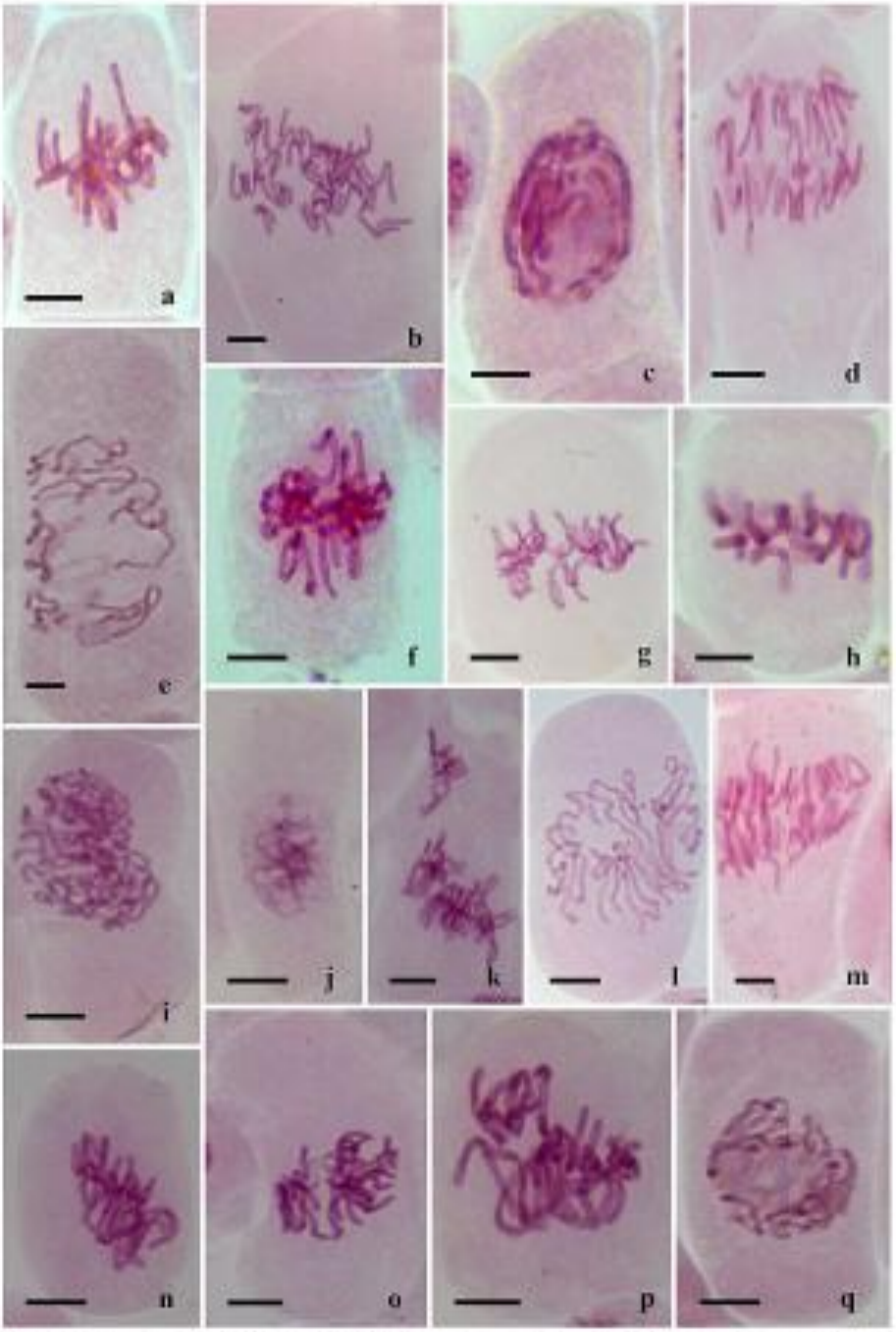



**Plate 13: Chromosomal aberrations induced by extracts of *Monocyclon* spp. in *A. cepa* at metaphase a** - Tropokinesis showing partial C-metaphase, **b** - Polyploid cell, **c** - Chained metaphase showing stickiness, **d** - Scattered and misoriented chromosomes, **e** - Scattered metaphase, **f** - Stellate metaphase, **g** - Tropokinesis showing displacement of chromosome, **h** - Sticky metaphase in a hypoploid cell, **i** - Abnormal condensation at early metaphase, **j** - Chromosome erosion, **k** - Displaced chromosome groups, **l** - Exposure of chromosome scaffold, **m** - Vagrants in polyploid cell, **n** - C- metaphase, **o** - Misorientation of chromosomes, **p** - Partial C- metaphase, **q** - Early ball metaphase showing lesion, Bar - 10 µm

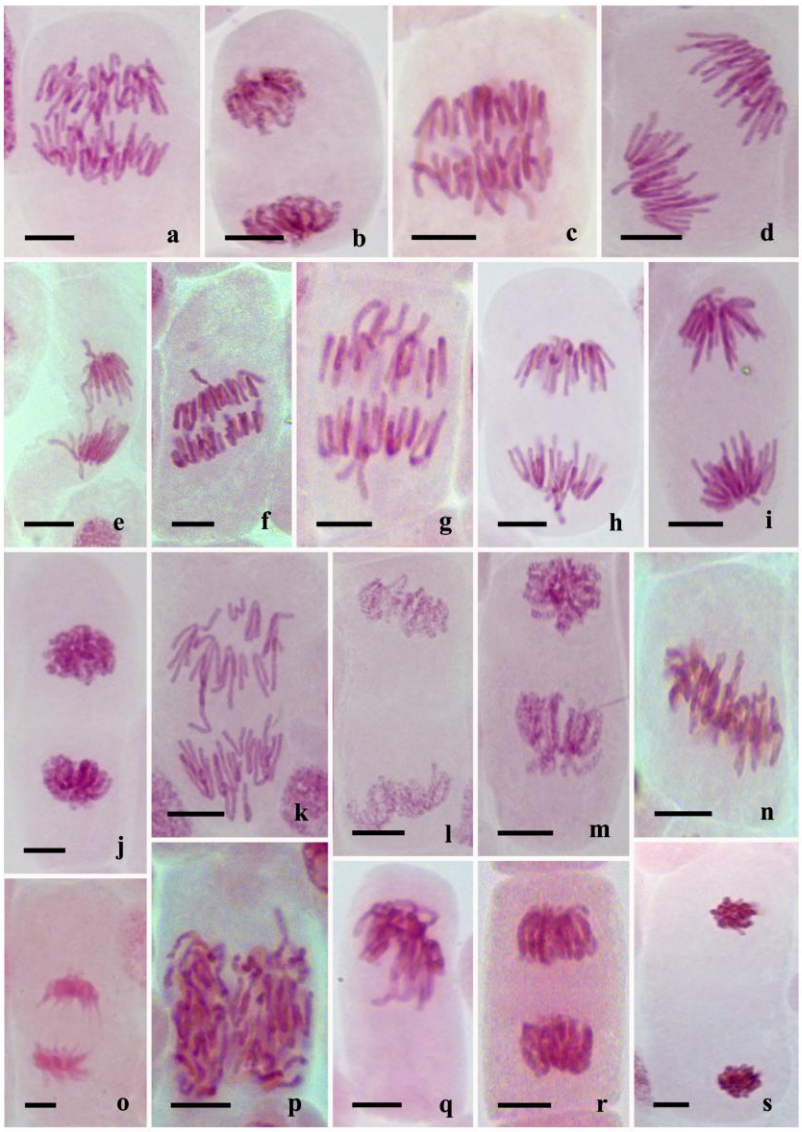



**Plate 14: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at anaphase a -** Anaphase in a hyperploid cell, **b -** Shift in MTOC, chromosome gaps and pulverization, **c -** Bridges and laggards, **d -** C-anaphase, **e -** Chromosome bridge, **f -** Diagonal anaphase showing early movement, **g -** Distrubed anaphase, **h -** Exposure of chromosome scaffold, **i -** Hemistellate anaphase, **j -** Partial chromosomal clumping, **k -** Lagging chromosome and fragments, **l -** Pulverized anaphase, **m -** Pulverized stellate anaphase, **n -** Diagonal stathmo anaphase, **o -** Sticky multiple bridges, **p -** Binucleate cell showing double anaphase with bridges, **q -** Unipolar movement of chromosome, **r -** Partial coagulated anaphase, **s -** Stellate anaphase, Bar - 10 µm

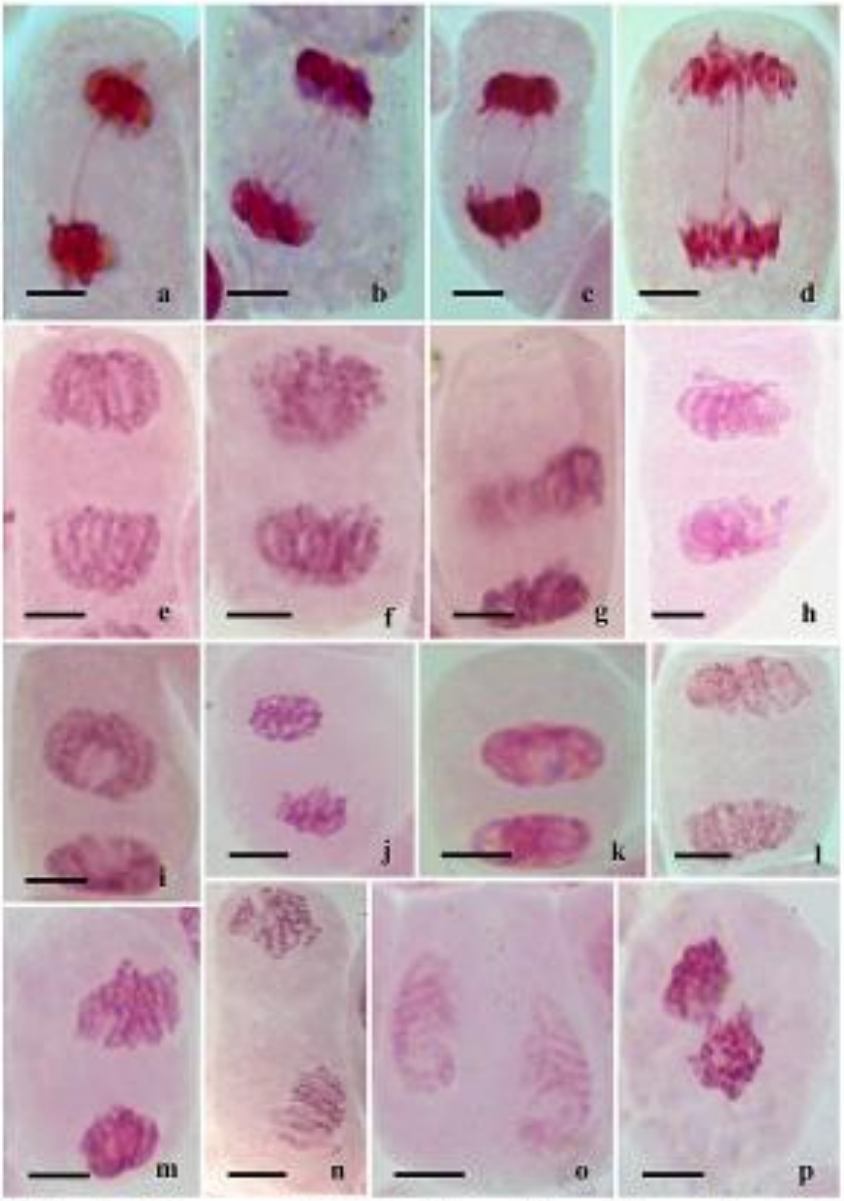



**Plate 15: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at anaphase and telophase a-d** Chromosomal bridges at anaphase, **e** - Chromosome erosion at telophase, **f** - Chromosome gaps, **g** - Cytoplasmic vacuolation and unequal separation of chromosomes, **h** - Early cell plate formation, **i** - Macro and micro cell formation showing single nuclear lesion, **j** - Misorientation of chromosome, **k** - Nuclear erosion and lesion, **l** - Pulverized chromosomes, **m** - Pulverized chromatin after unequal separation, **n** - Pulverized chromosomes showing oblique cell plate and displacement, **o** - Pulverized telophase after equatorial separation, **p** - Stellate telophase showing persistent bridge, Bar - 10 µm

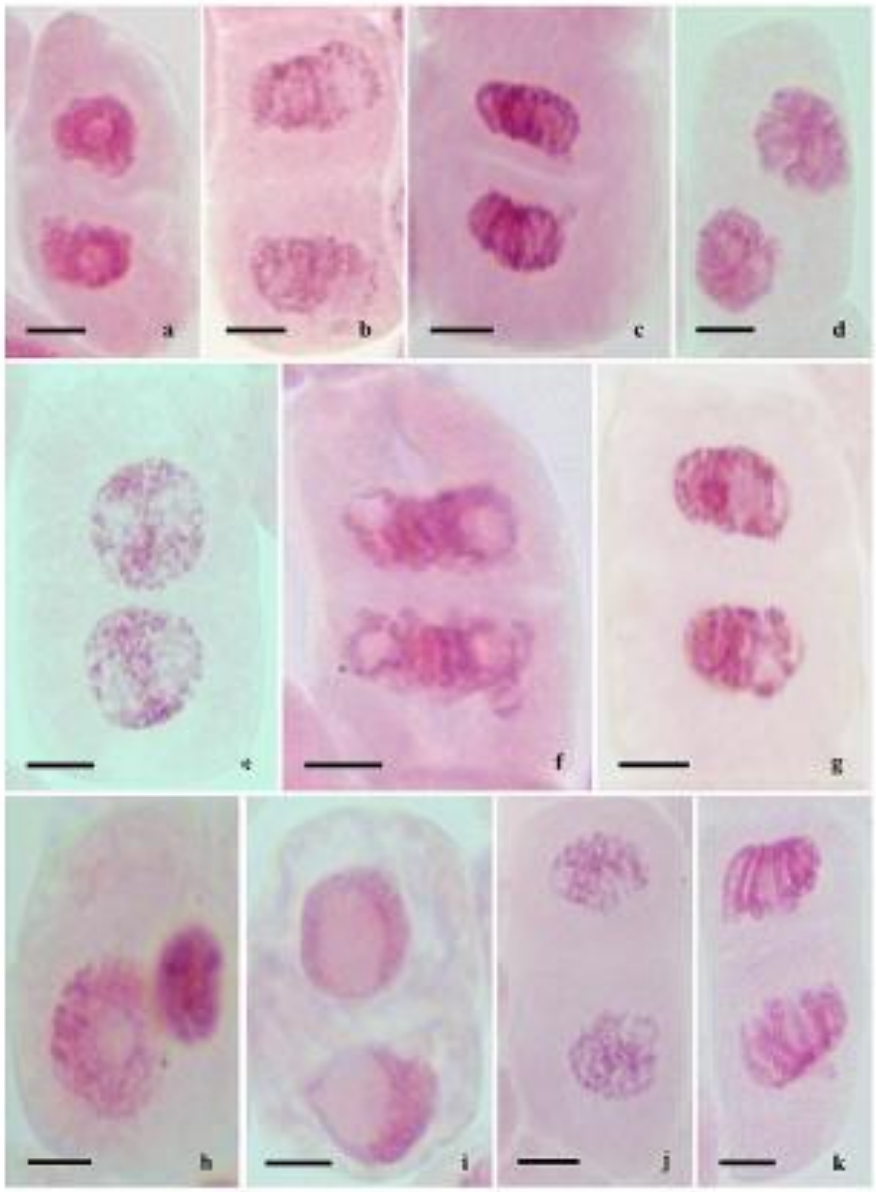



**Plate 16: Chromosomal aberrations induced by extracts of *Memecylon* spp. in *A. cepa* at cytokinesis a** - Aberrant cell wall formation at cytokinesis with single nuclear lesion, **b** - Chromatin erosion, **c** - Sticky cytokinesis showing lesion, **d** - Diagonal cell plate formation showing displacement, **e, f** - Double nuclear lesion at late cytokinesis, **g** - Formation of single lesion, **h** - Macro and micro cell showing abnormal cell division at late cytokinesis, **i** - Nuclear erosion, nuclear lesion and nuclear peak at late cytokinesis, **j** - Pulverized chromation, **k** - Unequal and oblique cell plate formation, Bar - 10 µm

*randerianum* fruit extract ranges from 34.52 ± 3.87 to 83.83 ± 1.28 and that of positive control is 80.53 ± 3.11 to 81.83 ± 5.76% (**Figures 26 b, 27 b, 28 b**). Here also the exposure time has no role in toxicity level. The concentration dependent cytotoxicity effect was much prominent in fruit extract of *M. randerianum* as compared to its leaf extract. The fruit extract shows a lower mitotic index and higher abnormality percentage, which is pointing to their potential cytotoxicity.

In *M. umbellatum* leaf extract, the number of dividing cells index ranges from 33.08 ± 4.18 to 74.18 ± 8.11% and the abnormality % from 32.85 ± 3.81 to 90.25 ± 2.74%. It was found to be higher when compared to the positive control (80.53 ± 3.11 to 81.83 ± 5.76%). The ½ hr, 2 hr and 24 hr exposure periods show a dosage dependent cytotoxicity. The decrease in mitotic index is positively correlated with increasing concentration of plant extracts. The highest mitotic index was shown in ½ hr treatment period in 12.5 µg/mL extract of *M. umbellatum* leaf (**Figures 26 a, 27 a, 28 a**). The highest abnormality percentage was shown at 1 hr exposure period in 100 µg/mL leaf extract. *M. umbellatum* fruit extract shows a mitotic index that ranges from 30.76 ± 7.00 to 81.56 ± 3.06%. The abnormality percentage ranges from 23.08 ± 3.25 to 91.73 ± 1.41%. The mitotic index observed at different time intervals are as follows; in ½ hr it ranges from 30.76 ± 7.0 to 77.95 ± 5.10%, 2 hr from 37.28 ± 2.83 to 81.56 ± 3.06% and 24 hr from 50.28 ± 5.31 to 80.01 ± 3.06%. The abnormality percentage increases with increasing concentration (**Figures 26 b, 27 b, 28 b)**. These two parameters have no impact with the time periods of treatment.

Several chromosomal aberrations resulted during the cytotoxic assay. It is classified into clastogenic and aneugenic aberrations (**Plates 10-16)**. The abnormal cells are easily distinguished from the normal stages of cell division **(Plate 9).** The clastogenic aberrations are the abnormal effects induced on the

genetic material and the aneugenic ones interfere with mitotic spindle formation. Several multiple aberrations are also noticed. In clastogenic aberrations, stickiness, pulverization, chromosomal clumping, chromosome gaps, nuclear lesions, nuclear erosions, nuclear disintegration, exposure of chromosome scaffold, giant cell formation, coagulated chromosomes, chromosome bridges *etc.*, are observed. Hypoploid condition, stellate chromosomes, lagging chromosome, C-mitosis, macro and micro cell formation, ball shaped chromosome arrangement, polyploidy, induction of vagrants *etc.*, may occur due to aneugenic aberrations.

Over viewing these results, it may be concluded that a dosage dependent cytotoxic effect was offered by the selected plant extracts and the time has no role in toxic effects. As the concentration increases a decline in mitotic index was observed. In the case of abnormality percentage, it is directly related to the concentration of plant extracts. When comparing these parameters, the selected fruits sample shows better toxic potential than its leaf extracts. Among the tested fruit samples, *M. umbellatum* shows the highest abnormal cell counts from 23.08 ± 3.25 to 91.73 ± 1.41% followed by *M. grande* fruit extract from 27.15 ± 4.29 to 90.72 ± 1.45%. *M. umbellatum* fruit extract is also having a low mitotic index as compared to the other extracts from 30.76 ± 7.00 to 81.56 ± 3.06%. The chromosomal aberrations are also indicating the toxic potential of selected plant extracts and conclude that all the selected extracts possess significant cytotoxicity.

## c) ANTIPROLIFERATIVE ACTIVITY OF *MEMECYLON* SPECIES

### 1) Cytotoxicity assay on MCF-7 cell lines

The antiproliferative activity of selected *Memecylon* species was tested by MTT assay using human breast cancer cell line, MCF-7. MTT assay is used for the colorimetric determination of toxic potential of extracts. The selected concentrations of the six plant extracts studied are 100 µg/mL, 50

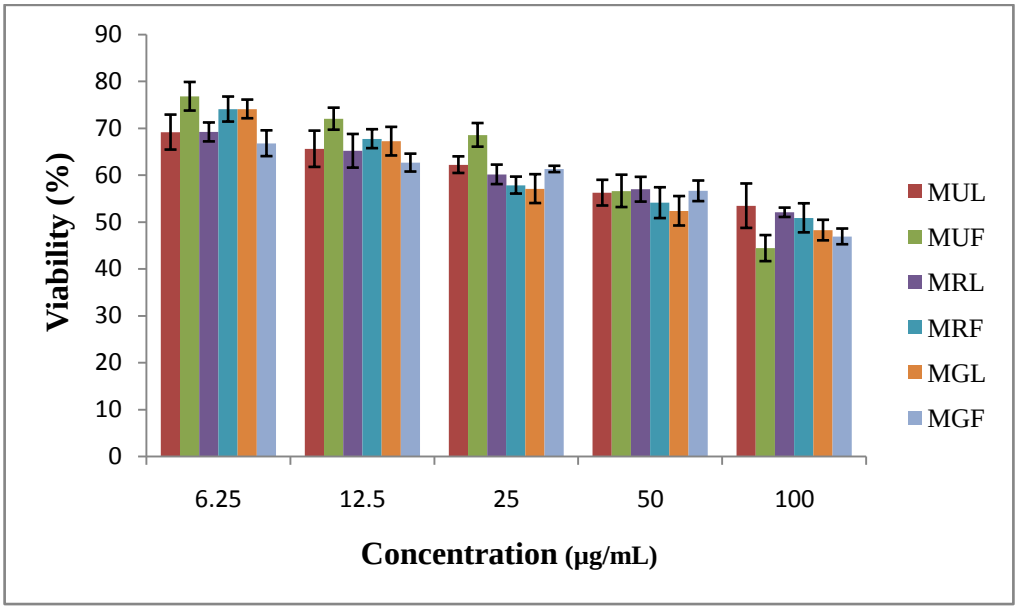



**Figure 29: Evaluation of cytotoxic potential of methanolic extracts of selected species of *Memecylon* on MCF-7 cells using MTT assay** MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits.

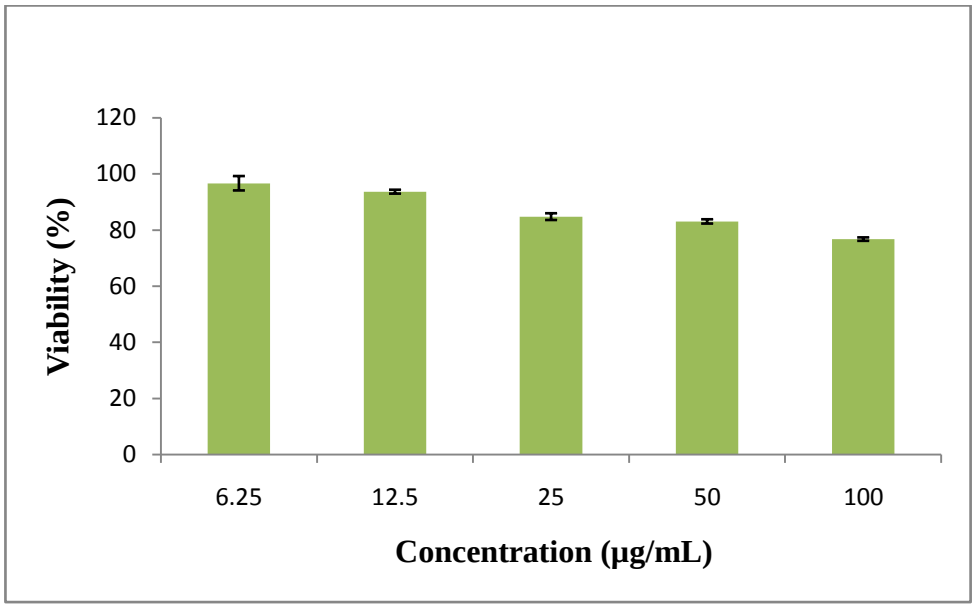



**Figure 30: Effect of methanolic extract of *Memecylon umbellatum* fruits on L929 cells**

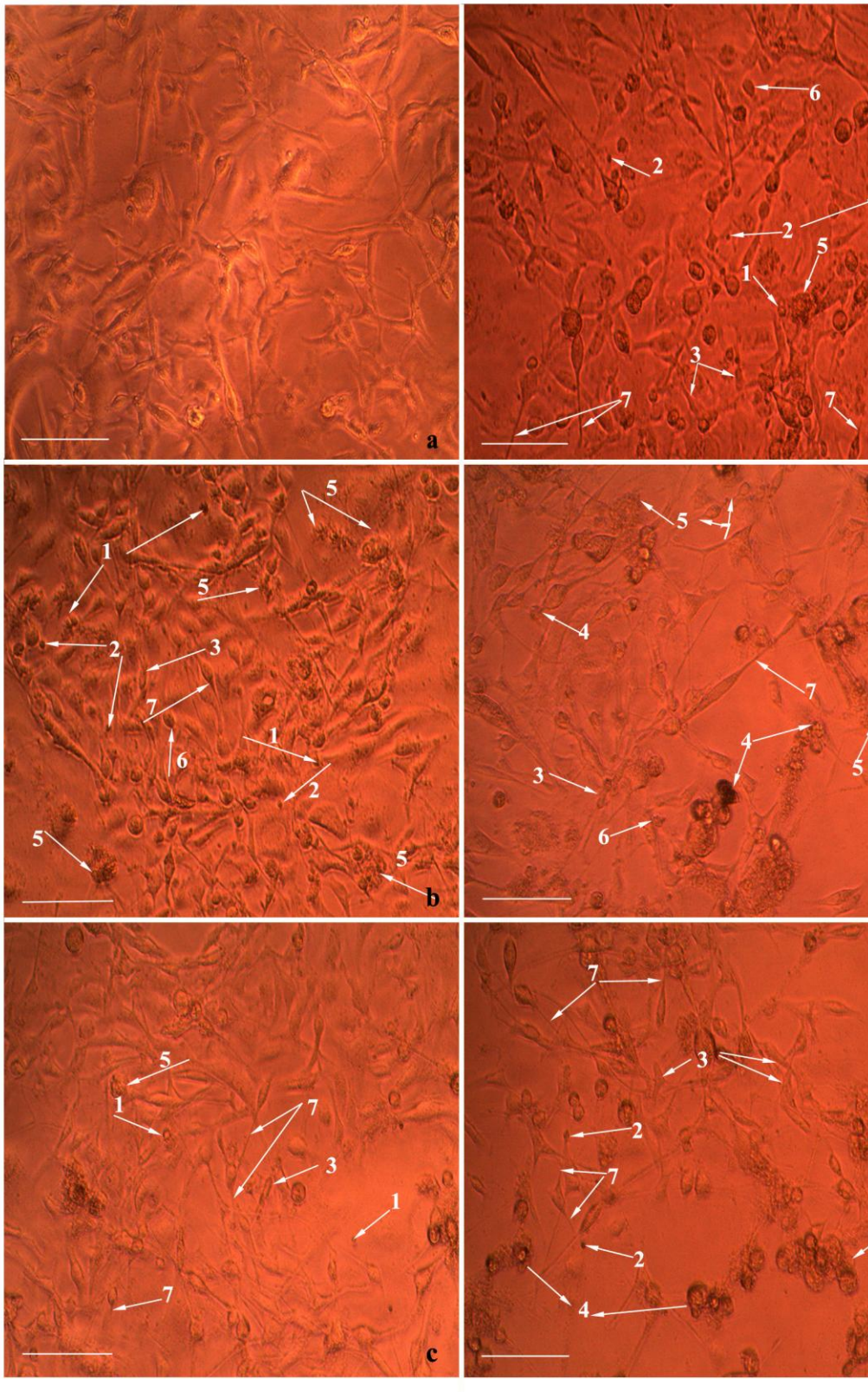



**Plate 22: Cytotoxic effects of methanolic fruits extract of *M. umbellatum* on MC**
**cell lines. a**. Control **b**. 6.25 µg/mL **c**. 12.5 µg/mL **d**. 25 µg/mL **e**. 50 µg/mL **f**.

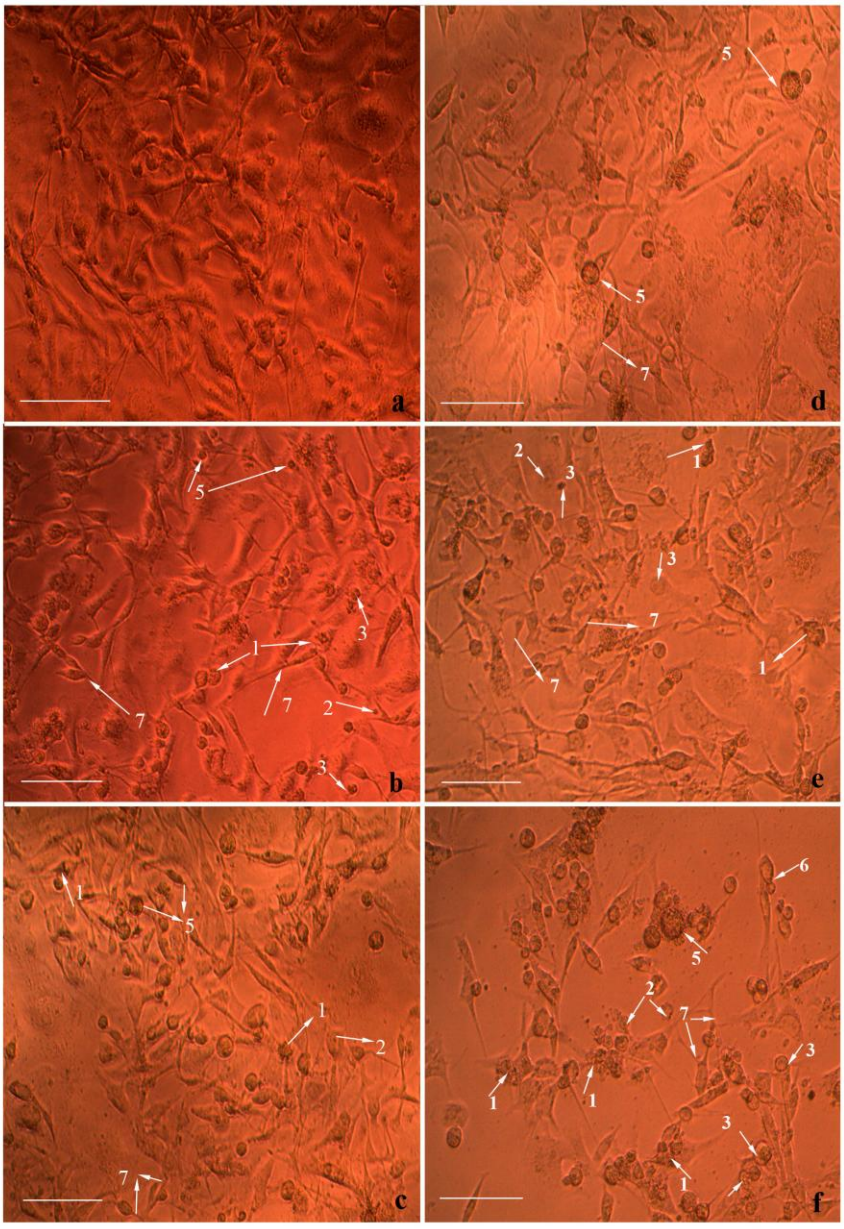



**Plate 21: Cytotoxic effects of methanolic leaves extract of *M. umbellatum* on MCF-7 cell lines**. **a-** Control, **b-** 6.25 µg/mL, **c-** 12.5 µg/mL, **d-** 25 µg/mL, **e-** 50 µg/mL, **f-** 100 µg/mL. Arrows indicating apoptotic signals 1. Nuclear fragmentation 2. Condensed nuclei 3. Cell shrinkage 4. Membrane blebbing 5. Apoptotic bodies 6. Budding 7. Echinoid spikes. Bar 100 µm

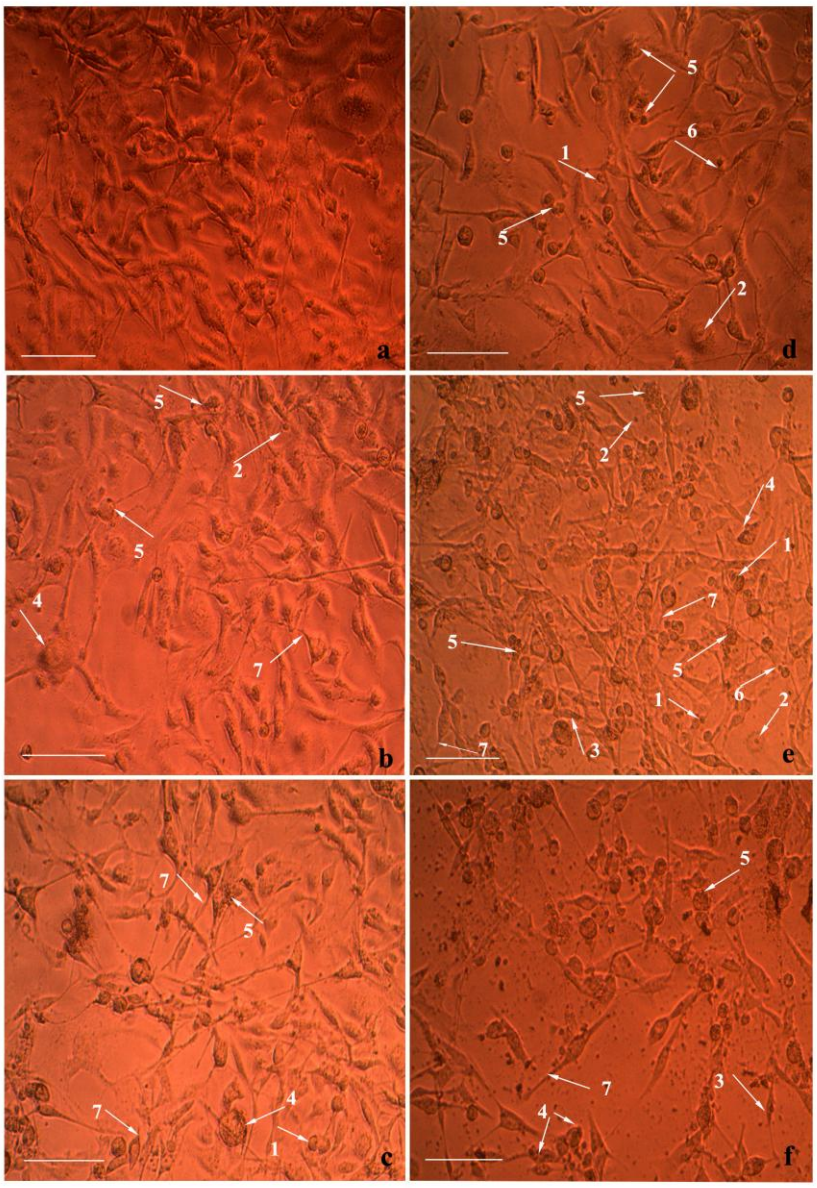



**Plate 20: Cytotoxic effects of methanolic fruits extract of *M. randerianum* on MCF-7 cell lines.** **a**- Control, **b**- 6.25 µg/mL, **c**- 12.5 µg/mL, **d**- 25 µg/mL, **e**- 50 µg/mL, **f**- 100 µg/mL. Arrows indicating apoptotic signals 1. Nuclear fragmentation 2. Condensed nuclei 3. Cell shrinkage 4. Membrane blebbing 5. Apoptotic bodies 6. Budding 7. Echinoid spikes. Bar 100 µm

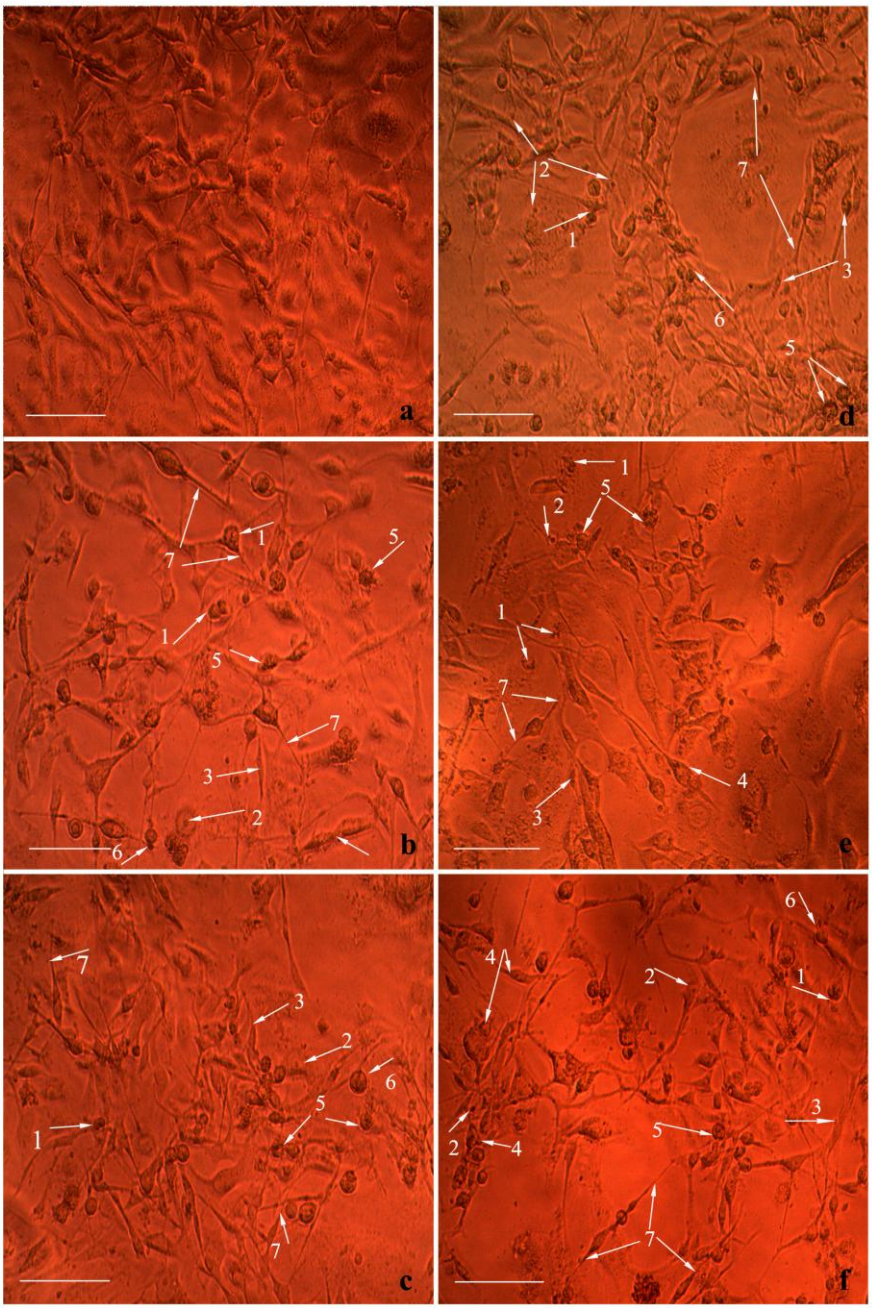



**Plate 19: Cytotoxic effects of methanolic leaves extract of *M. randerianum* on MCF-7 cell lines. a**- Control, **b**- 6.25 µg/mL, **c**- 12.5 µg/mL, **d**- 25 µg/mL, **e**- 50 µg/mL, **f**- 100 µg/mL. Arrows indicating apoptotic signals 1. Nuclear fragmentation 2. Condensed nuclei 3. Cell shrinkage 4. Membrane blebbing 5. Apoptotic bodies 6. Budding 7. Echinoid spikes. Bar 100 µm

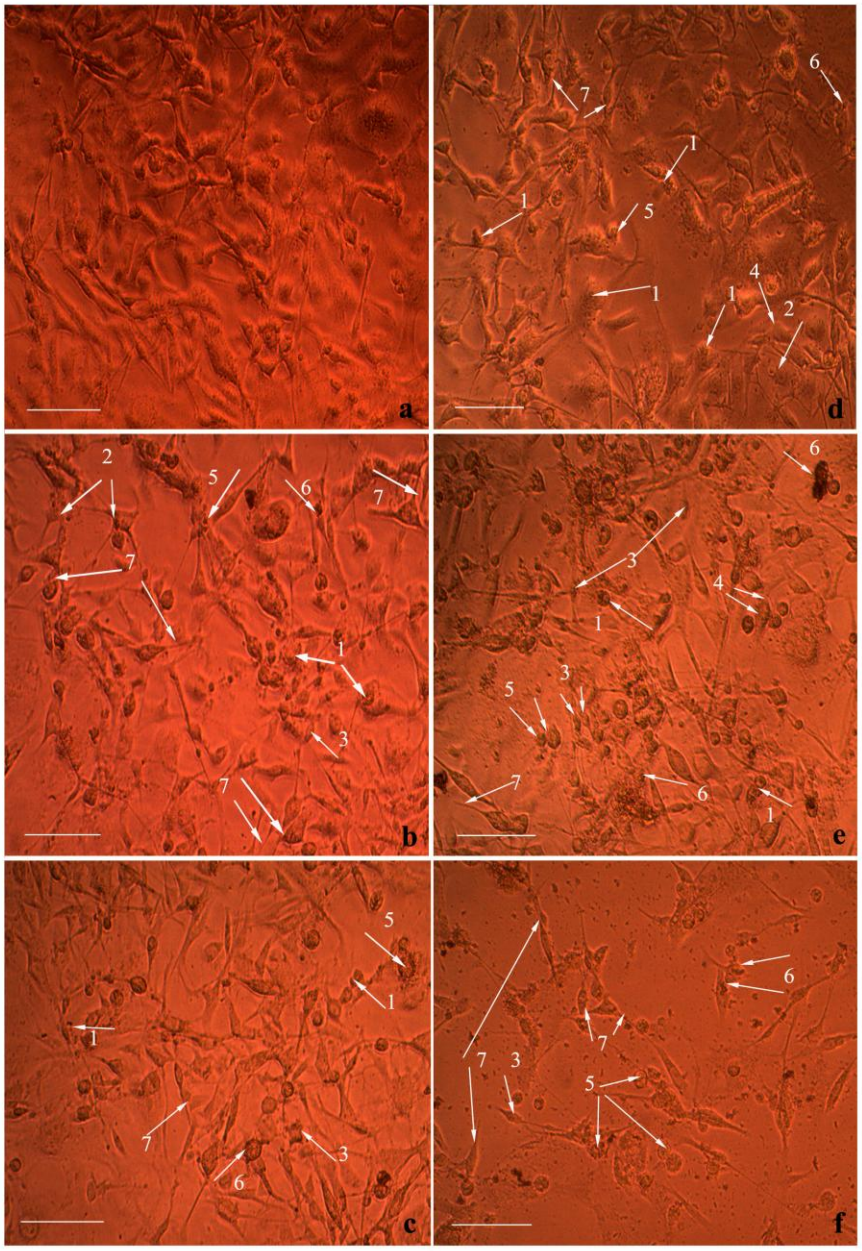



**Plate 18: Cytotoxic effects of methanolic fruits extract of *M. grande* on MCF-7 cell lines. a**- Control, **b**- 6.25 µg/mL, **c**- 12.5 µg/mL, **d**- 25 µg/mL, **e**- 50 µg/mL, **f**- 100 µg/mL. Arrows indicating apoptotic signals 1. Nuclear fragmentation 2. Condensed nuclei 3. Cell shrinkage 4. Membrane blebbing 5. Apoptotic bodies 6. Budding 7. Echinoid spikes. Bar 100 µm

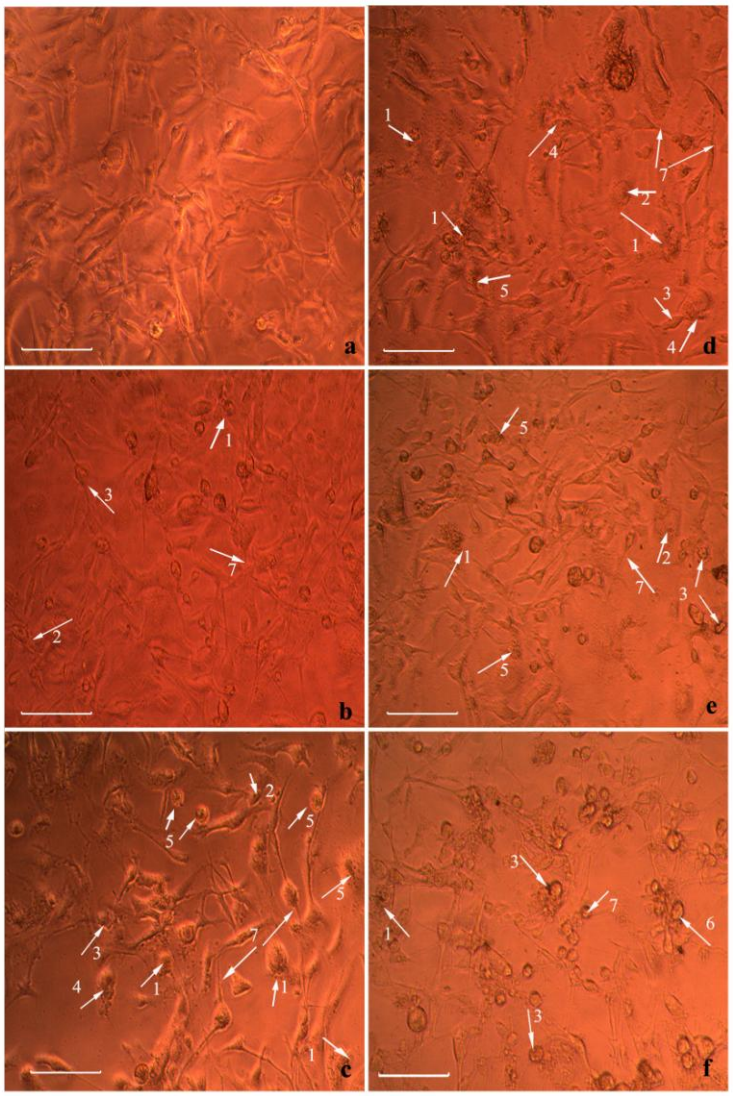



**Plate 17: Cytotoxic effects of methanolic leaves extract of *M. grande* on MCF-7 cell lines. a**- 6.25 µg/mL, **b**- 12.5 µg/mL, **c**- 25 µg/mL, **d**- 50 µg/mL, **e**- 50 µg/mL, **f**- 100 µg/mL. Arrows indicating apoptotic signals 1. Nuclear fragmentation 2. Condensed nuclei 3. Cell shrinkage 4. Membrane blebbing 5. Apoptotic bodies 6. Budding 7. Echinoid spikes. Bar 100 µm

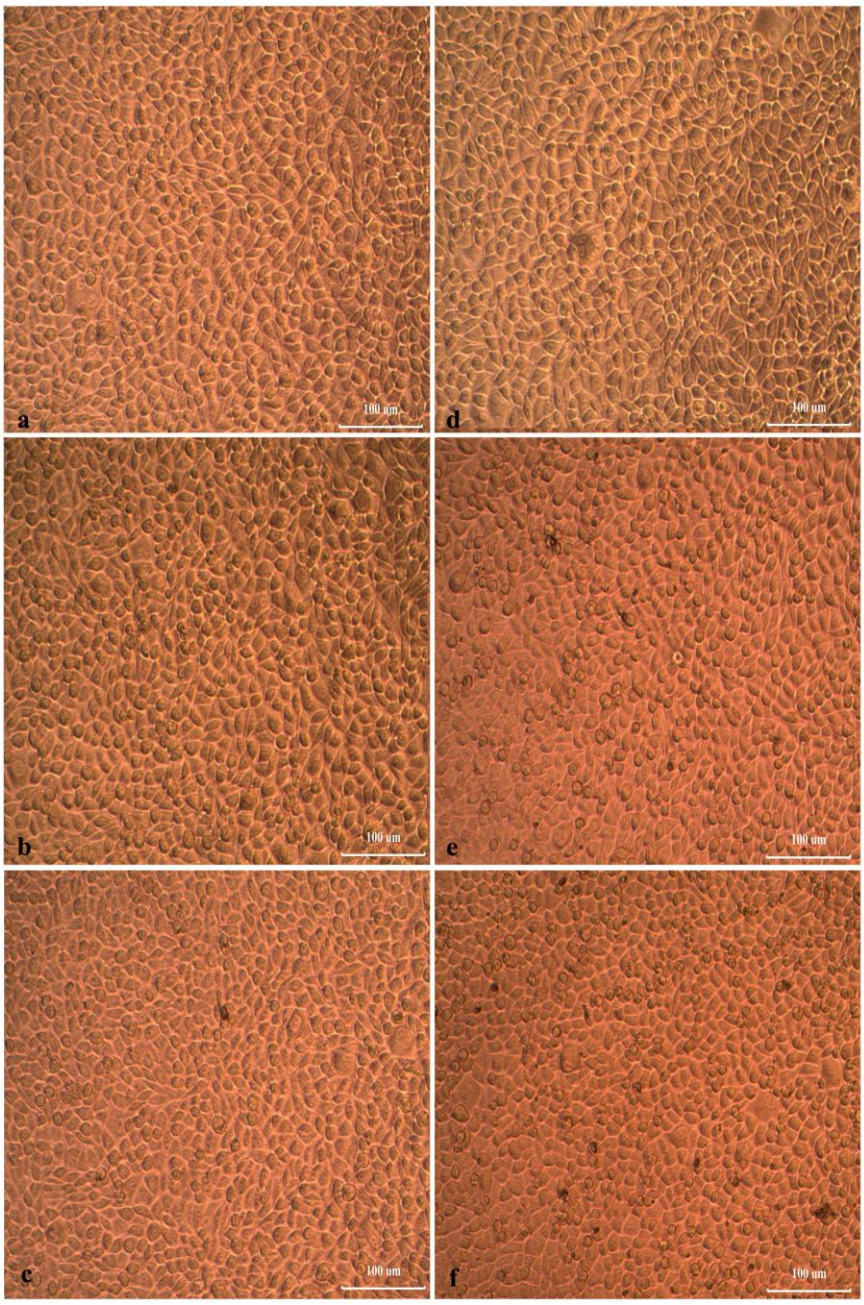



**Plate 23: Effect of methanolic fruits extract of *M. umbellatum* on L929 cells**. **a-** Control, **b-** 6.25 µg/mL, **c-** 12.5 µg/mL, **d-** 25 µg/mL, **e-** 50 µg/mL, **f-** 100 µg/mL

µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL and time period for the experiments was set for 24 hrs. The direct microscopic observation reveals the toxic potential of plant extracts. The aberrations like formation of membrane blebs, apoptotic bodies, nuclear condensation, membrane distortion, formation of echinoid spikes, budding, fragmentation and cell shrinkage are clearly visible in the MCF-7 cell lines, which form the hallmarks of cell death (**Plates 17-22**).

The percentage viability of cell lines treated with different plant extracts is determined through the absorbance value. The plant with the highest antiproliferative effect on MCF-7 cell line shows the lowest viability percentage as well. A dose dependent cytotoxicity was observed in it. The highest antiproliferative activity was shown by *M. umbellatum* fruit extract, ranging from 76.8 ± 2.75 to 44.4 ± 1.68% in a concentration gradient from 6.25 µg/mL to 100 µg/mL (**Figure 29**). The $LD_{50}$ value was calculated as 78.48 ± 0.8 µg/mL (**Table 11**). It is followed by *M. grande* fruit extract, which shows maximum antiproliferative activity of 46.93 ± 4.74% at 100 µg/mL concentration. At the same time *M. grande* leaf extract shows viability percentage of 48.28 ± 2.78 at 100 µg/mL. In the case of *M. umbellatum* leaf extract, it shows 53.48 ± 2.19 percentage of activity, which is the lowest value among the tested extracts. $LD_{50}$ value was found to be 110.85 ± 6.25 µg/mL, the highest $LD_{50}$ value among the tested six plant samples. A moderate antiproliferative efficacy was shown by *M. randerianum* leaf and fruit extracts. The leaf extract of *M. randerianum* possess remarkable antiproliferative activity of 52.08 ± 1.00% at 100 µg/mL concentration and that of fruit extract is 50.89 ± 3.10%. The overall results point out that the fruit extracts of selected samples show highest antiproliferative potential as compared to their corresponding leaf extracts. The $LD_{50}$ concentration of plant extracts are calculated by using ED50 PLUS V1.0 software represented in **Table 11**. The $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48

± 0.8 µg/mL of *M. umbellatum* fruit extract was selected for further anticancerous studies.

**Table 11: The effect of methanolic extracts of selected species of *Memecylon* in MTT assays**

| $LD_{50}$ value (µg/mL) | | | | | |
|---|---|---|---|---|---|
| **MGL** | **MGF** | **MRL** | **MRF** | **MUL** | **MUF** |
| 83.7 ± 0.67 | 79.14 ± 0.89 | 103.86 ± 0.64 | 89.38 ± 0.32 | 110.64 ± 0.43 | 78.48 ± 0.8 |

MGL: *Memecylon grande* leaves; MGF: *Memecylon grande* fruits; MRL: *Memecylon randerianum* leaves; MRF: *Memecylon randerianum* fruits; MUL: *Memecylon umbellatum* leaves; MUF: *Memecylon umbellatum* fruits

**2) Cytotoxic assay on L929 cell lines**

The most effective extract from the cytotoxic assay using MCF-7 cell line *ie.*, *M. umbellatum* fruit (MUF) extract was selected for further studies. Cytotoxicity studies using MCF-7 breast cancer cell line assay and *A. cepa* assay reveals the toxic potential of plant extracts. So the action of effective plant extract on normal cells must be evaluated. The MTT assay was carried on normal L929 (Fibroblast) cell line. A dose dependent viability percentage was resulted during the assay **(Figure 30)**. It ranges from 96.63 ± 2.56 to 76.72 ± 0.61% in a concentration gradient from 6.25 µg/mL to 100 µg/mL. The direct microscopic observation of L929 (Fibroblast) cell lines treated with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extracts show comparatively lesser cellular damages (**Plate 23).**

**3) Genotoxicity evaluation using comet assay**

Comet assay is a method for measuring the DNA damages in cells. It is a simple, sensitive and fast method to measure nuclear DNA damages. In this assay, MCF-7 cells treated with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract is used for the evaluation of its DNA damaging potential. The parameters namely comet length, tail length, tail DNA percentage, tail moment and olive tail moment were determined for the evaluation of DNA damages. The results obtained in this assay are termed as comet model of cells. The comet appeared with a distinct head and tail of which constitute relaxed loops and damaged DNA fragments (**Plate 24**). By measuring the above mentioned parameters, elevated levels of DNA damages can be observed. The percentage of DNA in tail shows much higher (48.08) value than that of control (23.85) (**Figure 31 a**). It is linearly related to the DNA breaking frequency induced by the plant extract. Similarly comet length and tail length are increased in MCF-7 cells treated with plant extract. The increase of comet length is from 10 px in control to 33.58 px in treated cells (**Figure 31 b**). The tail length ranges from 1.11 px to 5.92 px in control and treated sample (**Figure 31 c**). The intensity of the tail increases as the damage is enhanced. The tail moment and olive moment are also important parameters in comet assay. Olive tail moment is the product of the tail length and the fraction of total DNA in the tail. An elevated level of olive tail moment and tail moment were noticed during comet assay *ie.,* 3.43 tail moment and 2.53 olive tail moment (**Figures 31 d & e**). So comet assay confirms the DNA damaging potential of the plant extract.

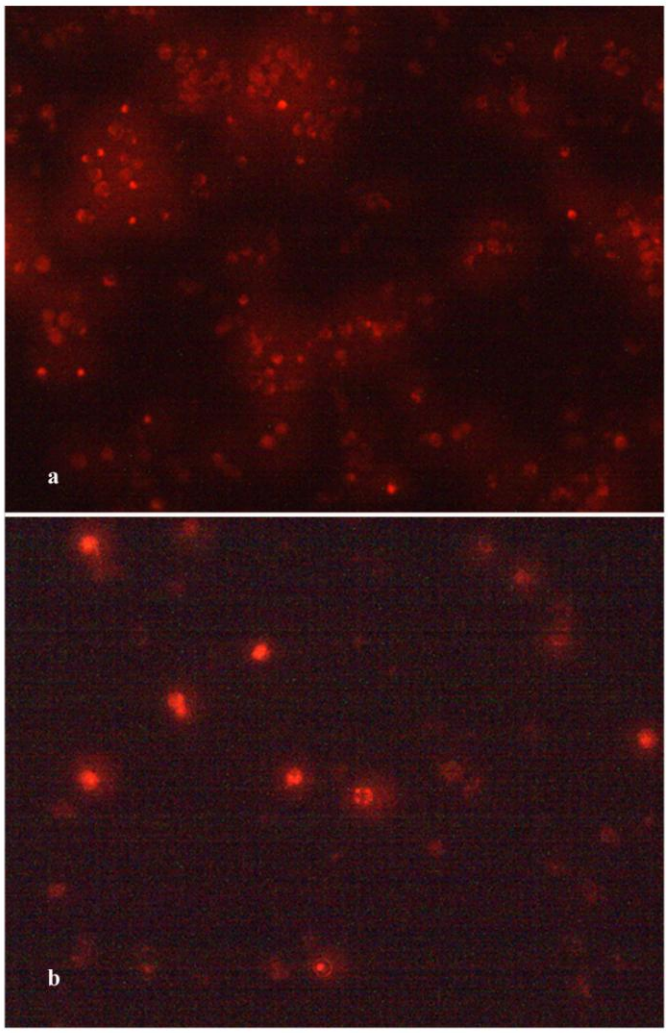



**Plate 24: DNA damage detection through comet assay on MCF-7 cells a-** control, **b- c**ells treated with *M. umbellatum* fruit extract

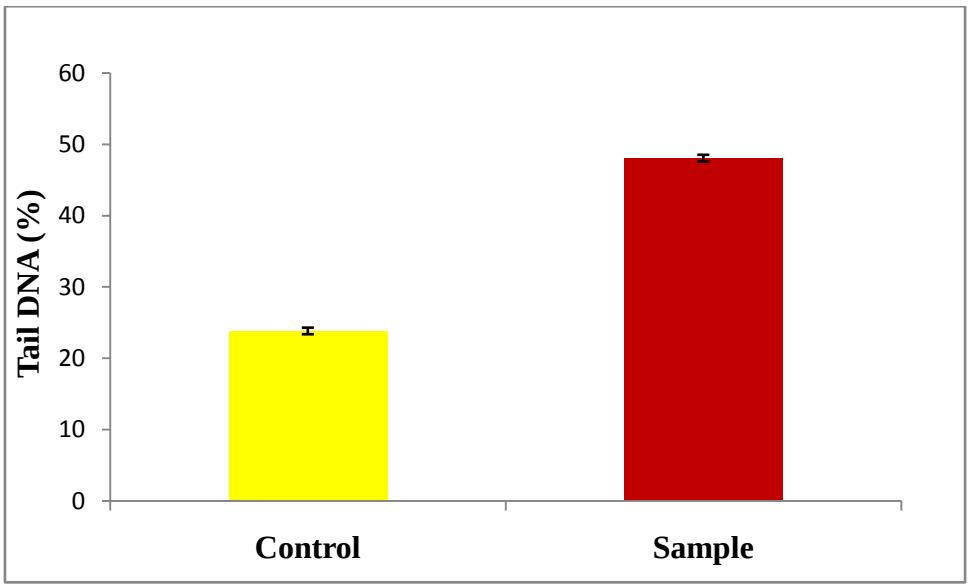



**Figure 31 a: Assessment of DNA damage induced by methanolic fruit extract of *M. umbellatum* on MCF-7 cells in comet assay showing Tail DNA percentage**

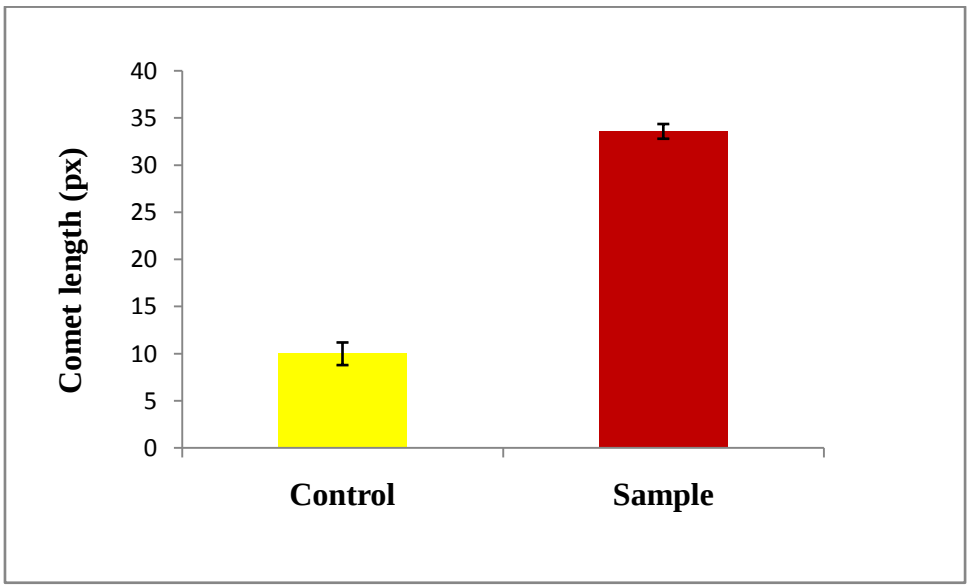



**Figure 31 b: Assessment of DNA damage induced by methanolic fruit extract of *M. umbellatum* on MCF-7 cells in comet assay showing Comet length**

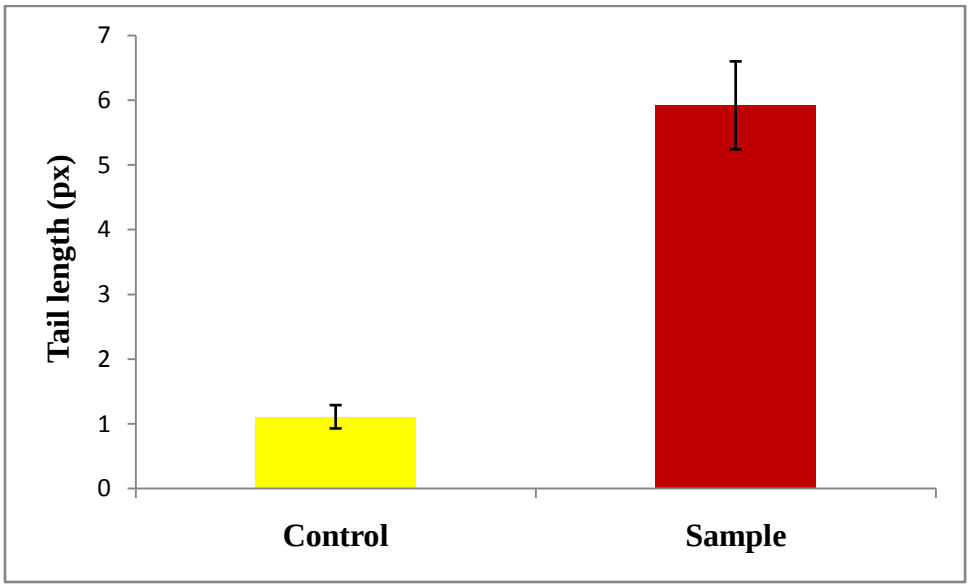



**Figure 31 c: Assessment of DNA damage induced by methanolic fruit extract of *M. umbellatum* on MCF-7 cells in comet assay showing Tail length**

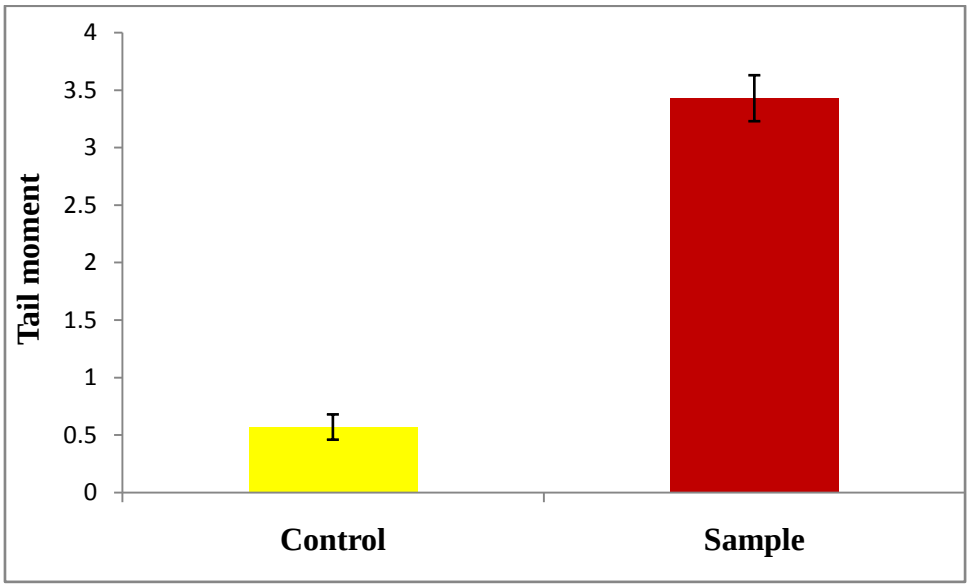



**Figure 31 d: Assessment of DNA damage induced by methanolic fruit extract of *M. umbellatum* on MCF-7 cells in comet assay showing Tail moment**

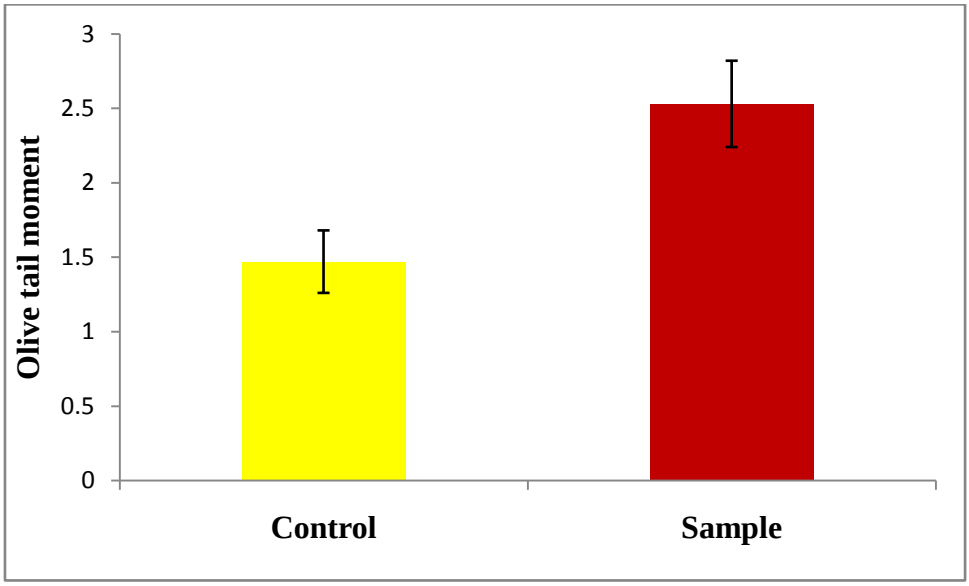



**Figure 31 e: Assessment of DNA damage induced by methanolic fruit extract of *M. umbellatum* on MCF-7 cells in comet assay showing Olive tail moment**

**4) Detection of apoptosis by double staining method**

The cytotoxic assay in *A. cepa*, MTT assay and comet assay reveals the cell damaging potential of the selected plant extract. The cell damaging potential or cell death inducing capability of the plant extract is further analyzed through the double staining method. It is a method to unveil the mechanism behind the cellular damages/cell death. Cell deaths are mainly of two types, apoptosis or necrosis. Apoptosis is the programmed cell death and necrosis is the unplanned cell death due to cellular injuries. A combination of acridine orange/ethidium bromide stains are eluted on the MCF-7 cell lines, which are treated with $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract. This staining method

enabled to distinguish the apoptotic or necrotic cells from the normal cells. The early apoptotic cells, late apoptotic cells and necrotic cells were distinguished from normal cells distinctly by their typical morphological features and variable staining patterns. The viable cells appeared in uniform green colour and without any membrane distortions. The plant extract treated cells have lost their viability and membrane integrity (**Plate 25)**. They are observed as orange coloured bodies. The non-viable cells appeared with membrane blebs, nuclear fragmentation, cell shrinkage and apoptotic bodies. These signs confirm the apoptotic potential of *M. umbellatum* fruit extract. The double staining method unveils that the cell death induced by the plant extract is through the apoptotic mechanism.

**5) Cell cycle analysis using flow cytometry**

Apoptotic effect of plant extract on cell cycle progression was analyzed through cell cycle analysis by using flow cytometry. MCF-7 cells were treated with *M. umbellatum* fruit extract (78.48 ± 0.8 µg/mL) for analyzing the cell cycle progression. The histogram represents the DNA count as well as population count of cells treated with the plant extract at various phases of cell cycle (**Figures 32 & 33**). The G0/G1 phase of cell cycle shows the highest amount of DNA content *ie.*, 72.9% in treated cells as compared to the untreated control cells, which shows DNA content of 42%. A subsequent reduction of DNA count was resulted in S and G2/M phases (**Figure 32 a & b**). S phase shows a DNA percentage of 19% and in G2/M phase it is 5.2%. In the case of cell population count, there is a scatter in untreated cells, while the treated cells show aggregation of cells. That means the progression of cell cycle was arrested in a particular phase of the cell cycle (**Figure 33 a & b**). The percentage of cell count in each phase of the cell cycle unveils the retardation of cell cycle progression (**Figure 34**). The G0/G1phase shows the highest cell count and subsequent reduction was observed in the following

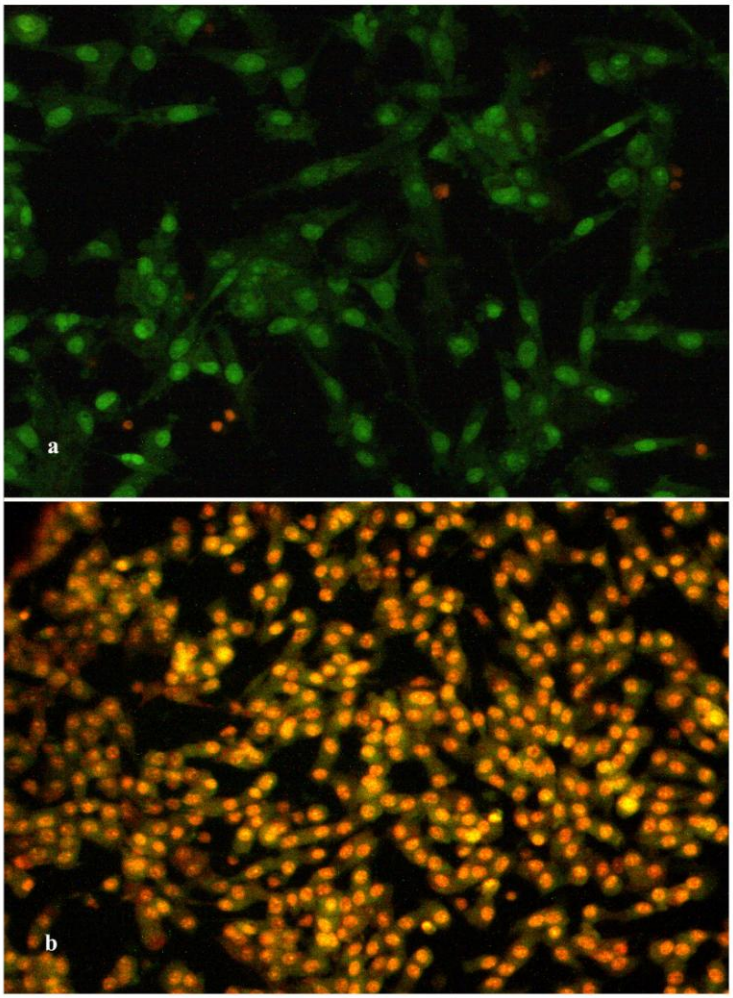



**Plate 25: Detection of apoptosis by AO/EB staining on MCF-7 cells. A-** Control. **B-** Cells treated with methanolic *M. umbellatum* fruit extract.

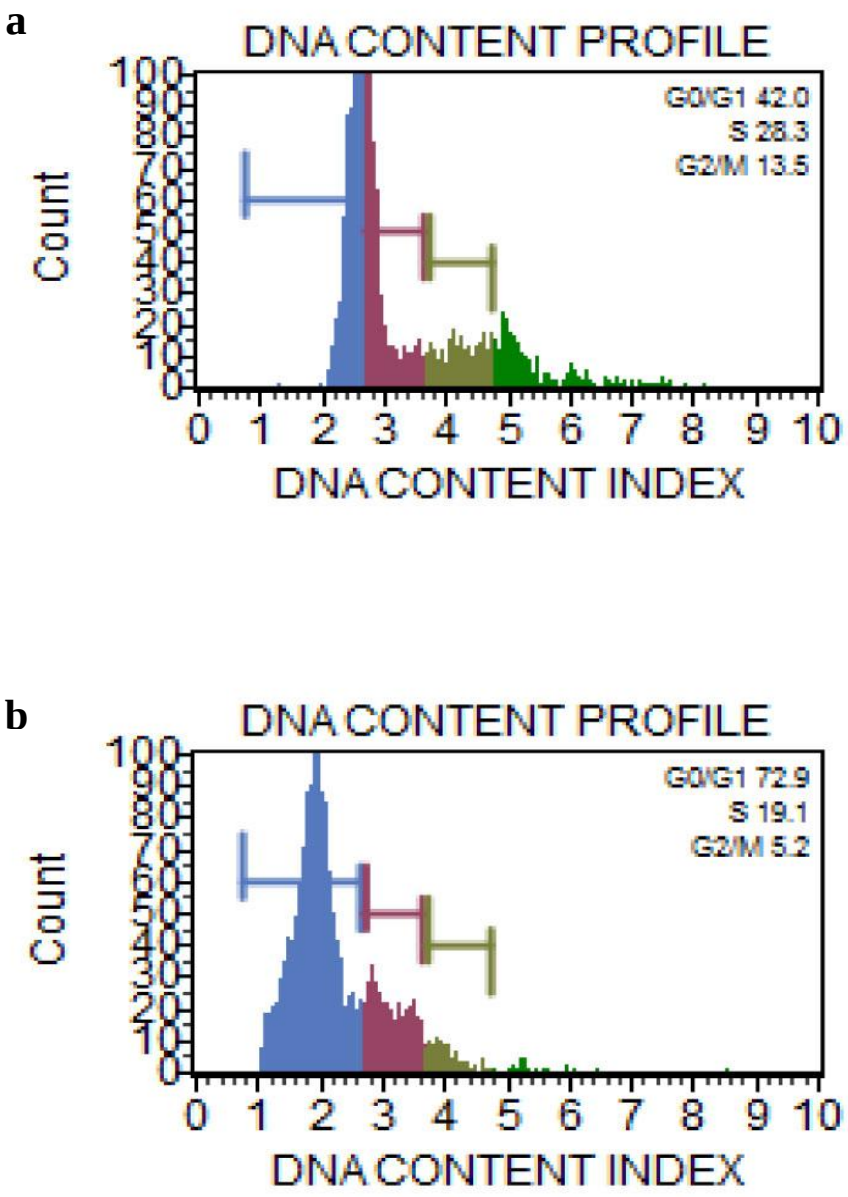



**Figure 32: Determination of cell cycle arrest in MCF-7 cells using flow cytometry** - **DNA content profile a -** Negative control, **b -** MCF-7 cells treated with *M. umbellatum* fruits extract

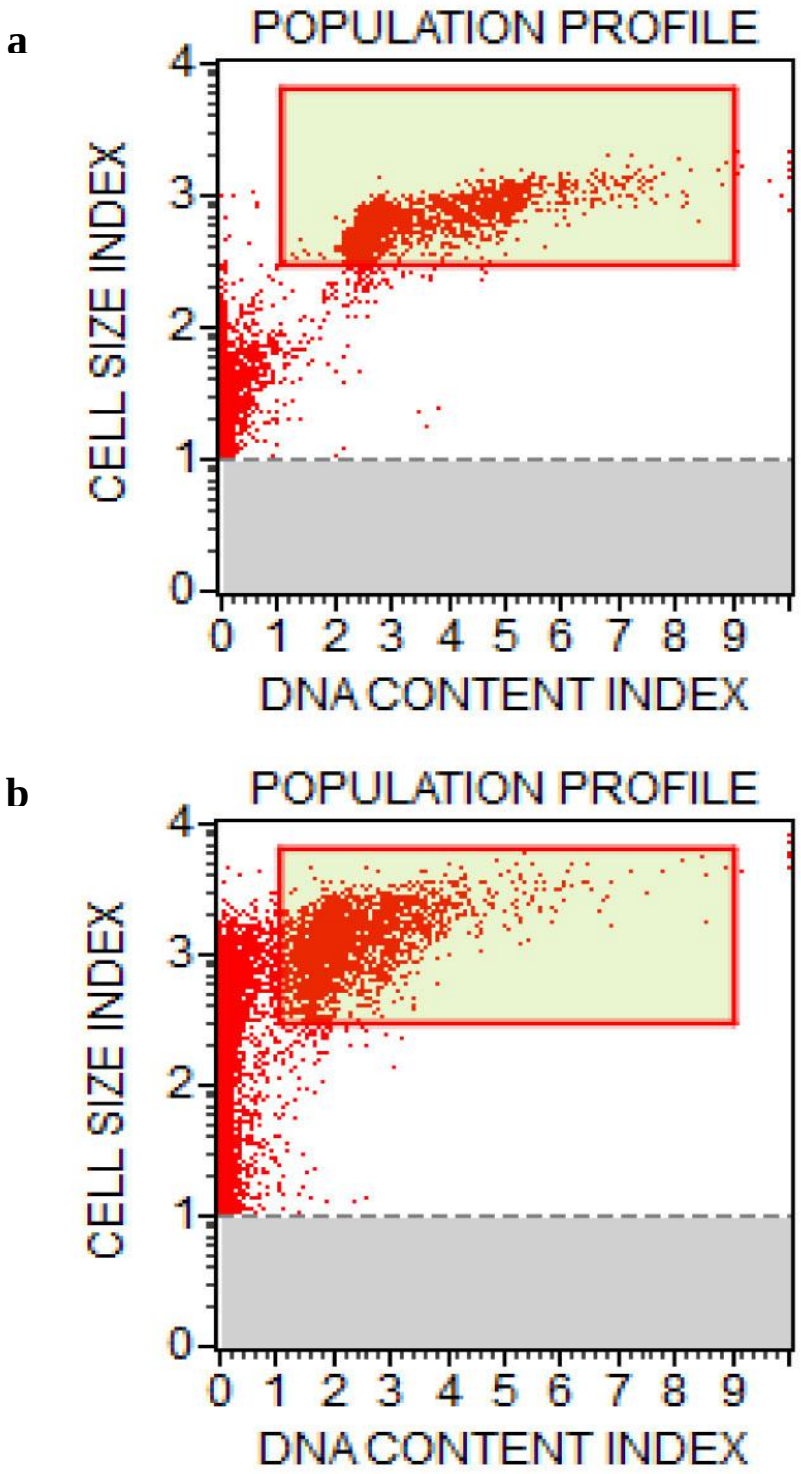



**Figure 33: Determination of cell cycle arrest of MCF-7 cells treated with methanolic fruits extract of *M. umbellatum* by flow cytometry - Population profile of MCF-7 cells a** - Negative control, **b** - *M. umbellatum* fruit extract treatment. The rectangle represents the cells of interest excluding the cellular debris.

Apoptotic cells are shown by their weaker staining affinity towards propidium iodide.

phases. So these results clearly indicate that the cell cycle arrest occurred at G0/G1 phase and the diminishing progression of cell cycle is due to the apoptotic mechanism induced by the plant extract.

**6) Gene expression study using RT- qPCR**

To substantiate the underlying mechanism of antiproliferative activity exhibited by the fruit extract of *M. umbellatum* on MCF-7 cells, the expression changes of genes which are known to be involved in cell cycle arrest and induction of apoptosis were examined. The expression pattern of p53 and p21 were studied by RT-qPCR and data were analysed according to $\Delta\Delta C_t$ method. The p53 and p21 genes regulate many downstream genes involved in the induction of cell cycle arrest, DNA repair and apoptosis. β-actin, a house keeping gene is used as the control. In Agarose gel electrophoresis, it is evident that a prominent expression of p53 and p21 was resulted (**Figure 35**). The intense fluorescence in gel electrophoresis clearly indicated that the treatment of MCF-7 cells with 78.48 ± 0.8 µg/mL of extract significantly induced an up-regulation in the expression of p53. The p53 gene can induce the expression of p21 gene. The combined action of these tumour suppressor genes can induce cell cycle arrest through the apoptotic mechanism. The expression fold analysis also proves the prominent expression of apoptotic genes. An expression fold change is the measure of changes in the expression level of a gene. Here the expression fold change of p53 is 1.86 over the control and that of p21 gene is 1.52 (**Figures 36 a & b**).

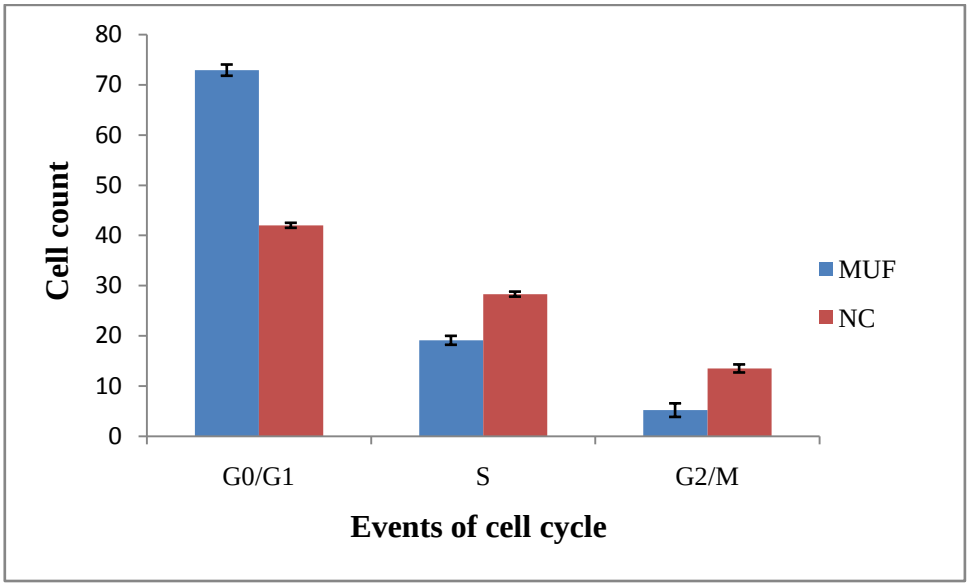
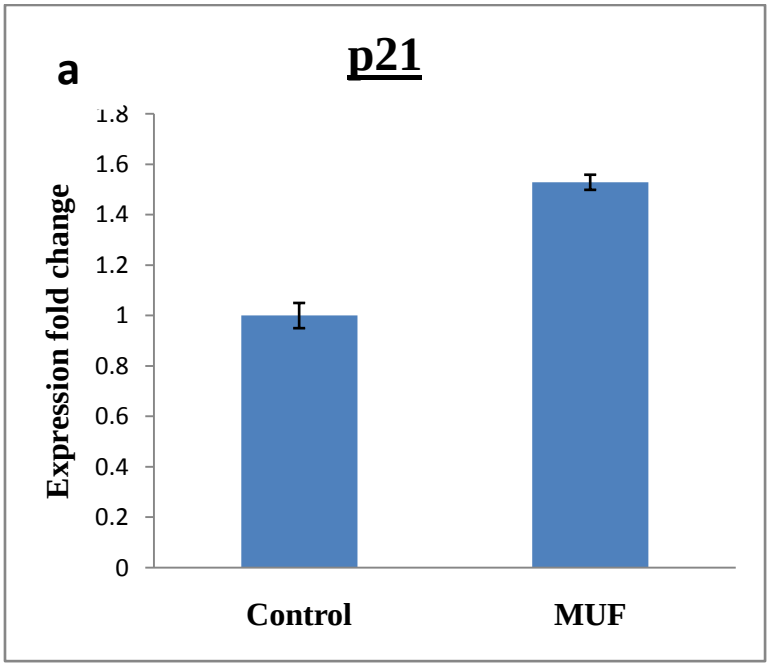



**Figure 34: Percentage of cell count of MCF-7 cells treated with fruit extract of *M. umbellatum***



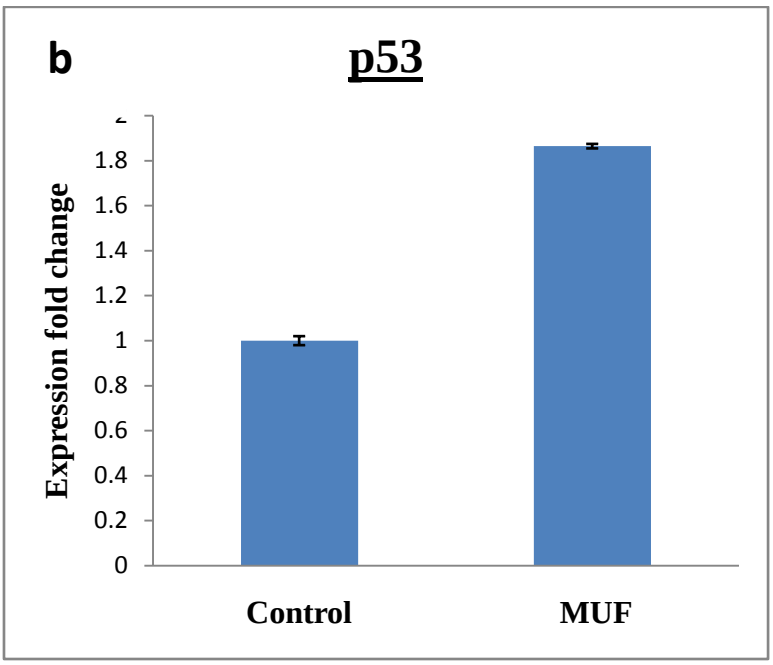



**Figure 36: Expression fold changes of apoptosis related genes in MCF-7 cells** MUF – *Memecylon umbellatum* fruit. **a -** p21, **b -** p53

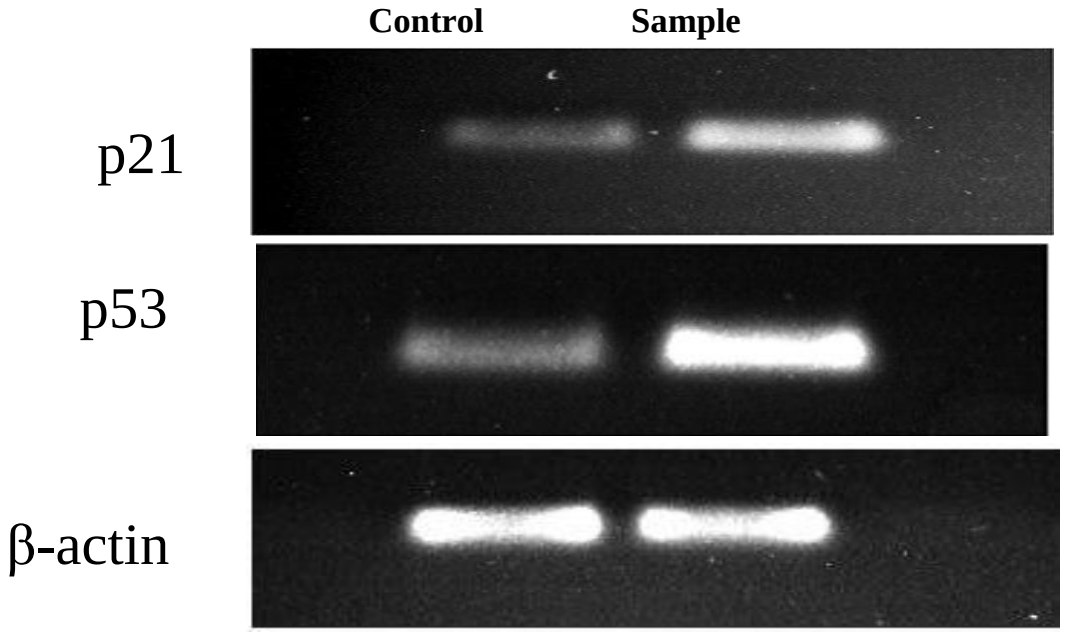



**Figure 35: Expression analysis of gene p21, p53 and β-actin using real time PCR**

**d) GREEN SYNTHESIS OF SILVER NANOPARTICLE**

Nanoparticle research is an intense scientific research area due to its potential application in the biomedical, optical and electronics field. Green synthesis of nanoparticles become a safe platform because they are free from toxic chemicals as well as contains natural capping agents. The present study highlights the evaluation of silver nanoparticle biosynthesis from selected *Memecylon* species. The silver nanoparticles were characterized through UV-Vis spectrophotometer and SEM analysis. The reduction of silver nitrate solution into silver nanoparticles after exposure to plant extracts is analyzed through the colour changes, surface plasmon resonance and shape of the nanoparticles.

The reduction of silver nitrate solution into silver nanoparticles by the action of plant extract that was resulted as the colour changes in the reaction tubes. It is the reducing capability of the plant extracts makes the silver nitrate solution into silver nanoparticles. It can be observed by the transformation of silver nitrate solution into a light yellowish brown or dark brown solution. *M. grande* leaf and fruit extracts were treated with silver nitrate solution resulting

in the formation of a brown colour solution, which indicates the presence of SNPs. The optimal conditions for the synthesis of SNPs using *M. grande* extracts are incubation period of 10 min., temperature at 80°C, 2 mM silver nitrate solution and pH 8.

*M. grande* fruit extract shows a brown colour change in the reaction tube whereas, *M. grande* leaf extract has a dark brown coloration in the reaction tube with an immediate reaction **(Plate 26 g, h)**. *M. randerianum* leaf extract possess a pale yellowish brown colour and its fruit extract is having a dark brown coloration in the reaction tube (**Plates 26 i; 27 g)**. The reaction mixture containing *M. umbellatum* leaf extract and silver nitrate solution produce a yellow coloured solution. The reducing capacity of *M. umbellatum* fruit extract produces a nanoparticle solution with brown colour (**Plate 27 h, i**).

UV-Vis spectroscopy (UV-Vis) is another relatively facile and low-cost characterization method of nanoparticle. The synthesized nanoparticles of selected *Memecylon* species were subjected to UV-Vis spectroscopy in a wavelength range of 200-700 nm. The synthesized nanoparticle of *M. grande* leaf extract, when subjected to UV-Vis spectroscopic analysis shows the maximum absorption peak at 440 nm. *M. grande* fruit extract possess a maximum absorption peak at 434 nm. The SNPs synthesized by both plant extracts show a broad peak area with an absorption value of 0.7 and 1.28 respectively **(Figure 37)**. The presence of a non specific peak is detected at 418 nm in *M. randerianum* leaf extract with absorption value of 0.3. The fruit extract of *M. randerianum* exhibit a specific broad peak at 432 nm with 1.71 absorption value. *M. umbellatum* leaf extract possess a non specific peak at 426 nm with 1.2 absorption value. A broad peak area at 468 nm with an absorption value of 0.33 was observed in *M. umbellatum* fruit extract. The range of 380-470 nm is characteristic λ max for AgNPs, so the peaks obtained from UV-Vis spectra confirm the presence of silver nanoparticles.

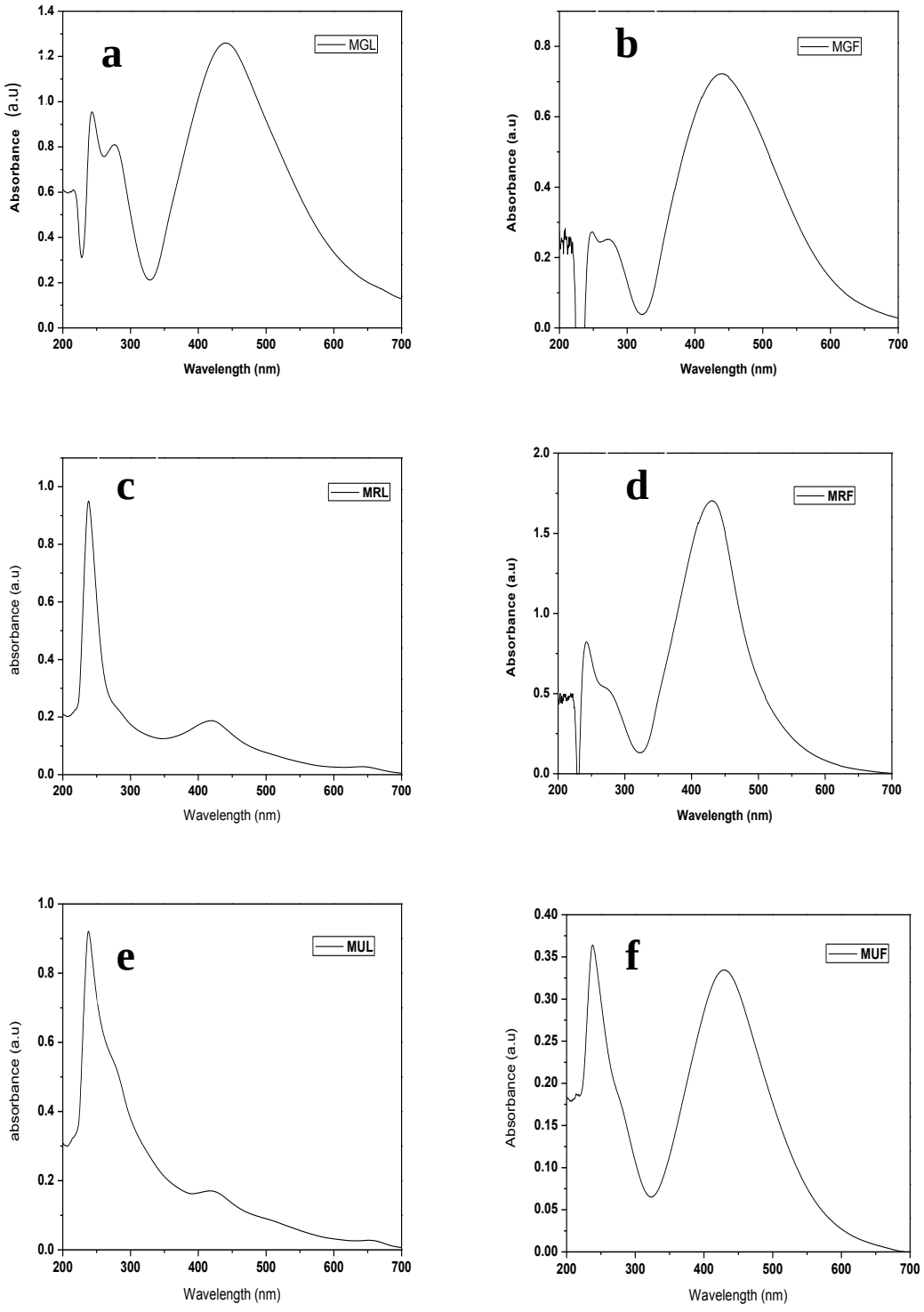



**Figure 37**: **UV-VIS spectra of silver nanoparticles synthesized by selected species of *Memecylon* a** - MGL: *Memecylon grande* leaves; **b** - MGF: *Memecylon grande* fruits; **c** - MRL: *Memecylon randerianum* leaves; **d** - MRF: *Memecylon randerianum* fruits; **e** - MUL: *Memecylon umbellatum* leaves; **f** - MUF: *Memecylon umbellatum* fruits.

The size and shape of synthesized nanoparticles are determined by Scanning Electron Microscopic analysis (SEM). In the present study, the nanoparticles of *M. grande* leaf extract is with a size of 20-30 nm and *M. grande* fruit extract possess 26-44 nm. The shape of the nanoparticle synthesized by the *M. grande* leaf extract is spherical (**Plate 28**) and that of *M. grande* fruit extract is with cubical shape (**Plate 29**). A perfect spherical shape with 20-32 nm sized nanoparticle was formed in the *M. randerianum* leaf extract mediated silver nanoparticle synthesis. Similarly a uniform size and morphology was exhibited by *M. randerianum* fruit extract also. They have spherical shape and with 20-28 nm size (**Plates 30, 31**). *M. umbellatum* leaf extract mediated silver nanoparticle synthesis contributes somewhat spherical shaped particles with 22-33 nm size. The fruit extract of *M. umbellatum* possesses almost spherical shaped silver nanoparticles with 26-35 nm size (**Plates 32, 33**).

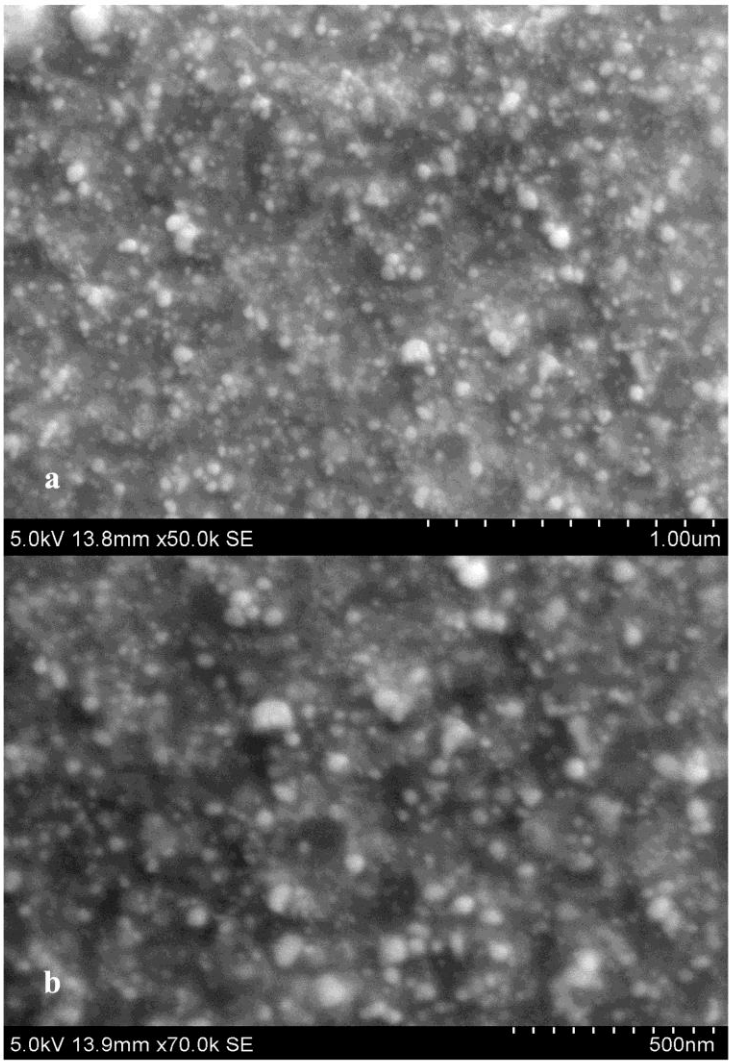



**Plate 28**: **Scanning electron micrographs of silver nanoparticles synthesized using *M. grande* leaves extract**. **a)** Low magnifcation **b)** Higher magnification

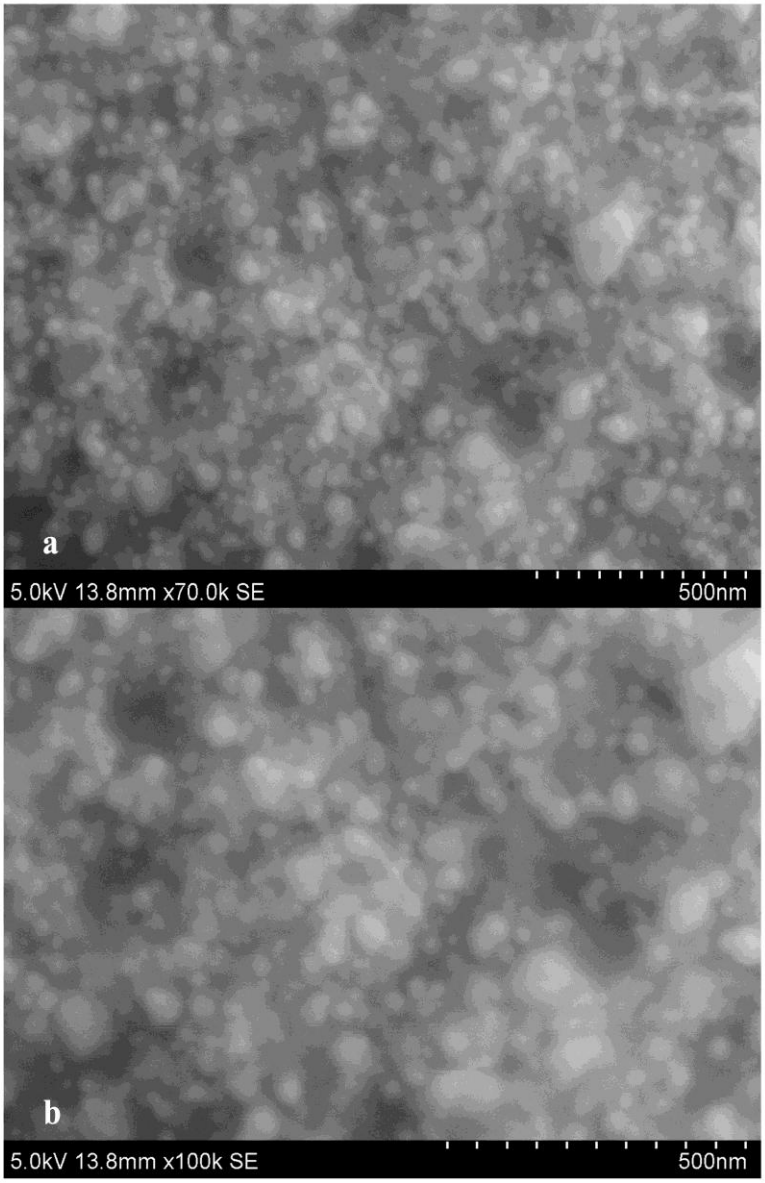



**Plate 29: Sanning electron micrographs of silver nanoparticles synthesized using *M. grande* fruits extract. a)** Low magnification **b)** Higher magnification

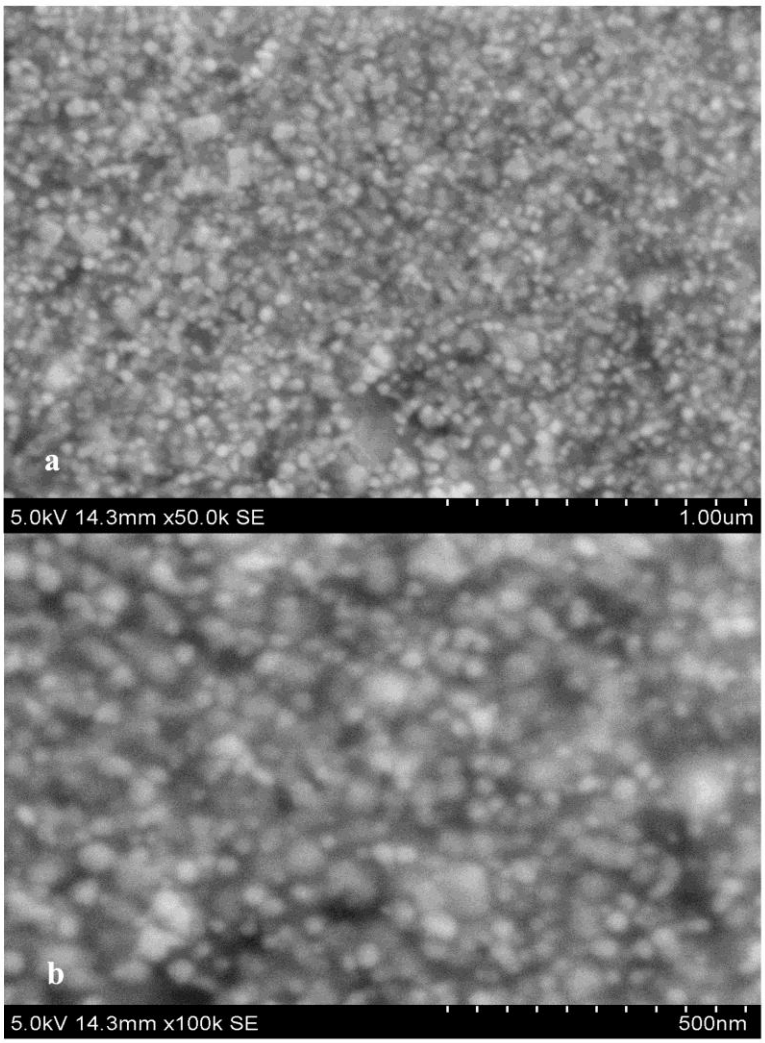



**Plate 30: Scanning electron micrographs of silver nanoparticles synthesized using *M. randerianum* leaves extract. a)** Low magnification **b)** Higher magnification

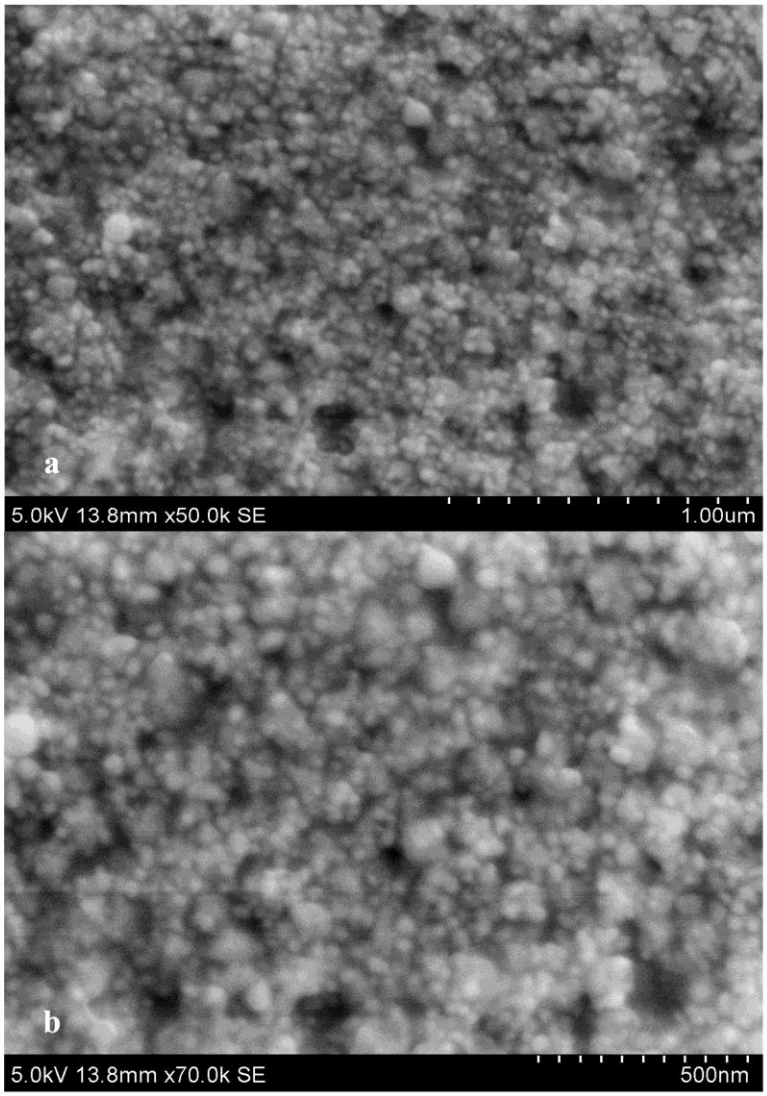



**Plate 31: Scanning electron micrographs of silver nanoparticles synthesized using *M. randerianum* fruits extract. a)** Low magnification **b)** Higher magnification

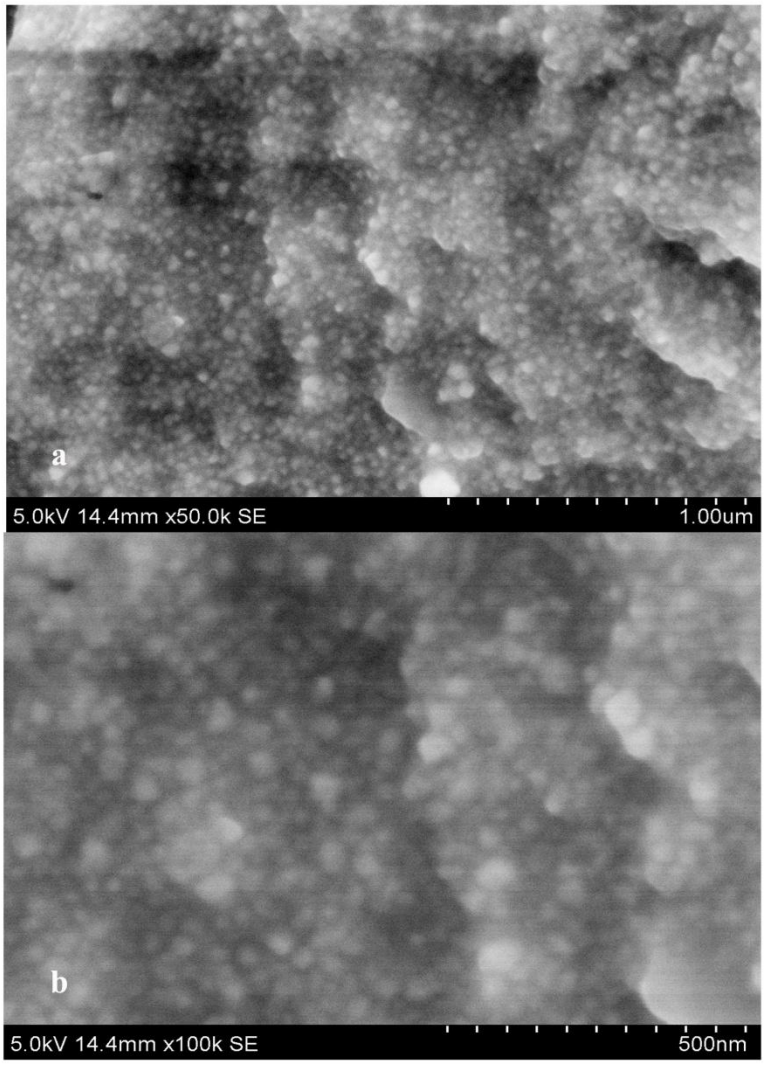



**Plate 32: Scanning electron micrographs of silver nanoparticles synthesized using *M. umbellatum* leaves extract. a)** Low magnification **b)** Higher magnification

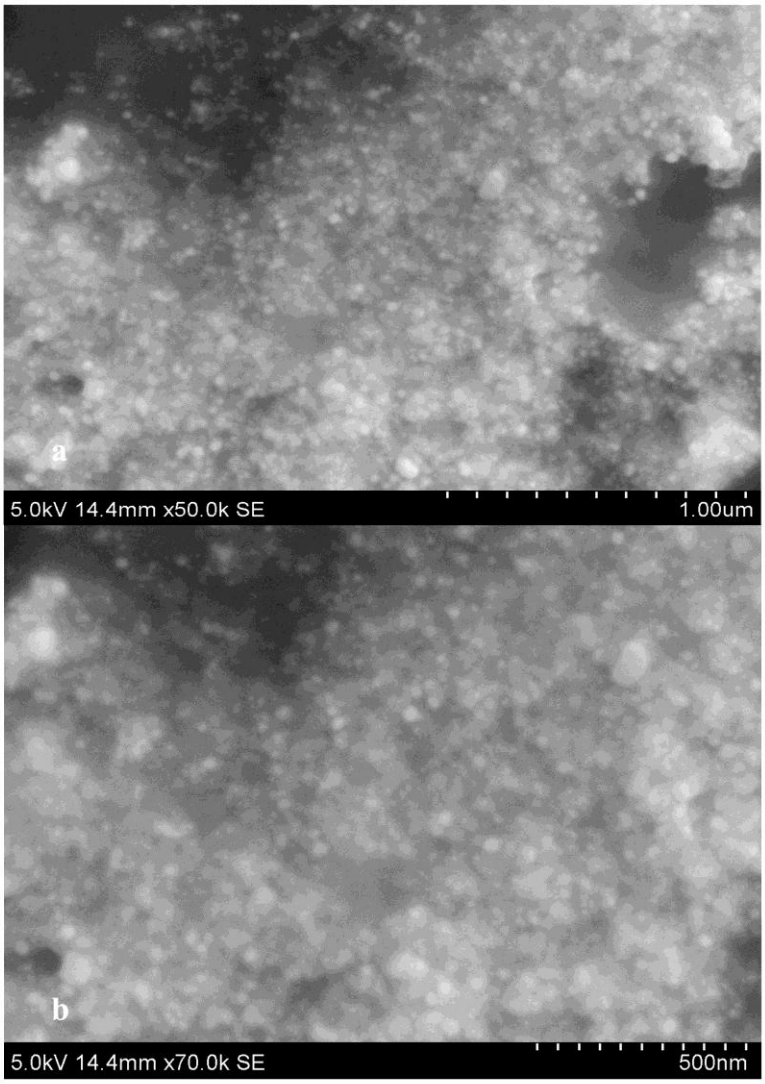



**Plate 33: Scanning electron micrographs of silver nanoparticles synthesized using *M. umbellatum* fruits extract. a)** Low magnification **b)** Higher magnification

# CHAPTER 5 DISCUSSION

## PHASE I- PHARMACOGNOSTIC PROFILING

Green technology and alternative eco-friendly products are a brand new thought to several people (Muller, 2017). The new lifestyle changes cause many perilous drawbacks, which opens a gateway for the search of new resolves. Thus nowadays the term “Green” becomes much popular. The major area under ‘Green’ consideration will be the medicinal field. Herbal medicines are a safe remedy for various human ailments because of it’s less side effects and low-cost treatments. So there is wide acceptance of the herbal medicinal system. The quality measurements of herbs are a challengeable stream, where the validations of herbs are more important prior to the usage. Adulterations become a curse in the herbal medicinal field, since they make quality and safety inconsistent. This will open a new approach to validate the quality assurance of herbs.

The collection of plant materials, authentification of specimens, analysis and formulation of drugs is the way to the discovery of the safer natural drugs. Here an attempt was done for the evaluation of pharmacognostic characters of the medicinally important genus *Memecylon*. Many systematic studies and new records are available on the genus *Memecylon*, but evaluations of micromorphological characters are trivial. The identification of *Memecylon* species becomes difficult due to the intraspecies morphological similarities. So the identification of species becomes much strenuous. The surface morphology of seeds or fruits, pharmacognostic evaluation and phytochemical analysis are the effective methods to rectify the

taxonomic difficulties in the authentification process and it opens a platform for the pharmaceutical analyses. Scanning electron microscopic analysis is the best way to analyze the surface features of the samples. The applications of SEM in vegetative and reproductive organs have great importance and impact on the systematic studies (Özcan, 2004). The functional purity of the plant sample is essential for the pharmaceutical trials. In the present study, purity of the sample was analyzed through the powder microscopy, SEM-EDX and ICPMS techniques.

Pharmacognosy is considered as a science of natural products. The term "natural product" may be applicable to the organism itself (plant, animal and microorganism) or any part of an organism (a leaf or flower of a plant, an isolated gland or other organ of an animal), and extract or pure substances (Orhan, 2014). It plays a pivotal role in drug preparation and therapies. Recently drug discovery from medicinal plants involves multifaceted approaches, combining botanical, computational, phytochemical, biological and molecular techniques. There are several examples of plant based drugs that are known to be indigenous to the medicinal system. Vincristine, vinblastine, morphine *etc.*, are few of them. The functional identity of the plant specimens that are targeted for the drug preparation should be analyzed. It is important to the specific bioactivity of the plant specimen. Nowadays, emphasis and focus of research in pharmacognosy have changed significantly, from focusing on identification of drugs, including the isolation of active principles, and more recently, the investigation of biological activity. Research into ethnobotany, ethnomedicine and ethnopharmacology has also become an important part of pharmacognosy (Sarker, 2012). While analyzing the application of pharmacognosy, it plays a crucial role in the identification of allied species or adulterants. The replacement of a drug with an allied species due to the unavailability of a particular crude drug on that particular season or its scarcity will critically abolish the bioactivity of the drug. It will

recall unwanted adverse effects of crude drugs. The substitution of medicinal plants with allied plants starts with the wrong identification of the plant specimen. The common vernacular name given to the different species will be misidentified by the people, which contribute to the chance of adulteration (Kumar, 2007). Unlike taxonomical identification, pharmacognostic studies offer the identification of powdered sample. In the powdered form of a sample, the morphological identity becomes lost and is easily prone to adulteration. At that time pharmacognostic techniques plays a key role. Adulteration and substitution are burning problems in herbal industry. So validation of functional purity of herbal medicine is very important.

There are several techniques employed in pharmaceutical field for the validation of herbal drugs. The validation of herbals may ensure the production of drugs with reproducible quality. The process validation is defined as "the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product" (FDA, 1987). The important standardization parameters used in pharmacognostic field includes organoleptic characters, macro and microscopic study, physico-chemical assays, phytochemical analysis, powder study and fluorescence analysis. Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity of plant specimen. The macroscopic study clearly emphasize on morphological identification and microscopic analysis, with the aid of a microscope. These are two common practices in pharmacognostic analysis. The powder microscopic analysis gave the characteristic features of powdered samples under microscopic evaluation. The parameters like moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values *etc*., are evaluated in physico-chemical analysis. Some constituents are visible only in fluorescent range in day light. So fluorescence

analysis is also a vital technique in pharmacognostic study (Chanda, 2014). Phytochemical analysis is an important part of pharmacological trails. Sometimes, it is considered as a separate branch and involves the metabolite profiling of plant samples. These techniques are validating the plant identity and standardization parameters for natural drug preparation.

The taxonomic profiling of *Memecylon* species are widely under consideration by many researchers. They are mainly focused on the morphological features of the plants. Macroscopic study handled by the taxonomic researchers incessantly discovering new species to our plant kingdom. It will always open the exploration of medicinally important species. The present work gave special emphasis on microscopic study of the selected *Memecylon* species. The phytochemical analyses are conducted as a separate section and included in the phase II studies. In microscopic study, powder analysis, scanning electron microscopy, energy dispersive X-ray analysis and ICP-MS analysis are carried out to reveal the pharmacognostic profile of the selected *Memecylon* species.

Powder microscopy acts as a diagnostic tool for the proper authentication of plant material. Several reports are available on the powder analysis of medicinally important plants (Najafi & Deokule, 2010; Kadam et al., 2012). In Ayurveda, 90% of the preparations are plant-based and hence the worthwhile usage of herbal medicines are promising candidates as the remedies of various human ailments. In most of the Ayurvedic preparations, the powdered samples of plant parts are used. So the authenticity of the powdered sample is very important. Powder microscopy is a simple and easiest method to analyze the powder sample and it is an essential step in the pharmacognostic evaluation of the plant sample. Microscopic techniques examine the structural and cellular features of herbs to determine their botanical origin. Microscopic evaluation is now an indispensable tool for the

identification of medicinal herbs and is one of the important parameters in modern science (Padmavathy et al., 2010a).

Here the powder samples of *Memecylon* leaves were characterized through their microscopic characters. It is light green coloured and odourless. Long trichosclereids, epidermal cells with tannin contents, paracytic stomata, thick walled fiber bundles, cluster crystals of calcium oxalate *etc*., are noticed and represented in **Plate 2**. The powder sample of *M. grande* fruits was brown coloured, odourless and slightly astringent (**Plate 3**). The characters found in the powders are epicarp cells, parenchyma cells with starch grains from mesocarp, stone cells from mesocarp, sclereids from endocarp, vessels with spiral and annular thickenings and rosette crystals. In the case of *M. randerianum* leaves powder sample is light green coloured, showing vessels with spiral and reticulate thickenings, fibre bundles, rosette crystals *etc*., (**Plate 4**). The fruits of *M. randerianum* are brown coloured, odourless with a characteristic taste (**plate 5**). It contains epicarp cells, stone cells, sclereids from endocarp, tracheids, fiber bundles, rosette crystals *etc*. The same type brown colored powder was also obtained in *M. umbellatum* fruits (**Plate 7**). The powder showed characters like epicarp cells, pitted parenchyma cells from mesocarp, stone cells, sclereids, spiral vessels, fibro-sclereids and rosette crystals. Leaves of *M. umbellatum* possess trichosclereids, mesophyll cells, parenchyma cells, vessels with reticulate and pitted thickenings, fibro-sclereids and rosette crystals (**Plate 6**).

These characters can be used to identify the plant specimen in Ayurvedic preparations. So we can easily identify the botanical origin of the plant specimen and clearly distinguish the presence of adulterants or the allied species. The microscopic evaluation of *M. umbellatum* leaves was done by Killedar et al. (2014b) and found the presence of lignified xylem with well-defined xylem fibers, vessels, and parenchyma. The presence of phloecentric

vascular bundles surrounded by endodermis and crystal sheath was also reported. *Memecylon* is an unexploited genus in pharmacognostic field. Only limited reports of literature are available on Melastomataceae family and *Memecylon* genus in the pharmacognosy field. Padmavathy et al. (2010a) evaluated the pharmacological profile of leaves and young stem of *M. umbellatum*. They analyzed parameters include macro-morphology, micromorphology, quantitative microscopy, physicochemical profile, powder analysis and fluorescence analysis. Dorababu et al. (2013) also established standards for *M. edule* leaves extract through the pharmacognostic study. The powder microscopic analysis confirms that the botanical origin of the plant samples is pure and devoid of foreign particles. So this result can be used as a standard reference for the identification of *Memecylon* fruits in future.

Scanning electron microscopy is a method for high resolution surface imaging using an electron beam having greater magnification and much larger depth of field. The fruit endocarp micromorphology and the entire seed morphology were studied by using a scanning electron microscope. The difference in electron emission in different areas provides the surface topography of the material. In this study, all the selected species show distinct morphological patterns. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships. In the case of *M. grande,* fruits show colliculate pattern in endocarp and the seed surface possesses tuberculate pattern (**Plate 8 a1-a4**). Scanning electron microscopic technique reveals that the fruit endocarp of *M. randerianum* has a pattern of ruminate reticulate type (**Plate 8 b1-b4**). The seed surface of *M. randerianum* is with a reticulate pattern. *M. umbellatum* fruit endocarp possesses a smoothened pattern and its seed surface shows a wrinkled pattern (**Plate 8 c1-c4**). The characteristic surface morphology becomes a useful tool in the identification process. Scanning electron microscopic analysis is a valuable tool in surface morphology analysis. The present study is a novel

report on the surface features of *Memecylon* species. The comparative seed morphology and pharmacognostic features of *Memecylon* species was initially reported by Ramya Sree and Thoppil (2020). In Melastomataceae, several reports are pointing towards the importance of SEM analysis in species delimitation. The seed morphology of 234 species distributed among 16 genera of the tribe Miconieae (Melastomataceae) was examined and documented with the use of scanning electron microscopy (Ocampo & Almeda, 2013). They had proposed a set of 37 morphological characters for describing size, general shape, raphal zone, appendage, testa characters and individual cell features of Miconieae seeds. In some cases, seed morphology corresponds with natural groups of species, which is of high phylogenetic importance (Martin & Michelangeli, 2009). The cladistic analysis of *Tococa* (Melastomataceae) was investigated using morphological data through SEM analysis (Michelangeli, 2000). Hence from the present study, the fruit endocarp and seed surface micromorphological and topographical data of *Memecylon* spp. obtained may prove to be distinguishing micromorphometrical markers for the identification of *Memecylon* spp.

Energy dispersive X-ray microanalysis (EDX) is a technique for analyzing elemental compositions at the microscopic level. For this purpose, scanning electron microscope (SEM) is equipped with an energy dispersive system having a quantitative electron probe for X-ray microanalysis. The SEM-EDX system can be applied to the surfaces of untreated specimens and, thus provides a vivid picture of elemental distribution in plant and animal material (Chen et al., 2014). Nowadays, it is used for the identification of single microbial cells exhibiting pathological conditions without following time-consuming microbiological cultivation methods (Khan et al., 2020). EDX analysis is a powerful tool in biomedical research and diagnosis of samples is well explained by Scimeca et al. (2018). The data generated by

EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed.

The present study is really focused on the elemental profiling of the selected *Memecylon* species. The elemental composition of *M. grande* fruits show that nitrogen content is maximum showing 91% and other elements are as follows; phosphorus 3.10%, potassium 1.53%, iron 1.41%, magnesium 0.87%, cobalt 0.63%, sodium 0.55%, copper 0.48%, calcium 0.35% and zinc 0.11% (**Figure 5 a**). In the case of *M. randerianum* fruit, nitrogen is the prominent element with 93% of the weight. Phosphorus 4.01%, potassium 1.15%, cobalt 0.90%, copper 0.49%, magnesium 0.22%, zinc 0.09%, calcium 0.09% and sodium 0.03% are the revealed composition of other elements (**Figure 5 b**). *M. umbellatum* fruit also possesses an elevated amount of nitrogen (93%) and all other elements in trace amounts like phosphorus 3.4%, potassium 1%, copper 0.95%, magnesium 0.67%, cobalt 0.34%, iron 0.22%, calcium 0.22%, zinc 0.11% and sodium 0.02% (**Figure 5 c**). This finding proves that *Memecylon* fruits are a reservoir of essential elements and it can be exploited in the pharmaceutical or nutritional field.

In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental composition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis was carried out. This technique gave the details of elements present in the sample in part per million units and determination of twelve elements were done *ie.,* aluminum (Al), arsenic (As), cobalt (Co), strontium (Sr), selenium (Se), zinc (Zn), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), barium (Ba) and manganese (Mn) **(Table 5)**. The standard reference concentrations of trace elements present in the adult human blood samples are noticed by Prashanth et al. (2015), because it is essential for the standardization of drugs**.** Most of the detected elements show vital biological functions**.** Some elements are the part of vitamins,

cofactors of enzymes, oxidative phosphorylation, fatty acids and cholesterol metabolism. It has been found that chromium causes significant increase in enzyme activity and serves as a stimulator in fatty acid and cholesterol biosynthesis from acetate molecule in the liver. It can also enhance sugar metabolism through the activation of insulin (Anderson, 1997). In the case of cobalt, it is the key factor of cobalamin (vitamin B12) and it has a role in the formation of amino acids and neurotransmitters. Although the biological function of nickel is still somewhat unclear in the human body, however nickel is found in higher concentration in the nucleic acids, particularly RNA and is thought to be involved in protein structure or function (Al-Fartusie & Mohssan, 2017). So the biological role of these trace elements is significant in regulating homeostasis and is vital for the prevention of free radical damage and various human ailments (WHO, 1973).

During the past decades, human beings are concerned about the nutritional status of the body. People are aware about the profound effect of micro and macronutrients on biological processes that range from whole-organism performance to the cellular function. According to the classification of trace elements, the group I which include carbon, hydrogen, oxygen, and nitrogen are the basic components of macromolecules such as carbohydrates, proteins, and lipids. Group II category includes nutritionally important minerals such as sodium, potassium, chloride, calcium, phosphorous, magnesium and sulfur. In group III, some essential trace elements like copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium are found (Prashanth et al., 2015). Copper plays an important role in the metabolism, mainly in the proper functioning of the enzymes and its deficiency may cause hypochromic anemia, joint pain neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility and osteoporosis (Campbell, 2001). Another most important element is iron, which is a prime portion of the blood cells and its deficiency is called anemia. Anemia is the

second most important cause of maternal mortality in India and 20% of mortality is directly related to anemia and another 50% is associated with other anemic side effects. In the case of zinc, it is essential for normal spermatogenesis and maturation, proper development of thymus, proper epithelialization in wound healing, taste sensation, and secretion of pancreatic and gastric enzymes (Watson, 1998).

Nickel is the cofactor of various enzyme catalyzed reactions. Nitrogenase enzyme, the key regulator of nitrogen assimilation in plants is catalyzed by nickel (Dmytryk et al., 2018). Nickel deficiency cause reduced growth rate and iron absorption rate in oraganisms. (Kumar & Trivedi, 2016). Molybdenum is an essential element for human body. It will help to neutralize sulfites in human body. Sulfites can induce allergic reactions and skin problems. Molybdenum catalyzes four major enzymes namely sulfite oxidase, xanthine oxidase, aldehyde oxidase, and mitochondrial amidoxime-reducing component (Novotny, 2011). The element strontium is closely related to calcium and they perform similar function, *ie.*, in bone formation (Specht et al., 2017). It can increase the bone density and used as a medicinal component in osteoporosis treatment (Kołodziejska et al., 2021). Manganese is an essential element in human body. It plays vital role in prevention of metabolic disorders and are known to be good free radical scavengers. It also reins the glucose and lipid metabolism in human body (Li & Yang, 2018). Melastomataceae members are known to be Al accumulators. The biological role of aluminium is closely related to tolerance capacity of the plant species. In the present study, the selected *Memecylon* species are good Al accumulators except *M. umbellatum* leaves extract. In *M. malabathricum* it has been suggested that Al is essential for its growth and the absence of the metal causes several morphological changes and chlorosis (Watanabe et al., 2006).

The element arsenic is known to have several therapeutic uses. The continuous exposure of arsenic on human body may lead to the development of many severe conditions. However, arsenic compounds are reported to be antitumor agents (Platanias, 2009). Selenium is a trace element found in human body that has anti- inflammatory, immune response and antioxidant effects. A new report on the beneficial effect of selenium was coined by Liu et al. (2021). It is effective in preventing RNA virus multiplication. The ICP-MS analysis thus proves that the selected *Memecylon* species are rich in bioactive elements needed for the human body. In trace quantity, all of them are essential for living organisms. The action of trace elements in the living system always depends upon the concentration of the elements (Mikulewicz et al., 2017).

Pharmacognostic profiling of selected *Memecylon* species gave a vivid picture of their powder characters, seed surface features and elemental composition. It can be considered as a valuable tool for identification of the *Memecylon* species. Powdered plant sample are the main raw material in the pharmaceutical preparations. So characterization of powdered sample is an antecedent important step in herbal drug preparation. Thus the present study thus contributes some pharmacognostic reference standards for the identification of *Memecylon* species in herbal medicinal system.

## PHASE II- PHYTOCHEMICAL CHARACTERIZATION

Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of several renewed systems of indigenous medicines such as Siddha, Ayurveda and Unani. Traditional systems of medicine rely on a single plant or combinations of more than one plant. Their efficacies depend upon the current knowledge about taxonomic features of plant species, plant parts and

biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites.

**a) Preliminary qualitative and quantitative phytochemical analysis**

Phytochemicals are naturally occurring bioactive chemicals found in plants. They possess various bioactivities, provide protection against diseases and damages, improve health conditions *etc*. Plants synthesize a wide range of chemical compounds which are classified on the basis of their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites are directly involved in growth and development of an organism. Examples are chlorophyll, amino acids, nucleotides, carbohydrates *etc*., which have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. The most important secondary metabolites are alkaloids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides (Geetha & Geetha, 2014). They are involved in the defensive mechanism of the organisms. In the present study various phytochemical constituents were identified in the selected *Memecylon* species through preliminary qualitative and quantitative phytochemical analysis, GC/MS and HR-LC/MS analysis.

Preliminary phytochemical analysis is the prime important step in phytochemistry. The methanolic extract of selected *Memecylon* species shows the presence of secondary metabolites like alkaloids, flavonoids, phenolics, steroids and tannins in all the extracts. The complete absence of resins was also observed (**Table 6**). In quantitative phytochemical analysis, considerable amount of major secondary metabolites like alkaloids, flavonoids, phenolics and terpenoids are noticed (**Table 7**). Phytochemicals are the basis of specific bioactivity of the plant species. The majority of natural products have certain biological properties, and they are used as medicines, insecticides, herbicides and perfumes or dye. For the last two to three decades, there has been a surge

of interest in plant foods as a source of phytochemicals, which could be beneficial in the prevention of chronic diseases like cancer, diabetes, heart disease, cataracts and gallstones (Rao, 2003). The secondary metabolites like alkaloids, terpenoids, phenolics *etc*., are the potential biochemicals and they have peculiar biological role that has been already reported. The biosynthesis of secondary metabolites is usually restricted to specific stages of plant development and during periods of stress. Some plant cells produce important secondary metabolites upon environment interactions or some are related to the reproductive mechanism of the plant (Mendoza & Silva, 2018). While considering the selected *Memecylon* species, considerable amount of phytochemicals are noticed and the bioactivity of the plant extracts depend on them. Basha et al. (2011) identified the presence of phytochemicals and reported its antimicrobial activity from aerial parts of *M. umbellatum*. Several previous phytochemical reports are validating the same result. *ie., Memecylon* species are a rich source of bioactive phytochemicals (Sivu et al., 2013; Murugesan et al., 2011). In this study, the biological activities of major secondary metabolites of three species of *Memecylon* are analyzed.

All over the World, several research groups have confirmed the vital role played by phytochemicals in reducing the risk of several diseases such as cancer and inflammatory conditions (Thangapazham et al., 2016). The usage of phytochemicals for the effective treatment of cancer was already reported (Kotecha et al., 2016; Liu et al., 2014). The protective mechanism of phytochemicals in stroke was reported by Kim and Karadeniz (2012). Many phytochemical compounds with anti-inflammatory, antioxidant and apoptotic effects have been widely studied (Feng et al., 2019; Husain et al., 2018; Son et al., 2008). The progressive usage of phytochemicals through diets as an effective method to cure the diseases was widely analyzed (Tan & Nishida, 2012). All these reports are pointing to the efficacy of the phytochemicals and the importance of their validation.

Alkaloids are natural compounds that show significant biological effects on animal models and also in human beings in very small doses. Atropine, morphine, quinine and vincristine are some of the important alkaloids used to treat a wide range of disease conditions from malaria to cancer. Alkaloids from many different plant species have other useful applications such as antiparasitic, antiplasmodial, anticorrosive, antioxidative, antibacterial, anti-HIV, and insecticidal activities (Kurek, 2019). In the present study, among the selected six extracts from three plants, *M. grande* fruit extract shows the highest amount of alkaloids, phenolics and terpenoids and *M. randerianum* fruit extract exhibits the lowest amount of alkaloids (32.17 ± 1.41 mg CE/g DW). The cytotoxic activity of alkaloids was noticed by Mat et al. (2000). The alkaloids isolated from *Brunsvigia radulosa* was tested against two strains of cultured *Plasmodium falciparum* and it's cytotoxicity was tested against BL6 mouse melanoma cells and shows a promising result (Campbell et al., 2000). In total flavonoid determination, *M. grande* leaf extract shows the highest content (215.96 ± 1.87 mg QE/g DW). Flavonoids are the largest group of phytoconstituents with more than 6000 varieties. They are classified into flavones, flavanones, isoflavones, catechins, and anthocyanins. The presence of flavonoids is responsible for the vivid colours in fruits and vegetables. It has many potential pharmacological roles *ie.,* antihepatotoxic, antiulcer and anti-inflammatory effects (Bors et al., 1990). Flavonoids also act as a secondary antioxidant defense system in plant tissues that are exposed to different abiotic and biotic stresses. Flavonoids are located in the nucleus of mesophyll cells and within the centers of ROS (Agati et al., 2012). The pharmacological and biochemical effects of flavonoids inhibit the action of various enzymes like cycloxygenase, phosphodiesterase, lipoxygenase *etc.* and also the hormones like androgens, estrogens and thyroids (Agrawal, 2011). Some of the flavonols like quercetin are found to inhibit cell growth of leukemia cells and EAC cells (Sorata et al.,

1984). Yoshida et al. (1990) has studied the effect of quercetin on cell cycle progression in human gastric cells. The flavonoid uptake has a direct action on the cell cycle of human colon adenocarcinoma cells, which was reported by Salucci et al. (2002).

Phenolic compounds are the main class of secondary metabolites in plants and are divided into phenolic acids and polyphenols. Many studies have shown a strong and positive correlation ($p \leq 0.05$) between the phenolic compound contents and the antioxidant potential of fruits and vegetables (Pinhero et al., 2016). In the present study, 370.28 ± 1.36 mg GAE/g DW phenolic content was noticed in *M. grande* fruit extracts. Similarly *M. randerianum* fruit extract possess 276.06 ± 1.12 mg GAE/g DW and *M. umbellatum* fruit extract with 60.83 ± 5.70 mg GAE/g DW as the phenolic content. The lowest amount of phenolic content was observed in *M. randerianum* leaf extract with 49.52 ± 4.72 mg GAE/g DW. Phenolics are considered as good antioxidant agents, because phenolic hydroxyl groups are good hydrogen donors. Hydrogen donating antioxidants can react with reactive oxygen and reactive nitrogen species (Pereira et al., 2009). In glioma cancer, cell proliferation can be effectively inhibited by the action of phenolic compounds (Lu et al., 2010). The wide utility of phenolic compounds was described by Olthof et al. (2001). The antibacterial property of phenolics was reported by Lou et al. (2012).

There are many reports regarding the bioactive terpenoids of plant origin (Macias et al., 2002; Li et al., 2009). Plant-based terpenoids have been used by humans in the food, pharmaceutical and chemical industries. Recently it has been exploited in the development of biofuel products (Tholl, 2015). Terpenoids have multifunctions such as the suppression of tumor proliferation, apoptosis inducing capacity and act as cation regulating channel (He et al., 1997; Roullet et al., 1997). In the preliminary quantitative

phytochemical analysis, terpenoids is the leading secondary metabolite in all the selected six plant extracts. It ranges from 127.5 ± 1.50 to 378.21 ± 1.02 mg LE/g DW. Terpenoids are used as potential chemopreventive and therapeutic agents in liver cancer treatment (Thoppil & Bishayee, 2011). Terpenes have anti-inflammatory effects by inhibiting various proinflammatory pathways in ear edema, chronic obstructive pulmonary disease, skin inflammation, and osteoarthritis (Rufino et al., 2014; Yu et al., 2016).

The presence of tannin was noticed in all selected plant samples. Tannins are a group of polyphenols. The effects of polyphenols in plants are reported by Zdunczyk et al. (2002). The antimicrobial activities of tannins are well studied. Tannins serve as a natural defense mechanism against fungi, yeasts, bacteria and viruses. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf life of certain foods, such as catfish fillets. Tannins have certain physiological effects, such as to accelerate blood clotting, decrease the serum lipid level, reduce blood pressure, produce liver necrosis and modulate immune responses. All these effects were dependent on the dosage and kind of tannins (Chung et al., 1998). A phlobaphene condensed tannins called as phlobatannins were noticed in *M. randerianum* leaf and fruit extracts and in *M. umbellatum* leaf extract. They have been reported to possess wound healing, anti-inflammatory, antioxidant and analgesic activities (Ayinde et al., 2007; Kumari & Jain, 2015). Coumarins are another group of polyphenols, which have a significant effect on physiological, bacteriostatic and antitumor activity (Rohini & Srikumar, 2014). Coumarins were noticed in the leaves extract of *M. randerianum* and *M. umbellatum*. Coumarins have potent anticancer activity *ie.,* it can be used against prostate cancer, renal cell carcinoma and leukemia (Finn et al., 2002). Glycosides are naturally occurring compounds with a wide range of medicinal and clinical applications. Both *M. grande* leaf and *M. umbellatum* fruit as well

as leaf extract show positive results in Keller Killiani test. Keller Killiani test is a method for determining cardiac glycosides. The anticancer property of glycosides against several cancer cell lines is reported by Khan et al. (2019). The potential biological roles of glycosides were discussed by various researchers, validating their significance (Kren & Martínková, 2001; Kren & Řezanka, 2008; La Ferla et al., 2011).

Saponins and steroids are the other phytochemicals observed during preliminary analysis. The presences of saponins are identified in all plant extracts except *M. umbellatum* fruit extract. Saponins are a class of substances with a rigid skeleton of at least four hydrocarbon rings to which sugars in groups of one or two are attached. Saponins have been proposed for the treatment of a variety of diseases, including diabetes, obesity and osteoporosis (Marrelli et al., 2016). It has anticancer properties through inhibiting cell proliferation, to counteract angiogenesis and to stimulate apoptosis (Kregiel et al., 2017). The plant steroids are known to be potential anti-inflammatory agents (Patel & Savjani, 2015). Phytosterols possess antioxidant activity, anti-inflammatory activity and anticancer activity against lungs, stomach, ovary and estrogen-dependent human breast cancer (Jain et al., 2019). The preliminary phytochemical analysis gave an insight on the secondary metabolite profile of selected *Memecylon* species. The biological role of these potential phytoconstituents leads to a detailed exploration to reveal the bioefficacy of *Memecylon* species. In the next section, a detailed phytochemical profile of *Memecylon* species is discussed.

**b) Phytochemical profiling by GC/MS**

One of the major criteria for the phytochemical validation is the characterization of bioactive compounds. Preliminary qualitative and quantitative analysis gave an insight on the occurrence of the phytoconstituents. Various preliminary phytochemical analyses confirm the

presence of these metabolites (Soumya et al., 2015). Gas chromatographic and liquid chromatographic assays are the common methods followed for identifying the biocomponents present in the sample. Gas chromatography-mass spectrometry is an analytical tool for the quantification of volatile compounds present in the plant extract. A total of 84 compounds were identified in the six samples of selected *Memecylon* species [**Table 8, Figures 13 (i-xvii**].

Fatty acid esters are the predominant group of compounds detected in *M. grande* methanolic extract. 9-Hexadecenoic acid methyl ester, propyl palmitate, methyl oleate, 10,13-octadecadienoic acid methyl ester, methyl 9-cis,11-trans-octadecadienoate, propyl oleate and methyl linoleate are detected in the GC/MS analysis of *M. grande* leaf extract. Fatty acid esters are used as bioadhesive agent and it has potential antioxidant activity (Hansen et al., 2001; Matsufuji et al., 1998). The antibacterial activity of poly unsaturated fatty acids and their ester derivatives against various oral pathogens like *Candida albicans*, *Streptococcus mutans* and *Porphyromonas gingivalis* was reported by Huang and Ebersole (2010). Fatty acid esters like methyl arachidate, methyl linoleate, methyl stearate and methyl myristate are common in the leaf and fruit extracts of *M. grande*. The fatty acid ester profile of *M. grande* fruit includes methyl octanoate, methyl pentadecanoate, ethyl 9-hexadecenoate, methyl elaidate and methyl palmitate. Fatty acid esters possess free radical scavenging activity and antiproliferative activity against human ductal breast epithelial tumor T47D, human breast adenocarcinoma MCF-7, human epithelial carcinoma HeLa, human epithelial colorectal adenocarcinoma Caco-2, human colorectal adenocarcinoma cell line HRT and human kidney carcinoma cell line A498 (Elagbar et al., 2016). The phytochemical reports from various *Memecylon* species are again validating these fatty acid profiles (Bharathi et al., 2017b; Uppu et al., 2018).

Palmitic acid, oleic acid and linoleic acid are found to be common in both leaf and fruit extracts. From the root extract of *M. umbellatum*, palmitic acid was isolated by Joshi et al. (2009b). Palmitic acid is a common fatty acid found in all selected plant extracts except *M. umbellatum* fruit extract. Fatty acids play an important role in cellular biological functions. The elevated fatty acid concentration can inhibit T-lymphocyte signaling and induce pancreatic B-cell apoptosis (Stulnig et al., 2000). Oleic acid (29.01%) is the prominent fatty acid noticed in *M. grande* leaf extract. Oleic acid is more steatogenic but less apoptotic than palmitic acid in hepatocyte cell cultures (Ricchi et al., 2009). Linoleic acid is known to be an anticancer agent. It reduces the risk of cancer in mice models as compared to control mice system (Ha et al., 1987). Numerous physiological activities were attributed to the conjugated linoleic acid. The trans-10, cis-12 isomers of linoleic acid inhibits lipoprotein lipase and stearoyl-coA desaturase, thereby reducing the uptake of lipids. The isomers like cis-9, cis-12, trans-11 and trans-10 conjugated linoleic acids are active in inhibiting carcinogenesis in animal models (Pariza et al., 2001).

Cholesterilene and campesterol acetate are steroid compounds detected in the leaves and fruit extracts of *M. grande*. Cholesterilene was found to exhibit wound healing activity, which was reported by Badiu et al. (2008). Antiangiogenesis activity of campesterol from *Chrysanthemum coronarium* was described by Choi et al. (2007). Oleamide is an organic compound found to be common in both extracts of *M. grande*, more over it was found to be the highest fraction of fruit extracts (31.27%). It is a fatty acid amide that can activate G-protein coupled, and other receptors to regulate a diversity of cellular and physiological functions throughout the body, including the reproductive, immune, nervous and cardiovascular systems (Hiley & Hoi, 2007). The antiepileptic and nephro-protective effect of oleamide was reported by Nam et al. (2017). Z, Z-6, 28-Heptatriacontadien-2-one is a ketone found in *M. grande* fruit extract. α-Amylase inhibition and antioxidant

activity of some marine algae was found to be due to Z, Z-6, 28-Heptatriacontadien-2-one (Unnikrishnan et al., 2015). This compound also contributes to the anti-inflammatory and larvicidal effect of the plant samples (Anupama et al., 2014; Pratheeba et al., 2015).

Stigmast-5-en-3-ol is observed in *M. grande* fruit extract and α-phytosterol is specific for *M. grande* leaf extract. These are sterol compounds identified in *M. grande*. The apoptotic and antiproliferative effects of stigmast-5-en-3-ol on human leukemia HL-60 and human breast cancer MCF-7 cells was reported by Fernando et al. (2018). It induces apoptosis mechanism through mitochondria mediated pathway. The insulin-like effect of stigmast-5-en-3-ol in stimulating glucose transport *in vitro* reveals the potential antidiabetic activity apart from its existing cholesterol lowering efficacy (Sujatha et al., 2010). The lowering of low density lipoprotein cholesterol is effective in reducing metabolic syndromes. It is also associated with increased cardiovascular disease. Phytosterol is effective in reducing cardio vascular diseases (Jones et al., 2000; Lerman et al., 2010). Phytosterol is an excellent candidate for cancer chemo-prevention, such as prostate cancer. The phytosterol intake was associated with a reduction in risk of 50% lung cancer that was reported in a case study in Uruguay (Shenouda et al., 2007; Mendilaharsu et al., 1998).

Among the selected *Memecylon* species, *M. randerianum* leaf extract possess highest amount of palmitic acid content (15.51%). The antitumor activity of palmitic acid was noted by Harada et al. (2002). Apoptosis induction ability of palmitic acid was analyzed through western blot analysis and it shows that it can down regulate apoptosis inhibitors like Bcl2 and up regulate apoptosis effecter, Bax. The other fatty acids noticed include lauric acid and octadecanoic acid. It was found to be 1.73 and 2.09% respectively. Lauric acid can modulate serum cholesterol levels and it is shown to be very

active against gram positive bacteria, a number of viruses and fungi. Lauric acid has the strongest antimicrobial activity among all saturated fatty acids (Dayrit, 2015). Octadecanoic acid can control inflammation reaction through the competitive inhibition of phospholipase A(2) (Aparna et al., 2012). *In vitro* studies of octadecanoic acid revealed that it is used as a pro-apoptotic signal for eliciting anti-inflammatory responses. Caspase-3 along with MMP2 and MMP9 affirms the anti-inflammatory properties. Molecular docking studies also show that octadecanoic acid has a strong binding affinity to MMP-2 (Manivannan et al., 2017).

Squalene is a natural dehydrotriterpenic hydrocarbon ($C_{30}H_{50}$) with six double bonds, an intermediate for the biosynthesis of phytosterol/cholesterol in plants/animals and humans, widespread in animal and plant kingdom. Anticancer activity and antioxidant potential of squalene was widely discussed (Huang et al., 2009). The interest in squalene was raised long ago, after the characterization of squalene in shark liver oil. Several studies exhibited that it has a wide spectrum of biological activities. Squalene was the third leading compound identified in *M. randerianum* leaf. It is also present in fruit extract of *M. randerianum* and leaf extract of *M. umbellatum*. Till date, anticancer, antioxidant, drug carrier, detoxifier, skin hydrating and emollient activities of squalene have been reported both in animal models and under *in vitro* environments (Kim & Karadeniz, 2012). Squalene is said to be a chemopreventive agent. The major activities underlying chemoprevention include inhibition of Ras farnesylation, modulation of carcinogen activities and antioxidant activity (Smith, 2000). Several epidemiological studies in breast, colon and pancreatic cancer shows that squalene uptake will diminish the risk of cancer and the tumor inhibiting role of squalene is a promising one (Newmark, 1997; Rao et al., 1998). Lupeol is a pentacyclic triterpene found in various species in the plant kingdom. This molecule exhibits a spectrum of pharmacological activities against various acute or chronic diseases, including

arthritis, renal disorders, diabetes, cancer, and microbial infections (Badshah et al., 2016; Alqahtani et al., 2013). The beneficial role of lupeol includes hepatoprotective, cardioprotective, anti-inflammatory and cancer chemo preventive activities, which was discussed by Patil (2018).

A diverse array of terpenoid compounds was detected in the GC/MS analysis. Agathenic acid and dihydroabietic acid are the diterpene compounds resulted through the analysis. Agathenic acid, a diterpenoid found in *M. randerianum* leaf extract, showed cytotoxic, antioxidant or antimicrobial activities alone or more often in synergism with other essential oil compounds. Labdane-type and abietane-type diterpenes have shown cytotoxicity against tumor cells and abietane-like compounds play an important role as antioxidants (Stanetic & Buchbauer, 2015). Antiulcer property of dehydroabietic acid was analyzed by Wada et al. (1985). Gastroprotective and cytotoxic effect of dehydroabietic acid derivatives was checked by Sepulveda et al. (2005).

Bicyclogermacrene (3.59%), γ-eudesmol (0.62%), farnesyl acetate (3.44%), ledol and virdiflorene are the sesquiterpenes observed in the leaf extract of *M. randerianum*. Antioxidant activity of bicyclogermacrene was reported by Yu et al. (2016). It also possesses potent cytotoxic activity (Grecco et al., 2015). The sesquiterpene, γ-eudesmol was mainly present in the wood oil of the plant species *Callitris collumellaris, C. intratropica*, eucalyptus oil, guava fruit oil *etc*. It exhibited potential cytotoxic activity against cancerous cells in liver by reducing the proliferation and causing the death of tumor cells by caspase-mediated apoptosis (Britto et al., 2012). Farnesyl acetate is a derivative of an isoprenoid compound of the Mevalonate pathway. It shows antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, E. faecium, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*. The cytotoxic activity was observed against

different cell lines that include malignant melanoma MeWo, colorectal adenocarcinoma HT29, promyelocytic leukemia HL60, gingival fibroblasts HFIG, skin keratinocytes HaCaT and rat small intestine epithelium IEC6 (Bonikowski et al., 2015). Larvicidal efficiency of virdiflorene was reported by Zhao et al. (2017) and Liu et al. (2014).

Phytol is a diterpene alcohol abundantly available in nature. The antinoceptive activity of phytol was effectively proven by Santos et al. (2013) and found that there is no change in motor functions of animals. The antinoceptive activity associated with antioxidant activity of phytol was also demonstrated by them. The antioxidant, apoptosis, antimicrobial, cytotoxic and anti-inflammatory effects of phytol prove that, it is a promising candidate in pharmaceutical field (Islam et al., 2018). The other terpene alcohols resulted in *M. randerianum* leaf extract by GC/MS analysis include maaliol (1.57%), β-eudesmol (4.58%) and α-cadinol (1.28%). Antinociceptive activity of maaliol was studied in *Valeriana wallichii* by Sah et al. (2012). It acts as an insect antifeedant component in *Senecio fistulosus* (Ruiz-Vásquez et al., 2019). β-Eudesmol inhibited angiogenesis in granuloma tissue in mice at 0.9 mol/kg (202 g/kg) (Tsuneki et al., 2005). Significant antihepatotoxic effects were exhibited by β-eudesmol against carbon tetrachloride-induced cytotoxicity in rat hepatocytes (Kiso et al., 1983). α-Cadinol was said to act as antifungal and hepatoprotective agent (Ho et al., 2011).

α-Angelica lactone is a five-membered unsaturated lactone, which is used as a flavoring agent and for fragrance. α-Angelica lactone is found in nature in almonds, coffee, raisins, cranberries, coconuts and soybeans. Tumor-inhibiting effect of α-angelica lactone was found by increasing the activity of the detoxifying enzyme glutathione-S-transferase (Nijhoff et al., 1993; Nijhoff et al., 1995; Van der Logt et al., 2003). The phenylpropene profile of *M. randerianum* leaf extract include chavicol, phenol, 2,4-bis(1-

phenylethyl) and methyl eugenol. Chavicol is also known as *p*-allylphenol. It is a natural phenylpropene, a type of organic compound. Chavicol is used as an odorant in perfumery. Phenol, 2,4-bis(1-phenylethyl), is a phenylpropanoid derivative. It's anti-inflammatory effect was studied by Chen et al. (2007). *In vitro* antimitotic, apoptotic and antiproliferative activity of this phenylpropanoid was examined in various studies (Melappa & Prakash, 2017; Muthulakshmi et al., 2012). Methyl eugenol is otherwise known as allylveratrol, which is a natural chemical compound classified as a phenylpropene, a type of phenylpropanoid. Methyl eugenol is found in a number of plants. The compound may have evolved in response to pathogens, as methyl eugenol and has some antifungal activity. It also repels many insects (Tan & Nishida, 2012). Eugenol is a phenolic compound, colorless to pale yellow and is an aromatic oily liquid extracted from clove, nutmeg, cinnamon, basil and bay leaf. Eugenol is found to be hepatotoxic (Thompson et al., 1998). It is also used as a local antiseptic and anaesthetic (Sell & Carlini, 1976). The recent scientific evidence supports that eugenol exerts beneficial effects on human health. The antimicrobial activity of eugenol is reported by Marchese et al. (2017). It also possesses anti-inflammatory activity through inhibited prostaglandin synthesis and reduced the tone of isolated gut muscle and myometrium in *in vivo* rat system (Bennett et al., 1988).

Friedlein is the prominent triterpene compound (31.3%) found in *M. randerianum* fruit extract. The antiviral efficacy of friedlein was tested against the NS3 helicase protein of hepatitis C virus. The computational screening method reveals that, it shows better drug-likeliness, activity and stability (Arumugam et al., 2013). The anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. was examined using *in vivo* models such as mouse and rat models. The tests like carrageenan-induced hind paw oedema, croton oil-induced ear oedema, acetic

acid-induced vascular permeability *etc.,* are employed to evaluate the inflammatory potential of the compound. Acetic acid-induced abdominal constriction response, formalin-induced paw licking response and the hot-plate test are the methods opted for the evaluation of analgesic effect of friedelin. The yeast-induced hyperthermia test in rats was the antipyretic evaluation method. All these test systems, evidently proved the potent anti-inflammatory, analgesic and antipyretic effects of friedelin (Antonisamy et al., 2011). The antimicrobial and cytotoxic effect of friedelin was noted by Mokoka et al. (2013). Squalene is another triterpenoid present in the plant extract with of *M. randerianum* fruit extract with 7.69%.

Stigmast-5-en-3-ol (12.8%) is the second lead compound in *M. randerianum* fruit extract. 4-Campestene-3-one and 4,22-stigmastadiene-3-one are the steroid components of *M. randerianum* fruit. Methyl palmitate and methyl stearate are the two fatty acid methyl ester compounds resulted in GC/MS analysis. Fatty acids like linoleic acid, octadecanoic acid and palmitic acid are also detected in the analysis. An organic compound quinic acid (0.67%) was also resulted through the analysis. The derivatives of quinic acid have antimicrobial and anti-inflammatory activity (Zanello et al., 2015; Zeng, 2010). Another organic compound noticed in the *M. randerianum* fruit extract is vitamin E. It possesses a wide range of bioactivities. β-Thujone and 3-thujanol are the monoterpenes detected in the fruit extract. The toxicity of thujone was extensively studied. It acts as a modulator of the $GABA_A$ receptor. Long term exposure lead to neurotoxicity (Pelkonen et al., 2013). The antifungal activity of β-thujone and α-thujone was tested against *Tiarosporella phaseolina*, *Fusarium moniliforme* and *Fusarium solani* (Farzaneh et al., 2006).

*M. umbellatum* leaves are a rich source of various bioactive metabolites. α-Phytosterol (17.72%) is the prominent compound detected

through GC/MS analysis. The cardio-protective and anti-inflammatory effects plant sterols in hyperlipidemic individuals were reported by Micallef and Garg (2009). The plant sterols commonly found in the diet are β-sitosterol, campesterol and stigmasterol. A clinical study indicates that the intake of phytosterols (2 g/day) is associated with a significant reduction (8-10%) in levels of low-density lipoprotein cholesterol (LDL-cholesterol) and lowering the risk of cardiovascular diseases (Cabral et al., 2017). Other important sterol compounds identified in the extract include campesterol and stigmasta- 5,22-dien-3-ol. As noted in *M. randerianum* leaf, campesterol is the bioactive component present in *M. umbellatum* leaf extract. The antimicrobial activity of stigmasta- 5,22-dien-3-ol was discussed by various researchers (Achika et al., 2016). 4,22-Stigmastadiene-3-one and 4-campestene-3-one is recognized as the steroid compounds in *M. umbellatum* leaf extract. 4,22-Stigmastadiene-3-one possess antimicrobial activity, which was identified by Singariya et al. (2013). 4-Campestene-3-one was identified in *Melia azedarach,* and it shows cytotoxic effects against several cancer cell lines such as, human colorectal carcinoma HT-29, breast cancer MCF-7, SK-BR-3 and kidney epithelial cell MDBK (Ervina, 2018).

Fatty acids like palmitic acid, octadecanoic acid and several fatty acid methyl esters such as methyl myristate, methyl palmitate, methyl heptadecanoate and methyl linoleate are also noticed in *Memecylon* spp. The fatty acids are known to be good antioxidant agents. They can reduce the cellular damages caused by oxidative stress. Oxidative stress can induce cell proliferation, cell division defects and cellular damages (Elagbar et al., 2016).

4-Vinylguaiacol and methyl 4-hydroxyphenyl acetate are the phenolic composition of the *M. umbellatum* leaf extract. 2-Methoxy-3-allylphenol is identified as a phenylpropanoid compound. Phenolic compounds have apoptosis inducing ability through the regulation of copper ion mobilization,

which can also interfere with chromatin during DNA fragmentation (Greenwell & Rahman, 2015). 4-Vinylguaiacol is used as a flavoring agent and it has good antioxidant property (Azadfar et al., 2015). It is a sinapic acid derivative and used as an antioxidant and antimutagenic agent, which suppresses carcinogenesis and the induction of inflammatory cytokines (Nićiforović & Abramovič, 2014). Methyl 4- hydroxyphenylacetic acid is known to give auxin-like effects in higher plants (Fries & Iwasaki, 1976). 2-Methoxy-3-allylphenol act as a cytotoxic, anti-inflammatory and antimicrobial agent (Loying et al., 2019). Curently, dietary phenolics have a great interest due to their antioxidative and possible anticarcinogenic activities.

α-Springene, neophytadiene and squalene are the revealed terpenes in *M. umbellatum* leaves. Neophytadiene is a good analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant compound (Raman et al., 2012). Squalene possesses a wide range of pharmacological activities that were already stated. Terpene alcohols like, phytol, 3,7,11,15-tetramethyl-2-hexadecen and isophytol that are revealed in GC/MS analysis. Hydroxymethylfurfural, levoglucosan, dihydroconiferyl alcohol and 1,1,10-trimethyl-6,9-epidioxydecalin are the organic compounds profiles identified in *M. umbellatum* leaf extract. Hydroxymethylfurfural (HMF) is a dehydration product of certain sugar moiety. It is considered as an important intermediate due to its rich chemistry and potential availability from carbohydrates sources. In recent years, considerable efforts have been made on the transformation of carbohydrate into HMF. Several biological activities of HMF is tested on *in vitro* and *in vivo* systems. The mutagenic effect has been assessed by the Ames test and found to be non-mutagenic in nature (Rosatella et al., 2011). Moreover, the presence of HMF protected the human liver cell line-LO2 against exposure to hydrogen peroxide, because it prevented nitric oxide production, caspase-3 activation and arrest of the cells

in the S-phase of the cell cycle (Ding et al., 2010). Levoglucosan is an active biocomponent in a variety of plant species such as *Aronia melanocarpa*, *Holigarna grahamii*, *Terminalia coriacea etc*. It has potent antioxidant and anti-diabetic properties. Dihydroconiferyl alcohol acts as a gibberellin synergist in hypocotyl elongation process (Shibata et al., 1975).

Vitamin E (α-Tocopherol) content in *M. umbellatum* leaf extract was found to be 5.73%. It is involved in the regulation of cellular signaling and gene expression. From the eight different forms of vitamin E, only α-tocopherol is retained in the body, because of the specific selection of *RRR*-α-tocopherol by the α-tocopherol transfer protein and their low rate of degradation and elimination was compared with the other vitamins. α-Tocopherol appears to be mostly involved in gene regulation (Brigelius-Flohé, 2006). The antioxidant activity of vitamin E is well known and it is a suitable candidate for adjuvant treatment of cancer (Valgimigli & Amorati, 2019; Constantinou et al., 2008). The anticancer activity of vitamin E against murine C6 glioma cells was reported by Mazzini et al. (2010). The anticancerous activity of vitamin E is executed through the antiangiogenesis process and it acts as a potent regulator of growth-factor-dependent signaling in endothelial cells (Miyazawa et al., 2009). Recently vitamin E based nanomedicines for oncological diseases have been reported and it was found to increase the tumor delivery of drugs and limiting the off-target uptake (Alavijeh & Akhbari, 2020).

2-Heptylacetate is a carboxylic acid ester, which possesses insecticidal activity (Nta et al., 2018; Nta & Oku, 2019). Isopentacosane and tetratetracontane are alkane group compounds detected in the GC/MS analysis. Methyl commate B is a triterpene glycoside only present in *M. umbellatum* leaf extract. It possesses antioxidant, antimutagenic and anticancerous activities (Gautam et al., 2020).

*M. umbellatum* fruit extract possess 12 bioactive compounds in which, 21.35% of the extract content was shared by an alkaloid, hordenine. It elicits plant defensive mechanism through jasmonate-dependent defense pathway (Ishiai et al., 2016). Pyruvate dehydrogenase kinase 3 (PDK3) inhibitors are an important target in lungs cancer therapy. Hordenine act as an inhibitor molecule of PDK3 through non-covalent interactions and induce conformational changes on them. It exhibits cytotoxic effects on lungs cancer cell lines with an admirable $IC_{50}$ value (Anwar et al., 2020). 2-Hydroxy-4-methyl pyrimidine and 4-methyl 2,5-dihydrofuran-2-one are the organic compounds noticed in *M. umbellatum* fruit extract. 2-Hydroxy-4-methyl pyrimidine possesses a wide range of activities. The pyrimidine compounds and its derivatives have a significant effect on microbes and most of them are antimicrobial agents. It also possess antioxidant, analgesic, anti-inflammatory and anticancerous activities (Sharma et al., 2014). 4-Methyl 2,5-dihydrofuran-2-one is a furan compound, extensively used in pharmaceutical field as a flavoring agent and it possesses insecticidal activity (Xia et al., 2011). Dehydromevalonic lactone is a building block of several natural compounds and can encompass antimicrobial property (Xavier et al., 2010). Methyl-3-methyl-5-oxooxolane-2-carboxylate has antifungal and antitumor activity which was reported by Guo et al. (2008). Lauric acid, myristic acid and octadecanoic acid form the fatty acid profile of *M. umbellatum* fruit extract. All these compounds have potent bioactivity. Myristic acid shows potent anti-inflammatory effect. The combined action of myristic acid and palmitic acid is effective against systemic candidiasis (Prasath et al., 2021).

Sundram et al. (1994) reveals that palmitic acid can reduce the cholesterol level than does a combination of lauric acid and myristic acid. GC/MS analysis describes the presence of lauric acid and myristic acid in *M. umbellatum* fruit extract. So it can be effectively used in lowering cholesterol level. These two fatty acid combinations have vital potential in preventing

prostatic hyperplasia (Babu et al., 2010). Two terpene compounds noticed in the GC/MS analysis are Mustakone and cyperenone. Mustakone isolated from *Cyperus articulatus* shows anti-plasmodial property, particularly active against *Plasmodium falciparum* (Rukunga et al., 2008). The antioxidant, antimicrobial and anticancer potential of mustakone was noticed by Gribner et al. (2020). Cyperenone is a sesquiterpene ketone, which shows cytotoxic and anti-inflammatory effects. It is also neuroprotective in function (Al-Snafi, 2016). Methyl myristate is a fatty acid methyl ester having antioxidant, cytotoxic and antifungal potential. It shows cytotoxic activity on human tumor cell lines like MCF-7, A549, CNE *etc*. (Su et al., 2013). Most of the fatty acid methyl esters are bioactive agents. They have potential antifungal and antioxidant activity reported by Pinto et al. (2017). While comparing the commercial antioxidants, fatty acid esters show better scavenging potential. It is active against 18 fungal strains, which are clinically important ones. Linoleoyl chloride, is a fatty acid derivative reported in *M. umbellatum* fruit extract. In *Kaempferia galanga* leaf extract, linoleoyl chloride is the major component. It is used as a medicine because it encompasses antinociceptive and anti-inflammatory potential (Bhuiyan et al., 2008).

GC/MS analysis of selected *Memecylon* species evidently proves that they are an affluent source of many bioactive phytochemicals. The above discussion substantiates the role of wide spectrum of bioactive phytochemicals. A total of 83 compounds were detected in the methanolic extract of selected species. The identified compounds belong to the classes of terpenoids, phenolics, fatty acids, fatty acid esters, steroids *etc*. *M. grande* fruit and leaf extracts were immensely rich in fatty acids and its esters. Fatty acids and its esters are usually associated with the oil containing plants, but here fatty acids and esters were seen in the methanolic extracts of all selected *Memecylon* species. *M. randerianum* extracts also possess significant amount of fatty acids, additionally. Moreover, it is rich in terpenoid compounds. In

the case of *M. umbellatum* extracts, steroids, alkaloids and fatty acids are the major phytoconstituents. A diverse array of phytoconstituents was present in the selected six plant extracts. Some organic compounds and trace compounds are also enlighten the bioactive potential of extracts. The presence of an immense array of compounds identified through the GC/MS analysis justifies the use of these plant extracts as herbal medicine.

**c) Phytochemical profiling by HR-LC/MS**

Non-volatile compositions of selected *Memecylon* species were revealed through the HR-LC/MS analysis. LC/MS analysis is a widely used technique in proteomics and metabolomics. It allows the broad screening of biomolecules. Liquid chromatography and mass spectrometry analysis enables the relative quantification of large amount of individual compounds from multiple samples (Katajamaa & Orešič, 2005). Electrospray ionization (ESI) is a most commonly used technique in LC/MS analysis. It is well suited for many metabolites and peptides. The liquid sample reaches the ionized states and it will be transferred to the mass spectrometer. To get suitable profile of samples, ionization was performed in positive and negative modes. MS with ESI method provides a wider platform to analyze biological molecules than in GC/MS analysis. It has a greater application in clinical biochemistry (Pitt, 2009).

A total of 48 compounds were identified in the methanolic extract of *Memecylon* species by HR-LC/MS analysis [**Table 9, Figures 17(i-xii)**]. HR-LC/MS analysis of leaf and fruit extract of *M. grande* together sum up to 21 compounds, *M. randerianum* 15 compounds and *M. umbellatum* 18 compounds. A diverse array of compounds was identified in *Memecylon* extracts and no compound was found to be common in all the six extracts. An alkaloid compound, rescinnamine was found to be common in all the selected fruit extracts. Ganglioside was found to be common in the fruit extract of *M.*

*grande* and *M. randerianum.* Swietenine, a terpenoid group of compound and biopeptides were commonly present in *M. grande* leaf, fruit and *M. randerianum* fruit extracts.

Lupanyl acid, aesculin, C16 sphinganine, 3-dehydro-6-deoxoteasterone and biopeptides were the major constituents of *M. grande* leaf extract. Lupanyl acid is a triterpenoid compound identified in the root of *Phyllanthus pulcher.* It exhibits cytotoxic effects in tumor cell lines, such as MCF-7, NCI-H460 and DU-145 (Bagalkotkar et al., 2011). It is identified as a cholinesterase inhibitory constituent in *Ficus bengalensis* (Riaz et al., 2012). Aesculin, a coumarin glycoside was identified in the present study. It is a common natural ingredient used in the Chinese medicine named *Cortex fraxini,* an inflammatory modulator in ulcerative colitis. The anti-inflammatory mechanism through the regulation of PPARγ and by inhibiting NF-κB pathways was noted by Tian et al. (2019). The antioxidant activity of aesculin has been reported by Witaicenis et al. (2014). C16 sphinganine has an important role in regulating apoptotic mechanism through TNF-α signal (Osawa et al., 2005). Sphingolipids are the biological building blocks and sometimes act as secondary messengers. Ceramides play an important role in cell cycle regulation and apoptotic mechanism (Jayadev et al., 1995). A terpenoid compound swietenine was found to be common in *M. grande* leaf, fruit and *M. randerianum* fruit extracts. It is isolated from *Swietenia macrophylla* seeds and shows significant hypoglycemic and hypolipidemic activity (Dewanjee et al., 2009). In cardiac hypertrophic condition, the lowering of the expression of Akt phosphorylation, ANP and BNP mRNA were noticed in swietenine treated mice models. It shows that swietenine might be a promising anti-hypertrophic agent against cardiac hypertrophy (Ding et al., 2020).

*M. grande* fruits are rich in diverse phytoconstituents. Tamarixetin, a flavonoid compound was ascertained during HR-LC/MS analysis. It is a derivative of quercetin, isolated from *Cyperus teneriffae.* It can act as a good anticancer agent. The cell cycle arrest and accumulation of cyclin B1, Bub1 and $p21^{Cip1/Waf_1}$associated with human leukemia was reported by Nicolini et al. (2014). Tamarixetin induces apoptosis and G2/M arrest in leukemia cells in a concentration and time dependent manner. In liver cancer cells, tamarixetin upregulated the expression of pro-apoptotic genes, Bax and caspase-3 and induce apoptosis through mitochondrial pathway (Xu et al., 2019). It also possesses anti-inflammatory potential (Park et al., 2018). Rescinnamine, an alkaloid compound isolated from *Rauwolfia serpentina* encompass antimicrobial, antioxidant and antimitotic potential, was observed in LC/MS analysis (Hemashekhar et al., 2019). *In silico* investigation on ZIKA virus inhibition reveals that rescinnamine shows significant results (Ahmed et al., 2020). Ganglioside GM1 is detected in the analysis and it acts as a neuronal regulator and prevents neurodegeneration. It plays an important role in maintaining the intercellular calcium homeostasis and subsequent cellular functions (Chiricozzi et al., 2020; Robert et al., 2011).

A terpenoid compound detected was madecassic acid with molecular weight 504.341. It is an active constituent of *Centella asiatica* having anti-colitis activity. It is also known to be a potential anticancer agent and shows cytotoxic effects on 26 different cancer cell lines (Valdeira et al., 2019). The antioxidant, anti-inflammatory and anti-diabetic properties of madecassic acid are well known (Yun et al., 2020). A carotene compound, 7, 8-didehydroastaxanthin, which was pinpointed in HR-LC/MS, is a colouring agent. It may protect cells against oxidative damages (Maoka et al., 2014). A vitamin D analogue, calcifedol was noticed in the present investigation. It is commonly used to manage vitamin D deficiency (Sosa Henríquez & Gómez de Tejada Romero, 2020). In COVID - 19 cases, the administration of

calcifedol will reduce the severity of the cases, because calcifedol can boost up the immune response of patients (Jungreis & Kellis, 2020). Dihydroxylycopene/OHRhodopin, is a carotene compound identified in the *M. grande* fruit extract. Ursolic acid, a potential terpenoid compound was also discerned during the HR-LC/MS analysis. The cytotoxic activity of ursolic acid against cancer cell lines was discussed by Ma et al. (2005). It has a wide spectrum of activity and is a good therapeutic agent. Antioxidant, anti-angiogenic, anti-inflammatory, anti metastatic *etc*., are the potential therapeutic roles of ursolic acid (Kashyap et al., 2016). Campestanol, a steroid group of compound was detected in the fruit extracts. It is known to be an antibacterial agent isolated from *Salvia jaminiana* root (Kabouche et al., 2005). The cholesterol reducing ability of campestanol was reported by Lichtenstein and Deckelbaum (2001). A limonoid compound, khayanthone was identified in HR/LC-MS analysis. It is a havanensin-class limonoids, isolated from the genus *Khaya* of Meliaceae. Limonoid compounds are known to be insecticidal agents and free radical scavengers (Tan & Luo, 2011; Mestry et al., 2020).

The leaf extract of *M. randerianum* shows a limited number of compounds in HR/LC-MS analysis. Among these a glycoside compound, bergenin was detected, which possesses diverse pharmacological activity. The antioxidant and antimicrobial activity was reported by Nazir et al. (2011). Bergenin have hepatoprotective, antiulcerogenic, immunomodulatory and anti-inflammatory activity (Patel et al., 2012). The anti-inflammatory activity was attributed by the inhibition of IL-1β and TNF-α production. 9,12,13-trihydroxy-10,15-octadecadienoic acid was a fatty acid derivative produced from linoleic acid by *Pseudomonas aeruginosa*. Trihydroxy fatty acids are produced in plants as a self defensive mechanism (Kim et al., 2000).

The first compound detected in *M. randerianum* fruit extract was an organic compound, violastyrene. The soluble guanylate cyclase is a receptor protein of nitric oxide. It is involved in the cell signaling pathways and associated with angiogenesis in tumor development. Violastyrene is considered as a soluble guanylate cyclase inhibitor and have a good antiangiogenic property (Petrova et al., 2020). In *Dalbergia saxatilis* leaves extract, the presence of violastyrene was noticed and it has protectant activity against cowpea pest, additionally it has insecticidal activity against mosquitoes and has antimicrobial activity (Okwute et al., 2009). The presence of rescinnamine was found to be common in the fruit extract of selected *Memecylon* species. In addition, another alkaloid compound detected in *M. randerianum* fruit was β-erythroidine. It shows curarizing property, that induces the muscular relaxation (Champtiaux et al., 2006). Glycerol palmitate obtained in the present study is a monoglyceride, derived from hexadecanoic acid. It has potent antioxidant activity, which was reported by Qadir et al. (2018). A steroid compound noticed in the present study was 6-deoxocastasterone. It is known to be a brassinosteroid that influences the plant growth and development. Cosmosiin, another phenolic compound detected and shows anticancer activity against HCEC, MCF-7 and Hep2 cell lines (Ahmed et al., 2017). It can up regulate ADAM10 (a disintegrin and metalloproteinase domain-containing protein), that is involved in the Alzheimer's disease therapy. Cosmosiin enhances the production of neurotoxic amyloid precursor that is normally depleted in Alzheimer's condition (Min et al., 2018). Cosmosiin act as an anticancer agent through the immune checkpoint inhibition in *Salvia plebeia* and acts upon the PD-1/PD-L1 interaction (Choi et al., 2020).

*M. umbellatum* leaf extract shows the presence of a sesquiterpene lactone, elephantopin in the LC-MS analysis. It is known to be a tumor inhibiting agent (Shukla et al., 2020). In *M. umbellatum* fruit extract, a diverse

array of chemical compounds was identified through the HR-LC/MS analysis. The presence of the polyphenolic compound chlorogenic acid; glycoside compound, amygdalin; terpenoid compound, deutzioside; quinone compound, idebenone; benzoquinone compound, embelin *etc*., are a few of them. Chlorogenic acid play several therapeutic roles, such as antioxidant, antimicrobial, antipyretic, anti-inflammatory *etc.* It is considered as a safe natural additive (Naveed et al., 2018). The health promoting characters of chlorogenic acid reveals that it is a promising food supplement (Santana-Gálvez et al., 2017). Amygdalin is commonly present in the members of Rosaceae. It possesses antitumor and anti-inflammatory activities and reduces blood glucose level (He et al., 2020). Several studies on the potential activity of amygdalin were conducted by various researchers (Jaswal et al., 2018; Liczbiński & Bukowska, 2018). So it validates the therapeutic role of *M. umbellatum* fruit extract. The anticancer mechanism of amygdalin is attributed through the cell cycle arrest, apoptosis and regulation of immune system (Shi et al., 2019).

Deutzioside is an iridoid compound, which belongs to monoterpene group of compounds and is found in many dietary folk medicines (Dinda, 2019). Stigmasta-7, 22 E, 25-trien-3beta-ol, a sterol and embelin was detected in the present study. Embelin is a bioactive natural compound under benzoquinone group, which was initially isolated from *Embelia ribes*. The wound healing activity of embelin was reported by Swamy et al. (2007). The anticancereous activities of embelin become an evaluable tool in cancer studies. It is an obstructor of X-linked inhibitor of the apoptosis protein (XIAP), an anti-apoptotic protein (Poojari, 2014). In hepatocarcinogenesis, embelin shows promising activity against *N*-nitrosodiethylamine (DENA) and phenobarbital (PB) induced tumorigenesis (Sreepriya & Bali, 2005). The antimicrobial and anticonvulsant activity of embelin was also reported (Chitra et al., 2003; Mahendran et al., 2011).

6b,11b,16a,17a,21-pentahydroxypregna-1,4-diene-3,20-dione16,17-acetonide is a terpenoid compound having anticancer activity, which was reported in *Cyathocline purpurea* (Javir et al., 2019). In addition to rescinnamine, another alkaloid, protoveratrine A was also detected in the analysis. It is known to be a steroidal alkaloid isolated at first from *Veratrum album* (Liliaceae) (Vengamma et al., 2019). It is used for the treatment of hypertension. The insecticidal activity and lowering of blood pressure are the beneficial roles of protoveratrine A (Akbar, 2017).

The presence of biopeptides was observed in the present investigation. Biopeptides are organic molecules formed by proteolysis and consist of two or more amino acids connected by covalent bonds. These are biologically active molecules with distinct nutritional and functional role in physiological processes of organisms. It has several biological functions such as antioxidative, antihypertensive, antidiabetic and immunomodulatory activities (Saadi et al., 2015). Biopeptides are specific sequence of amino acids with many health benefits and ameliorate disease conditions. It is considered as natural bioactive elements used as a drug, having nutraceutical value and as a food supplement (Montesano et al., 2020). The biopeptides of *Phalaris canariensis* L. was found to be antihypertensive, antidiabetic and with anti-obesity activity (Urbizo-Reyes et al., 2021). The presence of tripeptides is noticed in HR-LC/MS analysis of *Memecylon* species. The antioxidant activity of tripeptides with cysteine and tryptophan moieties was reported by Tian et al. (2015). Most of the tripeptides have antihypertensive activity, which regulate action of the angio-tensin converting enzyme (ACE). The anti-inflammatory, antimicrobial and antioxidant activities of biopeptides make them useful as food additives (Sánchez & Vázquez, 2017). Thus biopeptides are efficient bioactive components that can be targeted for drug preparation.

HR-LC/MS analysis of selected *Memecylon* species markedly proves that these plants are a rich source of many bioactivity phytochemicals, which belong to various classes of compounds. The bioactive reports of the phytoconstituents are again validating their usage as medicinal components. They are endowed with antioxidant, antibacterial, anticancerous and anti-inflammatory effects. The isolation and characterization of bioactive components critically influence their therapeutic uses. So the exploration of the individual phytocomponents present in the *Memecylon* species is essential for validating their specific bioactivity. The presence of an immense array of compounds identified through the GC/MS and HR-LC/MS analyses justifies the use of these plant extracts as herbal medicine.

## PHASE III- BIOACTIVITY STUDIES

### a) FREE RADICAL SCAVENGING ACTIVITY STUDIES

In aerobic organisms, mitochondria are the main generator of energy for the realization of its vital functions. It generates ATP through reactions of oxidation and reduction that attach tricarboxylic acid cycle with the electron transport chain. It occurs through the oxidation of the food and by the production of NADH and $FADH_2$ in different metabolic pathways, such as glycolysis, β-oxidation and the Krebs cycle. These reactions generate unpaired electrons in the form of free radicals or reactive oxygen species. They tend to stabilize themselves by sequestering electrons from other biomolecules. Thus they become unstable and therefore, are no longer able to perform their duties properly. It will alter the homeostasis and ultimately cause cell death (Aguilar et al., 2016). Free radicals are defined as "any chemical species capable of independent existence that contains one or more unpaired electrons". Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are common free radicals associated with cellular metabolism. The other biologically important free radicals are lipid hydroperoxide

(ROOH), lipid peroxyl radical ($ROO^{\bullet}$) and lipid alkoxyl radical ($RO^{\bullet}$), which are associated with membrane lipids; nitric oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$) and peroxynitrite ($ONOO^{-}$), which are reactive nitrogen species; and thiol radical ($RS^{\bullet}$), which has an unpaired electron on the sulfur atom (Kurutas, 2015).

Antioxidants are free radical scavengers and can neutralize the oxidative stress induced by the reactive oxygen species. Otherwise ROS can disrupt cellular mechanism and lead to severe pathological conditions and diseases like cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes *etc.* (Birben et al., 2012). Free radicals are unpaired and unstable, so unstable radicals tend to become paired with the biological macromolecules such as proteins, lipids and DNA to become stable. Thus it will cause protein and DNA damages (Gilgun-Sherki et al., 2002). Antioxidants are normally counter acting the effects created by the free radicals. The antioxidants are produced either endogenously or received from exogenous sources, which include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals like Se, Mn, Cu and Zn and vitamins like vitamin A, C and E. Glutathione, flavonoids, bilirubin, uric acid *etc*., possess promising antioxidant activity. In a healthy body, prooxidants and antioxidants maintain a ratio and a shift in this ratio towards prooxidants gives rise to oxidative stress (Irshad & Chaudhuri, 2002). However, reactive oxygen species mediate certain cellular functions like redox signaling and gene expression as well as defend against pathogens. Thus, the role of antioxidant systems is not to eliminate oxidants completely, but instead maintain them at an optimum level. The antioxidants are classified as enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants are converting oxidized metabolic products in a multi-step process to hydrogen peroxide ($H_2O_2$) and then to water using various cofactors. Non-enzymatic antioxidants intercept and terminate free radical

chain reactions, and it includes vitamin E, A, C, flavonoids, carotenoids, glutathione, plant polyphenols *etc.* (Moussa et al., 2020).

Antioxidants of natural origin like tea leaves, carrot, spinach, berries *etc*., are antioxidant sources of common people. There are several unknown sources of free radical scavengers. The present study focuses on the *in vitro* antioxidant activity of selected *Memecylon* species in DPPH, hydroxyl, nitric oxide and superoxide radical scavenging assays. A single assay is not adequate for the evaluation of antioxidant property of the samples. The assays developed to evaluate the antioxidant activity of plants and food constituents may vary. There are two general types of assays widely used for different antioxidant studies. One is an assay associated with lipid peroxidations, including the thiobarbituric acid assay (TBA), malonaldehyde/high-performance liquid chromatography (MA/HPLC) assay, malonaldehyde/gas chromatography (MA/GC) assay and conjugated diene assay. The second type assays are associated with electron or radical scavenging, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, ferric reducing/ antioxidant power (FRAP) assay, ferric thiocyanate (FTC) assay and aldehyde/carboxylic acid (ACA) assay (Moon & Shibamoto, 2009).

Generally *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple and inexpensive in comparison to other test models (Alam et al., 2013). DPPH is a stable free radical with pink colour which turns yellow when scavenged. Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence the absorbance decreases. The degree of discoloration is always related with the hydrogen donating ability of the antioxidants. In the present study, highest scavenging activity was shown by 200 µg/mL concentration of *M. grande*

fruit extract and exhibit an inhibition percentage of 75.77 ± 0.01 (**Figure 19**). The $IC_{50}$ value of *M. grande* fruit extract was found to be 83.91 ± 0.14 µg/mL. It is followed by the *M. umbellatum* fruit extract with the $IC_{50}$ value, 91.10 ± 0.12 µg/mL (**Table 10**). All the selected extracts show potent antioxidant activity *ie.,* more than 50% inhibition is being offered by the extracts. While comparing the species, the lowest activity was shown by the *M. grande* leaf extract of 64.37 ± 2.05%. All other extracts possess moderate range of scavenging potential. Previous reports of antioxidant activity of *Memecylon* species are available. The phytochemical analysis of selected *Memecylon* species, evidently propose that they are an immense source of bioactive phytochemicals. So the scavenging potential is the worthy contribution of the phytochemical constituents of *Memecylon*.

The hydroxyl radical ($^{\bullet}OH$) possesses an important role in cancer induction. It induces mutagenic effect, while interacting with the DNA molecule and resulting in DNA breakdown and cancer formation (Khan et al., 2013). It is the most reactive free radical. Hydroxyl radical interacts with micro- and macromolecules present in an organism and disrupt membrane and cellular proteins, lipids, DNA and RNA (Cederbaum, 2017). It can induce lipid peroxidation and damages on disulfide bonds of proteins, specifically fibrinogen, resulting in their unfolding and knotted refolding into unusual spatial configurations (Lipinski, 2011). Hydroxyl radicals are formed by incubating $Fe^{+3}$- EDTA premixture with ascorbic acid and $H_2O_2$, it is known to be the Fenton reaction. While analyzing the scavenging potential of *Memecylon* species, similar trends in DPPH assays are again reflected (**Figure 21**). *M. grande* fruit extract shows the highest scavenging potential (61.69 ± 0.52%) and the lowest effect was shown by its leaf extract (36.77 ± 0.62%). The $IC_{50}$ value indicates that *M. grande* fruit (1231 ± 0.48 µg/mL) extract is a better hydroxyl radical scavenger than the standard gallic acid (1347.51 ± 0.27 µg/mL) (**Table 10**). In the case of *M. randerianum* and *M.*

*umbellatum* extracts, a moderate level of scavenging activity was observed. Phenolic compounds are considered as effective scavengers of hydroxyl radical (Yıldırım et al., 2000). The phytochemical analysis is also validating the same. A promising amount of phenolic content was noticed in *Memecylon* species through quantitative phytochemical estimation and GC-MS analysis.

Nitric oxide radical ($^{\bullet}NO$) owns various biological functions. It has a crucial role in neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the beneficial role, it can act as an oxidant element through the interaction of superoxide and it forms peroxynitrite anion. It is a potential oxidant that can produce OH and NO (Patel Rajesh & Patel Natvar, 2011). In nitric oxide scavenging assay, similar range of activity was observed in *Memecylon* species. Griess assay was used to assess NO-inhibitory activity of the extracts. Here sodium nitro prusside in aqueous solution at physiological pH impulsively generates nitric oxide by the action of oxygen and produce nitrite ions that can be determined by using Griess reagent. Nitric oxide free radical has an important role in inflammatory responses. It activates nuclear factor κB (NF-κB), which induces the transcription of inflammatory cytokines and COX-2. Antioxidants can effectively block the transcription of inflammatory cytokines (Huang et al., 2001). *M. grande* fruit extract shows the highest scavenging activity of 76.85 ± 0.08% and lowest was found in *M. grande* leaf extract with 40.86 ± 0.20% (**Figure 23**). The $IC_{50}$ value of standard becomes low when compared with the sample concentrations. So nitric oxide radical scavenging potential of the selected extracts is in a moderate range.

Superoxide ($O_2^{\bullet-}$) is one of the strongest reactive oxygen species among the free radicals and can produce singlet oxygen. Here an estimate of the reduction rate of nitro blue tetrazolium (NBT) into a purple-colored formazan is measured (Fontana et al., 2001). Superoxide radical can induce

detrimental effects on the cell components. It induces lipid oxidation with the singlet oxygen production (Halliwell et al., 1987). Phytochemical components are always the responsible factor for the bioactivity. Flavonoids are considered as effective scavengers of superoxide radicals (Robak & Gryglewski, 1988). Super oxide radical scavenging ability of *Memecylon* species owns a similar trend as shown in other assays. But here the lowest activity was exhibited by *M. randerianum* leaf extract. The dose dependent scavenging activity become prominent in *M. grande* fruit extract with 72.17 ± 0.02% of activity (**Figure 25**). All the selected extracts show promising results. As compared with $IC_{50}$ value of standard (238.35 ± 0.03 µg/mL), *M. grande* fruit extract was exhibiting (698 ± 0.03 µg/mL) scavenging potential in a moderate range.

These *in vitro* assays show promising results in free radical scavenging activity. It might be helpful in preventing the oxidative stresses and associated malfunctions. All the selected extracts exhibit a moderate range of activity. While comparing all antioxidant assays, *M. grande* fruit extract was the leading scavenger of free radicals *ie.*, a remarkable hydroxyl radical scavenger. DPPH, superoxide and nitric oxide radical scavenging activity of all selected extracts prove to be valuable. The free radical scavenging activity of fruit extracts become more as compared with the leaves extract. It might be due to the diverse phytochemical composition of the plant parts. Several reports highlight that, the fruit samples show prominent antioxidant potential rather than the leaves, since the phenolic content of the fruits are much more in early stage of fruit development (Wang & Lin, 2000). Polyphenols are secondary metabolites with highest antioxidant potential, especially flavonoids offer an intriguing promise (Dimitrios et al., 2006). The bioactive phenols and several other compounds with antioxidant activity were noticed in the phytochemical analysis. The presence of bioactive compounds like

squalene, agathenic acid, bicyclogermacrene, phytol *etc.*, also contributes to the antioxidant potential of *Memecylon* species.

*M. grande* and *M. randerianum* extracts were found to be rich in diverse chemical constituents, especially immense amount of fatty acid and fatty acid esters. Fatty acids like palmitic acid, octadecanoic acid and several fatty acid methyl esters such as methyl myristate, methyl palmitate, methyl heptadecanoate and methyl linoleate are known to be good antioxidant agents. They can reduce the cellular damages caused by oxidative stress (Hansen et al., 2001). *M. randerianum* was also found to be rich in terpenoid compounds, which also contributes to its scavenging potential (Grassmann, 2005). In the case of *M. umbellatum* extracts, steroids, alkaloids, phenols and fatty acids are the major phytoconstituents.

Phytochemical profiling of selected *Memecylon* species markedly supports the antioxidant efficacy. From the preliminary quantitative phytochemical analysis, it is proved that selected plant extracts are rich in phytochemicals (**Table 7**). GC/MS and LC/MS analysis is again validating the same result. *Memecylon* species have significant potential to hunt free radicals and are rich in natural antioxidants. In light of these results, one can hope that the *Memecylon* fruits are a galore of natural antioxidants. However, the components and the mechanism responsible for the antioxidant activity are currently unclear. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activities of these extracts need to be assessed prior to clinical use.

### b) CYTOTOXIC SCREENING USING *ALLIUM CEPA*

*Allium cepa* is a model organism for genotoxicity/cytotoxicity studies. It is a common method of toxicity evaluation in plant materials. The toxic

ranges of industrial effluents from pesticide or textile areas are evaluated through this plant based assay (Wijeyaratne & Wickramasinghe, 2020). It is an easy and fast way to detect the toxic potential of plant specimens. So it will further lead to the development of plant based pharmaceuticals for various human ailments. The present study highlights the toxic potential of selected *Memecylon* species. In ½ hr, 2 hr and 24 hr of exposure period of treatments, a range of cellular aberrations is noticed (**Plates 10-16**). A normal untreated *A. cepa* root tip cells raised in distilled water and that grown in $H_2O_2$ medium are considered as negative and positive standards (**Plate 9**).

In cancer studies, target medicine of natural origin has great importance. Cytotoxic compounds are beneficial in proliferative studies. The prime step in cancer studies are the toxicity validation of test sample. *A. cepa* is one of the quick methods of toxicity validation. There are several plant systems that are used in cytotoxicity evaluation (**Table 2**). *Tradescantia, Vicia faba, Lactuca sativa etc*., are some other important test materials from the plant kingdom. *A. cepa* assay is considered as an efficient system, because it is directly related to the mammalian test system. In several environmental studies, *A. cepa* is considered as a satisfactory tool in environmental monitoring of xenobiotics, mutagens *etc*. (Leme & Marin-Morales, 2009). Cytogenotoxicity determination of different plant extracts and various chemicals using *A. cepa* root cells are still a common method. So it is always a strong and satisfactory step in toxicity determination (Salazar et al., 2020).

The toxicity efficacy of plant materials are always a fruitful pathway in therapeutics (Sammar et al., 2019). Mitotic index is a cellular measure of proliferation. It is the count of dividing cells in a group of cellular population and it determines the viability of the cell system. The lowering of the mitotic index is an indicator of retardation of protein synthesis, DNA synthesis and cell cycle arrest (Majewska et al., 2003). Chromosomal aberration is a sign of

the toxic potential of plant extracts. *A. cepa* treated with *Memecylon* extract, shows various chromosomal aberrations. Stickiness, stellate chromosomes, pulverization, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, lagging chromosome, nuclear disintegration, giant cell formation, coagulated chromosomes, C-mitosis *etc*., are the observed abnormalities found in *A. cepa* root tip cells. Chromosomal aberrations are mainly of two categories, clastogenic and aneugenic. The clastogenic abnormality affected the nuclear material of the test system, while aneugenic effect destructs the mitotic spindle machinery. The abnormality percentage and mitotic index of *Memecylon* species are given in **Figures 26, 27, 28**. Mitotic index and abnormality percentage are inversely related, mitotic index decreases with increasing concentration and the abnormality percentage increases with the increasing concentration of the plant extracts.

Among the selected six samples of plant extracts, *M. umbellatum* fruit extract shows the highest abnormal cell counts of 91.73 ± 1.41% and lowest mitotic index of 30.76 ± 7.00% at ½ hr exposure period of 100 µg/mL concentration. In *M. grande* leaf extract, 89.4 ± 2.29% aberrations were observed in 100 µg/mL sample treatment and 43.66 ± 3.84% was the lowest mitotic index at 24 hr treatment with a concentration of 100 µg/mL. *M. grande* fruit extract shows, 48.11 ± 7.39% as the lowest mitotic index and 90.72 ± 1.45% as the highest aberration percentage. In *M. umbellatum* leaf extract, the highest percentage of aberration noticed in 24 hr, 100 µg/mL experiment condition is 90.25 ± 2.74%. The reduced mitotic index observed at 2 hr, 100 µg/mL sample concentration was 32.85 ± 3.81%. *M. randerianum* leaf extract possess 82.39 ± 2.74% of aberration as the highest value at 2 hr, 100 µg/mL of concentration and the lowest mitotic index was at ½ hr, 100 µg/mL of concentration with 35.66 ± 5.58%. In the case of fruit extract of *M. randerianum*, 83.83 ± 1.28% is the highest aberration percentage observed at ½ hr and 34.52 ± 3.87% was the lowest mitotic index percentage at 24 hr.

Here all the tested concentrations of plant extracts cause mitodepressive effect and similar results were also noticed in various other studies (Khanna & Sharma, 2013; Lamsal et al., 2010). The mitodepressive effect may check the nuclear material synthesis, nucleoprotein formation and may affect the structure of chromosomes (Yuet Ping et al., 2012). While comparing the toxic potential of selected *Memecylon* species, 30-90% of aberrations were induced by the phytochemical constituents of the plant extract. It clearly indicates that the toxic potential of plant extracts is evidently dosage dependent. The mitotic delay and aberration percentage is directly related (Gudowska-Nowak et al., 2005). The toxicity potential of plant extracts is a clear indicator of developing target medicines in cancer therapy. So validation of toxicity level becomes the prime requirement of experiments. The mitotic index and abnormality percentage values give the first signs for the validation. The present investigation thus confirms that the selected species of *Memecylon* are potential cytotoxic agents and much more efficacy is shown by *M. umbellatum* fruit extract.

The decline of mitotic index and prominent aberration percentage are the key leads in toxicity determination. The reduction of mitotic index is due to the blockage of cell cycle or escalating the length of G2 phase or S phase or delaying the onset of prophase (Prokhorova et al., 2013). The cytotoxicity level of extracts is determined by their efficacy of lowering mitotic index. The mitotic index range of below 22% is considered as a lethal value and below 50% is a sub-lethal value for organisms. These are considered as the cytotoxic limit values as described by Prajitha and Thoppil (2016). The selected six plant extracts possess sub-lethal level of toxic effect on the *A. cepa* root tip cells. The mitodepressive and antiproliferative effects of plant extracts are confirmed through the lowered mitotic index parameter.

Various chromosomal aberrations were resulted in *A. cepa* assay. The toxic potential of selected extracts was clearly noticed in the chromosomal or nuclear abnormalities. The same aberrations were resulted in different plant extracts at various cell stages and in some cases, multiple abnormalities were also noticed. So the abnormalities in different cell cycle stages are documented in **Plates 10-16**, and for comparison, a normal cell cycle stages were provided in **Plate 9**. *M. umbellatum* fruit extract shows the highest abnormal cell counts of 91.73 ± 1.41%. In clastogenic aberrations, stickiness, pulverization, exposure of chromosome scaffold, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, nuclear disintegration, giant cell formation, coagulated chromosomes and chromosome bridges were detected. Hypoploid condition, stellate chromosomes, lagging chromosome, C-mitosis, macro and micro cell formation, ball shaped chromosome, polyploidy and induction of vagrants are noticed as aneugenic aberrations. While comparing these abnormalities, aneugenic aberrations are quite common.

The selected plant samples were found to induce a number of chromosomal aberrations, but specifically *M. randerianum* leaf extract was more potent in inducing C-mitosis. It is a distinct spindle damaging abnormality noticed in the cytotoxic assay. The C-mitotic activity of *M. randerianum* leaf extract was reported for the first time by Ramya Sree and Thoppil (2018). C-mitosis is the spindle abnormality observed during mitosis, so it disrupts the chromosomal movements and leads to aberrations like C-metaphase, C-anaphase, polyploidy *etc.* The spindle poisoning may occur due to the presence of colchicine like compounds in the leaf extract of *M. randerianum*. C-metaphase (**Plate 13 n**) is one of the main consequences of inactivation of spindle fibers, which cause delay in the division of centromere (Somashekar & Gowda, 1984). Partial inactivation of spindle fibers leading to partial C-mitosis was also observed during the study (**Plate 13 p**). The shift in microtubule organizing centers (MTOC) is resulted by the effect of C-mitosis

(**Plate 14 b**). MTOC is the assembling site of mitotic and meiotic spindle machinery. The active principles found in plant extract will affect the stability of microtubules and lead to shift in their position (Neelamkavil & Thoppil, 2018).

Formation of vagrants is the another frequently observed abnormality associated with C-mitosis. It is an indicator of spindle poisoning and cause unequal separation of chromosome groups (Rank, 2003). The spindle abnormality can induce lagging of chromosomes and leads to form laggards (Lera & Burkard, 2012). During anaphase, the formation of vagrants and laggards are common. The spindle distortions are reported in all the selected plant extracts. Polyploidy, chromosome rosette, scattered meta - and anaphases, unipolar movement of chromosomes, unequal chromosome groups, misorientation of chromosomes, diagonal and dislocated chromosomes are the vital abnormalities associated with it. Scattered meta- and anaphases may be the result of disturbances or inhibition of spindle formation (Tripathy & Rao, 2015). Polyploid cell (**Plate 13 b**) is a numerical aberration formed due to spindle abnormality. Similarly hypoploid cells were also resulted (**Plate 13 h**). Aberrant cell formation was frequently observed in the cell cycle stage of cytokinesis mainly, aberrant cell wall formation with single lesion, diagonal cell plate formation showing displacement, macro and micro cell and unequal and oblique cell plate formation (**Plate 16 a, d, h, k**). Stellate metaphase (**Plate 13 f**) and anaphase (**Plate 14 m**) were resulted by the clumping of chromosomes due to the spindle abnormality. Another chromosomal abnormality associated with spindle fibers resulted is tropokinesis. The abnormal orientation of the spindle midzone of the fibers is clearly noticed here (**Plate 13 a, g**). These diverse spindle abnormalities, clearly point towards the spindle poisoning ability of selected *Memecylon* species.

Ball metaphase is resulted by the destruction of spindle fibers and clumping of chromosomes and assumes the shape of a ball. The pulverization of chromosomes is due to the premature condensation of chromosomes (Rybaczek & Kowalewicz-Kulbat, 2011). Macro and micro cells formation resulted in various stages of cell cycles, may be due to the failure of normal organization and function of spindle apparatus as attributed by Adam and El-Ashry (2010). Coagulated prophase and anaphase were resulted in the present study. Here chromosomes seem to be adhering together to form an intact mass of aberrant chromosome group. It is due to depolymerization of deoxyribonucleic acid. The chemicals of plant extract can induce DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchange of the basic folded fibre units of chromatids and the stripping of the protein covering of DNA in chromosomes (Sumitha & Thoppil, 2016). Somatic pairing was noticed during the study (**Plate 12 l**). It is the pairing of homologous chromosomes in a somatic cell. Somatic pairing and chromosomal aberrations are related processes. Somatic pairing influences the frequency and type of the chromosome aberrations induced (Beçak et al., 2003). Stathmo anaphase is shown in **Plate 14 n**. Here the daughter chromosomes do not separate fully, but remain connected together by means of partial overlapping of their arms (Renjana & Thoppil, 2013). It is a spindle anomaly caused by the simultaneous multipolar and spindle poisoning activities of the extract. Chromosome scattering was resulted in the present investigation, which could be due to the interference of extract chemicals on the polymerization/de polymerization of the microtubular subunits (**Plate 13 d, e**).

Among the clastogenic aberrations induced by *Memecylon* extracts, nuclear lesions are a common one. It is a cytological evidence for the inhibitory action of the extracts on DNA biosynthesis (Akaneme & Iyioke, 2008). It is the first sign of the genetic material loss or degradation. Single or

double nuclear lesions are noticed prevalently in the present study (**Plates 10 c, e, g; 11 c; 16 i**). Chromosome stickiness is due to the cross-linkage of chromoproteins. It can also induce chromosome bridges and the subsequent failure of anaphase separation (Tkalec et al., 2009). The aberrations like chromosome gaps, nuclear lesions, erosions, nuclear breakage and nuclear disintegration are resulted in the *A. cepa* assay were clear indicators of apoptotic sign. So the cytotoxic results were further analyzed through the antiproliferative experiments. These strong cytological evidences prove the potential of these extracts of *Memecylon*. Nuclear or chromatin erosions are resulted in *A. cepa* assay. It is due to the extreme toxicity offered by the plant extract and the degradation of chromoproteins (Karaismailoglu, 2015). The fragmentation of chromatin is a sign of cell death or may lead to aneuploid condition (**Plate 11 b**). Binucleate cells are observed in the *A. cepa* assay (**Plates 10 a, b; 14 p**) which reveals that the plant extract can inhibit the cell cycle at certain points, particularly the prevention of the cytokinesis (Khanna & Sharma, 2013). Strap cell formation is also noticed during the study (**Plate 10 g, h**), which may be due to the abnormal cell enlargement.

Stickiness, bridges and fragments are chromatin dysfunctions that were found to be the frequent type of chromosomal aberrations in the study. Chromosome stickiness reflects toxic effects of plant materials, usually of an irreversible type, leading to cell death. The disorganization of chromatin, DNA agglomeration and complexity of interchromatin fibers are the main reasons for the chromosome stickiness (Mustafa & Suna Arikan, 2008). A disturbed balance in the quantity of histones or other proteins seems to be responsible for the change in structure of the nuclear chromatin (Hammann et al., 2020). The presence of sticky anaphase and metaphase reveals the chromatin remodeling potential of the plant extracts (**Plates 13 h; 14 o**). Increased stickiness also leads to the formation of sticky bridges in anaphase and telophase. Chromatin bridges could be attributed to the chromosomal

breakage and reunion of chromatids (**Plates 14 c, e, o, p; 15 a-d**). Chromosome bridges are formed due to the stickiness of the chromosomes and they can't separate apart in anaphase. It appears like bridges (Rad et al., 2011). Another reason proposed for bridge formation was due to the formation of dicentric chromosomes by breakage and reunion (Majewska et al., 2003). Chromosomal fragments are another notable chromosomal dysfunction. The chromosomal breaks and may be a part of anaphase bridge and the disturbances in microtubule assembly lead to fragment formation (**Plates 12 c; 14 k**).

In the present study, several multiple aberrations were observed *ie*., two or more aberrations occur simultaneously. Binucleate cell showing double and single nuclear lesions (**Plate 10 b**), sticky prophase showing single lesion (**Plate 11 g**), lesion and chromosome gaps at early metaphase (**Plate 12 p**), early ball metaphase showing lesion (**Plate 13 q**), pulverized stellate anaphase (**Plate 14 m**), stellate telophase showing persistent bridges (**Plate 15 p**), nuclear lesion, erosion and peak at cytokinesis (**Plate 16 i**) *etc*., are some of the multiple aberrations observed. A large number of cells with multiple aberrations are observed in the present study rather than single aberrations. The synergistic action of phytochemicals present in the extract is capable of inducing multiple disturbances in the normal cell cycle. Several reports are pointing that the cytotoxic effect of the plant species are the cumulative effect of the phytoconstituents present in it. The naturally occurring compounds such as flavanols, polyphenols, alkaloids and tannins have been involved in causing chromosomal damages (Carreon et al., 2002).

The *A. cepa* assay is considered to be an efficient test system because, the cytological evidences in the study have more application in cancer studies. It is the initial research that focuses on screening the ability of different plant

extracts for their cytotoxic and antiproliferative potentials. There are several studies which reveal that, the primary screening of cytotoxicity on *A. cepa* assay can be correlated with their antiproliferative efficacy against malignant cells (Isbilen et al., 2018; Abdullah et al., 2014). The cytotoxic efficacy of selected *Memecylon* extracts on *A. cepa* had revealed promising observations which can be further correlated with anticancer efficacy on mammalian test systems. In order to confirm the same *in vitro*, further anticancer study was conducted. It deals with the determination of the antiproliferative activity of the plant extracts on the human breast cancer cell line MCF-7.

## c) ANTIPROLIFERATIVE ACTIVITY OF *MEMECYLON* SPECIES

Anticancer drugs of natural origin have a great impact on the scientific community. They often search for a safe remedy for the most deleterious disease in the World. Agents, which deny the growth of cancer cells through apoptotic mechanism acquired a major interest in cancer research. The natural compounds of anticancer activity being reported will boost up the search for new ones. Vincristine, vinblastine, taxol *etc*., are few of them. Cytotoxicity is the key factor behind the control of unwanted cell proliferations. The herbal medicines with antiproliferative efficacy are proved to be cytotoxic agents. Hence, cytotoxic effects of plant extracts are targeted in antiproliferative studies. The present study thus shows promising results in cytotoxic screening using *A. cepa* root tip cells. Thus the remaining studies are focused on the anticancer properties of the selected species of *Memecylon*. The *in vitro* anticancer potential of *Memecylon* species are screened by using the breast cancer cell line, MCF-7. The selected concentrations of the six plant extracts are 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL and time period for the experiment was set for 24 hrs. The way of antiproliferative study was organized as follows: MTT assay was conducted for the cytotoxicity assessment. For determining the apoptotic mode of cell death,

acridine orange/ethidium bromide staining was performed. Subsequently the DNA damage at individual cell level was checked by comet assay. The influence of antiproliferative efficacy in cell cycle phases was analyzed by flow cytometry. The gene expression associated apoptotic pathways (p21, p53 and β- actin) were studied by RT-qPCR.

Breast cancer is the most common cancer in women. The increased number of population studies on breast cancer reveals an alarming signal that it continues to spread all over the World (Kalager et al., 2012; Abubakar et al., 2018). Naturally occurring plant compounds like curcumin, resveratrol, paclitaxel, docetaxel, quercetin *etc*., shows promising anticancerous property. It is less harmful to healthy cells and shows selective toxicity to abnormal cells. This might be the probable reason for the ample interest of herbal medicines. A number of natural anticancer drugs are discovered every year. From 1981 to 2014, 136 natural anticancer drugs are used globally, which are either natural or derivatives of natural products (Amaral, 2019). Several natural herbs are used to cure breast cancer (Shareef et al., 2016**)**. The present study gave special emphasis on the breast cancer inhibition ability of the selected *Memecylon* species.

Estrogen plays a major role in breast cancer induction. It can promote the transition of normal breast cells to malignant breast cells. Estrogen receptor positive is the major molecular signal of the breast cancer. So the herbal remedies for blocking estrogen receptors have great attention. Plant-based estrogen compounds mimic as the human estrogen analogue and can bind to the hormone receptor (Lamartiniere, 2000). It will create an imbalance in the molecular machinery, thereby checking breast cancer. Phytoestrogen rich products may prove to become a curative pathway in breast cancer. Phytoestrogen is mainly found in legumes and lignans. Lignans occur in seeds, nuts, whole grains, fruits and vegetables. The dietary intake of lignans

will reduce the risk of breast cancer (Rietjens et al., 2017). There are several reports on the herbal products that are used to cure breast cancer. Artemisinin and polyphenols isolated from *Artemisia annua* L., polyphenol-rich extracts of *Hibiscus sabdariffa* and aqueous extract of *Brucea javanica* are few of them (Laskar et al., 2020).

The first phase of anticancer study starts with the cytotoxicity evaluation of selected extracts on cell lines. In the previous section, in *A. cepa* assay it was noticed that the extracts prove to be cytotoxic. MTT assay is the common method used to determine the antiproliferative activity of compounds on cultured cells. It measures the mitochondrial metabolic rate and viable cell numbers (Li et al., 2009). The mechanism involved in MTT assay is as follows: the tetrazolium salt MTT is reduced to purple formazan crystal in the metabolically active cells by mitochondrial dehydrogenases (Shoemaker et al., 2004). In the present study antiproliferative efficacy of *Memecylon* species were tested using MCF-7 cell lines. The production of formazan is directly proportional to the number of viable cells. The highest antiproliferative activity was shown by *M. umbellatum* fruit extracts with 44.4 ± 1.68% (**Figure 29; Plate 22**). A dosage dependent antiproliferative activity was shown by all the selected plant extracts. 100 µg/mL was found to be the effective concentration of the plant sample. This is followed by *M. grande* fruits and leaves extracts with 46.93 ± 4.74 and 48.28 ± 2.78 viability percentages respectively (**Figure 29; Plates 17**, **18**). *M. randerianum* extracts possess moderate activity against MCF-7 cells. The overall results point out that the fruit extracts of selected samples show highest antiproliferative potential as compared to their corresponding leaf extracts. The $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract was selected for further anticancerous studies (**Table 11**). In *in vivo* toxicity studies, $LD_{50}$ is an important measure. It is considered as the safety value for evaluation of drugs in pharmaceutical industry

(Malmfors & Teiling, 1983). $LD_{50}$<1000 µg/mL was considered as an effective concentration of anticancer agents (Nguyen et al., 2020). Here *M. umbellatum* fruit extract prove to be with an appreciable $LD_{50}$ concentration for further anticancer studies. So it becomes the effective candidate for the next phase of the study.

The morphological variations that occur in MCF-7 cells treated with plant samples (**Plates 17-22**) form a clear indicator of toxic potential of the extracts. The aberrations like formation of membrane blebs, apoptotic bodies, nuclear condensation, membrane distortion, formation of echinoid spikes, budding, fragmentation and cell shrinkage are clearly visible in the MCF-7 cell lines. All these are the characteristic features indicating the occurrence of apoptosis. The chromatin/nuclear fragmentation associated with apoptosis could be the result of the inter-nucleosomal cleavage of DNA induced by the active phytoconstituents present in the plant extract (Liang et al., 2015). Apoptosis is characterized by a series of typical morphological features, such as fragmentation of chromatin, cell shrinkage, membrane-bound apoptotic body formation and rapid phagocytosis by neighboring cells (Saraste & Pulkki, 2000). The occurrence of the cell surface alterations is a clear indication of the apoptosis in animal cells (Thompson, 1995; Collins et al., 1997). An anticancer drug that has the potential to induce apoptosis is an effective step in the field of therapeutics. Microscopic observation of stained tissues shows the cells with unique morphological changes in the cells due to apoptosis, such as presence of condensed chromatin. In addition to that, cell surface morphology associated with apoptosis like echinoid spikes, budding, blebs *etc.* (Gown & Willingham, 2002) were also found in comparison with control. At the time of cell injury and blebs were formed that are balloon-like, quasi-spherical protrusions of the plasma membrane (Prajitha & Thoppil, 2017). Several morphological changes like blebs, budding, spikes (**Plates 17-22; 4, 6,** 7) and change in the cellular structures like condensation of nucleus

and chromatin, disappearance of chromatin mass and dissolved chromatin resulting in disappearance of nuclei (**Plates 17-22; 1, 2, 3, 5**) *etc*., were also observed.

The cytotoxic effect of the methanolic extract of *M. umbellatum* fruit was also analysed on the L929 cells in order to determine its effect on normal cells. The MTT assay was carried out on L929 (Fibroblast) cell line. The resultant effect of the sample extract on these cells can be related with that of normally dividing mammalian cells/tissues. A dose dependent viability percentage was resulted during the assay **(Figure 30)**. It ranges from 96.63 ± 2.56 to 76.72 ± 0.61% in a concentration gradient from 6.25 µg/mL to 100 µg/mL. No signs of apoptosis were noticed in the microscopic observation of cells (**Plate 23**). This indicates a mild and negligible cytotoxic effect of *M. umbellatum* fruit extract on the normal mammalian cells. The positive cytotoxicity assay in cancer cell line and negative result on normal L929 cell line enlighten the toxic potential of *M. umbellatum* fruit extract. The anticancer drug induces their toxic effects by apoptosis through the intrinsic pathway (Alshammari et al., 2020). Thus the next level of antiproliferative study was focused on the mechanisms behind the cytotoxic effect.

The genotoxicity potential of *M. umbellatum* fruit extract on MCF-7 is evaluated by the comet assay. The DNA damaging potential of plant extract was identified through this assay. So in order to find out the mechanism of toxic potential of plant extract, it's DNA damaging ability should form an effective parameter. Comet assay allows to measure single, double strand breaks, cross links and base damages (Olive & Banáth, 2006). It becomes a valuable technique for human cell biomonitoring and clinical studies. Apart from genotoxicity assays of radiations and certain chemicals, plant comet assay is also used in toxicity evaluation of nanoparticles (Santos et al., 2015). The kinetics of DNA damage recovery is a crucial part of therapeutic drug

formation. In the case of malignancy, induction of DNA damage is the vital step. The cells are treated with the chemical agents to remove all cellular proteins associated with the DNA. Then allow the DNA to unwind in alkaline/neutral conditions. The unwound DNA undergoes electrophoresis and the migration of the damaged DNA away from the nucleus was noticed. The staining with a DNA-specific fluorescent dye such as ethidium bromide is used to read the extent of damaging potential of the plant extracts.

In the assay, the DNA that is limited in the nucleus is termed as head and DNA that migrates out of the nucleus is called as tail DNA of the cells. The parameters namely comet length, tail length, tail DNA percentage, tail moment and olive tail moment were determined for the evaluation of DNA damages (**Plate 24**). The percentage of tail DNA is considered as the extent of the damages and it is the best measure of DNA damage potential (Møller et al., 2014). In the present investigation, cells treated with plant extract shows tail DNA percentage as 48.08 and that of control is 23.85% (**Figure 31 a**).The elevated level of comet length, tail length, tail moment and olive tail moment (**Figures 31 b, c, d, e)** confirms that *M. umbellatum* fruit extract has potent DNA damaging potential against MCF-7 breast cancer cell lines.

Tumour drug sensitivity test (DST) is an analytical method to check the most effective drugs that are used to treat tumours based on their sensitivity response. The molecular variances in tumour types become a barrier of lowering tumour DSTs. The reduced drug resistance and increased DSTs are efficient parameters of an anticancer drug. MTT assay is an *in vitro* DST. But it has some limitations, as it cannot distinguish apoptotic and necrotic cells. MTT assay gave an idea about the cytotoxicity level of test material and not regarding the mechanism of cell death. Comet assay gave an insight on the damaging potential of the extract. Acridine orange/ ethidium bromide (AO/EB) staining is a simple and accurate method that can be used

in tumour DSTs (Liu et al., 2015). Apoptosis and necrosis are the two basic mechanisms of cell death. In oncology research, studies are mainly focused on the genes and signals regulating the apoptosis. Apoptosis is a genetically regulated mechanism of cell death. It maintains normal homeostasis through the removal of damaged, physiologically redundant and abnormal cells (Carneiro & El-Deiry, 2020). The death of cells through disease or injury is termed as necrosis. It is caused by the factors outside the body and is an uncontrolled mechanism of cell death (Kanduc et al., 2002).

AO/EB staining was employed to analyze the induction of apoptotic nuclear damage in MCF-7 cell lines using the *M. umbellatum* fruit extract. In this analysis, the mechanism behind the cytotoxic effect of extracts can be recognised *ie.*, either apoptosis or necrosis. Apoptosis is associated with cell membrane damages and it has differential staining capability of the cells. In AO/EB staining, early and late apoptotic cells as well as necrotic cells are differently stained. The early apoptotic cells were noticed with greenish yellow nuclei, late apoptotic cells indicated condensed orange-red nuclei, while dead necrotic cells depicted red nuclei. AO can stain the normal cell membrane and emit green fluorescence in live cells whereas EB imparts stains on cells that had lost membrane integrity with orange - red coloured cells (Ribble et al., 2005). In the present study, it was clearly noticed that the untreated cells are observed as green fluorescence with normal nuclear morphology, where as *M. umbellatum* fruit extract treated cells appeared as orange coloured bodies with membrane damages (**Plate 25).** The double staining method unveils that the cell death induced by the plant extract is through the apoptotic mechanism.

Apoptotic effect of plant extract on cell cycle progression was analyzed through cell cycle analysis by using flow cytometry. In this study, MCF-7 cells were treated with *M. umbellatum* fruit extract (78.48 ± 0.8

µg/mL) to evaluate the cell cycle progression. The deregulation of cell cycle was frequently associated with cancer. The uncontrolled proliferation interrupts the cell cycle progression. So cell cycle regulators have importance in cancer therapy. Cyclin-dependent kinases with transcriptional functions are effectively targeted in BRCA1/BRCA2-mutant tumours (Otto & Sicinski, 2017). Mammalian cell cycle is a regulated process that gets progressed through G0/G1, S, G2 and M phases. The cell cycle progression is regulated by certain cyclins and cyclin dependent kinases (CDKs). The over expression of cell cycle proteins are allied with the cancer induction. Cyclin D, E, CDK4, CDK6 and CDK2 are overwhelmed in uncontrolled cell division. They are the key regulators of G0/G1 phase of the cell cycle (Vermeulen et al., 2003). The cell cycle progression from G0/G1 is initiated by serine/threonine kinases like, CDK4 and CDK6. The related action of cyclin D will enhance the activity of CDK4 and CDK6. The cyclin D/CDK4/6 activity will be hindered by the action of p21 and p27, the inhibitors of CDKs. Thus they prevent the G1-S transition. In breast cancer, the overexpression of cyclin D1 was noticed. The lack of cyclin D1 protein will prevent the mammary gland formation induced by certain oncogenes (Bowe et al., 2002). Several examples of cell cycle regulation through the inhibition of CDKs are reported. A mice bearing $Erbb2^{V664E}$ mammary tumour was triggered by inhibition of CDK4 and CDK6 kinases (Choi et al., 2012). The inhibition of CDK4 and CDK6 in mice is associated with tumor - specific apoptosis rather than senescence (Sawai et al., 2012). In the present study, G0/G1phase shows the highest cell count and subsequent reduction was observed in following phases (**Figure 34**). DNA count as well as population count of cells treated with the plant extract get decreased from G0 to M phases (**Figures 32 & 33**). So these results clearly point out that the cell cycle arrest occur at G0/G1 phase and the diminishing progression of cell cycle is due to the apoptotic mechanism induced by the plant extract.

The action of G0/G1 cell cycle regulators can prevent the proliferation of MCF-7 cells. Thus the decrease in cell counts as well as the population counts in successive phases of cell cycle is clearly pointing towards the cell cycle regulation capability of the plant extract. Liu et al. (2018) describes that the DNA count increased in G0/G1 phase is due to the DNA fragmentation associated with the MCF-7 cells. The CDKs activities become denied due to the presence of plant extract as compared to the negative control. So it might be useful in cancer therapy. CDKs are the target in various strategies of cancer treatment. Palbociclib, a CDK4/CDK6-selective inhibitor used in breast cancer treatment was the first successful clinical drug in this field. Similarly, ribociclib and abemaciclib are other two targeted CDK4/6 inhibitors used in breast cancer in combination with endocrine therapy. Palbociclib and letrozole are used in patients with advanced ER+ HER2- breast cancer (Lynce et al., 2018).

In MTT assay, comet assay, double staining test and cell cycle analysis, *M umbellatum* fruit extract shows a potent anticancerous effect. It has proved to be cytotoxic, DNA damaging and an executor of apoptosis against the breast cancer cell lines. The gene level approaches in cancer therapy become widely acceptable. The genetic divergence in cancer type and heterogeneity in populations are leads to new strategies investigated in the context of genetic pathways. Several case studies propose that genetic level studies are a more reliable method to convey effective medicine to cancer patients (Cheng et al., 2013). The gene expression studies gave an initiative for the disease management in cancer (Dopazo et al., 2001). In breast cancer studies, the action of p53 is widely studied. It plays multiple roles *ie.,* act as a transcriptional regulator, cell cycle arrestor and apoptosis inducer. An anticancer drug induces their effect through the genomic damages and facilitates apoptosis. p53 is an excellent executor of genomic damages and apoptosis. The action of p53 is regulated by another transcription activator

p21, and they merely activate anticancerous mechanism. p21 can induce the cell cycle arrest because it act as a CDK inhibitor (Elledge & Allred, 1998). Besides breast cancer, in many tumors p21 (WAF1/CIP1) is associated with p53 expression. The subcellular localization of p21 is a hall mark in breast cancer (Winters et al., 2001). In this study, the mRNA specific for the p21 and p53 along with that of a house keeping gene (β-actin) were isolated for which cDNA were prepared, amplified and examined after electrophoresis.

In the present investigation, MCF-7 cells with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract significantly induced an up-regulation in the expression of p53. The p53 gene can induce the expression of p21 gene (**Figure 35**). The vibrant action of p53 and p21 in association with cancer is widely discussed. p53 dependent p21 action in tumour cells are a prognostic way of apoptosis. p21 is a key regulator of apoptosis and DNA damages. The tumour inhibition of p21 is through the modulatory action of cyclins, transcription factors and proliferation of cell nuclear antigen (Shamloo & Usluer, 2019). The over expression of p21 that contributes towards tumour suppression through apoptosis in mice models are reported by Elliott et al. (2002). Recently the gene editing on p21 gene had become helpful in suppressing tumorigenesis. In the case of p21, deficient mice models with mice mammary tumour virus (MMTV) ras and myc, shows differences in their apoptosis levels. It is clearly indicated that p21 has a crucial role in apoptotic induction (Bearss et al., 2002). The action of p53 for maintaining the normal homeostasis of cells through Waf-1-mediated induction of G1 arrest or *Bax*-mediated apoptosis was reported by Keshava et al. (2002). The expression fold analysis also proves the prominent expression of apoptotic genes. It is the measurement of expression of genes. Here the expression fold change of p53 is 1.86 over the control and that of p21 gene is 1.52 (**Figures 36 a & b**). The positive value of expression fold changes indicates the up regulated mechanism of gene expression. In the present

study, the expression fold change clearly indicates that the p21 and p53 are key regulators in antiproliferative mechanism. The antiproliferative activity of *M. umbellatum* fruit extract significantly proves to be useful towards drug preparation. The phytochemical analysis reveals that *Memecylon species* has a diverse array of phytoconstituents present in it. In the case of *M. umbellatum* fruit extract, the presence of alkaloids, terpenoids, fatty acids *etc*., are noticed. Phytochemicals are the backbone of bioactivity. In cancer studies, phytochemicals are an effective target of clinical trials (Choudhari et al., 2020). The apoptotic signs noted in MTT assay, double staining test, DNA damaging potential and cell cycle arrest proved to be the satisfactory leads in the present study. Thus in the present investigation, cell line studies, comet assay, AO/EB staining, cell cycle analysis and gene expression studies evidently prove that *M. umbellatum* fruit extract has potent antiproliferative activity.

## d) GREEN SYNTHESIS OF SILVER NANOPARTICLES

The present study investigates the green synthesis of silver nanoparticles from *Memecylon* fruit and leaf extract. The physical and chemical approaches are the common methods of metallic nanoparticle production. The evaporation-condensation and laser ablation are the most important physical approaches. The most common chemical approaches for the synthesis of silver NPs are chemical reduction by organic and inorganic reducing agents, UV-initiated photoreduction, microemulsion techniques and electrochemical synthetic methods (Iravani et al., 2014). The chemical and physical methods of NPs production have many limitations. They are toxic, energy consuming, expensive and are not suitable for biological applications. The emergence of a new area in green synthesis of NPs had been nurtured before few decades (Ghaffari-Moghaddam et al., 2014). The biogenic syntheses of nanoparticles are pure, non-toxic, cost effective, ecofriendly and

have better bioactivity. Silver is the common metal used for the synthesis of nanoparticle. Silver is a soft, white, lustrous transition metal possessing high electrical and thermal conductivity. Silver nanoparticles have received a great attention due to their physical, chemical, and biological properties that include catalytic activity and bactericidal effects (Firdhouse & Lalitha, 2015). The antibacterial activity of silver nanoparticle is extensively studied *ie.*, it is active against *Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa* and *Salmonella typhi (*Morones et al., 2005). It has exhibited a wide range of activity against both gram-positive and gram-negative multidrug resistant bacteria (Qais et al., 2019). Silver nanoparticles synthesized from *Abutilon indicum* showed a dose-dependent anti-cancer activity against human colon cancer at a very low concentration (Ahmed & Mustafa, 2019). The antioxidant, cytotoxic and anti-inflammatory activity of SNPs is already reported in several plants (Khatoon et al., 2018; Rao et al., 2018; Gondwal & Joshi, 2018).

The reducing capacity of plant extract is exploited in the biogenic synthesis of SNPs. The plant extracts are a rich source of many phytoconstituents, which act as stabilizing as well as capping agents for the synthesis of nanoparticles. The present study reveals the reducing power of *Memecylon* species. *M. grande* leaf and fruit extracts when treated with silver nitrate solution results in the formation of a brown colour solution, which indicates the presence of SNPs. *M. grande* fruit extract shows a brown colour change in the reaction tube whereas, *M. grande* leaf extract has a dark brown coloration in the reaction tube with an immediate reaction (**Plate 26 g, h**). The difference in colour tinge may be related to the variation in the intensity of nanoparticle synthesis (Bhagyanathan & Thoppil, 2018). *M. randerianum* leaf extract possess a pale yellowish brown colour and its fruit extract is having a dark brown coloration in the reaction tube (**Plates 26 i; 27 g)**. The reaction mixture that contains *M. umbellatum* leaf extract and silver nitrate solution

produce a yellow coloured solution. The reducing capacity of *M. umbellatum* fruit extract creates a nanoparticle solution with a brown colour (**Plate 27 h, i**). The colour changes in the reaction tube that contain SNPs are also due to the Surface Plasmon Resonance (SPR). SPR is a versatile technique for biological analysis and depends on the optical properties of the metal layer (Sadrolhosseini et al., 2012). The presence of different phytochemicals responsible for the reduction, stabilization and capping of silver nanoparticles is confirmed through UV-VIS spectroscopy.

UV-Vis spectroscopy (UV-Vis) is another relatively facile and low-cost characterization method of nanoparticles. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. NPs have optical properties that are sensitive to size, shape, concentration and agglomeration state, which makes UV-Vis spectroscopy an important tool for characterizing nanoparticles. Gold, silver and copper nanostructure sols exhibit characteristic UV-Vis extinction spectra due to the existence of a LSPR (Localized Surface Plasmon Resonance) signal in the visible part of the spectrum. Nano metals showed conspicuous spectral characteristics according to the surface plasmon resonance (SPR). Mutual vibrations of free electron resonance with light waves can influence the size and shape of the synthesized NPs. Consequently, the broadening of the SPR peak width is considered as an agreeable detector of the nano metal size and its polydispersity (Mukherjee et al., 2001; Behzadi et al., 2015). The synthesized nanoparticle of *M. grande* fruit extract subjected to UV-Vis spectroscopic analysis shows the maximum absorption peak at 434 nm. *M. grande* leaf extract possess a maximum absorption peak at 440 nm. The SNPs synthesized by both plant extracts show a broad peak area with an absorption values of 0.7 and 1.28 respectively (**Figure 37**). The presence of a non specific peak is detected at 418 nm in *M. randerianum* leaf extract with absorption value of 0.3. The fruit extract of *M. randerianum* exhibit a specific

broad peak at 432 nm with 1.71 absorption value. *M. umbellatum* leaf extract possess a non specific peak at 426 nm with 1.2 absorption value. A broad peak area at 468 nm with an absorption value of 0.33 was observed in *M. umbellatum* fruit extract (**Figure 37**). The range of 380–470 nm is the characteristic λ max for AgNPs, so the peaks obtained from UV-Vis spectra confirm the presence of silver nanoparticles (Kumar et al., 2016).

The intensity of the peak area is directly related to the concentration or the size of the nanoparticle synthesized in the sample solution. *M. umbellatum* and *M. randerianum* leaf extracts possess few weak signals, which indicate that the nanoparticle size become comparatively less as evaluated with other extracts. A narrow peak of absorption also occurs in 263 nm with an absorption value of 0.93 and a narrow peak was resulted in the 238 nm with absorption value of 0.92 respectively for these two leaf extracts.

According to Mie theory, as the particle size decreases, a shift in peaks to lower wave length ranges may occur (Alvarez et al., 1997). The size and shape of synthesized nanoparticles are determined by Scanning Electron Microscopic analysis (SEM). In the present study, *M. grande* leaf extract possess 20-30 nm sized nanoparticles and nanoparticle synthesized from *M. grande* fruit extract is 26-44 nm size. The shape of the nanoparticles synthesized by the *M. grande* fruit extract is cubical (**Plate 29**) and that of *M. grande* leaf extract is having a spherical shape (**Plate 28**). A perfect spherical shape with 20-32 nm sized nanoparticle was formed in the *M. randerianum* leaf extract mediated silver nanoparticle synthesis. Similarly a uniform size and morphology of nanoparticles was exhibited by *M. randerianum* fruit extract also. They have spherical shape with 20-28 nm size (**Plates 30, 31**). *M. umbellatum* leaf extract mediated silver nanoparticles synthesis contributes somewhat spherical shaped particles with 22-33 nm size. The fruit extract of *M. umbellatum* possess almost spherical shaped silver nanoparticles with 26-

35 nm size (**Plates 32, 33**). From, the UV-Vis spectroscopic results, the particle size of the leaf extract of *Memecylon* species was found to be comparatively smaller as compared to their corresponding fruit extracts.

There is a direct relationship between the size and shape of the nanoparticle on its biological activity. The size and shape of silver nanoparticle vary in different plant extracts (Hamouda et al., 2019). The spherical shaped nanoparticles have a high surface area to volume ratio. This property enhances their antimicrobial activity (Kumar et al., 2015). The smaller nanoparticle has more penetration power, whereas too smaller nanoparticle can create toxic effects on the cell as compared to larger sized nanoparticles. So the nanoparticles have a size-specific biological activity (Wang et al., 2017).

There are several factors delimiting the application of bioactive phytoconstituents. The usage of these phytochemicals directly in medicines is inhibited by their solubility rate, stability and bioavailability. The application of nanotechnology can overcome these difficulties. Phytochemical oral delivery system (PODS), which is a new approach can unload the phytochemical filled nanoparticles on the target system without any stability and solubility problems. The proper designing of PODS can enhance the phytochemical potential of commercial products, *ie.*, in foods, supplements and pharmaceuticals (McClements, 2020). The bioactive proteins and peptides are denatured in commercial products or gastrointestinal tract, because of their poor stability. So in order to overcome these issues, the nanoparticles with encapsulated form of bioactive proteins and peptide products are used (McClements, 2018). The present investigation thus concludes that *Memecylon* is an important candidate in green nanotechnology. The nanoparticles biosynthesized from the species of *Memecylon* can be used as the lead component in biomedical field.

In conclusion, the present study gave an insight on the pharmacognostic identification, phytochemical and bioactivity validation of selected *Memecylon* species. It strongly suggests that *Memecylon* species form a promising candidate in the pharmaceutical field. The findings of the study are summarized and the conclusions drawn were presented in the next chapter.

# CHAPTER 6 SUMMARY AND CONCLUSIONS

Plants are being used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternate source of medicine to maintain health and improve the quality of life. The present study deals with the exploration of *M. grande, M. umbellatum* and *M. randerianum* fruit and leaf extracts. The objectives of the present study are summarized as: 1) Pharmacognostic profiling 2) Phytochemical characterization and 3) Bioactivity analysis.

Herbal development occurs through the various step wise analytical processes. Pharmacognosy is one among the preliminary steps in it. It includes the analysis of functional purity of the plant sample. The herbal medicines often suffer with quality controversies because of similar species or varieties that are used as adulterants. Pharmacognostic analyses can rectify the taxonomic misinterpretation in the identification process. On the basis of botanical origin of selected species, phytoconstituents of the plant samples were analyzed through the preliminary tests and chromatographic techniques. The presence of diverse chemical constituents leads us to investigate the bioactivity potential of *Memecylon* species.

Major findings from the present study are summarized below:

**1) Pharmacognostic profiling**

Powder microscopy, SEM-EDX analysis and ICP-MS analyses gave a vivid picture of the pharmacognostic profile of the selected *Memecylon* species. In powder microscopic analysis, fruit and leaf extracts of *M. grande*, *M. randerianum* and *M. umbellatum* shows the presence of characteristic elements.

The powder microscopic analysis confirms that the botanical origin of these plant samples is pure. So this result can be used as a future reference for the identification of *Memecylon* species. Scanning electron microscopic analysis of fruit endocarp and entire seed of selected *Memecylon* fruits show a distinct morphological pattern. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships. *M. grande*, fruits show colliculate pattern in endocarp and seed surface possesses tuberculate pattern. In *M. randerianum* fruit, endocarp is with ruminate reticulate type pattern and seed surface with reticulate pattern. *M. umbellatum* fruit endocarp possesses a smoothened pattern and its seed surface shows a wrinkled pattern of appearance. EDX spectra of the selected *Memecylon* species reveal the elemental composition at the microscopic level. Nitrogen was found to be the prominent compound detected in *Memecylon* species. Phosphorus, potassium, iron, magnesium, cobalt and sodium were also noticed through EDX analysis. In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental composition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was carried out. The presence of aluminium, arsenic, cobalt, strontium, selenium, zinc, chromium, copper, molybdenum, nickel, barium and manganese were noticed. These findings suggest that *Memecylon* fruits are a reservoir of essential elements and it can be exploited in the pharmaceutical or nutritional field. Thus the pharmacognostic profiling offers future reference parameters for *Memecylon* identification. The herbal parts with consistent quality, without adulterants or invasive free particles make their performance in a proper way. So the functional purity demands the pharmaceutical potential. The powder microscopy, SEM-EDX analysis and ICP-MS analysis thus validates the drug making capability of the selected *Memecylon* species.

## 2) Phytochemical characterization

The preliminary phytochemical analysis is carried out to determine the presence or absence of phytochemicals in the samples. The methanolic extracts of leaf and fruit extracts of *Memecylon* spp. were subjected to qualitative and quantitative analysis. In qualitative phytochemical analysis, the presence of alkaloids, flavonoids, phenolics, steroids and tannins were confirmed in all the selected species. The presence of glycosides is revealed in the leaf and fruit samples of *M. umbellatum.* The complete absence of resins and anthraquinones was confirmed in all the selected species. The quantitative determinations of alkaloids, flavonoids, phenolics and terpenoids were conducted and found that all the selected extracts have considerable amounts of potential secondary metabolites. *M. grande* fruit extract possesses highest amount of alkaloids, phenolics and terpenoids. The amount of flavonoids was also found to be highest in *M. grande* leaf extract.

The identification of volatile phytoconstituents in selected *Memecylon* species was done through the GC/MS analysis. A total of 83 compounds were detected in the methanolic extract of selected species. The identified compounds belong to the classes of terpenoids, phenolics, fatty acids, fatty acid esters, steroids *etc.* The GC/MS analysis of *M. grande* leaf extract reveals the presence of 17 compounds. The major constituents were oleic acid, methyl oleate and palmitic acid. The presence of fatty acid esters are in significant amount also. Similarly 17 compounds were noticed in *M. grande* fruit extract. The fatty acid esters are found to be in highest amount, in which methyl elaidate was prominent. A total of 26 compounds were detected in the methanolic leaf extract of *M. randerianum.* Palmitic acid, agathenic acid, squalene, phytol and lupeol are the major ones. Terpenes were the predominant class of compounds present in the leaf extract. Friedlein and stigmast-5-en-3-ol were the terpenoid group of compounds observed in

highest amount in *M. randerianum* fruit extract. The presence of fatty acids and fatty acid methyl esters are also confirmed in a total of 14 compounds present in *M. randerianum* fruit extracts. *M. umbellatum* leaf extract encompasses 32 compounds. It includes phenols, terpenoids, steroids and organic compounds. A steroid compound, α-phytosterol occurred in highest amount. The fruit extract of *M. umbellatum* possesses 12 bioactive phytoconstituents. The presence of an alkaloid, hordenine was found to be prominent. The presence of carbohydrate lactones, fatty acid derivatives and fatty acid methyl esters are also noticed.

Non-volatile compositions of selected *Memecylon* species were identified through the HR-LC/MS analysis. The identified 48 compounds belong to the classes like terpenoids, steroids, fatty acids, biopeptides, hydroxyl benzoquinones, glycosides, alkaloids, esters, carotenes *etc*. A terpenoid compound swietenine was found to be common in *M. grande* leaf, fruit and *M. randerianum* fruit extracts. Lupanyl acid, aesculin, C16 sphinganine, 3-dehydro-6-deoxoteasterone and biopeptides are the major constituents of *M. grande* leaf extract. Tamarixetin, rescinnamine, madecassic acid, campestanol, khayanthone and carotene were detected in *M. grande* fruit extract. The presence of bergenin and 9,12,13-trihydroxy-10,15-octadecadienoic acid are noticed in *M. randerianum* leaf extract. Violastyrene, gibberellin A8-catabolite, rescinnamine, β-erythroidine, glycerol palmitate, 6-deoxocastasterone and cosmosiin hexaacetate were found in *M. randerianum* fruit extract. *M. umbellatum* fruit extract, possesses a diverse array of non-volatile chemical constituents, a total of 16 compounds were noticed in it. Whereas, *M. umbellatum* leaf extract possesses a limited number of compounds. Protoveratrine A, embelin, amygdalin and stigmasta-7, 22 E, 25-trien-3beta-ol are the major compounds in *M. umbellatum* fruit extract. While comparing the non-volatile composition of *Memecylon* species, fruit extract shows much more diverse phytoconstituents.

### 3) Bioactivity analysis

Free radical scavenging activity of the selected *Memecylon* species was analyzed through DPPH, hydroxyl, nitric oxide and superoxide radical scavenging assays. In DPPH assay, the highest scavenging activity was shown by 200 µg/mL concentration of *M. grande* fruit extract. *M. grande* fruit extract exhibit an inhibition percentage of 75.77 ± 0.01. This is followed by *M. umbellatum* fruit extract. In hydroxyl radical assays, *M. grande* fruit extract shows the highest activity *ie.,* 61.69 ± 0.52%, followed by *M. umbellatum* fruit extract with 53.46 ± 1.89%. Here the inhibitory concentration of the standard compound was higher as compared to the effective plant extract. So *M. grande* fruit extract is considered as a good hydroxyl radical scavenger. Nitric oxide assay also shows similar trends of scavenging potential. In superoxide radical scavenging assay, *M. grande* fruit extract shows the highest scavenging activity of 72.17 ± 0.02%. The lowest activity was shown by *M. randerianum* leaf extract with 47.36 ± 0.01%. In all the antioxidant assays, the selected plant species follows similar trends of activity. *M. grande* fruit extract has the highest scavenging potential and lowest in *M. grande* leaf extract. An exception was noted in superoxide radical assay, where lowest activity was shown by the *M. randerianum* leaf extract. *M. randerianum* and *M. umbellatum* exhibits a moderate range of activity in all the assays. The free radical scavenging activity of fruit extracts become more as compared with the leaf extracts. It might be due to the diverse phytochemical composition of the plant parts.

The cytotoxic potential of the selected *Memecylon* species is analyzed by using *A. cepa* root tip meristem. The toxic potential of plant extracts were analyzed through the assessment of mitotic index and aberration percentage. Dose dependent mitotic index and abnormality percentage were resulted, and found that time has no role in the cytotoxic effect of the plant extracts. The

decrease in mitotic index is correlated with increasing concentration of plant extracts and the abnormality percentage increases with increasing concentrations. Several chromosomal aberrations are resulted during the cytotoxic assay. Stickiness, pulverization, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, stellate chromosomes, lagging chromosomes, exposure of chromosome scaffold *etc*., are few of them. These results are pointing to the fact that *Memecylon* extracts has potential cytotoxic role and antiproliferative efficacy revealed by potential mitotic inhibition.

Antiproliferative activity of *Memecylon* was tested against human breast cancer cell line MCF-7. The selected concentration of six plant extracts are 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL and time period for the experiment was set for 24 hrs. The direct microscopic observation reveals the toxic potential of plant extracts. The aberrations like formation of membrane blebs, apoptotic bodies, nuclear condensation, formation of echinoid spikes, budding, fragmentation and cell shrinkage are clearly visible in the MCF-7 cell lines, which are indicating the hallmarks of cell death. A dose dependent cytotoxic effect was observed and the highest antiproliferative activity was shown by *M. umbellatum* fruit extract with 76.8 ± 2.75%. The overall results thus point out that the fruit extracts of selected samples show highest antiproliferative potential as compared to their corresponding leaf extracts. The $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract was selected for further anticancerous studies.

Cytotoxic assays using MCF-7 breast cancer cell lines and *A. cepa* assay reveal the toxic potential of plant extracts. In order to find out the non-toxic effect of the plant extract on normal cells, MTT assay was carried out on L929 (Fibroblast) cell line. A dose dependent viability percentage was resulted during the assay. It ranges from 96.63 ± 2.56 to 76.72 ± 0.61% in a

concentration gradient from 6.25 µg/mL to 100 µg/mL. The cellular damages are very fewer in normal L929 cells. The DNA damaging potential of *M. umbellatum* fruit extract was analyzed by performing comet assay. In this assay, MCF-7 cells are treated with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract. The parameters namely comet length, tail length, tail DNA percentage, tail moment and olive tail moment were determined for the evaluation of DNA damages. The elevated levels of parameters are observed through the assay. The DNA percentage in tail was found to be much higher (48.08) than that of the control (23.85). It is linearly related to the DNA breaking potential of the plant extract. The intensity of the tail increases as the damage is enhanced. The elevated levels of olive tail moment and tail movement were noticed during comet assay. So comet assay confirms the DNA damaging potential of the plant extracts of *Memecylon*.

The cytotoxic assay in *A. cepa*, MTT assay and comet assay, reveals the cell damaging potential of the selected active plant extract of *M. umbellatum*. The cell damaging potential or cell death inducing capability of the plant extract is further analyzed through the double staining method. It is a method that unveils the mechanisms behind the cellular damages/cell death. A combination of acridine orange/ethidium bromide stains are eluted on the MCF-7 cell lines, which are treated with $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract. This staining method enabled to visualize the apoptotic or necrotic cells from the normal cells. The plant extract treated cells have lost their viability and membrane integrity. They are observed as orange coloured bodies. The double staining method reveals that the cell death induced by the plant extract is through the apoptotic mechanism.

The apoptotic effect of the plant extract on cell cycle progression was analyzed through cell cycle analysis by using flow cytometry. Here MCF-7

cells were treated with the active *M. umbellatum* fruit extract. G0/G1 phase of the cell cycle shows the highest amount of DNA content. There is a subsequent reduction of DNA content, which was resulted in S and G2/M phases. In the case of cell population count, there is a scatter in untreated cells, while the treated cells show aggregation. That means that the progression of cell cycle was arrested in a particular phase of cell cycle. The percentage of cell count in each phase of the cell cycle unveils the retardation of cell cycle progression. The G0/G1phase shows the highest cell count and subsequent reduction was observed in following phases. So these results clearly point out that the cell cycle arrest occurs at G0/G1 phase and the diminishing count during the progression of cell cycle is due to the apoptotic mechanism induced by the plant extract.

To substantiate the underlying mechanism of antiproliferative activity exhibited by the fruit extract of *M. umbellatum* on MCF-7 cells, the expression changes of genes which are known to be involved in cell cycle arrest and induction of apoptosis were examined. The expression pattern of p53 and p21 were studied by RT-qPCR and the data were analysed according to $\Delta\Delta C_t$ method. p53 and p21 are genes that regulate many downstream genes involved in the induction of cell cycle arrest, DNA repair and apoptosis. β-actin, a house keeping gene was used as the control. The intense fluorescence in gel electrophoresis has clearly indicated that the treatment of MCF-7 cells with 78.48 ± 0.8 μg/mL of extract significantly induced an up-regulation in the expression of p53. The p53 gene can induce the expression of p21 gene. The expression fold analysis also proves the prominent expression of apoptotic genes induced by the active extract of *M. umbellatum*.

The present study also highlights the evaluation of silver nanoparticles biosynthesized from selected *Memecylon* species. Green synthesis of nanoparticles become a safe platform because they are free from toxic

chemicals as well as contains natural capping agents. The silver nanoparticles were characterized through UV-Vis spectrophotometer and SEM analysis. The reduction of silver nitrate solution into silver nanoparticles after treating with plant extracts is analyzed through the colour changes, surface plasmon resonance and shape of the nanoparticles. The reduction of silver nitrate solution into silver nanoparticles by the action of plant extract has resulted in the colour changes of the reaction tubes. The selected plant extracts show a yellow to brown colouration in the reaction tubes. The synthesized nanoparticles of selected *Memecylon* species are subjected to UV-Vis spectroscopy in a wavelength range of 200-700 nm. The synthesized nanoparticles of *M. grande* leaf extract subjected to UV-Vis spectroscopic analysis show the maximum absorption peak at 440 nm. *M. grande* fruit extract possess a maximum absorption peak at 434 nm and similarly *M. randerianum* leaf and fruit extracts at 418 nm and 432 nm respectively. In the case of *M. umbellatum* leaf extract, a narrow peak was resulted at 426 nm and *M. umbellatum* fruit extract shows a peak at 468 nm. The range of 380-470 nm is the characteristic λ max for AgNPs, so the peaks obtained from UV-Vis spectra confirm the presence of silver nanoparticles.

From the SEM analysis, the nanoparticle size of *M. grande* leaf extract was found to be 20-30 nm and *M. grande* fruit extract possess 26-44 nm sized particles. The shape of the nanoparticles synthesized by *M. grande* leaf extract is spherical and that of *M. grande* fruit extract is with a cubical shape. A perfect spherical shape with 20-32 nm sized nanoparticles was formed in the *M. randerianum* leaf extract mediated silver nanoparticle synthesis. Similarly a uniform size and morphology was exhibited by the nanoparticles biosynthesized by *M. randerianum* fruit extract also. They have spherical shape and are 20-28 nm in size. *M. umbellatum* leaf extract mediated silver nanoparticle synthesis contributes somewhat spherical shaped particle with 22-33 nm size. The fruit extract of *M. umbellatum* possess almost spherical

shaped silver nanoparticles with 26-35 nm size. By substantiating the UV-Vis spectroscopic results, the particle size of the leaf extract of *Memecylon* species is comparatively smaller as compared to their corresponding fruit extracts.

The present study thus highlights that *Memecylon* is a suitable candidate in pharmaceutical field. The first phase of the study gave standard pharmacognostic profiles of *Memecylon* spp. as reference tools for future perspectives. The wide spectra of phytochemicals and their potential bioactivities together with nanoparticle synthesis from selected plant extract point towards their efficiency as potential drugs. The selected plant extracts shows better performance in all bioactivity studies. The synergistic action of phytochemicals present in the plant extract contributes towards the cytotoxic, antioxidant and anticancerous activity. The most effective extract selected from the six plant extracts studied was *M. umbellatum* fruit extract. The major findings in the present study open a gateway for the selection of an amenable source of natural medicine.

**Deliverables**

- Pharmacognostic profiling of *Memecylon* by using powder microscopy, SEM-EDX and ICP-MS analysis was reported for the first time.
- Immense source of potential phytoconstituents were identified and revealed as phytochemical profile from the selected *Memecylon* species through GC-MS and HR-LC/MS analysis.
- Potential free radical scavenging activity was revealed in selected *Memecylon* species.
- Cytotoxic activity revealed using *A. cepa* assay proves to be a leading step towards further antiproliferative studies.

- The antiproliferative activity against MCF-7 cell lines, cell cycle analysis and gene expression studies enlighten the anticancer potential of *Memecylon* species.
- A new approach on green synthesis of silver nanoparticles from *Memecylon* species was established.

**Future perspectives**

- Isolation of bioactive components from *Memecylon* species.
- *In- vivo* studies on animal models for detailed exploration of antiproliferative mechanism.
- Biomedical exploration of biosynthesized nanoparticles.

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# APPENDICES

**Wagner's reagent** **Appendix 1**

Iodine : 1.27g

KI : 2g

Dissolve the above chemicals in 5 mL $H_2SO_4$ and make up to 100mL.

**Phosphate buffered saline (PBS)** **Appendix 2**

NaCl : 8g

KCl : 0.2g

$Na_2HPO_4$ : 1.44g

$KH_2PO_4$ : 0.2g

Dissolve in 1 L double distilled water and adjust pH to 7.4.

**Griess Reagent** **Appendix 3**

Naphthylethylenediamine HCl : 0.1% in distilled water

Sulfanilmide : 1% in 5% $H_3PO_4$

Mix both in 1:1 ratio

**Modified Carnoy's fluid** **Appendix 4**

Acetic acid : 10 mL

Ethanol : 30 mL

**Acetocarmine** **Appendix 5**

Carmine : 2g

Acetic acid : 100 mL of 45% acetic acid

The solution is heated to dissolve carmine and is filtered to remove undissolved stain.

**DMEM (Dulbecco's Modified Eagle's) medium** **Appendix 6**

Sodium bicarbonate : 1.85g

HEPES : 2.95g

DMEM powder : 1 packet

Distilled water : 1L

Vacuum sterilized and stored at 4°C

**Lysing solution (1000 mL)** **Appendix 7**

2.5 M NaOH : 146.1 g

EDTA : 37.2 g (for 100 mM solution)

Trizma base : 1.2 g (10 mM)

1% SDS : 10 g

Add ingredients to about 700 mL of distilled water and stir the mixture. Add 8g NaOH and allow the mixture to dissolve for about 20 min and adjust the pH to 10 using concentrated HCl or NaOH and store at room temperature. To this mixture, 10% DMSO and 1% Triton X 100 are added prior to use.

**Electrophoresis buffer** **Appendix 8**

Stock solutions:

10 N NaOH : 200 g/500 mL distilled water

200 mM EDTA : 14. 89 g/200 mL distilled water

pH : 13

Store the stock solutions at room temperature.

For 1X Buffer (make fresh buffer before each electrophoresis run) add 30 mL NaOH and EDTA, per 1L and mix well. Ensure pH as > 13 prior to use.

**Neutralization buffer** **Appendix 9**

0.4 M Tris : 48.5 g

The above quantity of Tris is added to 800 mL distilled water and pH adjusted to 7.5 with concentrated HCl. The final volume is made to 1000 mL with distilled water and stored at room temperature.

**Ethidium bromide** **Appendix 10**

Ethidium bromide : 20 µg/mL

Add 10 mg to 50 mL distilled water and store at room temperature (10X).

For making 1X stock, mix 1 mL with 9 mL of distilled water. Handle ethidium bromide with caution as it is a known carcinogen.

**TE (Tris-EDTA) buffer** **Appendix 11**

Tris HCl : 10 mM, pH 8

EDTA : 0.1 mM, pH 8

**Research publications**

1. **P. R. Ramya Sree.,** & Thoppil J. E. **(2018).** C-mitotic potential of aqueous leaf extract of *Memecylon randerianum* S. M. & M. R. Almeida. - a promising natural colchicine analog. *International Research Journal of Pharmacy*, *9* (11), 115-118.
2. **P. R. Ramya Sree.,** & Thoppil J. E. **(2021).** Comparative seed morphology, pharmacognostic, phytochemical and antioxidant potential of *Memecylon* L. fruits. *Turkish Journal of Pharmaceutical Science*, *18*(2), 213-222. **IF-1.1**
3. **P. R. Ramya Sree.,** & Thoppil J. E. **(2021).** An overview on breast cancer genetics and recent innovations: Literature survey. Breast Disease, *40*(3), 1-12. **IF-1.6**
4. **BOOK: P. R. Ramya Sree**., & Thoppil J. E. **(2019).** Exploration of *Memecylon randerianum* S. M. & A. R. Almeida. Lambert publishers, Germany, ISBN: 978-3-659-54946-5.
5. **BOOK CHAPTER**: **P. R. Ramya Sree.,** & Thoppil J. E. **(2020).** Ecological importance of Melastomataceae. In A. K. Sarkar (Ed.), *Organism and environment* (pp. 219-224). New Delhi: Educreation publishing, ISBN-978-93-89808-99-5.

**Paper presentations**

1. **P. R. Ramya Sree.,** & Thoppil J. E. (**2017).** "Phytochemical screening and Cytotoxic potential of *Memecylon umbellatum* Burm. f. fruit and leaf- A potential medicinal plant of central Western Ghats" in third International conference on frontiers of mass Spectrometry, School of Environmental Science & Inter University Instrumentation Centre, Mahatma Gandhi University, Kottayam, Kerala. (Poster presentation)
2. **P. R. Ramya Sree.,** & Thoppil J. E. (**2018**). "Evaluation of phytoconstituents and bioactivity screening of the methanolic leaf extract of *Memecylon umbellatum* Burm." in International conference on phytomedicine, Dept. of Botany, Bharathiyar University, Coimbatore. (Oral presentation)
3. **P. R. Ramya Sree.,** & Thoppil J. E. (**2018)**. "Cytotoxic and apoptotic activities of extract of *Memecylon umbellatum* L." in International biodiversity congress, Forest Research Institute, Dehradun. (Poster presentation)

4. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. "Phytochemical screening and cytotoxic potential of *Memecylon randerianum* S. M. and M. R Almeida" in MESMAC International conference on People First? Man, Machine, Milieu. MES College Mampad, Malappuram. (Oral presentation)
5. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. *Memecylon randerianum* SM & MR almeida - a promising natural colchicine analog." In Recent Innovations in biosustainability and environmental research, Department of Zoology, Annamalai University. (Oral presentation)
6. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. "Pharmacognostic, phytochemical and cytotoxic evaluation of fruits of *Memecylon* species" in XLII All India botanical conference of the Indian botanical society and national symposium on innovations and inventions in plant science research, Dept of Botany, University of Calicut. (Oral presentation)
7. **P. R. Ramya Sree.,** & Thoppil J. E. (**2020)**. "Pharmacognostic, phytochemical and antiproliferative evaluation of fruits of *Memecylon umbellatum* Burm. f"**.** Current trends and advances in biological sciences (CTAB 2020). Post Graduate Department of Botany and Biotechnology, Bishop Moore College, Mavelikara. (Oral presentation).

# CHAPTER 5 DISCUSSION

## PHASE I- PHARMACOGNOSTIC PROFILING

Green technology and alternative eco-friendly products are a brand new thought to several people (Muller, 2017). The new lifestyle changes cause many perilous drawbacks, which opens a gateway for the search of new resolves. Thus nowadays the term “Green” becomes much popular. The major area under ‘Green’ consideration will be the medicinal field. Herbal medicines are a safe remedy for various human ailments because of it’s less side effects and low-cost treatments. So there is wide acceptance of the herbal medicinal system. The quality measurements of herbs are a challengeable stream, where the validations of herbs are more important prior to the usage. Adulterations become a curse in the herbal medicinal field, since they make quality and safety inconsistent. This will open a new approach to validate the quality assurance of herbs.

The collection of plant materials, authentification of specimens, analysis and formulation of drugs is the way to the discovery of the safer natural drugs. Here an attempt was done for the evaluation of pharmacognostic characters of the medicinally important genus *Memecylon*. Many systematic studies and new records are available on the genus *Memecylon*, but evaluations of micromorphological characters are trivial. The identification of *Memecylon* species becomes difficult due to the intraspecies morphological similarities. So the identification of species becomes much strenuous. The surface morphology of seeds or fruits, pharmacognostic evaluation and phytochemical analysis are the effective methods to rectify the taxonomic difficulties in the authentification process and it opens a platform

for the pharmaceutical analyses. Scanning electron microscopic analysis is the best way to analyze the surface features of the samples. The applications of SEM in vegetative and reproductive organs have great importance and impact on the systematic studies (Özcan, 2004). The functional purity of the plant sample is essential for the pharmaceutical trials. In the present study, purity of the sample was analyzed through the powder microscopy, SEM-EDX and ICPMS techniques.

Pharmacognosy is considered as a science of natural products. The term "natural product" may be applicable to the organism itself (plant, animal and microorganism) or any part of an organism (a leaf or flower of a plant, an isolated gland or other organ of an animal), and extract or pure substances (Orhan, 2014). It plays a pivotal role in drug preparation and therapies. Recently drug discovery from medicinal plants involves multifaceted approaches, combining botanical, computational, phytochemical, biological and molecular techniques. There are several examples of plant based drugs that are known to be indigenous to the medicinal system. Vincristine, vinblastine, morphine *etc.*, are few of them. The functional identity of the plant specimens that are targeted for the drug preparation should be analyzed. It is important to the specific bioactivity of the plant specimen. Nowadays, emphasis and focus of research in pharmacognosy have changed significantly, from focusing on identification of drugs, including the isolation of active principles, and more recently, the investigation of biological activity. Research into ethnobotany, ethnomedicine and ethnopharmacology has also become an important part of pharmacognosy (Sarker, 2012). While analyzing the application of pharmacognosy, it plays a crucial role in the identification of allied species or adulterants. The replacement of a drug with an allied species due to the unavailability of a particular crude drug on that particular season or its scarcity will critically abolish the bioactivity of the drug. It will recall unwanted adverse effects of crude drugs. The substitution of medicinal

plants with allied plants starts with the wrong identification of the plant specimen. The common vernacular name given to the different species will be misidentified by the people, which contribute to the chance of adulteration (Kumar, 2007). Unlike taxonomical identification, pharmacognostic studies offer the identification of powdered sample. In the powdered form of a sample, the morphological identity becomes lost and is easily prone to adulteration. At that time pharmacognostic techniques plays a key role. Adulteration and substitution are burning problems in herbal industry. So validation of functional purity of herbal medicine is very important.

There are several techniques employed in pharmaceutical field for the validation of herbal drugs. The validation of herbals may ensure the production of drugs with reproducible quality. The process validation is defined as "the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product" (FDA, 1987). The important standardization parameters used in pharmacognostic field includes organoleptic characters, macro and microscopic study, physico-chemical assays, phytochemical analysis, powder study and fluorescence analysis. Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity of plant specimen. The macroscopic study clearly emphasize on morphological identification and microscopic analysis, with the aid of a microscope. These are two common practices in pharmacognostic analysis. The powder microscopic analysis gave the characteristic features of powdered samples under microscopic evaluation. The parameters like moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values *etc*., are evaluated in physico-chemical analysis. Some constituents are visible only in fluorescent range in day light. So fluorescence analysis is also a vital technique in pharmacognostic study (Chanda, 2014).

Phytochemical analysis is an important part of pharmacological trails. Sometimes, it is considered as a separate branch and involves the metabolite profiling of plant samples. These techniques are validating the plant identity and standardization parameters for natural drug preparation.

The taxonomic profiling of *Memecylon* species are widely under consideration by many researchers. They are mainly focused on the morphological features of the plants. Macroscopic study handled by the taxonomic researchers incessantly discovering new species to our plant kingdom. It will always open the exploration of medicinally important species. The present work gave special emphasis on microscopic study of the selected *Memecylon* species. The phytochemical analyses are conducted as a separate section and included in the phase II studies. In microscopic study, powder analysis, scanning electron microscopy, energy dispersive X-ray analysis and ICP-MS analysis are carried out to reveal the pharmacognostic profile of the selected *Memecylon* species.

Powder microscopy acts as a diagnostic tool for the proper authentication of plant material. Several reports are available on the powder analysis of medicinally important plants (Najafi & Deokule, 2010; Kadam et al., 2012). In Ayurveda, 90% of the preparations are plant-based and hence the worthwhile usage of herbal medicines are promising candidates as the remedies of various human ailments. In most of the Ayurvedic preparations, the powdered samples of plant parts are used. So the authenticity of the powdered sample is very important. Powder microscopy is a simple and easiest method to analyze the powder sample and it is an essential step in the pharmacognostic evaluation of the plant sample. Microscopic techniques examine the structural and cellular features of herbs to determine their botanical origin. Microscopic evaluation is now an indispensable tool for the

identification of medicinal herbs and is one of the important parameters in modern science (Padmavathy et al., 2010a).

Here the powder samples of *Memecylon* leaves were characterized through their microscopic characters. It is light green coloured and odourless. Long trichosclereids, epidermal cells with tannin contents, paracytic stomata, thick walled fiber bundles, cluster crystals of calcium oxalate *etc*., are noticed and represented in **Plate 2**. The powder sample of *M. grande* fruits was brown coloured, odourless and slightly astringent (**Plate 3**). The characters found in the powders are epicarp cells, parenchyma cells with starch grains from mesocarp, stone cells from mesocarp, sclereids from endocarp, vessels with spiral and annular thickenings and rosette crystals. In the case of *M. randerianum* leaves powder sample is light green coloured, showing vessels with spiral and reticulate thickenings, fibre bundles, rosette crystals *etc*., (**Plate 4**). The fruits of *M. randerianum* are brown coloured, odourless with a characteristic taste (**plate 5**). It contains epicarp cells, stone cells, sclereids from endocarp, tracheids, fiber bundles, rosette crystals *etc*. The same type brown colored powder was also obtained in *M. umbellatum* fruits (**Plate 7**). The powder showed characters like epicarp cells, pitted parenchyma cells from mesocarp, stone cells, sclereids, spiral vessels, fibro-sclereids and rosette crystals. Leaves of *M. umbellatum* possess trichosclereids, mesophyll cells, parenchyma cells, vessels with reticulate and pitted thickenings, fibro-sclereids and rosette crystals (**Plate 6**).

These characters can be used to identify the plant specimen in Ayurvedic preparations. So we can easily identify the botanical origin of the plant specimen and clearly distinguish the presence of adulterants or the allied species. The microscopic evaluation of *M. umbellatum* leaves was done by Killedar et al. (2014b) and found the presence of lignified xylem with well-defined xylem fibers, vessels, and parenchyma. The presence of phloecentric

vascular bundles surrounded by endodermis and crystal sheath was also reported. *Memecylon* is an unexploited genus in pharmacognostic field. Only limited reports of literature are available on Melastomataceae family and *Memecylon* genus in the pharmacognosy field. Padmavathy et al. (2010a) evaluated the pharmacological profile of leaves and young stem of *M. umbellatum*. They analyzed parameters include macro-morphology, micromorphology, quantitative microscopy, physicochemical profile, powder analysis and fluorescence analysis. Dorababu et al. (2013) also established standards for *M. edule* leaves extract through the pharmacognostic study. The powder microscopic analysis confirms that the botanical origin of the plant samples is pure and devoid of foreign particles. So this result can be used as a standard reference for the identification of *Memecylon* fruits in future.

Scanning electron microscopy is a method for high resolution surface imaging using an electron beam having greater magnification and much larger depth of field. The fruit endocarp micromorphology and the entire seed morphology were studied by using a scanning electron microscope. The difference in electron emission in different areas provides the surface topography of the material. In this study, all the selected species show distinct morphological patterns. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships. In the case of *M. grande,* fruits show colliculate pattern in endocarp and the seed surface possesses tuberculate pattern (**Plate 8 a1-a4**). Scanning electron microscopic technique reveals that the fruit endocarp of *M. randerianum* has a pattern of ruminate reticulate type (**Plate 8 b1-b4**). The seed surface of *M. randerianum* is with a reticulate pattern. *M. umbellatum* fruit endocarp possesses a smoothened pattern and its seed surface shows a wrinkled pattern (**Plate 8 c1-c4**). The characteristic surface morphology becomes a useful tool in the identification process. Scanning electron microscopic analysis is a valuable tool in surface morphology analysis. The present study is a novel

report on the surface features of *Memecylon* species. The comparative seed morphology and pharmacognostic features of *Memecylon* species was initially reported by Ramya Sree and Thoppil (2020). In Melastomataceae, several reports are pointing towards the importance of SEM analysis in species delimitation. The seed morphology of 234 species distributed among 16 genera of the tribe Miconieae (Melastomataceae) was examined and documented with the use of scanning electron microscopy (Ocampo & Almeda, 2013). They had proposed a set of 37 morphological characters for describing size, general shape, raphal zone, appendage, testa characters and individual cell features of Miconieae seeds. In some cases, seed morphology corresponds with natural groups of species, which is of high phylogenetic importance (Martin & Michelangeli, 2009). The cladistic analysis of *Tococa* (Melastomataceae) was investigated using morphological data through SEM analysis (Michelangeli, 2000). Hence from the present study, the fruit endocarp and seed surface micromorphological and topographical data of *Memecylon* spp. obtained may prove to be distinguishing micromorphometrical markers for the identification of *Memecylon* spp.

Energy dispersive X-ray microanalysis (EDX) is a technique for analyzing elemental compositions at the microscopic level. For this purpose, scanning electron microscope (SEM) is equipped with an energy dispersive system having a quantitative electron probe for X-ray microanalysis. The SEM-EDX system can be applied to the surfaces of untreated specimens and, thus provides a vivid picture of elemental distribution in plant and animal material (Chen et al., 2014). Nowadays, it is used for the identification of single microbial cells exhibiting pathological conditions without following time-consuming microbiological cultivation methods (Khan et al., 2020). EDX analysis is a powerful tool in biomedical research and diagnosis of samples is well explained by Scimeca et al. (2018). The data generated by

EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed.

The present study is really focused on the elemental profiling of the selected *Memecylon* species. The elemental composition of *M. grande* fruits show that nitrogen content is maximum showing 91% and other elements are as follows; phosphorus 3.10%, potassium 1.53%, iron 1.41%, magnesium 0.87%, cobalt 0.63%, sodium 0.55%, copper 0.48%, calcium 0.35% and zinc 0.11% (**Figure 5 a**). In the case of *M. randerianum* fruit, nitrogen is the prominent element with 93% of the weight. Phosphorus 4.01%, potassium 1.15%, cobalt 0.90%, copper 0.49%, magnesium 0.22%, zinc 0.09%, calcium 0.09% and sodium 0.03% are the revealed composition of other elements (**Figure 5 b**). *M. umbellatum* fruit also possesses an elevated amount of nitrogen (93%) and all other elements in trace amounts like phosphorus 3.4%, potassium 1%, copper 0.95%, magnesium 0.67%, cobalt 0.34%, iron 0.22%, calcium 0.22%, zinc 0.11% and sodium 0.02% (**Figure 5 c**). This finding proves that *Memecylon* fruits are a reservoir of essential elements and it can be exploited in the pharmaceutical or nutritional field.

In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental composition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis was carried out. This technique gave the details of elements present in the sample in part per million units and determination of twelve elements were done *ie.,* aluminum (Al), arsenic (As), cobalt (Co), strontium (Sr), selenium (Se), zinc (Zn), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), barium (Ba) and manganese (Mn) **(Table 5)**. The standard reference concentrations of trace elements present in the adult human blood samples are noticed by Prashanth et al. (2015), because it is essential for the standardization of drugs**.** Most of the detected elements show vital biological functions**.** Some elements are the part of vitamins,

cofactors of enzymes, oxidative phosphorylation, fatty acids and cholesterol metabolism. It has been found that chromium causes significant increase in enzyme activity and serves as a stimulator in fatty acid and cholesterol biosynthesis from acetate molecule in the liver. It can also enhance sugar metabolism through the activation of insulin (Anderson, 1997). In the case of cobalt, it is the key factor of cobalamin (vitamin B12) and it has a role in the formation of amino acids and neurotransmitters. Although the biological function of nickel is still somewhat unclear in the human body, however nickel is found in higher concentration in the nucleic acids, particularly RNA and is thought to be involved in protein structure or function (Al-Fartusie & Mohssan, 2017). So the biological role of these trace elements is significant in regulating homeostasis and is vital for the prevention of free radical damage and various human ailments (WHO, 1973).

During the past decades, human beings are concerned about the nutritional status of the body. People are aware about the profound effect of micro and macronutrients on biological processes that range from whole-organism performance to the cellular function. According to the classification of trace elements, the group I which include carbon, hydrogen, oxygen, and nitrogen are the basic components of macromolecules such as carbohydrates, proteins, and lipids. Group II category includes nutritionally important minerals such as sodium, potassium, chloride, calcium, phosphorous, magnesium and sulfur. In group III, some essential trace elements like copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium are found (Prashanth et al., 2015). Copper plays an important role in the metabolism, mainly in the proper functioning of the enzymes and its deficiency may cause hypochromic anemia, joint pain neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility and osteoporosis (Campbell, 2001). Another most important element is iron, which is a prime portion of the blood cells and its deficiency is called anemia. Anemia is the

second most important cause of maternal mortality in India and 20% of mortality is directly related to anemia and another 50% is associated with other anemic side effects. In the case of zinc, it is essential for normal spermatogenesis and maturation, proper development of thymus, proper epithelialization in wound healing, taste sensation, and secretion of pancreatic and gastric enzymes (Watson, 1998).

Nickel is the cofactor of various enzyme catalyzed reactions. Nitrogenase enzyme, the key regulator of nitrogen assimilation in plants is catalyzed by nickel (Dmytryk et al., 2018). Nickel deficiency cause reduced growth rate and iron absorption rate in oraganisms. (Kumar & Trivedi, 2016). Molybdenum is an essential element for human body. It will help to neutralize sulfites in human body. Sulfites can induce allergic reactions and skin problems. Molybdenum catalyzes four major enzymes namely sulfite oxidase, xanthine oxidase, aldehyde oxidase, and mitochondrial amidoxime-reducing component (Novotny, 2011). The element strontium is closely related to calcium and they perform similar function, *ie.*, in bone formation (Specht et al., 2017). It can increase the bone density and used as a medicinal component in osteoporosis treatment (Kołodziejska et al., 2021). Manganese is an essential element in human body. It plays vital role in prevention of metabolic disorders and are known to be good free radical scavengers. It also reins the glucose and lipid metabolism in human body (Li & Yang, 2018). Melastomataceae members are known to be Al accumulators. The biological role of aluminium is closely related to tolerance capacity of the plant species. In the present study, the selected *Memecylon* species are good Al accumulators except *M. umbellatum* leaves extract. In *M. malabathricum* it has been suggested that Al is essential for its growth and the absence of the metal causes several morphological changes and chlorosis (Watanabe et al., 2006).

The element arsenic is known to have several therapeutic uses. The continuous exposure of arsenic on human body may lead to the development of many severe conditions. However, arsenic compounds are reported to be antitumor agents (Platanias, 2009). Selenium is a trace element found in human body that has anti- inflammatory, immune response and antioxidant effects. A new report on the beneficial effect of selenium was coined by Liu et al. (2021). It is effective in preventing RNA virus multiplication. The ICP-MS analysis thus proves that the selected *Memecylon* species are rich in bioactive elements needed for the human body. In trace quantity, all of them are essential for living organisms. The action of trace elements in the living system always depends upon the concentration of the elements (Mikulewicz et al., 2017).

Pharmacognostic profiling of selected *Memecylon* species gave a vivid picture of their powder characters, seed surface features and elemental composition. It can be considered as a valuable tool for identification of the *Memecylon* species. Powdered plant sample are the main raw material in the pharmaceutical preparations. So characterization of powdered sample is an antecedent important step in herbal drug preparation. Thus the present study thus contributes some pharmacognostic reference standards for the identification of *Memecylon* species in herbal medicinal system.

## PHASE II- PHYTOCHEMICAL CHARACTERIZATION

Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of several renewed systems of indigenous medicines such as Siddha, Ayurveda and Unani. Traditional systems of medicine rely on a single plant or combinations of more than one plant. Their efficacies depend upon the current knowledge about taxonomic features of plant species, plant parts and

biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites.

**a) Preliminary qualitative and quantitative phytochemical analysis**

Phytochemicals are naturally occurring bioactive chemicals found in plants. They possess various bioactivities, provide protection against diseases and damages, improve health conditions *etc*. Plants synthesize a wide range of chemical compounds which are classified on the basis of their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites are directly involved in growth and development of an organism. Examples are chlorophyll, amino acids, nucleotides, carbohydrates *etc*., which have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. The most important secondary metabolites are alkaloids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides (Geetha & Geetha, 2014). They are involved in the defensive mechanism of the organisms. In the present study various phytochemical constituents were identified in the selected *Memecylon* species through preliminary qualitative and quantitative phytochemical analysis, GC/MS and HR-LC/MS analysis.

Preliminary phytochemical analysis is the prime important step in phytochemistry. The methanolic extract of selected *Memecylon* species shows the presence of secondary metabolites like alkaloids, flavonoids, phenolics, steroids and tannins in all the extracts. The complete absence of resins was also observed (**Table 6**). In quantitative phytochemical analysis, considerable amount of major secondary metabolites like alkaloids, flavonoids, phenolics and terpenoids are noticed (**Table 7**). Phytochemicals are the basis of specific bioactivity of the plant species. The majority of natural products have certain biological properties, and they are used as medicines, insecticides, herbicides and perfumes or dye. For the last two to three decades, there has been a surge

of interest in plant foods as a source of phytochemicals, which could be beneficial in the prevention of chronic diseases like cancer, diabetes, heart disease, cataracts and gallstones (Rao, 2003). The secondary metabolites like alkaloids, terpenoids, phenolics *etc*., are the potential biochemicals and they have peculiar biological role that has been already reported. The biosynthesis of secondary metabolites is usually restricted to specific stages of plant development and during periods of stress. Some plant cells produce important secondary metabolites upon environment interactions or some are related to the reproductive mechanism of the plant (Mendoza & Silva, 2018). While considering the selected *Memecylon* species, considerable amount of phytochemicals are noticed and the bioactivity of the plant extracts depend on them. Basha et al. (2011) identified the presence of phytochemicals and reported its antimicrobial activity from aerial parts of *M. umbellatum*. Several previous phytochemical reports are validating the same result. *ie., Memecylon* species are a rich source of bioactive phytochemicals (Sivu et al., 2013; Murugesan et al., 2011). In this study, the biological activities of major secondary metabolites of three species of *Memecylon* are analyzed.

All over the World, several research groups have confirmed the vital role played by phytochemicals in reducing the risk of several diseases such as cancer and inflammatory conditions (Thangapazham et al., 2016). The usage of phytochemicals for the effective treatment of cancer was already reported (Kotecha et al., 2016; Liu et al., 2014). The protective mechanism of phytochemicals in stroke was reported by Kim and Karadeniz (2012). Many phytochemical compounds with anti-inflammatory, antioxidant and apoptotic effects have been widely studied (Feng et al., 2019; Husain et al., 2018; Son et al., 2008). The progressive usage of phytochemicals through diets as an effective method to cure the diseases was widely analyzed (Tan & Nishida, 2012). All these reports are pointing to the efficacy of the phytochemicals and the importance of their validation.

Alkaloids are natural compounds that show significant biological effects on animal models and also in human beings in very small doses. Atropine, morphine, quinine and vincristine are some of the important alkaloids used to treat a wide range of disease conditions from malaria to cancer. Alkaloids from many different plant species have other useful applications such as antiparasitic, antiplasmodial, anticorrosive, antioxidative, antibacterial, anti-HIV, and insecticidal activities (Kurek, 2019). In the present study, among the selected six extracts from three plants, *M. grande* fruit extract shows the highest amount of alkaloids, phenolics and terpenoids and *M. randerianum* fruit extract exhibits the lowest amount of alkaloids (32.17 ± 1.41 mg CE/g DW). The cytotoxic activity of alkaloids was noticed by Mat et al. (2000). The alkaloids isolated from *Brunsvigia radulosa* was tested against two strains of cultured *Plasmodium falciparum* and it's cytotoxicity was tested against BL6 mouse melanoma cells and shows a promising result (Campbell et al., 2000). In total flavonoid determination, *M. grande* leaf extract shows the highest content (215.96 ± 1.87 mg QE/g DW). Flavonoids are the largest group of phytoconstituents with more than 6000 varieties. They are classified into flavones, flavanones, isoflavones, catechins, and anthocyanins. The presence of flavonoids is responsible for the vivid colours in fruits and vegetables. It has many potential pharmacological roles *ie.,* antihepatotoxic, antiulcer and anti-inflammatory effects (Bors et al., 1990). Flavonoids also act as a secondary antioxidant defense system in plant tissues that are exposed to different abiotic and biotic stresses. Flavonoids are located in the nucleus of mesophyll cells and within the centers of ROS (Agati et al., 2012). The pharmacological and biochemical effects of flavonoids inhibit the action of various enzymes like cycloxygenase, phosphodiesterase, lipoxygenase *etc.* and also the hormones like androgens, estrogens and thyroids (Agrawal, 2011). Some of the flavonols like quercetin are found to inhibit cell growth of leukemia cells and EAC cells (Sorata et al.,

1984). Yoshida et al. (1990) has studied the effect of quercetin on cell cycle progression in human gastric cells. The flavonoid uptake has a direct action on the cell cycle of human colon adenocarcinoma cells, which was reported by Salucci et al. (2002).

Phenolic compounds are the main class of secondary metabolites in plants and are divided into phenolic acids and polyphenols. Many studies have shown a strong and positive correlation ($p \leq 0.05$) between the phenolic compound contents and the antioxidant potential of fruits and vegetables (Pinhero et al., 2016). In the present study, 370.28 ± 1.36 mg GAE/g DW phenolic content was noticed in *M. grande* fruit extracts. Similarly *M. randerianum* fruit extract possess 276.06 ± 1.12 mg GAE/g DW and *M. umbellatum* fruit extract with 60.83 ± 5.70 mg GAE/g DW as the phenolic content. The lowest amount of phenolic content was observed in *M. randerianum* leaf extract with 49.52 ± 4.72 mg GAE/g DW. Phenolics are considered as good antioxidant agents, because phenolic hydroxyl groups are good hydrogen donors. Hydrogen donating antioxidants can react with reactive oxygen and reactive nitrogen species (Pereira et al., 2009). In glioma cancer, cell proliferation can be effectively inhibited by the action of phenolic compounds (Lu et al., 2010). The wide utility of phenolic compounds was described by Olthof et al. (2001). The antibacterial property of phenolics was reported by Lou et al. (2012).

There are many reports regarding the bioactive terpenoids of plant origin (Macias et al., 2002; Li et al., 2009). Plant-based terpenoids have been used by humans in the food, pharmaceutical and chemical industries. Recently it has been exploited in the development of biofuel products (Tholl, 2015). Terpenoids have multifunctions such as the suppression of tumor proliferation, apoptosis inducing capacity and act as cation regulating channel (He et al., 1997; Roullet et al., 1997). In the preliminary quantitative

phytochemical analysis, terpenoids is the leading secondary metabolite in all the selected six plant extracts. It ranges from 127.5 ± 1.50 to 378.21 ± 1.02 mg LE/g DW. Terpenoids are used as potential chemopreventive and therapeutic agents in liver cancer treatment (Thoppil & Bishayee, 2011). Terpenes have anti-inflammatory effects by inhibiting various proinflammatory pathways in ear edema, chronic obstructive pulmonary disease, skin inflammation, and osteoarthritis (Rufino et al., 2014; Yu et al., 2016).

The presence of tannin was noticed in all selected plant samples. Tannins are a group of polyphenols. The effects of polyphenols in plants are reported by Zdunczyk et al. (2002). The antimicrobial activities of tannins are well studied. Tannins serve as a natural defense mechanism against fungi, yeasts, bacteria and viruses. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf life of certain foods, such as catfish fillets. Tannins have certain physiological effects, such as to accelerate blood clotting, decrease the serum lipid level, reduce blood pressure, produce liver necrosis and modulate immune responses. All these effects were dependent on the dosage and kind of tannins (Chung et al., 1998). A phlobaphene condensed tannins called as phlobatannins were noticed in *M. randerianum* leaf and fruit extracts and in *M. umbellatum* leaf extract. They have been reported to possess wound healing, anti-inflammatory, antioxidant and analgesic activities (Ayinde et al., 2007; Kumari & Jain, 2015). Coumarins are another group of polyphenols, which have a significant effect on physiological, bacteriostatic and antitumor activity (Rohini & Srikumar, 2014). Coumarins were noticed in the leaves extract of *M. randerianum* and *M. umbellatum*. Coumarins have potent anticancer activity *ie.,* it can be used against prostate cancer, renal cell carcinoma and leukemia (Finn et al., 2002). Glycosides are naturally occurring compounds with a wide range of medicinal and clinical applications. Both *M. grande* leaf and *M. umbellatum* fruit as well

as leaf extract show positive results in Keller Killiani test. Keller Killiani test is a method for determining cardiac glycosides. The anticancer property of glycosides against several cancer cell lines is reported by Khan et al. (2019). The potential biological roles of glycosides were discussed by various researchers, validating their significance (Kren & Martínková, 2001; Kren & Řezanka, 2008; La Ferla et al., 2011).

Saponins and steroids are the other phytochemicals observed during preliminary analysis. The presences of saponins are identified in all plant extracts except *M. umbellatum* fruit extract. Saponins are a class of substances with a rigid skeleton of at least four hydrocarbon rings to which sugars in groups of one or two are attached. Saponins have been proposed for the treatment of a variety of diseases, including diabetes, obesity and osteoporosis (Marrelli et al., 2016). It has anticancer properties through inhibiting cell proliferation, to counteract angiogenesis and to stimulate apoptosis (Kregiel et al., 2017). The plant steroids are known to be potential anti-inflammatory agents (Patel & Savjani, 2015). Phytosterols possess antioxidant activity, anti-inflammatory activity and anticancer activity against lungs, stomach, ovary and estrogen-dependent human breast cancer (Jain et al., 2019). The preliminary phytochemical analysis gave an insight on the secondary metabolite profile of selected *Memecylon* species. The biological role of these potential phytoconstituents leads to a detailed exploration to reveal the bioefficacy of *Memecylon* species. In the next section, a detailed phytochemical profile of *Memecylon* species is discussed.

**b) Phytochemical profiling by GC/MS**

One of the major criteria for the phytochemical validation is the characterization of bioactive compounds. Preliminary qualitative and quantitative analysis gave an insight on the occurrence of the phytoconstituents. Various preliminary phytochemical analyses confirm the

presence of these metabolites (Soumya et al., 2015). Gas chromatographic and liquid chromatographic assays are the common methods followed for identifying the biocomponents present in the sample. Gas chromatography-mass spectrometry is an analytical tool for the quantification of volatile compounds present in the plant extract. A total of 84 compounds were identified in the six samples of selected *Memecylon* species [**Table 8, Figures 13 (i-xvii**].

Fatty acid esters are the predominant group of compounds detected in *M. grande* methanolic extract. 9-Hexadecenoic acid methyl ester, propyl palmitate, methyl oleate, 10,13-octadecadienoic acid methyl ester, methyl 9-cis,11-trans-octadecadienoate, propyl oleate and methyl linoleate are detected in the GC/MS analysis of *M. grande* leaf extract. Fatty acid esters are used as bioadhesive agent and it has potential antioxidant activity (Hansen et al., 2001; Matsufuji et al., 1998). The antibacterial activity of poly unsaturated fatty acids and their ester derivatives against various oral pathogens like *Candida albicans*, *Streptococcus mutans* and *Porphyromonas gingivalis* was reported by Huang and Ebersole (2010). Fatty acid esters like methyl arachidate, methyl linoleate, methyl stearate and methyl myristate are common in the leaf and fruit extracts of *M. grande*. The fatty acid ester profile of *M. grande* fruit includes methyl octanoate, methyl pentadecanoate, ethyl 9-hexadecenoate, methyl elaidate and methyl palmitate. Fatty acid esters possess free radical scavenging activity and antiproliferative activity against human ductal breast epithelial tumor T47D, human breast adenocarcinoma MCF-7, human epithelial carcinoma HeLa, human epithelial colorectal adenocarcinoma Caco-2, human colorectal adenocarcinoma cell line HRT and human kidney carcinoma cell line A498 (Elagbar et al., 2016). The phytochemical reports from various *Memecylon* species are again validating these fatty acid profiles (Bharathi et al., 2017b; Uppu et al., 2018).

Palmitic acid, oleic acid and linoleic acid are found to be common in both leaf and fruit extracts. From the root extract of *M. umbellatum*, palmitic acid was isolated by Joshi et al. (2009b). Palmitic acid is a common fatty acid found in all selected plant extracts except *M. umbellatum* fruit extract. Fatty acids play an important role in cellular biological functions. The elevated fatty acid concentration can inhibit T-lymphocyte signaling and induce pancreatic B-cell apoptosis (Stulnig et al., 2000). Oleic acid (29.01%) is the prominent fatty acid noticed in *M. grande* leaf extract. Oleic acid is more steatogenic but less apoptotic than palmitic acid in hepatocyte cell cultures (Ricchi et al., 2009). Linoleic acid is known to be an anticancer agent. It reduces the risk of cancer in mice models as compared to control mice system (Ha et al., 1987). Numerous physiological activities were attributed to the conjugated linoleic acid. The trans-10, cis-12 isomers of linoleic acid inhibits lipoprotein lipase and stearoyl-coA desaturase, thereby reducing the uptake of lipids. The isomers like cis-9, cis-12, trans-11 and trans-10 conjugated linoleic acids are active in inhibiting carcinogenesis in animal models (Pariza et al., 2001).

Cholesterilene and campesterol acetate are steroid compounds detected in the leaves and fruit extracts of *M. grande*. Cholesterilene was found to exhibit wound healing activity, which was reported by Badiu et al. (2008). Antiangiogenesis activity of campesterol from *Chrysanthemum coronarium* was described by Choi et al. (2007). Oleamide is an organic compound found to be common in both extracts of *M. grande*, more over it was found to be the highest fraction of fruit extracts (31.27%). It is a fatty acid amide that can activate G-protein coupled, and other receptors to regulate a diversity of cellular and physiological functions throughout the body, including the reproductive, immune, nervous and cardiovascular systems (Hiley & Hoi, 2007). The antiepileptic and nephro-protective effect of oleamide was reported by Nam et al. (2017). Z, Z-6, 28-Heptatriacontadien-2-one is a ketone found in *M. grande* fruit extract. α-Amylase inhibition and antioxidant

activity of some marine algae was found to be due to Z, Z-6, 28-Heptatriacontadien-2-one (Unnikrishnan et al., 2015). This compound also contributes to the anti-inflammatory and larvicidal effect of the plant samples (Anupama et al., 2014; Pratheeba et al., 2015).

Stigmast-5-en-3-ol is observed in *M. grande* fruit extract and α-phytosterol is specific for *M. grande* leaf extract. These are sterol compounds identified in *M. grande*. The apoptotic and antiproliferative effects of stigmast-5-en-3-ol on human leukemia HL-60 and human breast cancer MCF-7 cells was reported by Fernando et al. (2018). It induces apoptosis mechanism through mitochondria mediated pathway. The insulin-like effect of stigmast-5-en-3-ol in stimulating glucose transport *in vitro* reveals the potential antidiabetic activity apart from its existing cholesterol lowering efficacy (Sujatha et al., 2010). The lowering of low density lipoprotein cholesterol is effective in reducing metabolic syndromes. It is also associated with increased cardiovascular disease. Phytosterol is effective in reducing cardio vascular diseases (Jones et al., 2000; Lerman et al., 2010). Phytosterol is an excellent candidate for cancer chemo-prevention, such as prostate cancer. The phytosterol intake was associated with a reduction in risk of 50% lung cancer that was reported in a case study in Uruguay (Shenouda et al., 2007; Mendilaharsu et al., 1998).

Among the selected *Memecylon* species, *M. randerianum* leaf extract possess highest amount of palmitic acid content (15.51%). The antitumor activity of palmitic acid was noted by Harada et al. (2002). Apoptosis induction ability of palmitic acid was analyzed through western blot analysis and it shows that it can down regulate apoptosis inhibitors like Bcl2 and up regulate apoptosis effecter, Bax. The other fatty acids noticed include lauric acid and octadecanoic acid. It was found to be 1.73 and 2.09% respectively. Lauric acid can modulate serum cholesterol levels and it is shown to be very

active against gram positive bacteria, a number of viruses and fungi. Lauric acid has the strongest antimicrobial activity among all saturated fatty acids (Dayrit, 2015). Octadecanoic acid can control inflammation reaction through the competitive inhibition of phospholipase A(2) (Aparna et al., 2012). *In vitro* studies of octadecanoic acid revealed that it is used as a pro-apoptotic signal for eliciting anti-inflammatory responses. Caspase-3 along with MMP2 and MMP9 affirms the anti-inflammatory properties. Molecular docking studies also show that octadecanoic acid has a strong binding affinity to MMP-2 (Manivannan et al., 2017).

Squalene is a natural dehydrotriterpenic hydrocarbon ($C_{30}H_{50}$) with six double bonds, an intermediate for the biosynthesis of phytosterol/cholesterol in plants/animals and humans, widespread in animal and plant kingdom. Anticancer activity and antioxidant potential of squalene was widely discussed (Huang et al., 2009). The interest in squalene was raised long ago, after the characterization of squalene in shark liver oil. Several studies exhibited that it has a wide spectrum of biological activities. Squalene was the third leading compound identified in *M. randerianum* leaf. It is also present in fruit extract of *M. randerianum* and leaf extract of *M. umbellatum*. Till date, anticancer, antioxidant, drug carrier, detoxifier, skin hydrating and emollient activities of squalene have been reported both in animal models and under *in vitro* environments (Kim & Karadeniz, 2012). Squalene is said to be a chemopreventive agent. The major activities underlying chemoprevention include inhibition of Ras farnesylation, modulation of carcinogen activities and antioxidant activity (Smith, 2000). Several epidemiological studies in breast, colon and pancreatic cancer shows that squalene uptake will diminish the risk of cancer and the tumor inhibiting role of squalene is a promising one (Newmark, 1997; Rao et al., 1998). Lupeol is a pentacyclic triterpene found in various species in the plant kingdom. This molecule exhibits a spectrum of pharmacological activities against various acute or chronic diseases, including

arthritis, renal disorders, diabetes, cancer, and microbial infections (Badshah et al., 2016; Alqahtani et al., 2013). The beneficial role of lupeol includes hepatoprotective, cardioprotective, anti-inflammatory and cancer chemo preventive activities, which was discussed by Patil (2018).

A diverse array of terpenoid compounds was detected in the GC/MS analysis. Agathenic acid and dihydroabietic acid are the diterpene compounds resulted through the analysis. Agathenic acid, a diterpenoid found in *M. randerianum* leaf extract, showed cytotoxic, antioxidant or antimicrobial activities alone or more often in synergism with other essential oil compounds. Labdane-type and abietane-type diterpenes have shown cytotoxicity against tumor cells and abietane-like compounds play an important role as antioxidants (Stanetic & Buchbauer, 2015). Antiulcer property of dehydroabietic acid was analyzed by Wada et al. (1985). Gastroprotective and cytotoxic effect of dehydroabietic acid derivatives was checked by Sepulveda et al. (2005).

Bicyclogermacrene (3.59%), γ-eudesmol (0.62%), farnesyl acetate (3.44%), ledol and virdiflorene are the sesquiterpenes observed in the leaf extract of *M. randerianum*. Antioxidant activity of bicyclogermacrene was reported by Yu et al. (2016). It also possesses potent cytotoxic activity (Grecco et al., 2015). The sesquiterpene, γ-eudesmol was mainly present in the wood oil of the plant species *Callitris collumellaris, C. intratropica*, eucalyptus oil, guava fruit oil *etc*. It exhibited potential cytotoxic activity against cancerous cells in liver by reducing the proliferation and causing the death of tumor cells by caspase-mediated apoptosis (Britto et al., 2012). Farnesyl acetate is a derivative of an isoprenoid compound of the Mevalonate pathway. It shows antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, E. faecium, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*. The cytotoxic activity was observed against

different cell lines that include malignant melanoma MeWo, colorectal adenocarcinoma HT29, promyelocytic leukemia HL60, gingival fibroblasts HFIG, skin keratinocytes HaCaT and rat small intestine epithelium IEC6 (Bonikowski et al., 2015). Larvicidal efficiency of virdiflorene was reported by Zhao et al. (2017) and Liu et al. (2014).

Phytol is a diterpene alcohol abundantly available in nature. The antinoceptive activity of phytol was effectively proven by Santos et al. (2013) and found that there is no change in motor functions of animals. The antinoceptive activity associated with antioxidant activity of phytol was also demonstrated by them. The antioxidant, apoptosis, antimicrobial, cytotoxic and anti-inflammatory effects of phytol prove that, it is a promising candidate in pharmaceutical field (Islam et al., 2018). The other terpene alcohols resulted in *M. randerianum* leaf extract by GC/MS analysis include maaliol (1.57%), β-eudesmol (4.58%) and α-cadinol (1.28%). Antinociceptive activity of maaliol was studied in *Valeriana wallichii* by Sah et al. (2012). It acts as an insect antifeedant component in *Senecio fistulosus* (Ruiz-Vásquez et al., 2019). β-Eudesmol inhibited angiogenesis in granuloma tissue in mice at 0.9 mol/kg (202 g/kg) (Tsuneki et al., 2005). Significant antihepatotoxic effects were exhibited by β-eudesmol against carbon tetrachloride-induced cytotoxicity in rat hepatocytes (Kiso et al., 1983). α-Cadinol was said to act as antifungal and hepatoprotective agent (Ho et al., 2011).

α-Angelica lactone is a five-membered unsaturated lactone, which is used as a flavoring agent and for fragrance. α-Angelica lactone is found in nature in almonds, coffee, raisins, cranberries, coconuts and soybeans. Tumor-inhibiting effect of α-angelica lactone was found by increasing the activity of the detoxifying enzyme glutathione-S-transferase (Nijhoff et al., 1993; Nijhoff et al., 1995; Van der Logt et al., 2003). The phenylpropene profile of *M. randerianum* leaf extract include chavicol, phenol, 2,4-bis(1-

phenylethyl) and methyl eugenol. Chavicol is also known as *p*-allylphenol. It is a natural phenylpropene, a type of organic compound. Chavicol is used as an odorant in perfumery. Phenol, 2,4-bis(1-phenylethyl), is a phenylpropanoid derivative. It's anti-inflammatory effect was studied by Chen et al. (2007). *In vitro* antimitotic, apoptotic and antiproliferative activity of this phenylpropanoid was examined in various studies (Melappa & Prakash, 2017; Muthulakshmi et al., 2012). Methyl eugenol is otherwise known as allylveratrol, which is a natural chemical compound classified as a phenylpropene, a type of phenylpropanoid. Methyl eugenol is found in a number of plants. The compound may have evolved in response to pathogens, as methyl eugenol and has some antifungal activity. It also repels many insects (Tan & Nishida, 2012). Eugenol is a phenolic compound, colorless to pale yellow and is an aromatic oily liquid extracted from clove, nutmeg, cinnamon, basil and bay leaf. Eugenol is found to be hepatotoxic (Thompson et al., 1998). It is also used as a local antiseptic and anaesthetic (Sell & Carlini, 1976). The recent scientific evidence supports that eugenol exerts beneficial effects on human health. The antimicrobial activity of eugenol is reported by Marchese et al. (2017). It also possesses anti-inflammatory activity through inhibited prostaglandin synthesis and reduced the tone of isolated gut muscle and myometrium in *in vivo* rat system (Bennett et al., 1988).

Friedlein is the prominent triterpene compound (31.3%) found in *M. randerianum* fruit extract. The antiviral efficacy of friedlein was tested against the NS3 helicase protein of hepatitis C virus. The computational screening method reveals that, it shows better drug-likeliness, activity and stability (Arumugam et al., 2013). The anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. was examined using *in vivo* models such as mouse and rat models. The tests like carrageenan-induced hind paw oedema, croton oil-induced ear oedema, acetic

acid-induced vascular permeability *etc.,* are employed to evaluate the inflammatory potential of the compound. Acetic acid-induced abdominal constriction response, formalin-induced paw licking response and the hot-plate test are the methods opted for the evaluation of analgesic effect of friedelin. The yeast-induced hyperthermia test in rats was the antipyretic evaluation method. All these test systems, evidently proved the potent anti-inflammatory, analgesic and antipyretic effects of friedelin (Antonisamy et al., 2011). The antimicrobial and cytotoxic effect of friedelin was noted by Mokoka et al. (2013). Squalene is another triterpenoid present in the plant extract with of *M. randerianum* fruit extract with 7.69%.

Stigmast-5-en-3-ol (12.8%) is the second lead compound in *M. randerianum* fruit extract. 4-Campestene-3-one and 4,22-stigmastadiene-3-one are the steroid components of *M. randerianum* fruit. Methyl palmitate and methyl stearate are the two fatty acid methyl ester compounds resulted in GC/MS analysis. Fatty acids like linoleic acid, octadecanoic acid and palmitic acid are also detected in the analysis. An organic compound quinic acid (0.67%) was also resulted through the analysis. The derivatives of quinic acid have antimicrobial and anti-inflammatory activity (Zanello et al., 2015; Zeng, 2010). Another organic compound noticed in the *M. randerianum* fruit extract is vitamin E. It possesses a wide range of bioactivities. β-Thujone and 3-thujanol are the monoterpenes detected in the fruit extract. The toxicity of thujone was extensively studied. It acts as a modulator of the $GABA_A$ receptor. Long term exposure lead to neurotoxicity (Pelkonen et al., 2013). The antifungal activity of β-thujone and α-thujone was tested against *Tiarosporella phaseolina*, *Fusarium moniliforme* and *Fusarium solani* (Farzaneh et al., 2006).

*M. umbellatum* leaves are a rich source of various bioactive metabolites. α-Phytosterol (17.72%) is the prominent compound detected

through GC/MS analysis. The cardio-protective and anti-inflammatory effects plant sterols in hyperlipidemic individuals were reported by Micallef and Garg (2009). The plant sterols commonly found in the diet are β-sitosterol, campesterol and stigmasterol. A clinical study indicates that the intake of phytosterols (2 g/day) is associated with a significant reduction (8-10%) in levels of low-density lipoprotein cholesterol (LDL-cholesterol) and lowering the risk of cardiovascular diseases (Cabral et al., 2017). Other important sterol compounds identified in the extract include campesterol and stigmasta- 5,22-dien-3-ol. As noted in *M. randerianum* leaf, campesterol is the bioactive component present in *M. umbellatum* leaf extract. The antimicrobial activity of stigmasta- 5,22-dien-3-ol was discussed by various researchers (Achika et al., 2016). 4,22-Stigmastadiene-3-one and 4-campestene-3-one is recognized as the steroid compounds in *M. umbellatum* leaf extract. 4,22-Stigmastadiene-3-one possess antimicrobial activity, which was identified by Singariya et al. (2013). 4-Campestene-3-one was identified in *Melia azedarach,* and it shows cytotoxic effects against several cancer cell lines such as, human colorectal carcinoma HT-29, breast cancer MCF-7, SK-BR-3 and kidney epithelial cell MDBK (Ervina, 2018).

Fatty acids like palmitic acid, octadecanoic acid and several fatty acid methyl esters such as methyl myristate, methyl palmitate, methyl heptadecanoate and methyl linoleate are also noticed in *Memecylon* spp. The fatty acids are known to be good antioxidant agents. They can reduce the cellular damages caused by oxidative stress. Oxidative stress can induce cell proliferation, cell division defects and cellular damages (Elagbar et al., 2016).

4-Vinylguaiacol and methyl 4-hydroxyphenyl acetate are the phenolic composition of the *M. umbellatum* leaf extract. 2-Methoxy-3-allylphenol is identified as a phenylpropanoid compound. Phenolic compounds have apoptosis inducing ability through the regulation of copper ion mobilization,

which can also interfere with chromatin during DNA fragmentation (Greenwell & Rahman, 2015). 4-Vinylguaiacol is used as a flavoring agent and it has good antioxidant property (Azadfar et al., 2015). It is a sinapic acid derivative and used as an antioxidant and antimutagenic agent, which suppresses carcinogenesis and the induction of inflammatory cytokines (Nićiforović & Abramovič, 2014). Methyl 4- hydroxyphenylacetic acid is known to give auxin-like effects in higher plants (Fries & Iwasaki, 1976). 2-Methoxy-3-allylphenol act as a cytotoxic, anti-inflammatory and antimicrobial agent (Loying et al., 2019). Curently, dietary phenolics have a great interest due to their antioxidative and possible anticarcinogenic activities.

α-Springene, neophytadiene and squalene are the revealed terpenes in *M. umbellatum* leaves. Neophytadiene is a good analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant compound (Raman et al., 2012). Squalene possesses a wide range of pharmacological activities that were already stated. Terpene alcohols like, phytol, 3,7,11,15-tetramethyl-2-hexadecen and isophytol that are revealed in GC/MS analysis. Hydroxymethylfurfural, levoglucosan, dihydroconiferyl alcohol and 1,1,10-trimethyl-6,9-epidioxydecalin are the organic compounds profiles identified in *M. umbellatum* leaf extract. Hydroxymethylfurfural (HMF) is a dehydration product of certain sugar moiety. It is considered as an important intermediate due to its rich chemistry and potential availability from carbohydrates sources. In recent years, considerable efforts have been made on the transformation of carbohydrate into HMF. Several biological activities of HMF is tested on *in vitro* and *in vivo* systems. The mutagenic effect has been assessed by the Ames test and found to be non-mutagenic in nature (Rosatella et al., 2011). Moreover, the presence of HMF protected the human liver cell line-LO2 against exposure to hydrogen peroxide, because it prevented nitric oxide production, caspase-3 activation and arrest of the cells

in the S-phase of the cell cycle (Ding et al., 2010). Levoglucosan is an active biocomponent in a variety of plant species such as *Aronia melanocarpa*, *Holigarna grahamii*, *Terminalia coriacea etc*. It has potent antioxidant and anti-diabetic properties. Dihydroconiferyl alcohol acts as a gibberellin synergist in hypocotyl elongation process (Shibata et al., 1975).

Vitamin E (α-Tocopherol) content in *M. umbellatum* leaf extract was found to be 5.73%. It is involved in the regulation of cellular signaling and gene expression. From the eight different forms of vitamin E, only α-tocopherol is retained in the body, because of the specific selection of *RRR*-α-tocopherol by the α-tocopherol transfer protein and their low rate of degradation and elimination was compared with the other vitamins. α-Tocopherol appears to be mostly involved in gene regulation (Brigelius-Flohé, 2006). The antioxidant activity of vitamin E is well known and it is a suitable candidate for adjuvant treatment of cancer (Valgimigli & Amorati, 2019; Constantinou et al., 2008). The anticancer activity of vitamin E against murine C6 glioma cells was reported by Mazzini et al. (2010). The anticancerous activity of vitamin E is executed through the antiangiogenesis process and it acts as a potent regulator of growth-factor-dependent signaling in endothelial cells (Miyazawa et al., 2009). Recently vitamin E based nanomedicines for oncological diseases have been reported and it was found to increase the tumor delivery of drugs and limiting the off-target uptake (Alavijeh & Akhbari, 2020).

2-Heptylacetate is a carboxylic acid ester, which possesses insecticidal activity (Nta et al., 2018; Nta & Oku, 2019). Isopentacosane and tetratetracontane are alkane group compounds detected in the GC/MS analysis. Methyl commate B is a triterpene glycoside only present in *M. umbellatum* leaf extract. It possesses antioxidant, antimutagenic and anticancerous activities (Gautam et al., 2020).

*M. umbellatum* fruit extract possess 12 bioactive compounds in which, 21.35% of the extract content was shared by an alkaloid, hordenine. It elicits plant defensive mechanism through jasmonate-dependent defense pathway (Ishiai et al., 2016). Pyruvate dehydrogenase kinase 3 (PDK3) inhibitors are an important target in lungs cancer therapy. Hordenine act as an inhibitor molecule of PDK3 through non-covalent interactions and induce conformational changes on them. It exhibits cytotoxic effects on lungs cancer cell lines with an admirable $IC_{50}$ value (Anwar et al., 2020). 2-Hydroxy-4-methyl pyrimidine and 4-methyl 2,5-dihydrofuran-2-one are the organic compounds noticed in *M. umbellatum* fruit extract. 2-Hydroxy-4-methyl pyrimidine possesses a wide range of activities. The pyrimidine compounds and its derivatives have a significant effect on microbes and most of them are antimicrobial agents. It also possess antioxidant, analgesic, anti-inflammatory and anticancerous activities (Sharma et al., 2014). 4-Methyl 2,5-dihydrofuran-2-one is a furan compound, extensively used in pharmaceutical field as a flavoring agent and it possesses insecticidal activity (Xia et al., 2011). Dehydromevalonic lactone is a building block of several natural compounds and can encompass antimicrobial property (Xavier et al., 2010). Methyl-3-methyl-5-oxooxolane-2-carboxylate has antifungal and antitumor activity which was reported by Guo et al. (2008). Lauric acid, myristic acid and octadecanoic acid form the fatty acid profile of *M. umbellatum* fruit extract. All these compounds have potent bioactivity. Myristic acid shows potent anti-inflammatory effect. The combined action of myristic acid and palmitic acid is effective against systemic candidiasis (Prasath et al., 2021).

Sundram et al. (1994) reveals that palmitic acid can reduce the cholesterol level than does a combination of lauric acid and myristic acid. GC/MS analysis describes the presence of lauric acid and myristic acid in *M. umbellatum* fruit extract. So it can be effectively used in lowering cholesterol level. These two fatty acid combinations have vital potential in preventing

prostatic hyperplasia (Babu et al., 2010). Two terpene compounds noticed in the GC/MS analysis are Mustakone and cyperenone. Mustakone isolated from *Cyperus articulatus* shows anti-plasmodial property, particularly active against *Plasmodium falciparum* (Rukunga et al., 2008). The antioxidant, antimicrobial and anticancer potential of mustakone was noticed by Gribner et al. (2020). Cyperenone is a sesquiterpene ketone, which shows cytotoxic and anti-inflammatory effects. It is also neuroprotective in function (Al-Snafi, 2016). Methyl myristate is a fatty acid methyl ester having antioxidant, cytotoxic and antifungal potential. It shows cytotoxic activity on human tumor cell lines like MCF-7, A549, CNE *etc*. (Su et al., 2013). Most of the fatty acid methyl esters are bioactive agents. They have potential antifungal and antioxidant activity reported by Pinto et al. (2017). While comparing the commercial antioxidants, fatty acid esters show better scavenging potential. It is active against 18 fungal strains, which are clinically important ones. Linoleoyl chloride, is a fatty acid derivative reported in *M. umbellatum* fruit extract. In *Kaempferia galanga* leaf extract, linoleoyl chloride is the major component. It is used as a medicine because it encompasses antinociceptive and anti-inflammatory potential (Bhuiyan et al., 2008).

GC/MS analysis of selected *Memecylon* species evidently proves that they are an affluent source of many bioactive phytochemicals. The above discussion substantiates the role of wide spectrum of bioactive phytochemicals. A total of 83 compounds were detected in the methanolic extract of selected species. The identified compounds belong to the classes of terpenoids, phenolics, fatty acids, fatty acid esters, steroids *etc*. *M. grande* fruit and leaf extracts were immensely rich in fatty acids and its esters. Fatty acids and its esters are usually associated with the oil containing plants, but here fatty acids and esters were seen in the methanolic extracts of all selected *Memecylon* species. *M. randerianum* extracts also possess significant amount of fatty acids, additionally. Moreover, it is rich in terpenoid compounds. In

the case of *M. umbellatum* extracts, steroids, alkaloids and fatty acids are the major phytoconstituents. A diverse array of phytoconstituents was present in the selected six plant extracts. Some organic compounds and trace compounds are also enlighten the bioactive potential of extracts. The presence of an immense array of compounds identified through the GC/MS analysis justifies the use of these plant extracts as herbal medicine.

**c) Phytochemical profiling by HR-LC/MS**

Non-volatile compositions of selected *Memecylon* species were revealed through the HR-LC/MS analysis. LC/MS analysis is a widely used technique in proteomics and metabolomics. It allows the broad screening of biomolecules. Liquid chromatography and mass spectrometry analysis enables the relative quantification of large amount of individual compounds from multiple samples (Katajamaa & Orešič, 2005). Electrospray ionization (ESI) is a most commonly used technique in LC/MS analysis. It is well suited for many metabolites and peptides. The liquid sample reaches the ionized states and it will be transferred to the mass spectrometer. To get suitable profile of samples, ionization was performed in positive and negative modes. MS with ESI method provides a wider platform to analyze biological molecules than in GC/MS analysis. It has a greater application in clinical biochemistry (Pitt, 2009).

A total of 48 compounds were identified in the methanolic extract of *Memecylon* species by HR-LC/MS analysis [**Table 9, Figures 17(i-xii)**]. HR-LC/MS analysis of leaf and fruit extract of *M. grande* together sum up to 21 compounds, *M. randerianum* 15 compounds and *M. umbellatum* 18 compounds. A diverse array of compounds was identified in *Memecylon* extracts and no compound was found to be common in all the six extracts. An alkaloid compound, rescinnamine was found to be common in all the selected fruit extracts. Ganglioside was found to be common in the fruit extract of *M.*

*grande* and *M. randerianum.* Swietenine, a terpenoid group of compound and biopeptides were commonly present in *M. grande* leaf, fruit and *M. randerianum* fruit extracts.

Lupanyl acid, aesculin, C16 sphinganine, 3-dehydro-6-deoxoteasterone and biopeptides were the major constituents of *M. grande* leaf extract. Lupanyl acid is a triterpenoid compound identified in the root of *Phyllanthus pulcher.* It exhibits cytotoxic effects in tumor cell lines, such as MCF-7, NCI-H460 and DU-145 (Bagalkotkar et al., 2011). It is identified as a cholinesterase inhibitory constituent in *Ficus bengalensis* (Riaz et al., 2012). Aesculin, a coumarin glycoside was identified in the present study. It is a common natural ingredient used in the Chinese medicine named *Cortex fraxini,* an inflammatory modulator in ulcerative colitis. The anti-inflammatory mechanism through the regulation of PPARγ and by inhibiting NF-κB pathways was noted by Tian et al. (2019). The antioxidant activity of aesculin has been reported by Witaicenis et al. (2014). C16 sphinganine has an important role in regulating apoptotic mechanism through TNF-α signal (Osawa et al., 2005). Sphingolipids are the biological building blocks and sometimes act as secondary messengers. Ceramides play an important role in cell cycle regulation and apoptotic mechanism (Jayadev et al., 1995). A terpenoid compound swietenine was found to be common in *M. grande* leaf, fruit and *M. randerianum* fruit extracts. It is isolated from *Swietenia macrophylla* seeds and shows significant hypoglycemic and hypolipidemic activity (Dewanjee et al., 2009). In cardiac hypertrophic condition, the lowering of the expression of Akt phosphorylation, ANP and BNP mRNA were noticed in swietenine treated mice models. It shows that swietenine might be a promising anti-hypertrophic agent against cardiac hypertrophy (Ding et al., 2020).

*M. grande* fruits are rich in diverse phytoconstituents. Tamarixetin, a flavonoid compound was ascertained during HR-LC/MS analysis. It is a derivative of quercetin, isolated from *Cyperus teneriffae.* It can act as a good anticancer agent. The cell cycle arrest and accumulation of cyclin B1, Bub1 and $p21^{Cip1/Waf-1}$associated with human leukemia was reported by Nicolini et al. (2014). Tamarixetin induces apoptosis and G2/M arrest in leukemia cells in a concentration and time dependent manner. In liver cancer cells, tamarixetin upregulated the expression of pro-apoptotic genes, Bax and caspase-3 and induce apoptosis through mitochondrial pathway (Xu et al., 2019). It also possesses anti-inflammatory potential (Park et al., 2018). Rescinnamine, an alkaloid compound isolated from *Rauwolfia serpentina* encompass antimicrobial, antioxidant and antimitotic potential, was observed in LC/MS analysis (Hemashekhar et al., 2019). *In silico* investigation on ZIKA virus inhibition reveals that rescinnamine shows significant results (Ahmed et al., 2020). Ganglioside GM1 is detected in the analysis and it acts as a neuronal regulator and prevents neurodegeneration. It plays an important role in maintaining the intercellular calcium homeostasis and subsequent cellular functions (Chiricozzi et al., 2020; Robert et al., 2011).

A terpenoid compound detected was madecassic acid with molecular weight 504.341. It is an active constituent of *Centella asiatica* having anti-colitis activity. It is also known to be a potential anticancer agent and shows cytotoxic effects on 26 different cancer cell lines (Valdeira et al., 2019). The antioxidant, anti-inflammatory and anti-diabetic properties of madecassic acid are well known (Yun et al., 2020). A carotene compound, 7, 8-didehydroastaxanthin, which was pinpointed in HR-LC/MS, is a colouring agent. It may protect cells against oxidative damages (Maoka et al., 2014). A vitamin D analogue, calcifedol was noticed in the present investigation. It is commonly used to manage vitamin D deficiency (Sosa Henríquez & Gómez de Tejada Romero, 2020). In COVID - 19 cases, the administration of

calcifedol will reduce the severity of the cases, because calcifedol can boost up the immune response of patients (Jungreis & Kellis, 2020). Dihydroxylycopene/OHRhodopin, is a carotene compound identified in the *M. grande* fruit extract. Ursolic acid, a potential terpenoid compound was also discerned during the HR-LC/MS analysis. The cytotoxic activity of ursolic acid against cancer cell lines was discussed by Ma et al. (2005). It has a wide spectrum of activity and is a good therapeutic agent. Antioxidant, anti-angiogenic, anti-inflammatory, anti metastatic *etc*., are the potential therapeutic roles of ursolic acid (Kashyap et al., 2016). Campestanol, a steroid group of compound was detected in the fruit extracts. It is known to be an antibacterial agent isolated from *Salvia jaminiana* root (Kabouche et al., 2005). The cholesterol reducing ability of campestanol was reported by Lichtenstein and Deckelbaum (2001). A limonoid compound, khayanthone was identified in HR/LC-MS analysis. It is a havanensin-class limonoids, isolated from the genus *Khaya* of Meliaceae. Limonoid compounds are known to be insecticidal agents and free radical scavengers (Tan & Luo, 2011; Mestry et al., 2020).

The leaf extract of *M. randerianum* shows a limited number of compounds in HR/LC-MS analysis. Among these a glycoside compound, bergenin was detected, which possesses diverse pharmacological activity. The antioxidant and antimicrobial activity was reported by Nazir et al. (2011). Bergenin have hepatoprotective, antiulcerogenic, immunomodulatory and anti-inflammatory activity (Patel et al., 2012). The anti-inflammatory activity was attributed by the inhibition of IL-1β and TNF-α production. 9,12,13-trihydroxy-10,15-octadecadienoic acid was a fatty acid derivative produced from linoleic acid by *Pseudomonas aeruginosa*. Trihydroxy fatty acids are produced in plants as a self defensive mechanism (Kim et al., 2000).

The first compound detected in *M. randerianum* fruit extract was an organic compound, violastyrene. The soluble guanylate cyclase is a receptor protein of nitric oxide. It is involved in the cell signaling pathways and associated with angiogenesis in tumor development. Violastyrene is considered as a soluble guanylate cyclase inhibitor and have a good antiangiogenic property (Petrova et al., 2020). In *Dalbergia saxatilis* leaves extract, the presence of violastyrene was noticed and it has protectant activity against cowpea pest, additionally it has insecticidal activity against mosquitoes and has antimicrobial activity (Okwute et al., 2009). The presence of rescinnamine was found to be common in the fruit extract of selected *Memecylon* species. In addition, another alkaloid compound detected in *M. randerianum* fruit was β-erythroidine. It shows curarizing property, that induces the muscular relaxation (Champtiaux et al., 2006). Glycerol palmitate obtained in the present study is a monoglyceride, derived from hexadecanoic acid. It has potent antioxidant activity, which was reported by Qadir et al. (2018). A steroid compound noticed in the present study was 6-deoxocastasterone. It is known to be a brassinosteroid that influences the plant growth and development. Cosmosiin, another phenolic compound detected and shows anticancer activity against HCEC, MCF-7 and Hep2 cell lines (Ahmed et al., 2017). It can up regulate ADAM10 (a disintegrin and metalloproteinase domain-containing protein), that is involved in the Alzheimer's disease therapy. Cosmosiin enhances the production of neurotoxic amyloid precursor that is normally depleted in Alzheimer's condition (Min et al., 2018). Cosmosiin act as an anticancer agent through the immune checkpoint inhibition in *Salvia plebeia* and acts upon the PD-1/PD-L1 interaction (Choi et al., 2020).

*M. umbellatum* leaf extract shows the presence of a sesquiterpene lactone, elephantopin in the LC-MS analysis. It is known to be a tumor inhibiting agent (Shukla et al., 2020). In *M. umbellatum* fruit extract, a diverse

array of chemical compounds was identified through the HR-LC/MS analysis. The presence of the polyphenolic compound chlorogenic acid; glycoside compound, amygdalin; terpenoid compound, deutzioside; quinone compound, idebenone; benzoquinone compound, embelin *etc*., are a few of them. Chlorogenic acid play several therapeutic roles, such as antioxidant, antimicrobial, antipyretic, anti-inflammatory *etc.* It is considered as a safe natural additive (Naveed et al., 2018). The health promoting characters of chlorogenic acid reveals that it is a promising food supplement (Santana-Gálvez et al., 2017). Amygdalin is commonly present in the members of Rosaceae. It possesses antitumor and anti-inflammatory activities and reduces blood glucose level (He et al., 2020). Several studies on the potential activity of amygdalin were conducted by various researchers (Jaswal et al., 2018; Liczbiński & Bukowska, 2018). So it validates the therapeutic role of *M. umbellatum* fruit extract. The anticancer mechanism of amygdalin is attributed through the cell cycle arrest, apoptosis and regulation of immune system (Shi et al., 2019).

Deutzioside is an iridoid compound, which belongs to monoterpene group of compounds and is found in many dietary folk medicines (Dinda, 2019). Stigmasta-7, 22 E, 25-trien-3beta-ol, a sterol and embelin was detected in the present study. Embelin is a bioactive natural compound under benzoquinone group, which was initially isolated from *Embelia ribes*. The wound healing activity of embelin was reported by Swamy et al. (2007). The anticancereous activities of embelin become an evaluable tool in cancer studies. It is an obstructor of X-linked inhibitor of the apoptosis protein (XIAP), an anti-apoptotic protein (Poojari, 2014). In hepatocarcinogenesis, embelin shows promising activity against *N*-nitrosodiethylamine (DENA) and phenobarbital (PB) induced tumorigenesis (Sreepriya & Bali, 2005). The antimicrobial and anticonvulsant activity of embelin was also reported (Chitra et al., 2003; Mahendran et al., 2011).

6b,11b,16a,17a,21-pentahydroxypregna-1,4-diene-3,20-dione16,17-acetonide is a terpenoid compound having anticancer activity, which was reported in *Cyathocline purpurea* (Javir et al., 2019). In addition to rescinnamine, another alkaloid, protoveratrine A was also detected in the analysis. It is known to be a steroidal alkaloid isolated at first from *Veratrum album* (Liliaceae) (Vengamma et al., 2019). It is used for the treatment of hypertension. The insecticidal activity and lowering of blood pressure are the beneficial roles of protoveratrine A (Akbar, 2017).

The presence of biopeptides was observed in the present investigation. Biopeptides are organic molecules formed by proteolysis and consist of two or more amino acids connected by covalent bonds. These are biologically active molecules with distinct nutritional and functional role in physiological processes of organisms. It has several biological functions such as antioxidative, antihypertensive, antidiabetic and immunomodulatory activities (Saadi et al., 2015). Biopeptides are specific sequence of amino acids with many health benefits and ameliorate disease conditions. It is considered as natural bioactive elements used as a drug, having nutraceutical value and as a food supplement (Montesano et al., 2020). The biopeptides of *Phalaris canariensis* L. was found to be antihypertensive, antidiabetic and with anti-obesity activity (Urbizo-Reyes et al., 2021). The presence of tripeptides is noticed in HR-LC/MS analysis of *Memecylon* species. The antioxidant activity of tripeptides with cysteine and tryptophan moieties was reported by Tian et al. (2015). Most of the tripeptides have antihypertensive activity, which regulate action of the angio-tensin converting enzyme (ACE). The anti-inflammatory, antimicrobial and antioxidant activities of biopeptides make them useful as food additives (Sánchez & Vázquez, 2017). Thus biopeptides are efficient bioactive components that can be targeted for drug preparation.

HR-LC/MS analysis of selected *Memecylon* species markedly proves that these plants are a rich source of many bioactivity phytochemicals, which belong to various classes of compounds. The bioactive reports of the phytoconstituents are again validating their usage as medicinal components. They are endowed with antioxidant, antibacterial, anticancerous and anti-inflammatory effects. The isolation and characterization of bioactive components critically influence their therapeutic uses. So the exploration of the individual phytocomponents present in the *Memecylon* species is essential for validating their specific bioactivity. The presence of an immense array of compounds identified through the GC/MS and HR-LC/MS analyses justifies the use of these plant extracts as herbal medicine.

## PHASE III- BIOACTIVITY STUDIES

### a) FREE RADICAL SCAVENGING ACTIVITY STUDIES

In aerobic organisms, mitochondria are the main generator of energy for the realization of its vital functions. It generates ATP through reactions of oxidation and reduction that attach tricarboxylic acid cycle with the electron transport chain. It occurs through the oxidation of the food and by the production of NADH and $FADH_2$ in different metabolic pathways, such as glycolysis, β-oxidation and the Krebs cycle. These reactions generate unpaired electrons in the form of free radicals or reactive oxygen species. They tend to stabilize themselves by sequestering electrons from other biomolecules. Thus they become unstable and therefore, are no longer able to perform their duties properly. It will alter the homeostasis and ultimately cause cell death (Aguilar et al., 2016). Free radicals are defined as "any chemical species capable of independent existence that contains one or more unpaired electrons". Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are common free radicals associated with cellular metabolism. The other biologically important free radicals are lipid hydroperoxide

(ROOH), lipid peroxyl radical (ROO•) and lipid alkoxyl radical (RO•), which are associated with membrane lipids; nitric oxide (•NO), nitrogen dioxide ($•NO_2$) and peroxynitrite ($ONOO^-$), which are reactive nitrogen species; and thiol radical (RS•), which has an unpaired electron on the sulfur atom (Kurutas, 2015).

Antioxidants are free radical scavengers and can neutralize the oxidative stress induced by the reactive oxygen species. Otherwise ROS can disrupt cellular mechanism and lead to severe pathological conditions and diseases like cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes *etc.* (Birben et al., 2012). Free radicals are unpaired and unstable, so unstable radicals tend to become paired with the biological macromolecules such as proteins, lipids and DNA to become stable. Thus it will cause protein and DNA damages (Gilgun-Sherki et al., 2002). Antioxidants are normally counter acting the effects created by the free radicals. The antioxidants are produced either endogenously or received from exogenous sources, which include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals like Se, Mn, Cu and Zn and vitamins like vitamin A, C and E. Glutathione, flavonoids, bilirubin, uric acid *etc*., possess promising antioxidant activity. In a healthy body, prooxidants and antioxidants maintain a ratio and a shift in this ratio towards prooxidants gives rise to oxidative stress (Irshad & Chaudhuri, 2002). However, reactive oxygen species mediate certain cellular functions like redox signaling and gene expression as well as defend against pathogens. Thus, the role of antioxidant systems is not to eliminate oxidants completely, but instead maintain them at an optimum level. The antioxidants are classified as enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants are converting oxidized metabolic products in a multi-step process to hydrogen peroxide ($H_2O_2$) and then to water using various cofactors. Non-enzymatic antioxidants intercept and terminate free radical

chain reactions, and it includes vitamin E, A, C, flavonoids, carotenoids, glutathione, plant polyphenols *etc.* (Moussa et al., 2020).

Antioxidants of natural origin like tea leaves, carrot, spinach, berries *etc*., are antioxidant sources of common people. There are several unknown sources of free radical scavengers. The present study focuses on the *in vitro* antioxidant activity of selected *Memecylon* species in DPPH, hydroxyl, nitric oxide and superoxide radical scavenging assays. A single assay is not adequate for the evaluation of antioxidant property of the samples. The assays developed to evaluate the antioxidant activity of plants and food constituents may vary. There are two general types of assays widely used for different antioxidant studies. One is an assay associated with lipid peroxidations, including the thiobarbituric acid assay (TBA), malonaldehyde/high-performance liquid chromatography (MA/HPLC) assay, malonaldehyde/gas chromatography (MA/GC) assay and conjugated diene assay. The second type assays are associated with electron or radical scavenging, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, ferric reducing/ antioxidant power (FRAP) assay, ferric thiocyanate (FTC) assay and aldehyde/carboxylic acid (ACA) assay (Moon & Shibamoto, 2009).

Generally *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple and inexpensive in comparison to other test models (Alam et al., 2013). DPPH is a stable free radical with pink colour which turns yellow when scavenged. Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence the absorbance decreases. The degree of discoloration is always related with the hydrogen donating ability of the antioxidants. In the present study, highest scavenging activity was shown by 200 µg/mL concentration of *M. grande*

fruit extract and exhibit an inhibition percentage of 75.77 ± 0.01 (**Figure 19**). The $IC_{50}$ value of *M. grande* fruit extract was found to be 83.91 ± 0.14 µg/mL. It is followed by the *M. umbellatum* fruit extract with the $IC_{50}$ value, 91.10 ± 0.12 µg/mL (**Table 10**). All the selected extracts show potent antioxidant activity *ie.,* more than 50% inhibition is being offered by the extracts. While comparing the species, the lowest activity was shown by the *M. grande* leaf extract of 64.37 ± 2.05%. All other extracts possess moderate range of scavenging potential. Previous reports of antioxidant activity of *Memecylon* species are available. The phytochemical analysis of selected *Memecylon* species, evidently propose that they are an immense source of bioactive phytochemicals. So the scavenging potential is the worthy contribution of the phytochemical constituents of *Memecylon*.

The hydroxyl radical (˙OH) possesses an important role in cancer induction. It induces mutagenic effect, while interacting with the DNA molecule and resulting in DNA breakdown and cancer formation (Khan et al., 2013). It is the most reactive free radical. Hydroxyl radical interacts with micro- and macromolecules present in an organism and disrupt membrane and cellular proteins, lipids, DNA and RNA (Cederbaum, 2017). It can induce lipid peroxidation and damages on disulfide bonds of proteins, specifically fibrinogen, resulting in their unfolding and knotted refolding into unusual spatial configurations (Lipinski, 2011). Hydroxyl radicals are formed by incubating $Fe^{+3}$- EDTA premixture with ascorbic acid and $H_2O_2$, it is known to be the Fenton reaction. While analyzing the scavenging potential of *Memecylon* species, similar trends in DPPH assays are again reflected (**Figure 21**). *M. grande* fruit extract shows the highest scavenging potential (61.69 ± 0.52%) and the lowest effect was shown by its leaf extract (36.77 ± 0.62%). The $IC_{50}$ value indicates that *M. grande* fruit (1231 ± 0.48 µg/mL) extract is a better hydroxyl radical scavenger than the standard gallic acid (1347.51 ± 0.27 µg/mL) (**Table 10**). In the case of *M. randerianum* and *M.*

*umbellatum* extracts, a moderate level of scavenging activity was observed. Phenolic compounds are considered as effective scavengers of hydroxyl radical (Yıldırım et al., 2000). The phytochemical analysis is also validating the same. A promising amount of phenolic content was noticed in *Memecylon* species through quantitative phytochemical estimation and GC-MS analysis.

Nitric oxide radical ($^{\bullet}NO$) owns various biological functions. It has a crucial role in neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the beneficial role, it can act as an oxidant element through the interaction of superoxide and it forms peroxynitrite anion. It is a potential oxidant that can produce OH and NO (Patel Rajesh & Patel Natvar, 2011). In nitric oxide scavenging assay, similar range of activity was observed in *Memecylon* species. Griess assay was used to assess NO-inhibitory activity of the extracts. Here sodium nitro prusside in aqueous solution at physiological pH impulsively generates nitric oxide by the action of oxygen and produce nitrite ions that can be determined by using Griess reagent. Nitric oxide free radical has an important role in inflammatory responses. It activates nuclear factor κB (NF-κB), which induces the transcription of inflammatory cytokines and COX-2. Antioxidants can effectively block the transcription of inflammatory cytokines (Huang et al., 2001). *M. grande* fruit extract shows the highest scavenging activity of 76.85 ± 0.08% and lowest was found in *M. grande* leaf extract with 40.86 ± 0.20% (**Figure 23**). The $IC_{50}$ value of standard becomes low when compared with the sample concentrations. So nitric oxide radical scavenging potential of the selected extracts is in a moderate range.

Superoxide ($O_2^{\bullet-}$) is one of the strongest reactive oxygen species among the free radicals and can produce singlet oxygen. Here an estimate of the reduction rate of nitro blue tetrazolium (NBT) into a purple-colored formazan is measured (Fontana et al., 2001). Superoxide radical can induce

detrimental effects on the cell components. It induces lipid oxidation with the singlet oxygen production (Halliwell et al., 1987). Phytochemical components are always the responsible factor for the bioactivity. Flavonoids are considered as effective scavengers of superoxide radicals (Robak & Gryglewski, 1988). Super oxide radical scavenging ability of *Memecylon* species owns a similar trend as shown in other assays. But here the lowest activity was exhibited by *M. randerianum* leaf extract. The dose dependent scavenging activity become prominent in *M. grande* fruit extract with 72.17 ± 0.02% of activity (**Figure 25**). All the selected extracts show promising results. As compared with $IC_{50}$ value of standard (238.35 ± 0.03 µg/mL), *M. grande* fruit extract was exhibiting (698 ± 0.03 µg/mL) scavenging potential in a moderate range.

These *in vitro* assays show promising results in free radical scavenging activity. It might be helpful in preventing the oxidative stresses and associated malfunctions. All the selected extracts exhibit a moderate range of activity. While comparing all antioxidant assays, *M. grande* fruit extract was the leading scavenger of free radicals *ie.*, a remarkable hydroxyl radical scavenger. DPPH, superoxide and nitric oxide radical scavenging activity of all selected extracts prove to be valuable. The free radical scavenging activity of fruit extracts become more as compared with the leaves extract. It might be due to the diverse phytochemical composition of the plant parts. Several reports highlight that, the fruit samples show prominent antioxidant potential rather than the leaves, since the phenolic content of the fruits are much more in early stage of fruit development (Wang & Lin, 2000). Polyphenols are secondary metabolites with highest antioxidant potential, especially flavonoids offer an intriguing promise (Dimitrios et al., 2006). The bioactive phenols and several other compounds with antioxidant activity were noticed in the phytochemical analysis. The presence of bioactive compounds like

squalene, agathenic acid, bicyclogermacrene, phytol *etc.*, also contributes to the antioxidant potential of *Memecylon* species.

*M. grande* and *M. randerianum* extracts were found to be rich in diverse chemical constituents, especially immense amount of fatty acid and fatty acid esters. Fatty acids like palmitic acid, octadecanoic acid and several fatty acid methyl esters such as methyl myristate, methyl palmitate, methyl heptadecanoate and methyl linoleate are known to be good antioxidant agents. They can reduce the cellular damages caused by oxidative stress (Hansen et al., 2001). *M. randerianum* was also found to be rich in terpenoid compounds, which also contributes to its scavenging potential (Grassmann, 2005). In the case of *M. umbellatum* extracts, steroids, alkaloids, phenols and fatty acids are the major phytoconstituents.

Phytochemical profiling of selected *Memecylon* species markedly supports the antioxidant efficacy. From the preliminary quantitative phytochemical analysis, it is proved that selected plant extracts are rich in phytochemicals (**Table 7**). GC/MS and LC/MS analysis is again validating the same result. *Memecylon* species have significant potential to hunt free radicals and are rich in natural antioxidants. In light of these results, one can hope that the *Memecylon* fruits are a galore of natural antioxidants. However, the components and the mechanism responsible for the antioxidant activity are currently unclear. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activities of these extracts need to be assessed prior to clinical use.

## b) CYTOTOXIC SCREENING USING *ALLIUM CEPA*

*Allium cepa* is a model organism for genotoxicity/cytotoxicity studies. It is a common method of toxicity evaluation in plant materials. The toxic

ranges of industrial effluents from pesticide or textile areas are evaluated through this plant based assay (Wijeyaratne & Wickramasinghe, 2020). It is an easy and fast way to detect the toxic potential of plant specimens. So it will further lead to the development of plant based pharmaceuticals for various human ailments. The present study highlights the toxic potential of selected *Memecylon* species. In ½ hr, 2 hr and 24 hr of exposure period of treatments, a range of cellular aberrations is noticed (**Plates 10-16**). A normal untreated *A. cepa* root tip cells raised in distilled water and that grown in $H_2O_2$ medium are considered as negative and positive standards (**Plate 9**).

In cancer studies, target medicine of natural origin has great importance. Cytotoxic compounds are beneficial in proliferative studies. The prime step in cancer studies are the toxicity validation of test sample. *A. cepa* is one of the quick methods of toxicity validation. There are several plant systems that are used in cytotoxicity evaluation (**Table 2**). *Tradescantia, Vicia faba, Lactuca sativa etc*., are some other important test materials from the plant kingdom. *A. cepa* assay is considered as an efficient system, because it is directly related to the mammalian test system. In several environmental studies, *A. cepa* is considered as a satisfactory tool in environmental monitoring of xenobiotics, mutagens *etc*. (Leme & Marin-Morales, 2009). Cytogenotoxicity determination of different plant extracts and various chemicals using *A. cepa* root cells are still a common method. So it is always a strong and satisfactory step in toxicity determination (Salazar et al., 2020).

The toxicity efficacy of plant materials are always a fruitful pathway in therapeutics (Sammar et al., 2019). Mitotic index is a cellular measure of proliferation. It is the count of dividing cells in a group of cellular population and it determines the viability of the cell system. The lowering of the mitotic index is an indicator of retardation of protein synthesis, DNA synthesis and cell cycle arrest (Majewska et al., 2003). Chromosomal aberration is a sign of

the toxic potential of plant extracts. *A. cepa* treated with *Memecylon* extract, shows various chromosomal aberrations. Stickiness, stellate chromosomes, pulverization, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, lagging chromosome, nuclear disintegration, giant cell formation, coagulated chromosomes, C-mitosis *etc*., are the observed abnormalities found in *A. cepa* root tip cells. Chromosomal aberrations are mainly of two categories, clastogenic and aneugenic. The clastogenic abnormality affected the nuclear material of the test system, while aneugenic effect destructs the mitotic spindle machinery. The abnormality percentage and mitotic index of *Memecylon* species are given in **Figures 26, 27, 28**. Mitotic index and abnormality percentage are inversely related, mitotic index decreases with increasing concentration and the abnormality percentage increases with the increasing concentration of the plant extracts.

Among the selected six samples of plant extracts, *M. umbellatum* fruit extract shows the highest abnormal cell counts of 91.73 ± 1.41% and lowest mitotic index of 30.76 ± 7.00% at ½ hr exposure period of 100 µg/mL concentration. In *M. grande* leaf extract, 89.4 ± 2.29% aberrations were observed in 100 µg/mL sample treatment and 43.66 ± 3.84% was the lowest mitotic index at 24 hr treatment with a concentration of 100 µg/mL. *M. grande* fruit extract shows, 48.11 ± 7.39% as the lowest mitotic index and 90.72 ± 1.45% as the highest aberration percentage. In *M. umbellatum* leaf extract, the highest percentage of aberration noticed in 24 hr, 100 µg/mL experiment condition is 90.25 ± 2.74%. The reduced mitotic index observed at 2 hr, 100 µg/mL sample concentration was 32.85 ± 3.81%. *M. randerianum* leaf extract possess 82.39 ± 2.74% of aberration as the highest value at 2 hr, 100 µg/mL of concentration and the lowest mitotic index was at ½ hr, 100 µg/mL of concentration with 35.66 ± 5.58%. In the case of fruit extract of *M. randerianum*, 83.83 ± 1.28% is the highest aberration percentage observed at ½ hr and 34.52 ± 3.87% was the lowest mitotic index percentage at 24 hr.

Here all the tested concentrations of plant extracts cause mitodepressive effect and similar results were also noticed in various other studies (Khanna & Sharma, 2013; Lamsal et al., 2010). The mitodepressive effect may check the nuclear material synthesis, nucleoprotein formation and may affect the structure of chromosomes (Yuet Ping et al., 2012). While comparing the toxic potential of selected *Memecylon* species, 30-90% of aberrations were induced by the phytochemical constituents of the plant extract. It clearly indicates that the toxic potential of plant extracts is evidently dosage dependent. The mitotic delay and aberration percentage is directly related (Gudowska-Nowak et al., 2005). The toxicity potential of plant extracts is a clear indicator of developing target medicines in cancer therapy. So validation of toxicity level becomes the prime requirement of experiments. The mitotic index and abnormality percentage values give the first signs for the validation. The present investigation thus confirms that the selected species of *Memecylon* are potential cytotoxic agents and much more efficacy is shown by *M. umbellatum* fruit extract.

The decline of mitotic index and prominent aberration percentage are the key leads in toxicity determination. The reduction of mitotic index is due to the blockage of cell cycle or escalating the length of G2 phase or S phase or delaying the onset of prophase (Prokhorova et al., 2013). The cytotoxicity level of extracts is determined by their efficacy of lowering mitotic index. The mitotic index range of below 22% is considered as a lethal value and below 50% is a sub-lethal value for organisms. These are considered as the cytotoxic limit values as described by Prajitha and Thoppil (2016). The selected six plant extracts possess sub-lethal level of toxic effect on the *A. cepa* root tip cells. The mitodepressive and antiproliferative effects of plant extracts are confirmed through the lowered mitotic index parameter.

Various chromosomal aberrations were resulted in *A. cepa* assay. The toxic potential of selected extracts was clearly noticed in the chromosomal or nuclear abnormalities. The same aberrations were resulted in different plant extracts at various cell stages and in some cases, multiple abnormalities were also noticed. So the abnormalities in different cell cycle stages are documented in **Plates 10-16**, and for comparison, a normal cell cycle stages were provided in **Plate 9**. *M. umbellatum* fruit extract shows the highest abnormal cell counts of 91.73 ± 1.41%. In clastogenic aberrations, stickiness, pulverization, exposure of chromosome scaffold, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, nuclear disintegration, giant cell formation, coagulated chromosomes and chromosome bridges were detected. Hypoploid condition, stellate chromosomes, lagging chromosome, C-mitosis, macro and micro cell formation, ball shaped chromosome, polyploidy and induction of vagrants are noticed as aneugenic aberrations. While comparing these abnormalities, aneugenic aberrations are quite common.

The selected plant samples were found to induce a number of chromosomal aberrations, but specifically *M. randerianum* leaf extract was more potent in inducing C-mitosis. It is a distinct spindle damaging abnormality noticed in the cytotoxic assay. The C-mitotic activity of *M. randerianum* leaf extract was reported for the first time by Ramya Sree and Thoppil (2018). C-mitosis is the spindle abnormality observed during mitosis, so it disrupts the chromosomal movements and leads to aberrations like C-metaphase, C-anaphase, polyploidy *etc.* The spindle poisoning may occur due to the presence of colchicine like compounds in the leaf extract of *M. randerianum*. C-metaphase (**Plate 13 n**) is one of the main consequences of inactivation of spindle fibers, which cause delay in the division of centromere (Somashekar & Gowda, 1984). Partial inactivation of spindle fibers leading to partial C-mitosis was also observed during the study (**Plate 13 p**). The shift in microtubule organizing centers (MTOC) is resulted by the effect of C-mitosis

(**Plate 14 b**). MTOC is the assembling site of mitotic and meiotic spindle machinery. The active principles found in plant extract will affect the stability of microtubules and lead to shift in their position (Neelamkavil & Thoppil, 2018).

Formation of vagrants is the another frequently observed abnormality associated with C-mitosis. It is an indicator of spindle poisoning and cause unequal separation of chromosome groups (Rank, 2003). The spindle abnormality can induce lagging of chromosomes and leads to form laggards (Lera & Burkard, 2012). During anaphase, the formation of vagrants and laggards are common. The spindle distortions are reported in all the selected plant extracts. Polyploidy, chromosome rosette, scattered meta - and anaphases, unipolar movement of chromosomes, unequal chromosome groups, misorientation of chromosomes, diagonal and dislocated chromosomes are the vital abnormalities associated with it. Scattered meta- and anaphases may be the result of disturbances or inhibition of spindle formation (Tripathy & Rao, 2015). Polyploid cell (**Plate 13 b**) is a numerical aberration formed due to spindle abnormality. Similarly hypoploid cells were also resulted (**Plate 13 h**). Aberrant cell formation was frequently observed in the cell cycle stage of cytokinesis mainly, aberrant cell wall formation with single lesion, diagonal cell plate formation showing displacement, macro and micro cell and unequal and oblique cell plate formation (**Plate 16 a, d, h, k**). Stellate metaphase (**Plate 13 f**) and anaphase (**Plate 14 m**) were resulted by the clumping of chromosomes due to the spindle abnormality. Another chromosomal abnormality associated with spindle fibers resulted is tropokinesis. The abnormal orientation of the spindle midzone of the fibers is clearly noticed here (**Plate 13 a, g**). These diverse spindle abnormalities, clearly point towards the spindle poisoning ability of selected *Memecylon* species.

Ball metaphase is resulted by the destruction of spindle fibers and clumping of chromosomes and assumes the shape of a ball. The pulverization of chromosomes is due to the premature condensation of chromosomes (Rybaczek & Kowalewicz-Kulbat, 2011). Macro and micro cells formation resulted in various stages of cell cycles, may be due to the failure of normal organization and function of spindle apparatus as attributed by Adam and El-Ashry (2010). Coagulated prophase and anaphase were resulted in the present study. Here chromosomes seem to be adhering together to form an intact mass of aberrant chromosome group. It is due to depolymerization of deoxyribonucleic acid. The chemicals of plant extract can induce DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchange of the basic folded fibre units of chromatids and the stripping of the protein covering of DNA in chromosomes (Sumitha & Thoppil, 2016). Somatic pairing was noticed during the study (**Plate 12 l**). It is the pairing of homologous chromosomes in a somatic cell. Somatic pairing and chromosomal aberrations are related processes. Somatic pairing influences the frequency and type of the chromosome aberrations induced (Beçak et al., 2003). Stathmo anaphase is shown in **Plate 14 n**. Here the daughter chromosomes do not separate fully, but remain connected together by means of partial overlapping of their arms (Renjana & Thoppil, 2013). It is a spindle anomaly caused by the simultaneous multipolar and spindle poisoning activities of the extract. Chromosome scattering was resulted in the present investigation, which could be due to the interference of extract chemicals on the polymerization/de polymerization of the microtubular subunits (**Plate 13 d, e**).

Among the clastogenic aberrations induced by *Memecylon* extracts, nuclear lesions are a common one. It is a cytological evidence for the inhibitory action of the extracts on DNA biosynthesis (Akaneme & Iyioke, 2008). It is the first sign of the genetic material loss or degradation. Single or

double nuclear lesions are noticed prevalently in the present study (**Plates 10 c, e, g; 11 c; 16 i**). Chromosome stickiness is due to the cross-linkage of chromoproteins. It can also induce chromosome bridges and the subsequent failure of anaphase separation (Tkalec et al., 2009). The aberrations like chromosome gaps, nuclear lesions, erosions, nuclear breakage and nuclear disintegration are resulted in the *A. cepa* assay were clear indicators of apoptotic sign. So the cytotoxic results were further analyzed through the antiproliferative experiments. These strong cytological evidences prove the potential of these extracts of *Memecylon*. Nuclear or chromatin erosions are resulted in *A. cepa* assay. It is due to the extreme toxicity offered by the plant extract and the degradation of chromoproteins (Karaismailoglu, 2015). The fragmentation of chromatin is a sign of cell death or may lead to aneuploid condition (**Plate 11 b**). Binucleate cells are observed in the *A. cepa* assay (**Plates 10 a, b; 14 p**) which reveals that the plant extract can inhibit the cell cycle at certain points, particularly the prevention of the cytokinesis (Khanna & Sharma, 2013). Strap cell formation is also noticed during the study (**Plate 10 g, h**), which may be due to the abnormal cell enlargement.

Stickiness, bridges and fragments are chromatin dysfunctions that were found to be the frequent type of chromosomal aberrations in the study. Chromosome stickiness reflects toxic effects of plant materials, usually of an irreversible type, leading to cell death. The disorganization of chromatin, DNA agglomeration and complexity of interchromatin fibers are the main reasons for the chromosome stickiness (Mustafa & Suna Arikan, 2008). A disturbed balance in the quantity of histones or other proteins seems to be responsible for the change in structure of the nuclear chromatin (Hammann et al., 2020). The presence of sticky anaphase and metaphase reveals the chromatin remodeling potential of the plant extracts (**Plates 13 h; 14 o**). Increased stickiness also leads to the formation of sticky bridges in anaphase and telophase. Chromatin bridges could be attributed to the chromosomal

breakage and reunion of chromatids (**Plates 14 c, e, o, p; 15 a-d**). Chromosome bridges are formed due to the stickiness of the chromosomes and they can't separate apart in anaphase. It appears like bridges (Rad et al., 2011). Another reason proposed for bridge formation was due to the formation of dicentric chromosomes by breakage and reunion (Majewska et al., 2003). Chromosomal fragments are another notable chromosomal dysfunction. The chromosomal breaks and may be a part of anaphase bridge and the disturbances in microtubule assembly lead to fragment formation (**Plates 12 c; 14 k**).

In the present study, several multiple aberrations were observed *ie*., two or more aberrations occur simultaneously. Binucleate cell showing double and single nuclear lesions (**Plate 10 b**), sticky prophase showing single lesion (**Plate 11 g**), lesion and chromosome gaps at early metaphase (**Plate 12 p**), early ball metaphase showing lesion (**Plate 13 q**), pulverized stellate anaphase (**Plate 14 m**), stellate telophase showing persistent bridges (**Plate 15 p**), nuclear lesion, erosion and peak at cytokinesis (**Plate 16 i**) *etc*., are some of the multiple aberrations observed. A large number of cells with multiple aberrations are observed in the present study rather than single aberrations. The synergistic action of phytochemicals present in the extract is capable of inducing multiple disturbances in the normal cell cycle. Several reports are pointing that the cytotoxic effect of the plant species are the cumulative effect of the phytoconstituents present in it. The naturally occurring compounds such as flavanols, polyphenols, alkaloids and tannins have been involved in causing chromosomal damages (Carreon et al., 2002).

The *A. cepa* assay is considered to be an efficient test system because, the cytological evidences in the study have more application in cancer studies. It is the initial research that focuses on screening the ability of different plant

extracts for their cytotoxic and antiproliferative potentials. There are several studies which reveal that, the primary screening of cytotoxicity on *A. cepa* assay can be correlated with their antiproliferative efficacy against malignant cells (Isbilen et al., 2018; Abdullah et al., 2014). The cytotoxic efficacy of selected *Memecylon* extracts on *A. cepa* had revealed promising observations which can be further correlated with anticancer efficacy on mammalian test systems. In order to confirm the same *in vitro*, further anticancer study was conducted. It deals with the determination of the antiproliferative activity of the plant extracts on the human breast cancer cell line MCF-7.

## c) ANTIPROLIFERATIVE ACTIVITY OF *MEMECYLON* SPECIES

Anticancer drugs of natural origin have a great impact on the scientific community. They often search for a safe remedy for the most deleterious disease in the World. Agents, which deny the growth of cancer cells through apoptotic mechanism acquired a major interest in cancer research. The natural compounds of anticancer activity being reported will boost up the search for new ones. Vincristine, vinblastine, taxol *etc*., are few of them. Cytotoxicity is the key factor behind the control of unwanted cell proliferations. The herbal medicines with antiproliferative efficacy are proved to be cytotoxic agents. Hence, cytotoxic effects of plant extracts are targeted in antiproliferative studies. The present study thus shows promising results in cytotoxic screening using *A. cepa* root tip cells. Thus the remaining studies are focused on the anticancer properties of the selected species of *Memecylon*. The *in vitro* anticancer potential of *Memecylon* species are screened by using the breast cancer cell line, MCF-7. The selected concentrations of the six plant extracts are 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL and time period for the experiment was set for 24 hrs. The way of antiproliferative study was organized as follows: MTT assay was conducted for the cytotoxicity assessment. For determining the apoptotic mode of cell death,

acridine orange/ethidium bromide staining was performed. Subsequently the DNA damage at individual cell level was checked by comet assay. The influence of antiproliferative efficacy in cell cycle phases was analyzed by flow cytometry. The gene expression associated apoptotic pathways (p21, p53 and β- actin) were studied by RT-qPCR.

Breast cancer is the most common cancer in women. The increased number of population studies on breast cancer reveals an alarming signal that it continues to spread all over the World (Kalager et al., 2012; Abubakar et al., 2018). Naturally occurring plant compounds like curcumin, resveratrol, paclitaxel, docetaxel, quercetin *etc*., shows promising anticancerous property. It is less harmful to healthy cells and shows selective toxicity to abnormal cells. This might be the probable reason for the ample interest of herbal medicines. A number of natural anticancer drugs are discovered every year. From 1981 to 2014, 136 natural anticancer drugs are used globally, which are either natural or derivatives of natural products (Amaral, 2019). Several natural herbs are used to cure breast cancer (Shareef et al., 2016**)**. The present study gave special emphasis on the breast cancer inhibition ability of the selected *Memecylon* species.

Estrogen plays a major role in breast cancer induction. It can promote the transition of normal breast cells to malignant breast cells. Estrogen receptor positive is the major molecular signal of the breast cancer. So the herbal remedies for blocking estrogen receptors have great attention. Plant-based estrogen compounds mimic as the human estrogen analogue and can bind to the hormone receptor (Lamartiniere, 2000). It will create an imbalance in the molecular machinery, thereby checking breast cancer. Phytoestrogen rich products may prove to become a curative pathway in breast cancer. Phytoestrogen is mainly found in legumes and lignans. Lignans occur in seeds, nuts, whole grains, fruits and vegetables. The dietary intake of lignans

will reduce the risk of breast cancer (Rietjens et al., 2017). There are several reports on the herbal products that are used to cure breast cancer. Artemisinin and polyphenols isolated from *Artemisia annua* L., polyphenol-rich extracts of *Hibiscus sabdariffa* and aqueous extract of *Brucea javanica* are few of them (Laskar et al., 2020).

The first phase of anticancer study starts with the cytotoxicity evaluation of selected extracts on cell lines. In the previous section, in *A. cepa* assay it was noticed that the extracts prove to be cytotoxic. MTT assay is the common method used to determine the antiproliferative activity of compounds on cultured cells. It measures the mitochondrial metabolic rate and viable cell numbers (Li et al., 2009). The mechanism involved in MTT assay is as follows: the tetrazolium salt MTT is reduced to purple formazan crystal in the metabolically active cells by mitochondrial dehydrogenases (Shoemaker et al., 2004). In the present study antiproliferative efficacy of *Memecylon* species were tested using MCF-7 cell lines. The production of formazan is directly proportional to the number of viable cells. The highest antiproliferative activity was shown by *M. umbellatum* fruit extracts with 44.4 ± 1.68% (**Figure 29; Plate 22**). A dosage dependent antiproliferative activity was shown by all the selected plant extracts. 100 µg/mL was found to be the effective concentration of the plant sample. This is followed by *M. grande* fruits and leaves extracts with 46.93 ± 4.74 and 48.28 ± 2.78 viability percentages respectively (**Figure 29; Plates 17**, **18**). *M. randerianum* extracts possess moderate activity against MCF-7 cells. The overall results point out that the fruit extracts of selected samples show highest antiproliferative potential as compared to their corresponding leaf extracts. The $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract was selected for further anticancerous studies (**Table 11**). In *in vivo* toxicity studies, $LD_{50}$ is an important measure. It is considered as the safety value for evaluation of drugs in pharmaceutical industry

(Malmfors & Teiling, 1983). $LD_{50}$<1000 µg/mL was considered as an effective concentration of anticancer agents (Nguyen et al., 2020). Here *M. umbellatum* fruit extract prove to be with an appreciable $LD_{50}$ concentration for further anticancer studies. So it becomes the effective candidate for the next phase of the study.

The morphological variations that occur in MCF-7 cells treated with plant samples (**Plates 17-22**) form a clear indicator of toxic potential of the extracts. The aberrations like formation of membrane blebs, apoptotic bodies, nuclear condensation, membrane distortion, formation of echinoid spikes, budding, fragmentation and cell shrinkage are clearly visible in the MCF-7 cell lines. All these are the characteristic features indicating the occurrence of apoptosis. The chromatin/nuclear fragmentation associated with apoptosis could be the result of the inter-nucleosomal cleavage of DNA induced by the active phytoconstituents present in the plant extract (Liang et al., 2015). Apoptosis is characterized by a series of typical morphological features, such as fragmentation of chromatin, cell shrinkage, membrane-bound apoptotic body formation and rapid phagocytosis by neighboring cells (Saraste & Pulkki, 2000). The occurrence of the cell surface alterations is a clear indication of the apoptosis in animal cells (Thompson, 1995; Collins et al., 1997). An anticancer drug that has the potential to induce apoptosis is an effective step in the field of therapeutics. Microscopic observation of stained tissues shows the cells with unique morphological changes in the cells due to apoptosis, such as presence of condensed chromatin. In addition to that, cell surface morphology associated with apoptosis like echinoid spikes, budding, blebs *etc.* (Gown & Willingham, 2002) were also found in comparison with control. At the time of cell injury and blebs were formed that are balloon-like, quasi-spherical protrusions of the plasma membrane (Prajitha & Thoppil, 2017). Several morphological changes like blebs, budding, spikes (**Plates 17-22; 4, 6,** 7) and change in the cellular structures like condensation of nucleus

and chromatin, disappearance of chromatin mass and dissolved chromatin resulting in disappearance of nuclei (**Plates 17-22; 1, 2, 3, 5**) *etc*., were also observed.

The cytotoxic effect of the methanolic extract of *M. umbellatum* fruit was also analysed on the L929 cells in order to determine its effect on normal cells. The MTT assay was carried out on L929 (Fibroblast) cell line. The resultant effect of the sample extract on these cells can be related with that of normally dividing mammalian cells/tissues. A dose dependent viability percentage was resulted during the assay **(Figure 30)**. It ranges from 96.63 ± 2.56 to 76.72 ± 0.61% in a concentration gradient from 6.25 µg/mL to 100 µg/mL. No signs of apoptosis were noticed in the microscopic observation of cells (**Plate 23**). This indicates a mild and negligible cytotoxic effect of *M. umbellatum* fruit extract on the normal mammalian cells. The positive cytotoxicity assay in cancer cell line and negative result on normal L929 cell line enlighten the toxic potential of *M. umbellatum* fruit extract. The anticancer drug induces their toxic effects by apoptosis through the intrinsic pathway (Alshammari et al., 2020). Thus the next level of antiproliferative study was focused on the mechanisms behind the cytotoxic effect.

The genotoxicity potential of *M. umbellatum* fruit extract on MCF-7 is evaluated by the comet assay. The DNA damaging potential of plant extract was identified through this assay. So in order to find out the mechanism of toxic potential of plant extract, it's DNA damaging ability should form an effective parameter. Comet assay allows to measure single, double strand breaks, cross links and base damages (Olive & Banáth, 2006). It becomes a valuable technique for human cell biomonitoring and clinical studies. Apart from genotoxicity assays of radiations and certain chemicals, plant comet assay is also used in toxicity evaluation of nanoparticles (Santos et al., 2015). The kinetics of DNA damage recovery is a crucial part of therapeutic drug

formation. In the case of malignancy, induction of DNA damage is the vital step. The cells are treated with the chemical agents to remove all cellular proteins associated with the DNA. Then allow the DNA to unwind in alkaline/neutral conditions. The unwound DNA undergoes electrophoresis and the migration of the damaged DNA away from the nucleus was noticed. The staining with a DNA-specific fluorescent dye such as ethidium bromide is used to read the extent of damaging potential of the plant extracts.

In the assay, the DNA that is limited in the nucleus is termed as head and DNA that migrates out of the nucleus is called as tail DNA of the cells. The parameters namely comet length, tail length, tail DNA percentage, tail moment and olive tail moment were determined for the evaluation of DNA damages (**Plate 24**). The percentage of tail DNA is considered as the extent of the damages and it is the best measure of DNA damage potential (Møller et al., 2014). In the present investigation, cells treated with plant extract shows tail DNA percentage as 48.08 and that of control is 23.85% (**Figure 31 a**).The elevated level of comet length, tail length, tail moment and olive tail moment (**Figures 31 b, c, d, e)** confirms that *M. umbellatum* fruit extract has potent DNA damaging potential against MCF-7 breast cancer cell lines.

Tumour drug sensitivity test (DST) is an analytical method to check the most effective drugs that are used to treat tumours based on their sensitivity response. The molecular variances in tumour types become a barrier of lowering tumour DSTs. The reduced drug resistance and increased DSTs are efficient parameters of an anticancer drug. MTT assay is an *in vitro* DST. But it has some limitations, as it cannot distinguish apoptotic and necrotic cells. MTT assay gave an idea about the cytotoxicity level of test material and not regarding the mechanism of cell death. Comet assay gave an insight on the damaging potential of the extract. Acridine orange/ ethidium bromide (AO/EB) staining is a simple and accurate method that can be used

in tumour DSTs (Liu et al., 2015). Apoptosis and necrosis are the two basic mechanisms of cell death. In oncology research, studies are mainly focused on the genes and signals regulating the apoptosis. Apoptosis is a genetically regulated mechanism of cell death. It maintains normal homeostasis through the removal of damaged, physiologically redundant and abnormal cells (Carneiro & El-Deiry, 2020). The death of cells through disease or injury is termed as necrosis. It is caused by the factors outside the body and is an uncontrolled mechanism of cell death (Kanduc et al., 2002).

AO/EB staining was employed to analyze the induction of apoptotic nuclear damage in MCF-7 cell lines using the *M. umbellatum* fruit extract. In this analysis, the mechanism behind the cytotoxic effect of extracts can be recognised *ie.*, either apoptosis or necrosis. Apoptosis is associated with cell membrane damages and it has differential staining capability of the cells. In AO/EB staining, early and late apoptotic cells as well as necrotic cells are differently stained. The early apoptotic cells were noticed with greenish yellow nuclei, late apoptotic cells indicated condensed orange-red nuclei, while dead necrotic cells depicted red nuclei. AO can stain the normal cell membrane and emit green fluorescence in live cells whereas EB imparts stains on cells that had lost membrane integrity with orange - red coloured cells (Ribble et al., 2005). In the present study, it was clearly noticed that the untreated cells are observed as green fluorescence with normal nuclear morphology, where as *M. umbellatum* fruit extract treated cells appeared as orange coloured bodies with membrane damages (**Plate 25).** The double staining method unveils that the cell death induced by the plant extract is through the apoptotic mechanism.

Apoptotic effect of plant extract on cell cycle progression was analyzed through cell cycle analysis by using flow cytometry. In this study, MCF-7 cells were treated with *M. umbellatum* fruit extract (78.48 ± 0.8

µg/mL) to evaluate the cell cycle progression. The deregulation of cell cycle was frequently associated with cancer. The uncontrolled proliferation interrupts the cell cycle progression. So cell cycle regulators have importance in cancer therapy. Cyclin-dependent kinases with transcriptional functions are effectively targeted in BRCA1/BRCA2-mutant tumours (Otto & Sicinski, 2017). Mammalian cell cycle is a regulated process that gets progressed through G0/G1, S, G2 and M phases. The cell cycle progression is regulated by certain cyclins and cyclin dependent kinases (CDKs). The over expression of cell cycle proteins are allied with the cancer induction. Cyclin D, E, CDK4, CDK6 and CDK2 are overwhelmed in uncontrolled cell division. They are the key regulators of G0/G1 phase of the cell cycle (Vermeulen et al., 2003). The cell cycle progression from G0/G1 is initiated by serine/threonine kinases like, CDK4 and CDK6. The related action of cyclin D will enhance the activity of CDK4 and CDK6. The cyclin D/CDK4/6 activity will be hindered by the action of p21 and p27, the inhibitors of CDKs. Thus they prevent the G1-S transition. In breast cancer, the overexpression of cyclin D1 was noticed. The lack of cyclin D1 protein will prevent the mammary gland formation induced by certain oncogenes (Bowe et al., 2002). Several examples of cell cycle regulation through the inhibition of CDKs are reported. A mice bearing $Erbb2^{V664E}$ mammary tumour was triggered by inhibition of CDK4 and CDK6 kinases (Choi et al., 2012). The inhibition of CDK4 and CDK6 in mice is associated with tumor - specific apoptosis rather than senescence (Sawai et al., 2012). In the present study, G0/G1phase shows the highest cell count and subsequent reduction was observed in following phases (**Figure 34**). DNA count as well as population count of cells treated with the plant extract get decreased from G0 to M phases (**Figures 32 & 33**). So these results clearly point out that the cell cycle arrest occur at G0/G1 phase and the diminishing progression of cell cycle is due to the apoptotic mechanism induced by the plant extract.

The action of G0/G1 cell cycle regulators can prevent the proliferation of MCF-7 cells. Thus the decrease in cell counts as well as the population counts in successive phases of cell cycle is clearly pointing towards the cell cycle regulation capability of the plant extract. Liu et al. (2018) describes that the DNA count increased in G0/G1 phase is due to the DNA fragmentation associated with the MCF-7 cells. The CDKs activities become denied due to the presence of plant extract as compared to the negative control. So it might be useful in cancer therapy. CDKs are the target in various strategies of cancer treatment. Palbociclib, a CDK4/CDK6-selective inhibitor used in breast cancer treatment was the first successful clinical drug in this field. Similarly, ribociclib and abemaciclib are other two targeted CDK4/6 inhibitors used in breast cancer in combination with endocrine therapy. Palbociclib and letrozole are used in patients with advanced ER+ HER2- breast cancer (Lynce et al., 2018).

In MTT assay, comet assay, double staining test and cell cycle analysis, *M umbellatum* fruit extract shows a potent anticancerous effect. It has proved to be cytotoxic, DNA damaging and an executor of apoptosis against the breast cancer cell lines. The gene level approaches in cancer therapy become widely acceptable. The genetic divergence in cancer type and heterogeneity in populations are leads to new strategies investigated in the context of genetic pathways. Several case studies propose that genetic level studies are a more reliable method to convey effective medicine to cancer patients (Cheng et al., 2013). The gene expression studies gave an initiative for the disease management in cancer (Dopazo et al., 2001). In breast cancer studies, the action of p53 is widely studied. It plays multiple roles *ie.,* act as a transcriptional regulator, cell cycle arrestor and apoptosis inducer. An anticancer drug induces their effect through the genomic damages and facilitates apoptosis. p53 is an excellent executor of genomic damages and apoptosis. The action of p53 is regulated by another transcription activator

p21, and they merely activate anticancerous mechanism. p21 can induce the cell cycle arrest because it act as a CDK inhibitor (Elledge & Allred, 1998). Besides breast cancer, in many tumors p21 (WAF1/CIP1) is associated with p53 expression. The subcellular localization of p21 is a hall mark in breast cancer (Winters et al., 2001). In this study, the mRNA specific for the p21 and p53 along with that of a house keeping gene (β-actin) were isolated for which cDNA were prepared, amplified and examined after electrophoresis.

In the present investigation, MCF-7 cells with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract significantly induced an up-regulation in the expression of p53. The p53 gene can induce the expression of p21 gene (**Figure 35**). The vibrant action of p53 and p21 in association with cancer is widely discussed. p53 dependent p21 action in tumour cells are a prognostic way of apoptosis. p21 is a key regulator of apoptosis and DNA damages. The tumour inhibition of p21 is through the modulatory action of cyclins, transcription factors and proliferation of cell nuclear antigen (Shamloo & Usluer, 2019). The over expression of p21 that contributes towards tumour suppression through apoptosis in mice models are reported by Elliott et al. (2002). Recently the gene editing on p21 gene had become helpful in suppressing tumorigenesis. In the case of p21, deficient mice models with mice mammary tumour virus (MMTV) ras and myc, shows differences in their apoptosis levels. It is clearly indicated that p21 has a crucial role in apoptotic induction (Bearss et al., 2002). The action of p53 for maintaining the normal homeostasis of cells through Waf-1-mediated induction of G1 arrest or *Bax*-mediated apoptosis was reported by Keshava et al. (2002). The expression fold analysis also proves the prominent expression of apoptotic genes. It is the measurement of expression of genes. Here the expression fold change of p53 is 1.86 over the control and that of p21 gene is 1.52 (**Figures 36 a & b**). The positive value of expression fold changes indicates the up regulated mechanism of gene expression. In the present

study, the expression fold change clearly indicates that the p21 and p53 are key regulators in antiproliferative mechanism. The antiproliferative activity of *M. umbellatum* fruit extract significantly proves to be useful towards drug preparation. The phytochemical analysis reveals that *Memecylon species* has a diverse array of phytoconstituents present in it. In the case of *M. umbellatum* fruit extract, the presence of alkaloids, terpenoids, fatty acids *etc*., are noticed. Phytochemicals are the backbone of bioactivity. In cancer studies, phytochemicals are an effective target of clinical trials (Choudhari et al., 2020). The apoptotic signs noted in MTT assay, double staining test, DNA damaging potential and cell cycle arrest proved to be the satisfactory leads in the present study. Thus in the present investigation, cell line studies, comet assay, AO/EB staining, cell cycle analysis and gene expression studies evidently prove that *M. umbellatum* fruit extract has potent antiproliferative activity.

## d) GREEN SYNTHESIS OF SILVER NANOPARTICLES

The present study investigates the green synthesis of silver nanoparticles from *Memecylon* fruit and leaf extract. The physical and chemical approaches are the common methods of metallic nanoparticle production. The evaporation-condensation and laser ablation are the most important physical approaches. The most common chemical approaches for the synthesis of silver NPs are chemical reduction by organic and inorganic reducing agents, UV-initiated photoreduction, microemulsion techniques and electrochemical synthetic methods (Iravani et al., 2014). The chemical and physical methods of NPs production have many limitations. They are toxic, energy consuming, expensive and are not suitable for biological applications. The emergence of a new area in green synthesis of NPs had been nurtured before few decades (Ghaffari-Moghaddam et al., 2014). The biogenic syntheses of nanoparticles are pure, non-toxic, cost effective, ecofriendly and

have better bioactivity. Silver is the common metal used for the synthesis of nanoparticle. Silver is a soft, white, lustrous transition metal possessing high electrical and thermal conductivity. Silver nanoparticles have received a great attention due to their physical, chemical, and biological properties that include catalytic activity and bactericidal effects (Firdhouse & Lalitha, 2015). The antibacterial activity of silver nanoparticle is extensively studied *ie.*, it is active against *Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa* and *Salmonella typhi (*Morones et al., 2005). It has exhibited a wide range of activity against both gram-positive and gram-negative multidrug resistant bacteria (Qais et al., 2019). Silver nanoparticles synthesized from *Abutilon indicum* showed a dose-dependent anti-cancer activity against human colon cancer at a very low concentration (Ahmed & Mustafa, 2019). The antioxidant, cytotoxic and anti-inflammatory activity of SNPs is already reported in several plants (Khatoon et al., 2018; Rao et al., 2018; Gondwal & Joshi, 2018).

The reducing capacity of plant extract is exploited in the biogenic synthesis of SNPs. The plant extracts are a rich source of many phytoconstituents, which act as stabilizing as well as capping agents for the synthesis of nanoparticles. The present study reveals the reducing power of *Memecylon* species. *M. grande* leaf and fruit extracts when treated with silver nitrate solution results in the formation of a brown colour solution, which indicates the presence of SNPs. *M. grande* fruit extract shows a brown colour change in the reaction tube whereas, *M. grande* leaf extract has a dark brown coloration in the reaction tube with an immediate reaction (**Plate 26 g, h**). The difference in colour tinge may be related to the variation in the intensity of nanoparticle synthesis (Bhagyanathan & Thoppil, 2018). *M. randerianum* leaf extract possess a pale yellowish brown colour and its fruit extract is having a dark brown coloration in the reaction tube (**Plates 26 i; 27 g)**. The reaction mixture that contains *M. umbellatum* leaf extract and silver nitrate solution

produce a yellow coloured solution. The reducing capacity of *M. umbellatum* fruit extract creates a nanoparticle solution with a brown colour (**Plate 27 h, i**). The colour changes in the reaction tube that contain SNPs are also due to the Surface Plasmon Resonance (SPR). SPR is a versatile technique for biological analysis and depends on the optical properties of the metal layer (Sadrolhosseini et al., 2012). The presence of different phytochemicals responsible for the reduction, stabilization and capping of silver nanoparticles is confirmed through UV-VIS spectroscopy.

UV-Vis spectroscopy (UV-Vis) is another relatively facile and low-cost characterization method of nanoparticles. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. NPs have optical properties that are sensitive to size, shape, concentration and agglomeration state, which makes UV-Vis spectroscopy an important tool for characterizing nanoparticles. Gold, silver and copper nanostructure sols exhibit characteristic UV-Vis extinction spectra due to the existence of a LSPR (Localized Surface Plasmon Resonance) signal in the visible part of the spectrum. Nano metals showed conspicuous spectral characteristics according to the surface plasmon resonance (SPR). Mutual vibrations of free electron resonance with light waves can influence the size and shape of the synthesized NPs. Consequently, the broadening of the SPR peak width is considered as an agreeable detector of the nano metal size and its polydispersity (Mukherjee et al., 2001; Behzadi et al., 2015). The synthesized nanoparticle of *M. grande* fruit extract subjected to UV-Vis spectroscopic analysis shows the maximum absorption peak at 434 nm. *M. grande* leaf extract possess a maximum absorption peak at 440 nm. The SNPs synthesized by both plant extracts show a broad peak area with an absorption values of 0.7 and 1.28 respectively (**Figure 37**). The presence of a non specific peak is detected at 418 nm in *M. randerianum* leaf extract with absorption value of 0.3. The fruit extract of *M. randerianum* exhibit a specific

broad peak at 432 nm with 1.71 absorption value. *M. umbellatum* leaf extract possess a non specific peak at 426 nm with 1.2 absorption value. A broad peak area at 468 nm with an absorption value of 0.33 was observed in *M. umbellatum* fruit extract (**Figure 37**). The range of 380–470 nm is the characteristic λ max for AgNPs, so the peaks obtained from UV-Vis spectra confirm the presence of silver nanoparticles (Kumar et al., 2016).

The intensity of the peak area is directly related to the concentration or the size of the nanoparticle synthesized in the sample solution. *M. umbellatum* and *M. randerianum* leaf extracts possess few weak signals, which indicate that the nanoparticle size become comparatively less as evaluated with other extracts. A narrow peak of absorption also occurs in 263 nm with an absorption value of 0.93 and a narrow peak was resulted in the 238 nm with absorption value of 0.92 respectively for these two leaf extracts.

According to Mie theory, as the particle size decreases, a shift in peaks to lower wave length ranges may occur (Alvarez et al., 1997). The size and shape of synthesized nanoparticles are determined by Scanning Electron Microscopic analysis (SEM). In the present study, *M. grande* leaf extract possess 20-30 nm sized nanoparticles and nanoparticle synthesized from *M. grande* fruit extract is 26-44 nm size. The shape of the nanoparticles synthesized by the *M. grande* fruit extract is cubical (**Plate 29**) and that of *M. grande* leaf extract is having a spherical shape (**Plate 28**). A perfect spherical shape with 20-32 nm sized nanoparticle was formed in the *M. randerianum* leaf extract mediated silver nanoparticle synthesis. Similarly a uniform size and morphology of nanoparticles was exhibited by *M. randerianum* fruit extract also. They have spherical shape with 20-28 nm size (**Plates 30, 31**). *M. umbellatum* leaf extract mediated silver nanoparticles synthesis contributes somewhat spherical shaped particles with 22-33 nm size. The fruit extract of *M. umbellatum* possess almost spherical shaped silver nanoparticles with 26-

35 nm size (**Plates 32, 33**). From, the UV-Vis spectroscopic results, the particle size of the leaf extract of *Memecylon* species was found to be comparatively smaller as compared to their corresponding fruit extracts.

There is a direct relationship between the size and shape of the nanoparticle on its biological activity. The size and shape of silver nanoparticle vary in different plant extracts (Hamouda et al., 2019). The spherical shaped nanoparticles have a high surface area to volume ratio. This property enhances their antimicrobial activity (Kumar et al., 2015). The smaller nanoparticle has more penetration power, whereas too smaller nanoparticle can create toxic effects on the cell as compared to larger sized nanoparticles. So the nanoparticles have a size-specific biological activity (Wang et al., 2017).

There are several factors delimiting the application of bioactive phytoconstituents. The usage of these phytochemicals directly in medicines is inhibited by their solubility rate, stability and bioavailability. The application of nanotechnology can overcome these difficulties. Phytochemical oral delivery system (PODS), which is a new approach can unload the phytochemical filled nanoparticles on the target system without any stability and solubility problems. The proper designing of PODS can enhance the phytochemical potential of commercial products, *ie.*, in foods, supplements and pharmaceuticals (McClements, 2020). The bioactive proteins and peptides are denatured in commercial products or gastrointestinal tract, because of their poor stability. So in order to overcome these issues, the nanoparticles with encapsulated form of bioactive proteins and peptide products are used (McClements, 2018). The present investigation thus concludes that *Memecylon* is an important candidate in green nanotechnology. The nanoparticles biosynthesized from the species of *Memecylon* can be used as the lead component in biomedical field.

In conclusion, the present study gave an insight on the pharmacognostic identification, phytochemical and bioactivity validation of selected *Memecylon* species. It strongly suggests that *Memecylon* species form a promising candidate in the pharmaceutical field. The findings of the study are summarized and the conclusions drawn were presented in the next chapter.

# CHAPTER 6 SUMMARY AND CONCLUSIONS

Plants are being used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternate source of medicine to maintain health and improve the quality of life. The present study deals with the exploration of *M. grande, M. umbellatum* and *M. randerianum* fruit and leaf extracts. The objectives of the present study are summarized as: 1) Pharmacognostic profiling 2) Phytochemical characterization and 3) Bioactivity analysis.

Herbal development occurs through the various step wise analytical processes. Pharmacognosy is one among the preliminary steps in it. It includes the analysis of functional purity of the plant sample. The herbal medicines often suffer with quality controversies because of similar species or varieties that are used as adulterants. Pharmacognostic analyses can rectify the taxonomic misinterpretation in the identification process. On the basis of botanical origin of selected species, phytoconstituents of the plant samples were analyzed through the preliminary tests and chromatographic techniques. The presence of diverse chemical constituents leads us to investigate the bioactivity potential of *Memecylon* species.

Major findings from the present study are summarized below:

**1) Pharmacognostic profiling**

Powder microscopy, SEM-EDX analysis and ICP-MS analyses gave a vivid picture of the pharmacognostic profile of the selected *Memecylon* species. In powder microscopic analysis, fruit and leaf extracts of *M. grande*, *M. randerianum* and *M. umbellatum* shows the presence of characteristic elements.

The powder microscopic analysis confirms that the botanical origin of these plant samples is pure. So this result can be used as a future reference for the identification of *Memecylon* species. Scanning electron microscopic analysis of fruit endocarp and entire seed of selected *Memecylon* fruits show a distinct morphological pattern. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships. *M. grande*, fruits show colliculate pattern in endocarp and seed surface possesses tuberculate pattern. In *M. randerianum* fruit, endocarp is with ruminate reticulate type pattern and seed surface with reticulate pattern. *M. umbellatum* fruit endocarp possesses a smoothened pattern and its seed surface shows a wrinkled pattern of appearance. EDX spectra of the selected *Memecylon* species reveal the elemental composition at the microscopic level. Nitrogen was found to be the prominent compound detected in *Memecylon* species. Phosphorus, potassium, iron, magnesium, cobalt and sodium were also noticed through EDX analysis. In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental composition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was carried out. The presence of aluminium, arsenic, cobalt, strontium, selenium, zinc, chromium, copper, molybdenum, nickel, barium and manganese were noticed. These findings suggest that *Memecylon* fruits are a reservoir of essential elements and it can be exploited in the pharmaceutical or nutritional field. Thus the pharmacognostic profiling offers future reference parameters for *Memecylon* identification. The herbal parts with consistent quality, without adulterants or invasive free particles make their performance in a proper way. So the functional purity demands the pharmaceutical potential. The powder microscopy, SEM-EDX analysis and ICP-MS analysis thus validates the drug making capability of the selected *Memecylon* species.

**2) Phytochemical characterization**

The preliminary phytochemical analysis is carried out to determine the presence or absence of phytochemicals in the samples. The methanolic extracts of leaf and fruit extracts of *Memecylon* spp. were subjected to qualitative and quantitative analysis. In qualitative phytochemical analysis, the presence of alkaloids, flavonoids, phenolics, steroids and tannins were confirmed in all the selected species. The presence of glycosides is revealed in the leaf and fruit samples of *M. umbellatum.* The complete absence of resins and anthraquinones was confirmed in all the selected species. The quantitative determinations of alkaloids, flavonoids, phenolics and terpenoids were conducted and found that all the selected extracts have considerable amounts of potential secondary metabolites. *M. grande* fruit extract possesses highest amount of alkaloids, phenolics and terpenoids. The amount of flavonoids was also found to be highest in *M. grande* leaf extract.

The identification of volatile phytoconstituents in selected *Memecylon* species was done through the GC/MS analysis. A total of 83 compounds were detected in the methanolic extract of selected species. The identified compounds belong to the classes of terpenoids, phenolics, fatty acids, fatty acid esters, steroids *etc.* The GC/MS analysis of *M. grande* leaf extract reveals the presence of 17 compounds. The major constituents were oleic acid, methyl oleate and palmitic acid. The presence of fatty acid esters are in significant amount also. Similarly 17 compounds were noticed in *M. grande* fruit extract. The fatty acid esters are found to be in highest amount, in which methyl elaidate was prominent. A total of 26 compounds were detected in the methanolic leaf extract of *M. randerianum.* Palmitic acid, agathenic acid, squalene, phytol and lupeol are the major ones. Terpenes were the predominant class of compounds present in the leaf extract. Friedlein and stigmast-5-en-3-ol were the terpenoid group of compounds observed in

highest amount in *M. randerianum* fruit extract. The presence of fatty acids and fatty acid methyl esters are also confirmed in a total of 14 compounds present in *M. randerianum* fruit extracts. *M. umbellatum* leaf extract encompasses 32 compounds. It includes phenols, terpenoids, steroids and organic compounds. A steroid compound, α-phytosterol occurred in highest amount. The fruit extract of *M. umbellatum* possesses 12 bioactive phytoconstituents. The presence of an alkaloid, hordenine was found to be prominent. The presence of carbohydrate lactones, fatty acid derivatives and fatty acid methyl esters are also noticed.

Non-volatile compositions of selected *Memecylon* species were identified through the HR-LC/MS analysis. The identified 48 compounds belong to the classes like terpenoids, steroids, fatty acids, biopeptides, hydroxyl benzoquinones, glycosides, alkaloids, esters, carotenes *etc*. A terpenoid compound swietenine was found to be common in *M. grande* leaf, fruit and *M. randerianum* fruit extracts. Lupanyl acid, aesculin, C16 sphinganine, 3-dehydro-6-deoxoteasterone and biopeptides are the major constituents of *M. grande* leaf extract. Tamarixetin, rescinnamine, madecassic acid, campestanol, khayanthone and carotene were detected in *M. grande* fruit extract. The presence of bergenin and 9,12,13-trihydroxy-10,15-octadecadienoic acid are noticed in *M. randerianum* leaf extract. Violastyrene, gibberellin A8-catabolite, rescinnamine, β-erythroidine, glycerol palmitate, 6-deoxocastasterone and cosmosiin hexaacetate were found in *M. randerianum* fruit extract. *M. umbellatum* fruit extract, possesses a diverse array of non-volatile chemical constituents, a total of 16 compounds were noticed in it. Whereas, *M. umbellatum* leaf extract possesses a limited number of compounds. Protoveratrine A, embelin, amygdalin and stigmasta-7, 22 E, 25-trien-3beta-ol are the major compounds in *M. umbellatum* fruit extract. While comparing the non-volatile composition of *Memecylon* species, fruit extract shows much more diverse phytoconstituents.

**3) Bioactivity analysis**

Free radical scavenging activity of the selected *Memecylon* species was analyzed through DPPH, hydroxyl, nitric oxide and superoxide radical scavenging assays. In DPPH assay, the highest scavenging activity was shown by 200 µg/mL concentration of *M. grande* fruit extract. *M. grande* fruit extract exhibit an inhibition percentage of 75.77 ± 0.01. This is followed by *M. umbellatum* fruit extract. In hydroxyl radical assays, *M. grande* fruit extract shows the highest activity *ie.,* 61.69 ± 0.52%, followed by *M. umbellatum* fruit extract with 53.46 ± 1.89%. Here the inhibitory concentration of the standard compound was higher as compared to the effective plant extract. So *M. grande* fruit extract is considered as a good hydroxyl radical scavenger. Nitric oxide assay also shows similar trends of scavenging potential. In superoxide radical scavenging assay, *M. grande* fruit extract shows the highest scavenging activity of 72.17 ± 0.02%. The lowest activity was shown by *M. randerianum* leaf extract with 47.36 ± 0.01%. In all the antioxidant assays, the selected plant species follows similar trends of activity. *M. grande* fruit extract has the highest scavenging potential and lowest in *M. grande* leaf extract. An exception was noted in superoxide radical assay, where lowest activity was shown by the *M. randerianum* leaf extract. *M. randerianum* and *M. umbellatum* exhibits a moderate range of activity in all the assays. The free radical scavenging activity of fruit extracts become more as compared with the leaf extracts. It might be due to the diverse phytochemical composition of the plant parts.

The cytotoxic potential of the selected *Memecylon* species is analyzed by using *A. cepa* root tip meristem. The toxic potential of plant extracts were analyzed through the assessment of mitotic index and aberration percentage. Dose dependent mitotic index and abnormality percentage were resulted, and found that time has no role in the cytotoxic effect of the plant extracts. The

decrease in mitotic index is correlated with increasing concentration of plant extracts and the abnormality percentage increases with increasing concentrations. Several chromosomal aberrations are resulted during the cytotoxic assay. Stickiness, pulverization, chromosomal clumping, chromosome gaps, nuclear lesions, erosions, stellate chromosomes, lagging chromosomes, exposure of chromosome scaffold *etc*., are few of them. These results are pointing to the fact that *Memecylon* extracts has potential cytotoxic role and antiproliferative efficacy revealed by potential mitotic inhibition.

Antiproliferative activity of *Memecylon* was tested against human breast cancer cell line MCF-7. The selected concentration of six plant extracts are 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL and time period for the experiment was set for 24 hrs. The direct microscopic observation reveals the toxic potential of plant extracts. The aberrations like formation of membrane blebs, apoptotic bodies, nuclear condensation, formation of echinoid spikes, budding, fragmentation and cell shrinkage are clearly visible in the MCF-7 cell lines, which are indicating the hallmarks of cell death. A dose dependent cytotoxic effect was observed and the highest antiproliferative activity was shown by *M. umbellatum* fruit extract with 76.8 ± 2.75%. The overall results thus point out that the fruit extracts of selected samples show highest antiproliferative potential as compared to their corresponding leaf extracts. The $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract was selected for further anticancerous studies.

Cytotoxic assays using MCF-7 breast cancer cell lines and *A. cepa* assay reveal the toxic potential of plant extracts. In order to find out the non-toxic effect of the plant extract on normal cells, MTT assay was carried out on L929 (Fibroblast) cell line. A dose dependent viability percentage was resulted during the assay. It ranges from 96.63 ± 2.56 to 76.72 ± 0.61% in a

concentration gradient from 6.25 µg/mL to 100 µg/mL. The cellular damages are very fewer in normal L929 cells. The DNA damaging potential of *M. umbellatum* fruit extract was analyzed by performing comet assay. In this assay, MCF-7 cells are treated with 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract. The parameters namely comet length, tail length, tail DNA percentage, tail moment and olive tail moment were determined for the evaluation of DNA damages. The elevated levels of parameters are observed through the assay. The DNA percentage in tail was found to be much higher (48.08) than that of the control (23.85). It is linearly related to the DNA breaking potential of the plant extract. The intensity of the tail increases as the damage is enhanced. The elevated levels of olive tail moment and tail movement were noticed during comet assay. So comet assay confirms the DNA damaging potential of the plant extracts of *Memecylon*.

The cytotoxic assay in *A. cepa*, MTT assay and comet assay, reveals the cell damaging potential of the selected active plant extract of *M. umbellatum*. The cell damaging potential or cell death inducing capability of the plant extract is further analyzed through the double staining method. It is a method that unveils the mechanisms behind the cellular damages/cell death. A combination of acridine orange/ethidium bromide stains are eluted on the MCF-7 cell lines, which are treated with $LD_{50}$ concentration of the most active plant extract *ie.*, 78.48 ± 0.8 µg/mL of *M. umbellatum* fruit extract. This staining method enabled to visualize the apoptotic or necrotic cells from the normal cells. The plant extract treated cells have lost their viability and membrane integrity. They are observed as orange coloured bodies. The double staining method reveals that the cell death induced by the plant extract is through the apoptotic mechanism.

The apoptotic effect of the plant extract on cell cycle progression was analyzed through cell cycle analysis by using flow cytometry. Here MCF-7

cells were treated with the active *M. umbellatum* fruit extract. G0/G1 phase of the cell cycle shows the highest amount of DNA content. There is a subsequent reduction of DNA content, which was resulted in S and G2/M phases. In the case of cell population count, there is a scatter in untreated cells, while the treated cells show aggregation. That means that the progression of cell cycle was arrested in a particular phase of cell cycle. The percentage of cell count in each phase of the cell cycle unveils the retardation of cell cycle progression. The G0/G1phase shows the highest cell count and subsequent reduction was observed in following phases. So these results clearly point out that the cell cycle arrest occurs at G0/G1 phase and the diminishing count during the progression of cell cycle is due to the apoptotic mechanism induced by the plant extract.

To substantiate the underlying mechanism of antiproliferative activity exhibited by the fruit extract of *M. umbellatum* on MCF-7 cells, the expression changes of genes which are known to be involved in cell cycle arrest and induction of apoptosis were examined. The expression pattern of p53 and p21 were studied by RT-qPCR and the data were analysed according to $\Delta\Delta C_t$ method. p53 and p21 are genes that regulate many downstream genes involved in the induction of cell cycle arrest, DNA repair and apoptosis. β-actin, a house keeping gene was used as the control. The intense fluorescence in gel electrophoresis has clearly indicated that the treatment of MCF-7 cells with 78.48 ± 0.8 μg/mL of extract significantly induced an up-regulation in the expression of p53. The p53 gene can induce the expression of p21 gene. The expression fold analysis also proves the prominent expression of apoptotic genes induced by the active extract of *M. umbellatum*.

The present study also highlights the evaluation of silver nanoparticles biosynthesized from selected *Memecylon* species. Green synthesis of nanoparticles become a safe platform because they are free from toxic

chemicals as well as contains natural capping agents. The silver nanoparticles were characterized through UV-Vis spectrophotometer and SEM analysis. The reduction of silver nitrate solution into silver nanoparticles after treating with plant extracts is analyzed through the colour changes, surface plasmon resonance and shape of the nanoparticles. The reduction of silver nitrate solution into silver nanoparticles by the action of plant extract has resulted in the colour changes of the reaction tubes. The selected plant extracts show a yellow to brown colouration in the reaction tubes. The synthesized nanoparticles of selected *Memecylon* species are subjected to UV-Vis spectroscopy in a wavelength range of 200-700 nm. The synthesized nanoparticles of *M. grande* leaf extract subjected to UV-Vis spectroscopic analysis show the maximum absorption peak at 440 nm. *M. grande* fruit extract possess a maximum absorption peak at 434 nm and similarly *M. randerianum* leaf and fruit extracts at 418 nm and 432 nm respectively. In the case of *M. umbellatum* leaf extract, a narrow peak was resulted at 426 nm and *M. umbellatum* fruit extract shows a peak at 468 nm. The range of 380-470 nm is the characteristic λ max for AgNPs, so the peaks obtained from UV-Vis spectra confirm the presence of silver nanoparticles.

From the SEM analysis, the nanoparticle size of *M. grande* leaf extract was found to be 20-30 nm and *M. grande* fruit extract possess 26-44 nm sized particles. The shape of the nanoparticles synthesized by *M. grande* leaf extract is spherical and that of *M. grande* fruit extract is with a cubical shape. A perfect spherical shape with 20-32 nm sized nanoparticles was formed in the *M. randerianum* leaf extract mediated silver nanoparticle synthesis. Similarly a uniform size and morphology was exhibited by the nanoparticles biosynthesized by *M. randerianum* fruit extract also. They have spherical shape and are 20-28 nm in size. *M. umbellatum* leaf extract mediated silver nanoparticle synthesis contributes somewhat spherical shaped particle with 22-33 nm size. The fruit extract of *M. umbellatum* possess almost spherical

shaped silver nanoparticles with 26-35 nm size. By substantiating the UV-Vis spectroscopic results, the particle size of the leaf extract of *Memecylon* species is comparatively smaller as compared to their corresponding fruit extracts.

The present study thus highlights that *Memecylon* is a suitable candidate in pharmaceutical field. The first phase of the study gave standard pharmacognostic profiles of *Memecylon* spp. as reference tools for future perspectives. The wide spectra of phytochemicals and their potential bioactivities together with nanoparticle synthesis from selected plant extract point towards their efficiency as potential drugs. The selected plant extracts shows better performance in all bioactivity studies. The synergistic action of phytochemicals present in the plant extract contributes towards the cytotoxic, antioxidant and anticancerous activity. The most effective extract selected from the six plant extracts studied was *M. umbellatum* fruit extract. The major findings in the present study open a gateway for the selection of an amenable source of natural medicine.

**Deliverables**

- Pharmacognostic profiling of *Memecylon* by using powder microscopy, SEM-EDX and ICP-MS analysis was reported for the first time.
- Immense source of potential phytoconstituents were identified and revealed as phytochemical profile from the selected *Memecylon* species through GC-MS and HR-LC/MS analysis.
- Potential free radical scavenging activity was revealed in selected *Memecylon* species.

- Cytotoxic activity revealed using *A. cepa* assay proves to be a leading step towards further antiproliferative studies.
- The antiproliferative activity against MCF-7 cell lines, cell cycle analysis and gene expression studies enlighten the anticancer potential of *Memecylon* species.
- A new approach on green synthesis of silver nanoparticles from *Memecylon* species was established.

**Future perspectives**

- Isolation of bioactive components from *Memecylon* species.
- *In- vivo* studies on animal models for detailed exploration of antiproliferative mechanism.
- Biomedical exploration of biosynthesized nanoparticles.

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# APPENDICES

**Wagner's reagent** **Appendix 1**

Iodine : 1.27g

KI : 2g

Dissolve the above chemicals in 5 mL $H_2SO_4$ and make up to 100mL.

**Phosphate buffered saline (PBS)** **Appendix 2**

NaCl : 8g

KCl : 0.2g

$Na_2HPO_4$ : 1.44g

$KH_2PO_4$ : 0.2g

Dissolve in 1 L double distilled water and adjust pH to 7.4.

**Griess Reagent** **Appendix 3**

Naphthylethylenediamine HCl : 0.1% in distilled water

Sulfanilmide : 1% in 5% $H_3PO_4$

Mix both in 1:1 ratio

**Modified Carnoy's fluid** **Appendix 4**

Acetic acid : 10 mL

Ethanol : 30 mL

**Acetocarmine** **Appendix 5**

Carmine : 2g

Acetic acid : 100 mL of 45% acetic acid

The solution is heated to dissolve carmine and is filtered to remove undissolved stain.

**DMEM (Dulbecco's Modified Eagle's) medium** **Appendix 6**

Sodium bicarbonate : 1.85g

HEPES : 2.95g

DMEM powder : 1 packet

Distilled water : 1L

Vacuum sterilized and stored at 4°C

**Lysing solution (1000 mL)** **Appendix 7**

2.5 M NaOH : 146.1 g

EDTA : 37.2 g (for 100 mM solution)

Trizma base : 1.2 g (10 mM)

1% SDS : 10 g

Add ingredients to about 700 mL of distilled water and stir the mixture. Add 8g NaOH and allow the mixture to dissolve for about 20 min and adjust the pH to 10 using concentrated HCl or NaOH and store at room temperature. To this mixture, 10% DMSO and 1% Triton X 100 are added prior to use.

**Electrophoresis buffer** **Appendix 8**

Stock solutions:

10 N NaOH : 200 g/500 mL distilled water

200 mM EDTA : 14. 89 g/200 mL distilled water

pH : 13

Store the stock solutions at room temperature.

For 1X Buffer (make fresh buffer before each electrophoresis run) add 30 mL NaOH and EDTA, per 1L and mix well. Ensure pH as > 13 prior to use.

**Neutralization buffer** **Appendix 9**

0.4 M Tris : 48.5 g

The above quantity of Tris is added to 800 mL distilled water and pH adjusted to 7.5 with concentrated HCl. The final volume is made to 1000 mL with distilled water and stored at room temperature.

**Ethidium bromide** **Appendix 10**

Ethidium bromide : 20 µg/mL

Add 10 mg to 50 mL distilled water and store at room temperature (10X).

For making 1X stock, mix 1 mL with 9 mL of distilled water. Handle ethidium bromide with caution as it is a known carcinogen.

**TE (Tris-EDTA) buffer** **Appendix 11**

Tris HCl : 10 mM, pH 8

EDTA : 0.1 mM, pH 8

**Research publications**

1. **P. R. Ramya Sree.,** & Thoppil J. E. **(2018).** C-mitotic potential of aqueous leaf extract of *Memecylon randerianum* S. M. & M. R. Almeida. - a promising natural colchicine analog. *International Research Journal of Pharmacy, 9* (11), 115-118.

2. **P. R. Ramya Sree.,** & Thoppil J. E. **(2021).** Comparative seed morphology, pharmacognostic, phytochemical and antioxidant potential of *Memecylon* L. fruits. *Turkish Journal of Pharmaceutical Science*, *18*(2), 213-222. **IF-1.1**

3. **P. R. Ramya Sree.,** & Thoppil J. E. **(2021).** An overview on breast cancer genetics and recent innovations: Literature survey. Breast Disease, *40*(3), 1-12. **IF-1.6**

4. **BOOK: P. R. Ramya Sree**., & Thoppil J. E. **(2019).** Exploration of *Memecylon randerianum* S. M. & A. R. Almeida. Lambert publishers, Germany, ISBN: 978-3-659-54946-5.

5. **BOOK CHAPTER**: **P. R. Ramya Sree.,** & Thoppil J. E. **(2020).** Ecological importance of Melastomataceae. In A. K. Sarkar (Ed.), *Organism and environment* (pp. 219-224). New Delhi: Educreation publishing, ISBN-978-93-89808-99-5.

**Paper presentations**

1. **P. R. Ramya Sree.,** & Thoppil J. E. (**2017).** "Phytochemical screening and Cytotoxic potential of *Memecylon umbellatum* Burm. f. fruit and leaf- A potential medicinal plant of central Western Ghats" in third International conference on frontiers of mass Spectrometry, School of Environmental Science & Inter University Instrumentation Centre, Mahatma Gandhi University, Kottayam, Kerala. (Poster presentation)

2. **P. R. Ramya Sree.,** & Thoppil J. E. (**2018**). "Evaluation of phytoconstituents and bioactivity screening of the methanolic leaf extract of *Memecylon umbellatum* Burm." in International conference

on phytomedicine, Dept. of Botany, Bharathiyar University, Coimbatore. (Oral presentation)

3. **P. R. Ramya Sree.,** & Thoppil J. E. (**2018)**. "Cytotoxic and apoptotic activities of extract of *Memecylon umbellatum* L." in International biodiversity congress, Forest Research Institute, Dehradun. (Poster presentation)

4. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. "Phytochemical screening and cytotoxic potential of *Memecylon randerianum* S. M. and M. R Almeida" in MESMAC International conference on People First? Man, Machine, Milieu. MES College Mampad, Malappuram. (Oral presentation)

5. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. *Memecylon randerianum* SM & MR almeida - a promising natural colchicine analog." In Recent Innovations in biosustainability and environmental research, Department of Zoology, Annamalai University. (Oral presentation)

6. **P. R. Ramya Sree.,** & Thoppil J. E. (**2019)**. "Pharmacognostic, phytochemical and cytotoxic evaluation of fruits of *Memecylon* species" in XLII All India botanical conference of the Indian botanical society and national symposium on innovations and inventions in plant science research, Dept of Botany, University of Calicut. (Oral presentation)

7. **P. R. Ramya Sree.,** & Thoppil J. E. (**2020)**. "Pharmacognostic, phytochemical and antiproliferative evaluation of fruits of *Memecylon umbellatum* Burm. f"**.** Current trends and advances in biological sciences (CTAB 2020). Post Graduate Department of Botany and Biotechnology, Bishop Moore College, Mavelikara. (Oral presentation).