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EXTRACTION OF ESSENTIAL OILS FROM *Fagaropsis angolensis*, *Callistemon citrinus* AND *Melaleuca armillaris* AND ASSESSMENT OF THEIR ANTIMICROBIAL AND INSECTICIDAL ACTIVITIES

BY: ALMAZ SEMATU

OCTOBER, 2021

JIMMA, ETHIOPIA

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A THESIS SUBMITTED TO SCHOOL OF POST GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (ORGANIC)

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DECLARATION

I hereby declare that this thesis submitted for the degree of Master of Science in chemistry (organic stream) at Jimma University, College of Natural Sciences, Jimma, Ethiopia, is my own original work and have not been submitted previously to any university. The resources and materials used in this work have been duly acknowledged.

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ABBREVIATION AND ACRONYMS

AV	Acid Value
DMSO	Dimethyl Sulfoxide
EO_S	Essential Oils
GC-MS	Gas Chromatographic-Mass Spectroscopy
GC	Gas Chromatography
MAE	Microwave-Assisted Extraction
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
MS	Mass-Spectrometry
PDA	Potato Dextrose Agar
SFE	Supercritical Fluid Extraction
SFME	Solvent Free Microwave Assisted Extraction
SV	Saponification Value

ABSTRACT

There are several bioactive plants in Ethiopia that can produce essential oils. However, their antimicrobial and insecticidal activities of these plants are not widely assessed. Therefore, the aim of this study was to assess some biological activity from essential oil of Fagaropsis angolensis seed, Callistemon citrinus and Melaleuca armillaris leaves from Jimma and surroundings, Ethiopia. The hydro-distillation extraction method was used to extract essential oils, and the constituents of essential oil were identified by using Gas chromatography mass spectrometry analysis. The antimicrobial activities against bacteria (Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium and Escherichia coli), fungi (Candida albicans) using broth micro-dilution bioassay and insecticidal activities against insect (termites) using no-choice bioassay were assessed. The yield of essential oils was 2.58%, 1.00% and 2.29% for Fagaropsis angolensis seed, Callistemon citrinus leaves and Melaleuca armillaris leaves respectively. Acid values were 2, 4 and 4 for Fagaropsis angolensis seed, Callistemon citrinus and Melaleuca armillaris leaves essential oil were observed respectively. Saponification value 80.50, 72.45 and 72.45 of the essential oil were observed for Fagaropsis angolensis seed, Callistemon citrinus and Melaleuca armillaris leaves essential oil respectively. The major components identified from Fagaropsis angolensis seed were Bicyclo (3.1.0) hexane, 4-methylene-1-(1-methylethyl) - (sabinene) (72.29%) and Terpinen-4-ol (8.06%). From Callistemon citrinus leaves were Acetic acid, chloro-ethyl ester (13.61%), and Eucalyptol (1, 8-cineol) (72.46%). From Melaleuca armillaris leaves were Eucalyptol (80.08%) and α-terpineol (8.66%). The antimicrobial activities of essential oils of Fagaropsis angolensis seed were with MIC 0.40 ± 0.29 against (B cereus, S aureus and S typhimurium), 0.14 ± 0.09 against (E. coli) and 1 ± 0 against (C albicans), Callistemon citrinus leaves MIC 3.64 ± 2.57 against (E. coli), 1.21 ± 0.86 against (B cereus, S aureus and S typhimurium), and 1 ± 0 against (C albicans) and Melaleuca armillaris leaves MIC 3.64 ± 2.57 against (E. coli), 3.64 ± 2.57 against (B cereus and S aureus), 10.93 ± 7.72 against (S typhimurium), and 1 ± 0 against (C albicans). Moreover, all the tested oils were bacteriostatic and fungistatic against all tested strains. The 100 % mortality of termites was observed for 5% essential oils with short time of exposure from10min to 3 hours.

***Keywords**: Fagaropsis angolensis, callistemon citrinus, Melaleuca armillaris, Essential oils, biological activities*

1 INTRODUCTION

Essential oils are natural, complex mixture of compounds characterized by a strong odor and are formed as secondary metabolites [1–3]. They are highly volatile, contains terpenes, non-terpenes and terpene derivatives [4]. In nature, essential oils play an important role in the protection of the plants as antibacterial, antivirals, antifungals, and insecticidal. They are produced in plants part and serves as chemical signals to control and regulate their own environments [5]. The major plant families that are well known for their ability to produce EOs of medicinal and industrial values include *Alliaceae*, *Apiaceae*, *Asteraceae*, *Lamiaceae*, *Myrtaceae*, *Piperaceae*, *Cupressaceae*, *Lauraceae*, *Pinaceae*, *Zingiberaceae*, and *Rutaceae* [1–3, 6]. All the essential oil (EO) can be obtained from many different parts of plants, including flowers, leaves, fruits, seeds, roots, bark, bulbs, and dried flower buds [1, 7].

The extraction methods of essential oils are based on the purpose of uses; water distillation, steam distillation, solvent extraction, and supercritical fluid extractions. Some of them are steam distillation for pharmaceuticals and foods industries, and supercritical fluid extraction for perfumes industries. Based on the type of extraction method employed, the chemical profile of essential oil products can vary in the number of molecules but also stereo-chemical types of molecules. The extraction products can vary in quality, quantity, and in composition according to climate, soil composition, plant type, plant organ, and plant age [8]. So, in order to obtain in constant composition and stereochemistry, they have to be extracted under the same condition [8]. The gas chromatography mass spectrometry (GC-MS) is a method for identification of essential oil components by comparison of their relative retention time and their mass spectra (MS). It is still the most widely applicable method for analysis of essential oils [9]. The essential oils have been widely used in pharmaceutical, agricultural, cosmetic and food industries due to their antimicrobial, antioxidant and other biological properties [10].

1.1 Statement of the problem

The microorganisms and insects are the main cause of human health problem in world. Insects can affect human being directly by transmitting disease or indirectly by destroying all wood product [11]. Traditionally, people have used essential oil containing plants to inhibit diseases caused by microbes and insects [12]. Essential oils have been known antimicrobial agents and have ability

to inhibit the growth of microbes [13]. Compounds obtained from essential oils have been investigated for their antimicrobials and insecticidal activities and many of them used as model for the development of active components to control insects and microbes.

Controlling bacteria, fungi and insects by help of plant product is highly important to improve quality of human life especially in developing country. However, the antimicrobial and insecticidal activities as well as the chemical composition of essential oils of *Callistemon citrinus* and *Melaleuca armillaris* leaves are rarely reported in the literature. Therefore, the aim of study is to investigate the antimicrobial and insecticidal activities of essential oils from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves and analyses of their chemical compositions by using GC-MS system. The research questions were:

- Which essential oil can exhibit remarkable antimicrobial and insecticidal activities?
- What composition of essential oils resulted from the selected plants?

1.2 Objective of the study

1.2.1 General objective

The general objective of this study was to characterize essential oils from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves and to assess their antimicrobial and insecticidal activities.

1.2.2 Specific objectives

The specific objectives of study were to:

- ✓ Determine yield of essential oil from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves
- ✓ Determine some of the physico-chemical properties of essential oils isolated
- ✓ Identify the chemical composition of essential oils using Gas chromatography mass spectrometry.
- ✓ Assess the antimicrobial and insecticidal activities of the extracted essential oils against bacteria (*Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium and Escherichia coli), Candida albicans* and insect termite

2 LITERATURE REVIEW

2.1 Essential oils

The plants have been used by humans as ethno-medicines for a long time due to the presence of the important essential oils [14]. About 3000 EOs have been identified and about 300 types of EOs are being used in perfumery due to high aroma and fragrance. They are secondary metabolites, and are important for plant defense mechanism; hence, they have various medicinal properties including antimicrobial activity. Essential oils (EOs) are liquids, volatile compounds and widely used as medicine, perfumes, cosmetics, and as food preservatives [5]. They are highly concentrated complex molecular substances obtained from the essence rich in natural flavors and are present in the specialized cells or glands of certain plants parts [15].

Essential oil may contain several hundred chemical compounds and this complex mixture of compounds gives the oil its characteristic fragrance and flavor. They are normally not getting rancid, however; they generally react with water and oxygen. Plants contain volatile oil in all their parts in different concentrations. The synthesis and accumulation of essential oils occur in plant organs, namely stems, bark, seeds, fruits, leaves and roots. However, more commonly they are found in flowers and leaves in varying amounts [16].

2.1.1 Distribution of essential oils in natural sources

The production of essential oils in plants is generally associated with the presence of specialized secretory cells. After the formation within the plant cells, these oils are also released into the atmosphere by secreting cells. Essential oils are biosynthesized in specialized secreting cells types, such as osmophores, Glandular Trichomes, conical-papillate cells, ducts and cavities, present on different parts of these plants. These cells have a central role in essential oil biosynthesis, accumulation, and secretion into the atmosphere; as a result, they are the natural factories for essential oil synthesis [17, 18]. The granulocrine and eccrine mechanisms are two different mechanisms of secretion, proposed to be responsible for the secretion of essential oils and both mechanisms could exist for different compounds and plants [18].

The term osmophore, where "osmo- "means "odor" and "-phore" means "bearing", was to describe an enclosed area of floral tissue that is specialized in scent emission. Osmophores, also called floral fragrance glands, are specialized clusters of cells in flowers, and are distributed on sepals and petals to attract insect pollinators. Osmophores consist of a multilayered glandular epithelium with homogeneous layers of cells. These cells contain dense cytoplasm, enormous deposits of starch, or other storage compounds within the mesophyll. These deposits are usually missing in epidermis cells. This generates a distinction between the production and the emission layer. The Osmophore cells can be found on the whole epidermis of petals in more than 200 species [19].

2.1.2 Chemical composition of essential oils

Plant essential oils are usually the complex mixture of natural compounds. The constituents in essential oils are terpenes, terpenoids, and aromatic compounds (Fig. 1). Terpenes are the most common class of chemical compounds found in essential oils. They are made from isoprene units, which are called a "terpene unit." Essential oils consist of mainly monoterpenes (C10) and sesquiterpenes (C15), which are hydrocarbons with the general formula (C5H8) n. The diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentrations [20, 21]

The chemical composition of the essential oil often changes between different plant parts. Several factors affect essential oil composition, including the tissue variation (secretory cells and excretion cavities), the ontogenetic phase, and the growing location of the plant, type of soil condition, and other environmental factors that impact plant growth and development [22]. The essential oils are mixtures of known and unknown compounds. They may contain terpene of hydrocarbons, alcohols, aldehydes, ketones, phenols and esters [9]. Some compounds of essential oils are Phenols: thymol, eugenol, and carvacrol, chavicol, and thymol so on, Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, and so on. Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, and so on. Aldehydes: citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, and so on. Ketones: carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, and so on. Esters: bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, and so on. Oxides: 1, 8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, and so on. Lactones: bergaptene, nepetalactone, psoralen, aesculatine, citroptene, and so on. Ethers: 1, 8-cineole, anethole, elemicin, myristicin, and so on[20, 21].

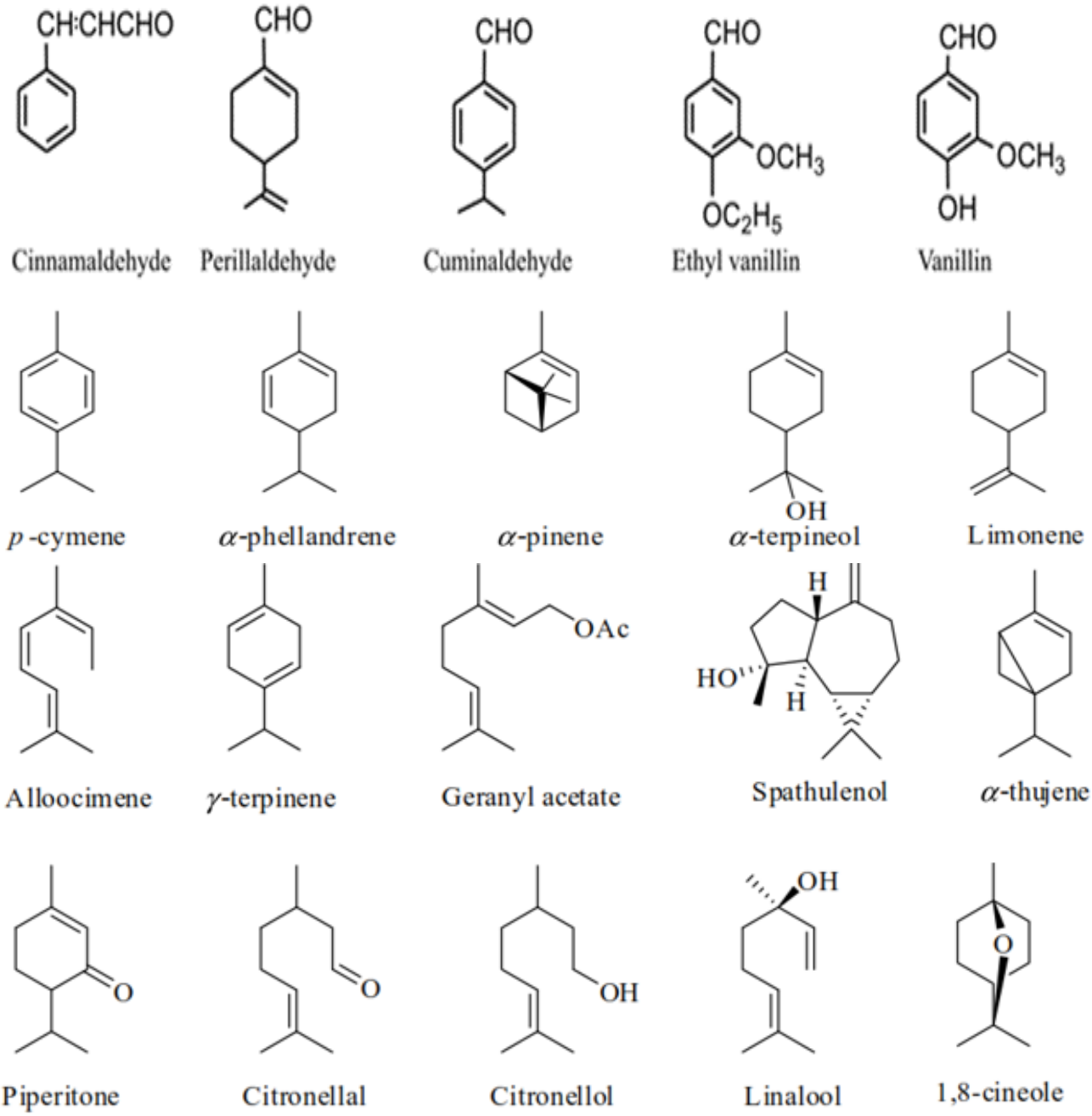


Figure 1: Structure of essential oils components from different plants

2.1.3 Biosynthesis of essential oils

The essential oils are comprised of highly functionalized chemical classes including monoterpenoids, sesquiterpenoids, and phenylpropanoids [19]. They consist of organic volatile compounds which belong to various chemical classes: alcohols, aldehydes, ketones, esters, phenols, and mainly the terpenes [23]. Terpenes are hydrocarbon consisting of isoprene unit having

five carbon molecules (Fig 2). Due to number of isoprene unit the terpenes are named in different ways like hemiterpene (C5), monoterpene (C10), sesquiterpene (C15), diterpene (C20) and sesterpene (C25) with having different number of isoprene unit. The isoprene unit are obtained biosynthetically via mevalonate and methylerythritophosphate (non-mevalonate) phathway [2, 18].

2.1.3.1. Mevalonate pathway biosynthesis

The IPP biosynthesis of the acetate or mevalonate pathway is widely accepted (Fig. 2). This cytosolic IPP pathway involves the two-step condensation of three molecules of acetyl-CoA catalyzed by thiolase and hydroxymethylglutaryl-CoA synthase. The resulting product, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), is subsequently reduced by HMG-CoA reductase in two coupled reactions that form mevalonic acid. Two sequential ATP-dependent phosphorylations of mevalonic acid and a subsequent phosphorylation/elimination-assisted decarboxylation yield IPP [24].

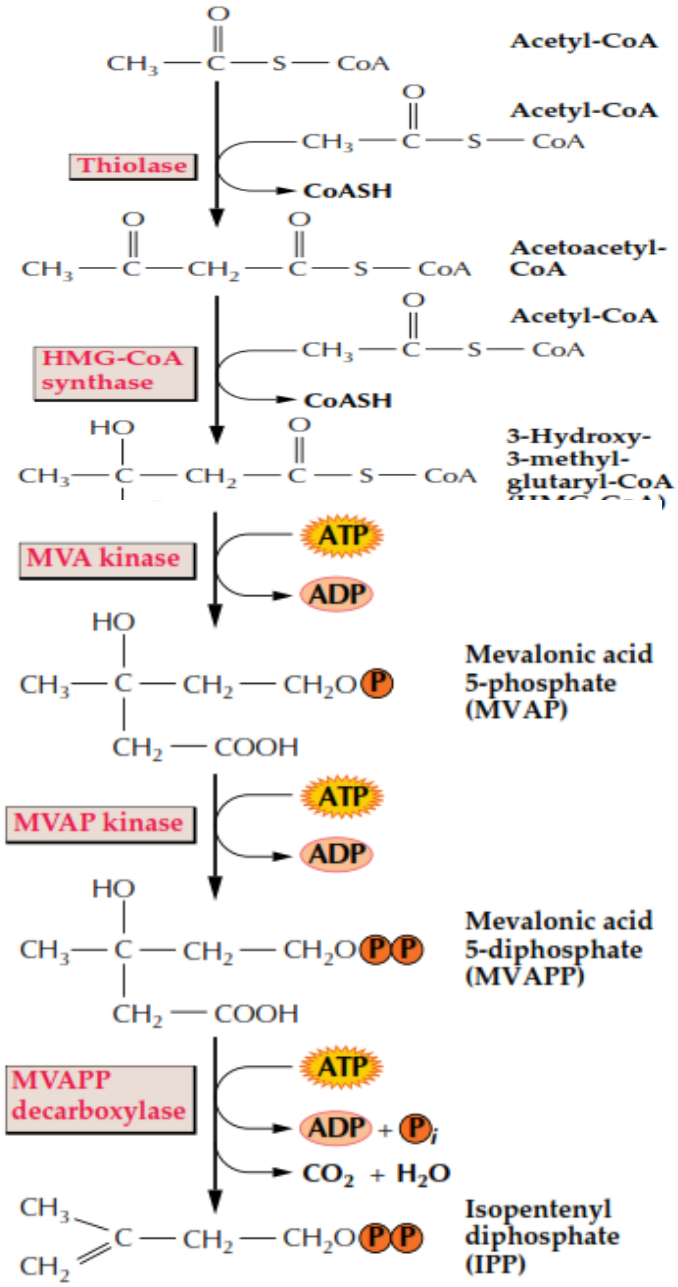


Figure 2: Mevalonate pathway biosynthesis

2.1.3.2 Non-mevalonate (MEP) pathway biosynthesis

In this pathway, pyruvate reacts with thiamine pyrophosphate (TPP) to yield a two-carbon fragment, hydroxyethyl-TPP, which condenses with glyceraldehyde 3 phosphate (Fig. 3) for similar TPP-mediated C2 transfers catalyzed by transketolase. TPP is released to form a five-carbon intermediate, 1-deoxy-D-xylulose -5-phosphate, which is rearranged and reduced to form 2-C-methylD-erythritol-4phosphate and subsequently transformed to yield IPP [24].

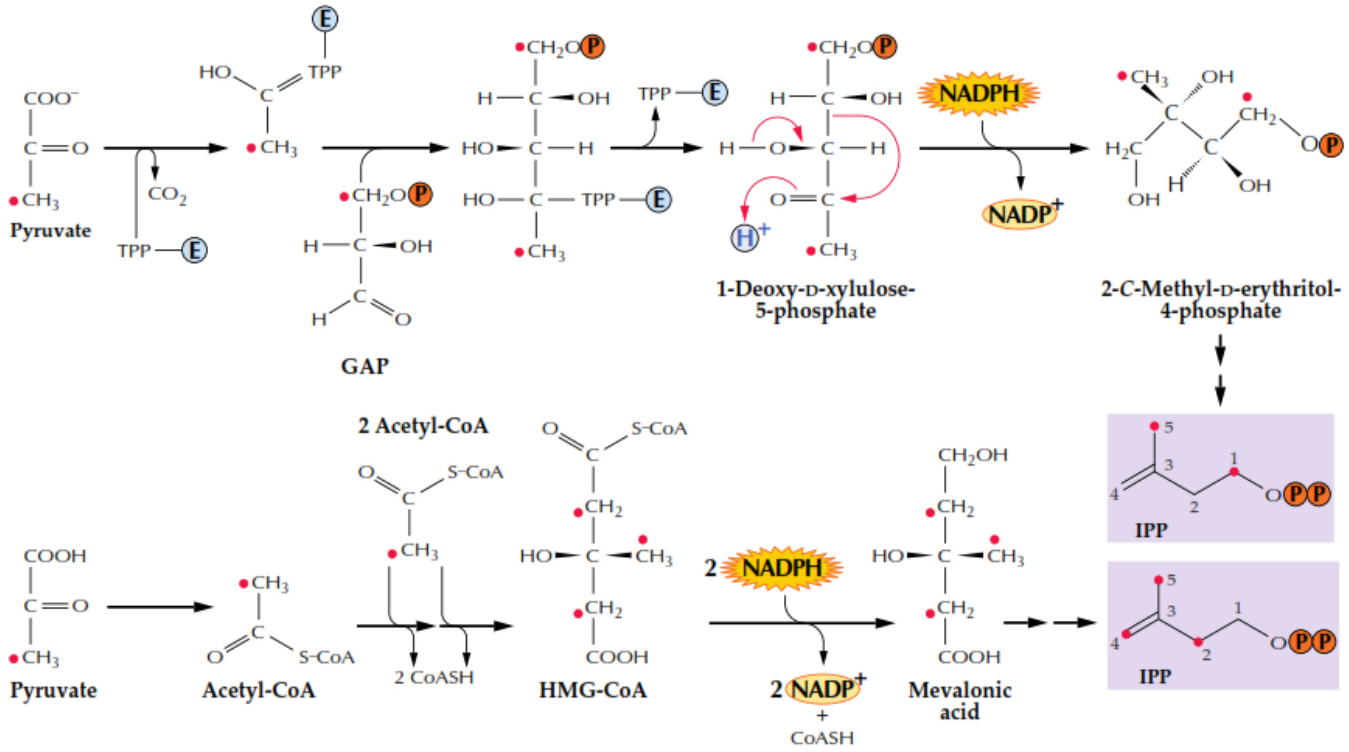


Figure 3: non-mevalonate pathway biosynthesis

2.1.3.3 Biosynthesis of 1, 8-cineol, sabinene, and α-terpineol

The terpenes are formed by the enzyme class of terpene synthases, which convert the prenyl diphosphates to the basic terpene olefins or alcohols. A factor contributing to the terpene structure diversity is the unique feature of terpene synthases to form multiple products from one substrate. One terpene synthase can generate complex terpene blends with over 50 compounds. The reaction pathway starts with the ionization of the 2, 3-(E)-GPP substrate in the presence of divalent cations, mostly magnesium (Mg^{2+}). A magnesium cluster causes the elimination of the diphosphate moiety which leads to the geranyl cation, a carbocationic intermediate that is highly reactive.

Acyclic monoterpenes arise directly after the ionization through water binding or deprotonation. The water capture leads to geraniol or linalool, while deprotonation results in myrcene. The (E)-configuration of GPP at the 2, 3-double bond prevents the formation of cyclic products. Therefore, cyclization reactions require a rearrangement of GPP to linalyl diphosphate (LPP), enabled by the transfer of the diphosphate moiety to C-3. This allows the rotation of the new-risen 2, 3-single bond and the conformation of LPP from the transoid to the cisoid form. The following 6, 1-cyclization of the cisoid LPP is initiated by another ionization leading to the linalyl cation. The cyclization results in the α-terpinyl cation, the central intermediate and origin of all cyclic monoterpenes. The array of diverse cyclic monoterpenes is then formed after hydride shifts, endocyclizations, proton loss, and water capture and rearrangements (Fig. 4)

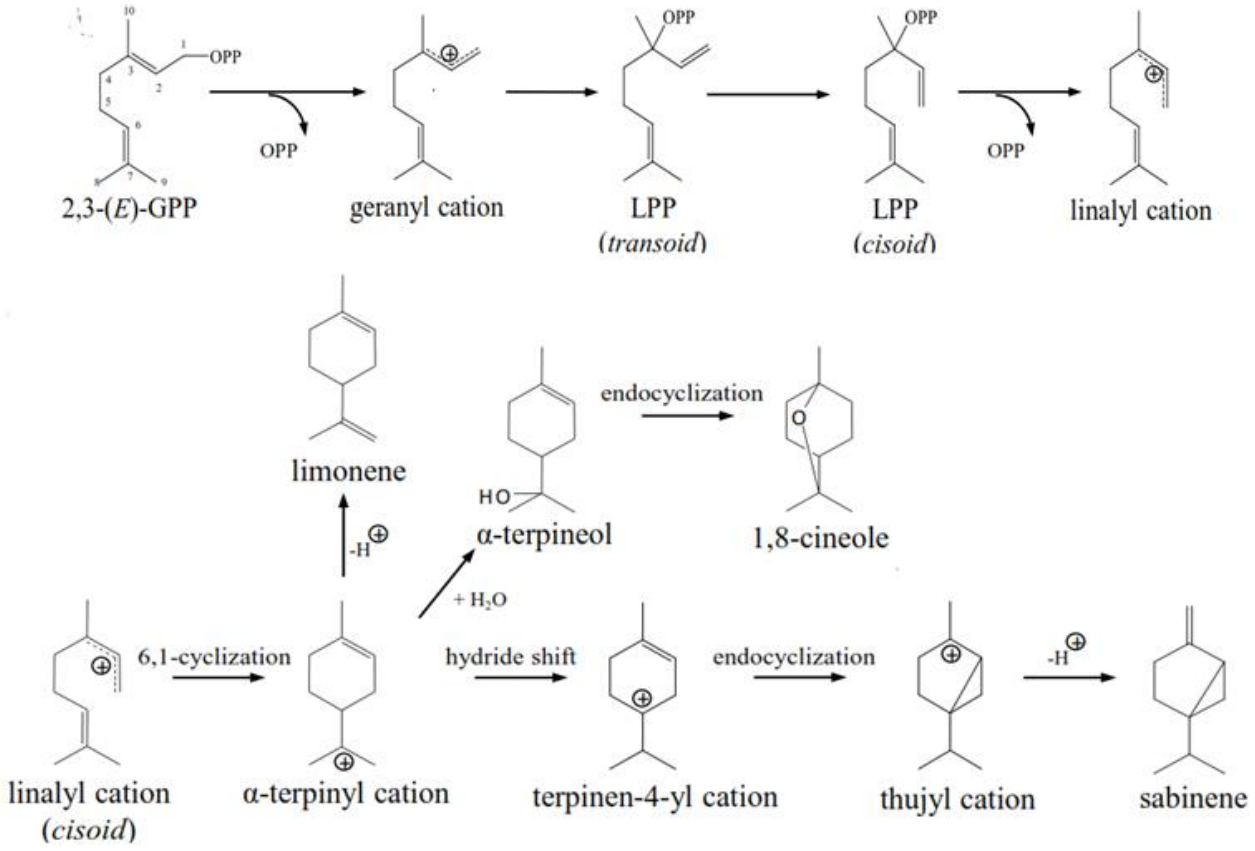


Figure 4: Biosynthesis of essential oils components

2.1.4 Extraction method of essential oils

There are several methods for extracting the essential oils from the natural sources. These include hydro-distillation, steam distillation, solvent extraction, microwave extraction, cold press extraction and supercritical fluid extraction [25]. Hydro-distillation or steam distillation is the most widely utilized physical method for isolating essential oils [9].

2.1.4.1 Water Distillation (hydro-distillation) Extraction

Hydro-distillation processes involve direct distillation of mixture of sample with water. The device for hydro-distillation can be of different type and one of that is the Clevenger type apparatus which constitute of round bottomed flask into which plant material fully immersed in water is placed and allowed to boil from few minutes to 3-4 hours depending on nature of plant materials. Then vapor generated which carries volatile constituents is subsequently allowed to condense when it joins condenser vertically mounted via graduated volumetric cylinder used as reservoir for condensates and then the essential oil will soon get separated. Separation of the oil from water will be achieved by slowly draining the liquid through the opening equipped with the stopcock. Hydro-distillation based extraction of essential oils has several disadvantages such as high chance to lose oxygenated components associated with their high aqueous solubility and longer experimental time that increase chance of formation of artifacts through hydrolysis of thermo labile constituents like esters , polymerization of acyclic monoterpenes hydrocarbons and aldehydes [26, 27].

2.1.4.2 Steam Distillation Extraction

In steam distillation method, the botanical material is placed in and steam is forced over the material to release the aroma molecules from the plant material [28]. The steam contains the essential oil is passed through a cooling system to condense essential oil and water [27]. In this process, the plant material is not in direct contact with the heat source to avoid damaging the essential oil [29]. The main advantage in this process, the plant material is not in direct contact with the heat source to avoid damaging the essential oil. The main disadvantage in this process are unsafe, time consuming due to low pressure steam, poor quality oil, improper condensation, incomplete oil separation, less recovery, Poor material construction and excessive pollution [30].

2.1.4.3 Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) due to its unique heating mechanism (based on friction), reasonable cost, and good performance under atmospheric conditions, leads to higher extraction

yields, shorter extraction times, and higher selectivity. Moreover, MAE can also be considered superior to supercritical fluid extraction (SFE) in terms of simplicity and operation cost. To take advantage of microwave heating, researchers have combined microwaves with conventional methods developing new methods [31] such as microwave-assisted solvent extraction, vacuum microwave hydro-distillation, microwave hydro-distillation, compressed air microwave distillation, and microwave-accelerated steam distillation. Microwave–assisted hydro-distillation extraction process had less energy demanding and more sustainable. One of the most promising and the most used methods of the modified MAE for essential oils is solvent free microwave-assisted extraction (SFME). SFME significantly reduces the extraction times compared with conventional methods ranging from a few hr. to 20–30min for essential oil extraction [32].

2.1.4.4 Solvent Extraction

Solvent extraction is solid-liquid extraction that can be used to extract essential oils of that are thermally labile [31]. In this process the plant material is placed into a solvent bath dissolves their volatile constituents such as hexane, ethanol, petroleum ether, and methanol. After separation of the liquid mixture the essential oil will be separated through evaporation or distillation. Solvents that are commonly used for solvent extraction are [33]. The main advantage of extraction over distillation is that a lower temperature is used during the process, therefore reducing the risk of chemical changes due to high temperatures, which are used during distillation. Solvent extraction is also relatively fast process since the diffusion rates are influenced by temperature; it is possible to increase the speed of the process by using hot solvents. The disadvantage of solvent extraction are essential oil solvent with the oil [32], low extraction efficiency [13] the loss of essential oil from the solvent at the time of the evaporation, relatively high solvent consumption and often unsatisfactory reproducibility [1]. This process is the most commonly used in the cosmetic industry [31].

2.1.4.5 Cold-Pressing Extraction

Cold-pressing is mechanical extraction where by a plant sample is pressed at low temperatures and pressure. Then essential oil which rises to the surface is separated from the material by filtration or distillation. Cold pressing is one of the best methods used for most carrier oils and essential oils. It is mainly used for extracting essential oils from citrus fruit. This method is extremely fast, cheap

and does not pollute the extracts, but it does not provide a way of selectively extracting essential oils [31] and oils extracted have a relatively short shelf life [1].

2.1.4.6 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is the process of separating oils from plant parts using supercritical fluids as the extracting solvent. Apart from having relatively low critical pressure (74bars) and temperature (32°C), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [28], but sometimes modified by co-solvents such as ethanol or methanol. The oil extracts prepared by SFE yielded a higher antioxidant activity and produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of the equipment's and it is not easily handled [1]. Since; the essential oils possess most often aroma profiles that almost identical to the raw materials from which they have been extracted. Therefore they are often used in the flavor and fragrance and in food industry[34].

2.1.5 Method for characterization of essential oils

2.1.5.1 Gas chromatographic –Mass spectrometry method analysis (GC-MS)

Gas chromatography Mass spectrometry analysis is a method which combines the features of gas chromatography, and mass spectrometry is a chemical analysis instrument used to separate and identify individual constituents found within a given essential oil since; essential oils are highly volatile (Fig 1). The gas chromatography (GC) portion separates the chemical mixture into pure chemicals, after the sample has passed through the GC, the chemical pulses continue to the mass spectrometer (MS) which help to identify the chemicals separated [35].

Each chemical constituent of an essential oil pass through the GC instrument within different times is registered to produces some type of peak, from very short to very tall. A GC report reveals the peaks of different chemical constituents within given oil, it does not, however, name the specific chemical constituent. Mass spectrometry which allows for the detection of chemical constituents by separating ions by their unique mass and identifies specific compounds registered on the gas chromatography report. Different molecules have different masses, and this fact is used to determine what molecules are present in a sample [2].

2.1.5.2 Principle of GC-MS analysis

The gas chromatography is basically a temperature-controlled oven designed to hold and heat the GC column. The Carrier gas, usually nitrogen, helium, or hydrogen is used to sweep the injected sample onto and down the column where the separation occurs and then out into the mass spectrometer interface. The interface may serve only as a transfer line to carry the pressurized GC output into the evacuated ion source of the mass spectrometer. A jet separator interface can also serve as a sample concentrator by eliminating much of the carrier gas. It can permit carrier gas displacement by a second gas more compatible with the desired analysis, that is, carbon dioxide for chemically induced ionization for molecular weight analysis. It can be used to split the GC output into separate streams that can be sent to a secondary detector for simultaneous analysis by a completely different, complimentary method.

The mass spectrometer has three basic sections: an ionization chamber, the analyzer, and the ion detector. In the evacuated ionization chamber, the sample is bombarded with electrons or charged molecules to produce ionized sample molecules. These are swept into the high vacuum analyzer where they are focused electrically then selected in the quadrupole rods. The direct current signal charging apposing poles of the quadrupole rods creates a standing magnetic field in which the ions are aligned. Individual masses are selected from this field by sweeping it with a radio frequency (RF) signal. As different RF frequencies are reached, different mass/charge ratio (m/z) ions can escape the analyzer and reach the ion detector. By sweeping from higher to lower frequency, the available range of m/z ions are released one at a time to the detector, producing a mass spectrum (Fig. 5). On entering the ion detector, the ions are deflected onto a cascade plate where the signal is multiplied and then sent to the data system as an ion current versus m/z versus time [36].

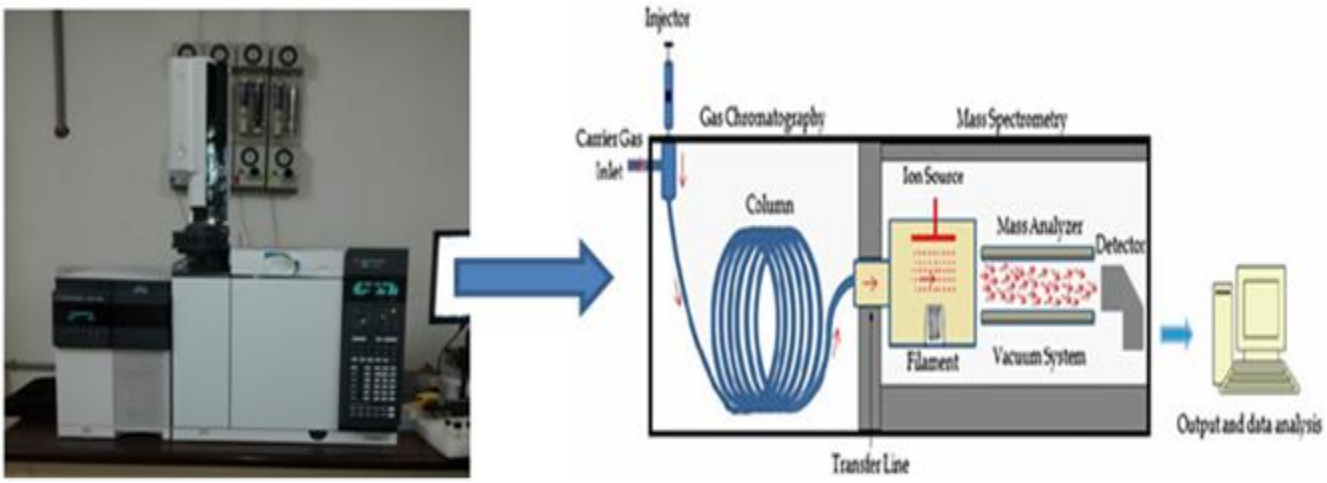


Figure 5: GC-MS machine and its internal view

2.1.6 Physicochemical properties of essential oils

The essential oils have physical properties namely, color, characteristic odor, viscosity and high refractive index. The chemical properties like acid value, saponification value and iodine value of essential oils used to decide their utilization in eating, pharmaceuticals and industrial making [37]. Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, resistance to pouring, resistance to flow. It determines the rheological proprieties of oils. The refractive index is the degree of the deflection of a beam of light that occurs when it passes from one transparent medium to the other. It increases with the length of chains and with the number of carbon atoms present. Therefore, the refractive index determines evidences that the sample might be unsaturated long carbon chain. The iodine value is a useful tool in predicting the drying properties of oils. The high iodine value of oils indicates the high content of unsaturation and suggests that the oils may be used as drying agent for the manufacturing of oil paints, varnishes, cosmetics and also as cocking oil manufacturing index. The iodine value is also an index of assessing the ability of oil to go rancid. It is also used for determining the level of oxidative deterioration of the oil by enzymatic or chemical oxidation.

Acid value is an index of oil which is used to determine the quality, age, edibility and suitability of oil for industrial use such as paint. This value is used to measure the extent of glycerides in the oil, which have been decomposed by lipase and other physical factors such as light and heat. Saponification value is an index of average molecular mass of various fatty acids in oil samples.

The lower value of saponification means molecular weight of fatty acids is lower and has lower limit of use in industry. The saponification value suggests the use of oil in production of liquid soap, shampoos and lather shaving creams [38]. The saponification value is the amount (mg) of KOH/NaOH required to saponify 1g of oils. It is a measure of the average molecular weight of all the fatty acids present in the oils. In saponification, triglycerides of fatty acids are hydrolyzed with alkali produce glycerol and alkali salts of fatty acids. This process is significant in making of soap (Fig. 6) [39].

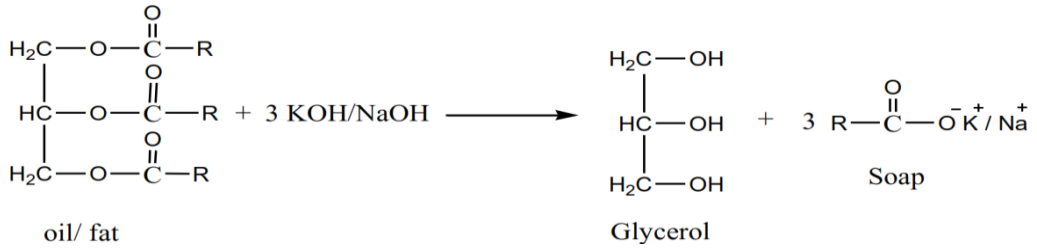


Figure 6: Process of oil/fat changed to soap within presence of alkali

2.1.7 Application of essential oils

2.1.7.1 Food

EOs are primarily used in food industry for food preservation due to their natural antimicrobial contents and show strong inhibitory activities against pathogenic bacteria [5]. Foods containing antimicrobial compounds have extended shelf-life. These antimicrobial compounds reduce the growth rate and feasibility of microbes in both unprocessed and processed foods. Antimicrobial activity of essential oils is determined by its chemical structure, composition, functional group [40], lipophilicity and the hydrophobicity of their major functional groups and ranked as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons [41, 42].

Essential oils having phenolic groups [40], terpenoids and phenylpropenes [41, 42] are most effective against microbes and used in food packaging to enhance the shelf life of food products [43]. Properties of the food matrix that may affect the activity of EO and extracts include pH, temperature, amount of oxygen, as well as water, protein, and fat content. High fat content appears to decrease the antimicrobial activity by potentially providing protection for the bacteria. Many have proposed the use of EOs and plant extracts as natural alternatives to chemicals commonly used in the food industry to prevent or reduce contamination by foodborne pathogens [40].

2.1.7.2 Medicine

The medicinal properties of plants from essential oil bearing family are known from ancient times due to their essential oils [44]. Aromatherapy is the therapeutic use of fragrances or volatiles to cure prevent diseases, infection, and indisposition by means of inhalation. Inhalation of essential oils or their individual volatile terpenes has a significant role in controlling the central nervous system [2]. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both pathogenic and non-pathogenic diseases. For the treatment of non-pathogenic diseases for instance, the vapor of lavender essential oil or its main component linalool may also be applicable to the treatment of menopausal disorder through inhalation. Garlic essential oil significantly lowered serum cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases.

Oral administration of combination of oregano, cinnamon, cumin, and other essential oils decreases systolic blood pressure in rats. Some essential oils may degenerate diabetes, for instance rosemary essential oil showed hyperglycemic and insulin release inhibitory effect in diabetic rabbits. Essential oils and their components showed cancer suppressive inactivity when tested on human cancer cells lines including glioma, tumors, breast cancer, and leukemia. Treatment of human leukemia cells with eucalyptus oil showed morphological changes (fragmentation of DNA) indicating an induction of apoptosis [45].

2.1.7.2.1 Mode of action of essential oils

The mechanisms by which essential oils can inhibit microorganisms involve different mode of action are described in the three phases. Firstly, EO spreading on the cell wall of microbes to enhances the membrane permeability which leads to loss of cellular components. Secondly, an acidification inside the cell which blocks the production of cellular energy due to the ion loss, the collapse of proton pumps and reduction of membrane potential. Lastly the destruction of genetic materials, that results in the death of bacteria and fungi [41, 42]. They may target various cell structures or chemical pathways, such as cell wall degradation, membrane damage, dissipation of the proton motive force permeabilization of the membranes is associated with loss of ions, reduction of membrane potential, collapse of proton pump and depletion of the adenosine triphosphate pool (Fig.7). They can coagulate the cytoplasm and cause damages to lipids and proteins [46] and causes the impairment of bacterial enzyme system and cell respiration [47].

The essential oils of their lipophilic fraction react with the lipid parts of the cell membranes and they can modify the activity of the calcium ion channels and the volatile oils saturate the membranes [48]. The Gram-negative bacteria are less susceptible to the antimicrobial action of EOs than Gram-positive organisms due to the presence of a protective outer membrane; however, a number of studies have shown various EOs to be effective on Gram-negative bacteria [40]. Essential oils with high concentrations of thymol and carvacrol usually inhibit Gram-positive more than Gram-negative pathogenic bacteria. However, the essential oil of *Achillea clavennae* exhibited strong antibacterial activity against the Gram negative *Haemophilus influenzae* and *Pseudomonas aeruginosa* respiratory pathogens, while Gram positive *Streptococcus pyogenes* was the most resistant to the oil.

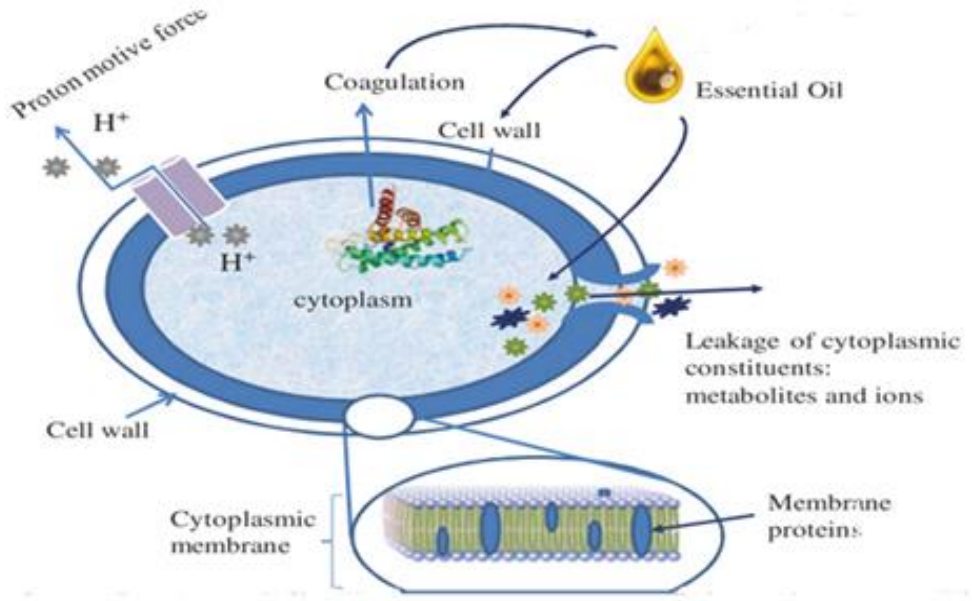


Figure 7: Mechanism of Essential oils

2.1.7.3 Cosmetics industry

Cosmetics defined as "any substance or mixture intended to be placed in contact with the various external parts of the human body or mainly to cleaning, perfuming, changing appearance, protecting, moisturizing, drying, keeping in good condition or correcting body odors. Essential oils are widely combined in cosmetic products and perfume due to the variety of their properties mainly their pleasant odor. They contain compounds with varied chemical structure, effects, skin

sensitivity and irritations symptoms may arise after their application. Essential oils are considered as safe and nontoxic when used at low concentrations. Due to the antimicrobial and antifungal impact of essential oils, they can be used as chemical preservative as an active agent (e.g., rosemary oil, eucalyptus oil) in the cosmetic preparations such as creams, gels and ointments. However, essential oils do not only have positive effects; it can be sources of potential allergens [49, 50].

2.1.8 Safety issues associated with essential oils

Essential oils are complex mixtures of compounds and they are extensively used in food and cosmetic industries. Several compounds present in essential oils and generally recognized as safe, however, a series of adverse reactions have been reported after their use either by internal or external routes. Chemical complexity of essential oils is challenging when investigating which individual components are responsible for certain unwanted effects. Some plants essential oils are potentially irritant to the skin [51].

A scientifically-based guide has been developed to evaluate the safety of essential oils, for their intended use. The safety of the intake of the essential oil is evaluated in the context of data on absorption, metabolism, and toxicology of members of the congeneric group [34]. Depending on the analytical method, it is possible to detect and identify constituents of essential oil. However, there will remain a number of volatile constituents detected but not identified. Further analysis may reduce the level of unknown's constituents such that it no longer raises a safety concern. The toxicity data could be generated for the essential oil to provide an adequate margin of safety for intake of the unidentified constituents present in that essential oil. The safety factor typically used to ensure a margin of safety may not be relevant, if the essential oil is widely consumed in food [52].

2.2 Overview on damages caused by microbes / pests, management strategies and the associated problems

2.2.1 Problems caused by microbes / pests

Microbes are microscopic organisms such as bacteria, fungi, protozoa, viruses, and algae that are found almost everywhere on earth. Some microbes are commensal; others are mutualistic, while some are infectious agents. All microbes play significant roles in immunity and function in areas of modulation, metabolism, and drug interaction in the body. Bacteria are unicellular prokaryotic

organisms medically classified based on their shapes as spiral, curved, bacilli, cylindrical, rod-shaped, cocci or spherical bacteria. According the quantity of peptidoglycan in their cell walls, bacteria can be classified as Gram-positive and Gram-negative [53].

Gram-negative bacteria, such as *Escherichia coli*, *Salmonella*, and *Proteus mirabilis*, are generally known to cause numerous diseases [53]. *Escherichia coli* and *Salmonella* bacteria are group of *Enterobacteriaceae* with the characteristic of becoming resistant to the most common antibiotics. The *Salmonella* has changed its characteristics worldwide, becoming the etiologic agent of many peculiar pathological processes such as cancer development, inflammatory process, and immune-pathogenesis [54].

The pathogenic *Salmonella* and *Escherichia coli* can infect humans, domestic animals, and most wild animals. However, the majorities of *Escherichia coli* strains are non-pathogenic, normally exist in the healthy human intestine, and are required to maintain proper conditions for the function of other beneficial microorganisms of the gut [55]. *Escherichia coli* and *Salmonella* can survive in both soil and in plants. Rarely, they raise conditions leading to food produce contamination. Salmonella spp. can survive and grow on the surface of mature, intact tomatoes at ambient temperature [56].

Gram-positive bacteria such as *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens* are responsible for causing intoxications in food. The other bacteria include *Acinetobacter, Alcaligenes, Aeromonas, Flavobacterium, Arcobacter, Lactococcus, Pseudomonas, Serratia, Shigella, Listeria, Yersinia, Campylobacter, Citrobacter, Vibrio Enterobacter, Micrococcus, Enterococcus, Paenibacillus, Corynebacterium, Staphylococcus,* and *Weissella* cause infections, harm and food spoilage. Some bacteria strains of *Lactobacillus, Bifidobacterium, Erwinia,* and *Streptococcus* are necessary in digestion, decomposition, and the production of food such as cheese, bread, and yoghurt [53]. Around 250 different foodborne diseases are caused by bacteria ; among bacteria that cause foodborne disease are typically gram positive pathogen such as *Bacillus cereus* and *Staphylococcus aureus* [57]. These pathogens cause illness through preformed toxin production in improperly handled foods. Gram positive bacteria are more susceptible than gram negative pathogenic bacteria[58].

Fungi, molds and yeast are classified into the kingdom fungi. Molds are filamentous multicellular characterized by cottony or fuzzy appearance on the surface of food. It requires little moisture and survives in temperatures within 25–30°C and with low pH levels. For instance, *Camembert, Botrytis cinerea* and *Roquefort* are useful in the production of foods and serves as a catalyst (enzymes) such as cheese, bread or citric acid used in soft drinks. Yeasts like *Candida*, *Cryptococcus, Saccharomyces, Brettanomyces,* and *Debaryomyces* are most commonly used in the fermentation of sugars to ethanol and carbon dioxide [53]. Some fungal infections are highly lethal and disproportionately affect vulnerable patients, such as neonates and cancer patients. *Candida albicans* is the most common fungal pathogen in most clinical settings. The morphological flexibility of *Candida albicans* plays a crucial role in several aspects of infection and host recognition. *Candida albicans* can cause two major types of infections in humans: superficial infections, such as skin, oral, or vaginal candidiasis, and life-threatening systemic infections [59].

2.2.2 Management strategies problems caused by microbes

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which is determined using various methods such as disk diffusion, broth dilutions, and time kill assays or survival curves, are used to define the antimicrobial activity against a particular organism [40]. The broth micro-dilution methods are the standard method used to compare the inhibition efficiency of antimicrobial agents. The main advantage of micro-dilution method is to estimate the concentration of the test compounds in the broth suspension. In the broth micro dilution, the minimal inhibitory concentration (MIC) is usually the lowest concentration that can able to inhibit any visible microbial growth.

The turbidity is most frequently used and it allows determining whether an extract of plants has microbicidal or microbistatic action at tested concentration. The minimal bactericidal and fungicidal concentration (MBC and MFC) are the lowest concentration of an antibiotic, expressed in mg/L, which under defined in vitro conditions reduces by 99.9%, within a defined period of time and determined by plating out samples of completely inhibited dilution culture and assessing growth (static) or no growth (cidal) after incubation [46]. More precise data were obtained through the determination of bacteriostatic (MIC) and bactericidal (MBC) concentrations since; it is more

sensitive than the agar disc diffusion technique, which was used only as a screening tool to eliminate those essential oils with no significant inhibitory properties against the pathogens [60].

2.2.3 Management strategies problems caused by insects

The interest in botanical insecticides has increased because of environmental concerns and insect populations becoming resistant to conventional chemicals. Botanical insecticides are naturally occurring insecticides that are derived from plants. The insecticidal activity of essential oils and plant extracts against different pests and insects has been evaluated [61]. Essential oils are important source of bioactive chemicals and provide interesting alternatives to conventional insecticides due to their limited persistence on the environment; low mammalian toxicity, low probability of generate resistance [62] and they possess ovicidal, larvicidal, and repellent properties against various insect species.

The insecticidal constituents of many essential oils are monoterpenoids [61] and they are highly volatile and lipophilic compounds that can rapidly infiltrate insects and produce physiological effects [63]. EOs have a great potential as insecticides and repellents [62] and depends on its chemical composition and of the sensitivity of the target pest to its active compounds [12]. Many essential oils are known to possess a variety of bio-efficiencies such as repellency, prevention, a reduced sweetness, growth inhibition through an altered protein availability, enzyme inhibition, and direct toxicity. Certain essential oils and their constituents are capable of manifesting insecticidal activity against different insect species [64].

2.3 Overview of plants included in these study

2.3.1 Callistemon citrinus

The *Callistemon citrinus* is a flowering plant belonging to the *Myrtaceae* family and most widely cultivated species among the 34 species of *Callistemon* genus. The plant is a small tree, which grows up to 7.5 m tall, with crimson flowers and dark red anthers [5]. The genus found mostly only on the Australia and endemic to Australia. They are commonly referred as bottlebrush, because of their flowers are ranged in spikes that can up to 12 cm long, brush like flowers resembling a traditional bottlebrush. It had a character as traditional folk medicine for its anti-cough, anti-bronchitis, and insecticidal effects.

Callistemon citrinus is essential oil containing plant with complex mixtures of natural compounds. The volatile compounds identified were lipids, terpenoids, ketones, phenols and oxygenated derivatives with multiple biological activities such as antimicrobial, insecticidal and antioxidant properties [65]. The major chemical components from its essential oil such as 1,8-cineole, α-pinene, and α-terpineol, along with other in a lower amount such as, ß-pinene, α-pinene, and linalool, which were made to exhibit antifungal and bacteriostatic activities [66]. Its leaf essential oils from different parts of the world have found 1, 8-cineol to be the major component. *Callistemon citrinus* leaves (Fig. 8c) essential oil has been studied to show several pharmacological effects [5]

2.3.2 Melaleuca armillaris

Melaleuca armillaris is one of the *Melaleuca* species belongs to the *myrtaceae* family [67]. It is commonly known as Honey Bracelet Myrtle and grow in the form of large bush or small tree [68]. The *Melaleuca* genus is known as tea tree plants and it can reach 7m in height. They have thin bark and long pointed leaves that when broken it can emit strong aroma. They can be cut after 15 month of cultivation and cropped each year and native to Australia and Indian Ocean Islands [67]. The investigation of its essential oil by GC-MS revealed the presence of 1, 8-cineol as major components [68]. The *Melaleuca armillaris* leaves (Fig. 8b) essential oil revealed good antimicrobial against *Staphylococcus. aureus* [69].

2.3.3 Fagaropsis angolensis

Fagaropsis angolensis is a tree that belongs to the *Rutaceae* family of flowering plants. The different parts of the plant are traditionally used for the treatment of back pain, joint-aches, malaria, male sterility, and cancer. The seeds of the plant are also chewed for malaria. In Ethiopia the stem bark crude extract is widely used in the treatment of malaria, pneumonia, amoebiasis, and diarrhea. Traditionally, the plant is also applied in veterinary uses to treat diarrhea, and wounds in cattle. The phytochemicals like polyphenols, flavonoids, terpenoids, steroids, alkaloids, and glycosides were identified from crude extract stem bark of *Fagaropsis angolensis*. The *Fagaropsis angolensis* possessed antimicrobial, anticancer, anti-leishmanial, antifungal, and anti-trypanosomal activities. The stem bark extracts of *Fagaropsis angolensis* showed significant in vitro anti-plasmodial activity against strains of *P. falciparum* [70].

The stem bark crude extract of *Fagaropsis angolensis* possess the strongest antimicrobial activity inhibiting growth of *Staphylococcus aureus* and *Candida albicans* with minimum inhibitory concentrations of 64 and 32 µg/ml, respectively [71]. The seed of *Fagaropsis angolensis* (Fig 8a) is used as spice in Gimbo district and put in milk and coffee to flavor them. If a person takes a milk and coffee spiced with this seed, then he/she would never prefer to drink without being spiced with this fruit. The bark of *Fagaropsis angolensis* is mixed with salt and fed to cows so that the milk production improves and taste better. It is also believed that when the bark of this plant is mixed with salt and given to cattle, the cattle's resistances to diseases will be improved and will be very healthy and productive [72]. The other informants reported that, if the seed of this plant given to newly child born mothers until nine days of the child was born to control breast cancer for future and to improve the milk quantity and quality.

a) b) c)

Figure 8: The plant parts used in this study

3 MATERIALS AND METHOD

3.1 Chemicals and equipment'

Analytical grade solvents and reagents such as hydrochloric acid, sulfuric acid, hexane, sodium hydroxide, ferric chloride, dimethyl sulfoxide (DMSO), anhydrous sodium sulfate, methanol, distilled water, gentamycin, clotrimazole, ferric chloride, sodium hydroxide, n-hexane, Wagner reagent, ethyl acetate, petroleum ether, Malathion and ethanol were used in this study.

The equipment's used were test tubes, Whatman No. 2, 8.5, syringe, glass petri-dish, desiccator, rack, cooler, heatmantile, dropper, balance, beakers, round bottom flask, condenser, scissor, labeling papers, cotton, incubator and micro-pippet. For the antibacterial and antifungal activities *Bacillus cereus* and *Staphylococcus aureus* (gram positive bacteria), *E. coli* and *Salmonella typhimurium* (gram negative bacteria), *Candida albicans* (fungi) were used.

For the insecticidal activity evaluation selected insect termites (Isoptera) were used. Mueller Hinton broth (MHB) and potato dextrose agar (PDA) as culture media were used for bacteria and fungi respectively. Dimethyl Sulfoxide (DMSO) was also used as a negative control. Gentamycin and clotrimazole were used as positive control for bacteria and fungi respectively.

3.2 Collection of plant materials

The fresh plant materials were collected during their flowering period; *Fagaropsis angolensis* seed (Yaaye aafo in keffigna) from Gimbo district, *Callistemon citrinus* and *Melaleuca armillaris* leaves (Fig 7) from Jimma town (Jimma University main campus); Southwest Ethiopia in April 2021. The plant specimens collected were authenticated by Mr. Melaku Wondafrash, a plant taxonomist at the Ethiopian National Herbarium, Addis Ababa University and specimens were deposited with voucher numbers AS001, AS002 and AS003 for *Callistemon citrinus*, *Melaleuca armillaris* leaves and *Fagaropsis angolensis* seed respectively

3.3 Extraction of essential oils

Essential oils from selected plant parts (fresh samples) were extracted using hydro-distillation using a Clevenger-type apparatus for 3 h (Fig 9). The fresh plants (310g *Fagaropsis angolensis* seed, 350g of *Callistemon citrinus* leaves and 350g of *Melaleuca armillaris* leaves) were used. After the condensed material was cooled, the oil and water were separated dried with anhydrous

sodium sulfate and filtration, then Percentage yields of the oils were calculated were stored in a refrigerator at 4 °c until use them for further analysis.

Percent yield of EO =Volume of oil/Total weight of fresh sample×100

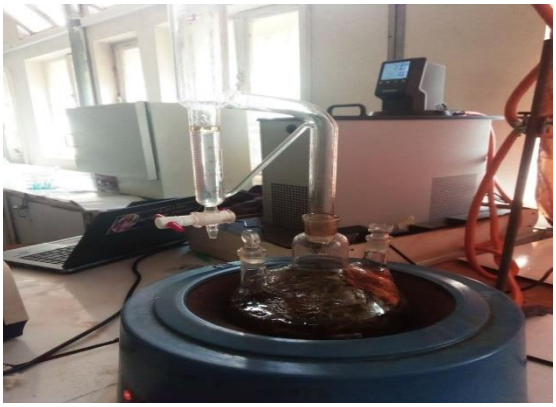


Figure 9: Hydro-distillation extraction of the essential oils

3.4 Characterization of essential oils

3.4.1 Physicochemical characterization of essential oils

The physicochemical analysis of essential oils like color, odor, acid value and saponification value were carried following method described in [38].

- To determine acid value 1g of essential oil sample was weighed in a conical flask. Then 5 ml of ethanol was added to the conical flask and boiled in a water bath for 10 minutes. The solution was shaken well to dissolve the free fatty acids and add about 1ml of phenolphthalein indicator. The hot solution was titrated against the 0.5N sodium hydroxide taken in the burette. The appearance of permanent pale pink color is the end point [73]. The acid value determines the quality storage of essential oils. The higher the acid value the lower the storage quality of essential oils [39]. The acid value of oil sample was calculated using the following formula,

$$\text{Acid value} = \frac{40 \times V \times N\ NaOH}{W}$$

V=volume of NaOH, N=normality of NaOH, W=weight of oils (g) [73]

- To determine saponification value 1g of essential oil was weighted into flask and 12.5 ml of alcoholic sodium hydroxide (Na (OH)) was added. A condenser was attached to the flask and heated for 15min and shaken periodically. Then 0.5ml of 1 % phenolphthalein indicator was added and was titrated with 0.5 N HCl until it reached the end point where it turned colorless. A blank titration was carried out at the same time and under the same condition. The Saponification value was calculated [38].

$$SV = b\text{-}a\ /m \quad (8.05)$$

Where SV = saponification value, b = volume of HCl required by blank, a = volume of HCl required by sample, m = mass of sample

3.4.2 GC-MS analysis of essential oils

Essential oil components were identified using GC-MS system (Agilent technologies 7820A) at Addis Ababa University, College of Natural and Computational Sciences, Chemistry Department, Addis Ababa, Ethiopia. The GC system is coupled to a mass detector (5977E MSD) and HP-88 column (30m x 0.25 mm x 0.25 µm film thickness) coated with 100% poly (dimethyl siloxane). Samples (1 µL) were injected and helium gas was used as carrier gas. The identification of the constituents was based on a comparison of their retention indices mass spectra with those stored in the mass spectral library (NIST) of the GC–MS data system.

3.4.3 Biological activity

3.4.3.1 Antimicrobial activity

The antimicrobial activity tests were carried using broth micro-dilution method at Microbiology laboratory, Department of biology, Jimma University following standard protocols described in Clinical and Laboratory Standards Institute guideline [74]. Gram-positive bacteria (*Bacillus cereus, ATCC 14579)* and *Staphylococcus aureus, ATCC 25923)*, Gram-negative bacteria (*Salmonella typhimurium, ATCC-13311* and *Escherichia coli, ATCC-25922)* and fungi (*Candida albicans, ATCC 60193)* were initially obtained from Health and Nutrition Research Institute, Addis Ababa.

3.4.3.1.1 The MIC determination of essential oils of study plants

For the determination of minimum inhibitory concentration (MIC) initially test tubes containing 1ml of sterile Mueller Hinton Broth (MHB) with final concentration of the essential oil (16.39, 5.46, 1.82, 0.61, 0.20, 0.07l, 0.02 and 0 µl/ml) and control drugs Gentamicin and Clotrimazole were prepared in duplicate for each EOs. Then 100 µl of a bacterial and fungal suspension s were added into each test tube by drawing equal volume of prepared samples. Finally, each test tubes were capped and incubated at 37°C for 24 h. The MICs were determined as the lowest concentration of test samples that doesn't show turbidity.

3.4.3.1.2 MBC and MFC determination of essential oils of study plants

For the determination of MBC / MFC test samples (essential oil/ control drugs) were determined by sub culturing of MIC tubes on Mueller hinter agar plates. The content of test tubes that was shown no visible microbial growth were streaked using sterile wire loop on broth plate free of bacteria and fungi and incubated at 37^{o}c for 24 h. The MBC/MFC's were determined as the lowest concentration of test samples that doesn't show visible growth on agar plate. The MBC and MFC test essential oil was done by following the method of Haloui, T.; Farah, 2015 [75] with some modification.

3.4.3.2 Insecticidal activity

The no-choice bioassay method was employed to evaluate the anti-termite activity of the plant essential oils [76]. First two-fold serial dilutions of each essential oil and Malathion were prepared in a test tube to achieve a final concentration of 5. Then filter paper (Whatman No. 2, 8.5 cm in diameter) was put inside of glass petri-dish and treated with 1 mL of each test concentration in duplicate and 30 active termites (20 workers and 10 soldiers) were introduced into each petri dishes put on each. To avoid desiccation of termite's small role of sterile cotton was placed inside the plate moistened regularly with distilled water. The mortality of termite was then evaluated after exposure time of 5 min, 10 min, 20 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h. Termite showing no sign of movement when slowly shaken the dish or pierced with sharp objects were scored as mortal or death. A piece of filter paper treated with distilled water only was used as negative control. Then mortality was calculated as percentage mean ± SD by using Microsoft excel 2007. Mortality of negative control should be less than 20%; for reliability of corrected mortality [77].

4 RESULTS AND DISCUSSION

4.1 Yield and physical characteristics of essential oils

The physical characteristics such as yield, color (Fig. 10), and odor of the essential oil of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves are given in (Table.1). The essential oil from fresh *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves were yield of 2.58 %, 1.00 %, and 2.29 % respectively. The difference in the yields was noted between the studied essential oil. These differences may be due to the difference in plants species, plants family, plants parts used and environmental location.

4.2 Physico-chemical properties of essential oils

The physico-chemical properties of acid value and saponification value of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves are given in (Table.3). Acid values were 2, 4 and 4 for *Fagaropsis angolensis* seed; *Callistemon citrinus* and *Melaleuca armillaris* leaves essential oil were observed respectively. Acid value determined in this study was used as a general indication of the condition storage of the oil. This is because an increase in acid value is guided by development of unpleasant flavors and odors. The higher the acid value of oil, the lower storage quality and vice-versa, this ensures that the extracted oil has an excellent storage quality. Since the acceptable acid value limited for edible oil is less than 10 [39]. Acid value is also used as an indicator for suitability of oil for use. The acid value of *Fagaropsis angolensis* seed essential oil was lower than the *Callistemon citrinus* and *Melaleuca armillaris* leaves essential oils because of the presence of low number of oxygenated monoterpenes. The greater is the oxygenated terpenes greater the formation of acid compounds due to hydrolysis reaction. Due to this the lower acid value and the oil had good quality [37].

Saponification value 80.50, 72.45, and 72.45 of the essential oil were observed for *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves essential oil respectively. The saponification value determined was revealed that it may contain short chain fatty acids. Saponification value of *Fagaropsis angolensis* seed was higher than the other two plants. The saponification value is a measure of the proportion of low molecular weight triacylglycerols in oil. The high value of saponification in this study indicates amount of molecular weight of fatty acids or number of ester bonds. Furthermore, saponification value indicates the average molecular mass

of fatty acid which is inversely proportional to the chain length of fatty acid in fats and oils [78]. This signifies that; a longer average fatty acid chain length, results in a smaller saponification value. Therefore, *Fagaropsis angolensis* seed has fatty acid with the shortest chain length followed by *Callistemon citrinus* and *Melaleuca armillaris* leaves essential oil being the longest. The *Callistemon citrinus* leaf essential oils from Nepal about acid value (1.02) and saponification values (1.86) and the oil yield (1 %) were reported [66]. From Northern Ethiopia the *Callistemon citrinus* leaf essential oils about acid value (4.37 ± 0.16), saponification value (14.02 ± 0.24) and oil yield (0.73 %) are reported [79] and it is almost similar result to the present study. The *Callistemon citrinus* leaf EO yield (1.20 %), percent of oil (92.00%) and major component 1, 8-cineol (61.20%) reported from South Africa [80] is supportive to our result. The essential oil of *Melaleuca armillaris* leaf studied from Argentina reveal 550 ml from 44.75 kg by method of steam distillation [68] and from Tunisia 0.65 % (dry mass) was reported [81]. There is no published report on the physicochemical properties of the essential oil of *Fagaropsis angolensis* seed. Therefore, the variation in physicochemical properties of studied plant compared to reported one were the plants differed in geographical location, plants harvesting time and oil storage conditions.

Table 1: The physico-chemical properties of essential oils of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves

Physico-chemical properties	**Plants scientific name**		
	Fagaropsis angolensis (seed)	*Callistemon citrinus* (leaves)	*Melaleuca armillaris* (leaves)
Color	Colorless	Colorless	Colorless
Odor	Pleasant	Pleasant	Pleasant
Acid- value	2	4	4
Saponification value	80.50	72.45	72.45

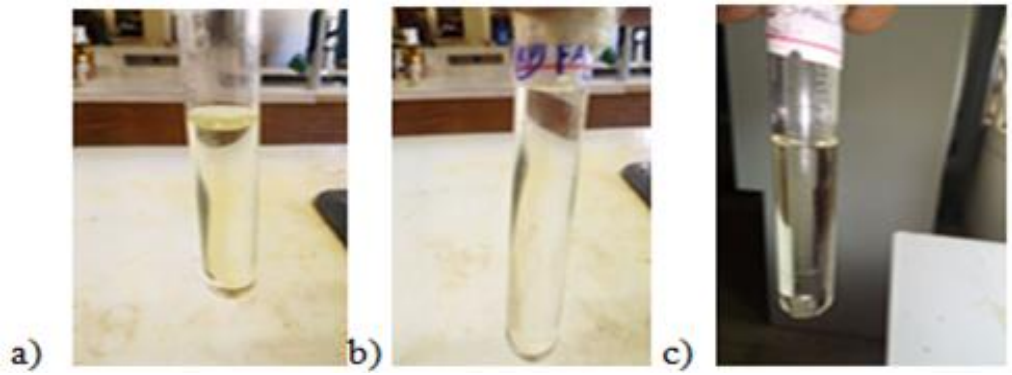


Figure 10: Essential oils from *Callistemon citrinus* leaves (a), *Fagaropsis angolensis* seed (b) and *Melaleuca armillaris* leaves (c)

4.3 Chemical constituents of essential oils of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves

The identified components, their retention time and area are summarized in (Table 2). Five components per each plant were identified accounting to 100%, 100% and 99.99% of the total identified components for *Callistemon citrinus*, *Melaleuca armillaris* and *Fagaropsis angolensis* respectively (Appendex 6). The major constituents of essential oils in three plants *Fagaropsis angolensis* seed, *Melaleoca armillaris* and *Callistemon citrinus* leaves were Bicyclo (3.1.0) hexane, 4-methylene-1-(1-methylethyl)- (72.29%), Eucalyptol (1,8-cineol) (80.08%), and Eucalyptol (1,8-cineol) (72.45%) respectively. Their structures were indicated in (Fig 11).

The finding of the present study showed that essential oil from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves were mainly composed of monoterpene. From Argentina the *Melaleuca armillaris* leaves essential oils the main components reported were 1,8-cineol (72.30%) , limonene (7.80%) and α-pinene (6.00%) [68]. From Tunisia the *Melaleuca armillaris* leaves essential oils cis-calamenene (19.00%), torreyol (15.10%) and dihydrocarveol (9.00%) were reported as major components [81] which is none of them were resulted in our study it may be due to geographical location difference.

Table 2: Chemical constituents identified from essential oils of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves

S. N	Name of compounds	*Fagaropsis angolensis*		*Melaleuca armillaris*		*Callistemon citrinus*	
		RT	Area (%)	RT	Area (%)	RT	Area (%)
1	Bicyclo (3.1.0) hexane, 4-methylene-1-(1-methylethyl) (sabinene) (6)	5.88	72.29	-	-	-	-
2	1,4-pentadiene (4)	6.69	6.42	-	-	-	-
3	p-cymene (1)	6.96	5.99	-	-	-	-
4	Terpinen-4-ol (7)	9.89	8.06	9.89	1.09	-	-
5	2-undecanone (3)	11.56	7.23	-	-	-	-
6	Methylene chloride (11)	-	-	5.14	4.83	-	-
7	D-limonene (9)	-	-	6.69	5.35	6.69	4.78
8	Eucalyptol(1,8-cineol) (2)	-	-	7.02	80.08	7.02	72.45
9	α-terpineol (8)	-	-	10.33	8.66	10.34	6.74
10	Acetic acid, chloro-ethyl ester (5)	-	-	-	-	4.95	2.42
11	2-chloro ethanol (10)	-	-	-	-	5.08	13.61
Monoterpenes (hydrocarbon)		78.28		5.35		4.78	
Oxygenated monoterpenes		15.29		89.83		79.19	
Others		6.42		4.83		16.03	
Total (%)		99.99		100		100	

(-) absence or not resulted

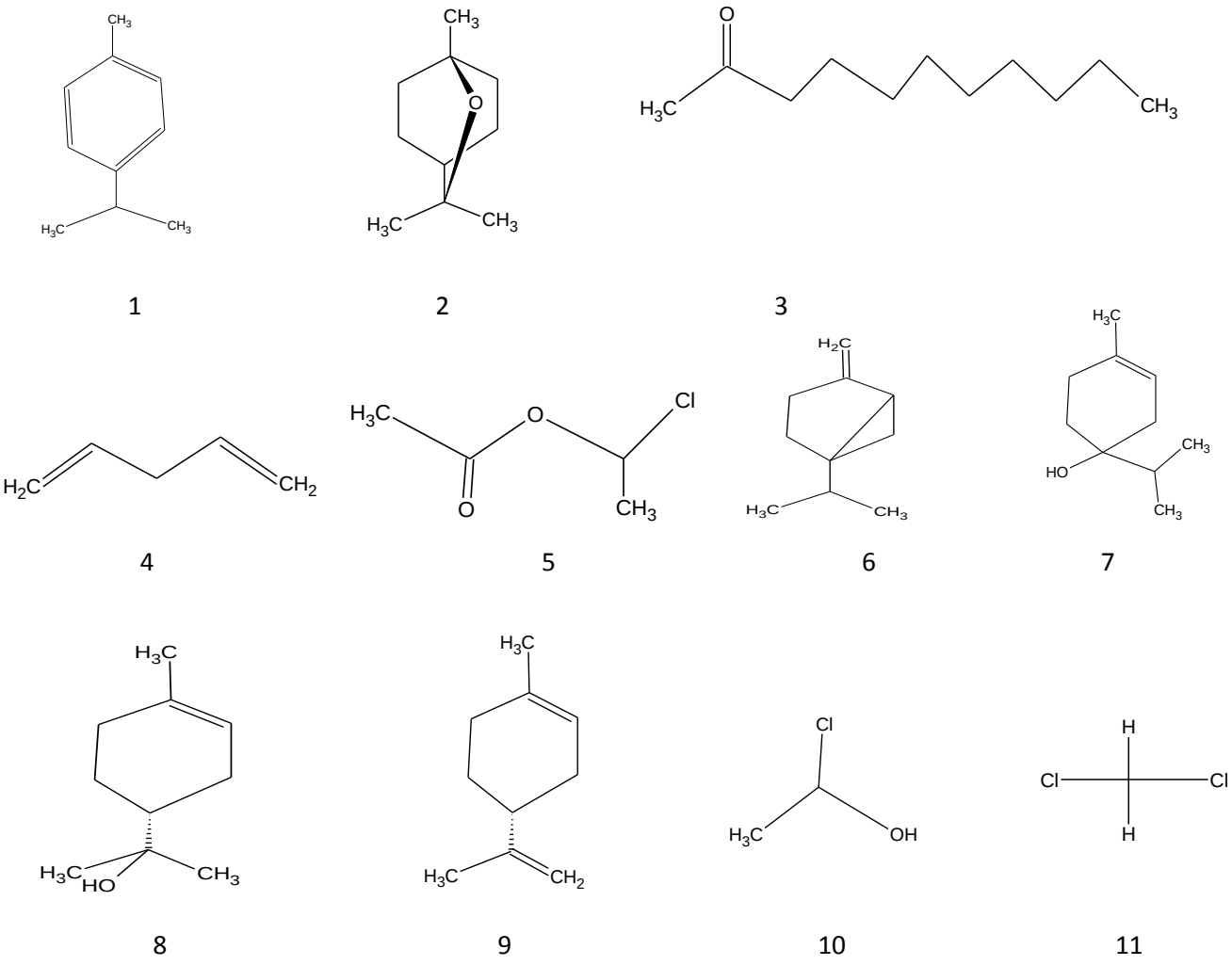


Figure 11: The structure of the constituents of essential oil of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves

4.4 Biological activities of essential oils

4.4.1 Antimicrobial activity

The three plants essential oils antimicrobial activities results were noted and showed the difference in an antimicrobial activity by MIC test (Table 3). Among the tested essential oils, *Fagaropsis angolensis* seed oil was showed the very highest microbial inhibition activity compared to *Callistemon citrinus* and *Melaleuca armillaris* leaves with MIC 0.40 ± 0.29 against (*B cereus, S aureus* and *S typhimurium*,) MIC 0.14 ± 0.09 against (*E. coli*) and MIC 1 ± 0 against (*C. albicans*).

The results also indicate that all the essential oils have bacteriostatic and fungistatic properties. Their antimicrobial activity order was *Fagaropsis angolensis* seed oil >> *Callistemon citrinus* leaves oil > *Melaleuca armillaris* leaves oils. The difference observed at the MIC concentration and antimicrobial activities was due to many factors such as environmental, plant family and species, plant parts and constituents' variation.

The essential oil constituents Bicyclo (3.1.0) hexane, 4-methylene-1-(1-methylethyl) - (sabinene) **(**72.29 %) (6), *p*-cymene (5.99%) resulted from *Fagaropsis angolensis* seed and their synergistic effect with other constituents may make it high in antimicrobial activity compared to *Callistemon citrinus* leaf and *Melaleuca armillaris* essential oils. Since; p-Cymene shows high affinity for microbial membranes and increased the antimicrobial activities of other compounds. However, it does not affect the membrane permeability, but it significantly decreases the enthalpy and melting temperature of the membrane [60]. The highest content of 1, 8-cineol may be the factor contributing to the antimicrobial activities of the *Callistemon citrinus* and *Melaleuca armillaris* leaves essential oils since; the compound has been recognized with the permeability of membrane of microorganism as antimicrobial activity. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity.

The MIC 1 ± 0 of *Callistemon citrinus* leaves essential oil against *Candida albicans* in our study is lower compared to result from central Vietnam MIC 16 µg/ml [82]. The dry leaves of *Callistemon citrinus* essential oil from Nepal was bacteriostatic against *S. aureus* and *E. coli* [66] and this support our result. The essential oil of *Melaleuca armillaris* leaves from Argentina; antibacterial against *S. aureus* with MIC 25µg/ml [68].

Table 3: MIC (µl/ml) of essential oils of *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves

Test sample	Bacterial strains				Fungal strain
	E. coli	*B. cereus*	*S. aureus*	*S.typhimurium*	*C. albicans*
Fagaropsis angolensis	0.14 ± 0.09	0.40 ± 0.29	0.40 ± 0.29	0.40 ± 0.29	1 ± 0
Callistemon citrinus	3.64 ± 2.57	1.21 ± 0.86	1.21 ± 0.86	1.21 ± 0.86	1 ± 0
Melaleuca armillaris	3.64 ± 2.57	3.64 ± 2.57	3.64 ± 2.57	10.93 ± 7.72	1 ± 0
Gentamycin	0.14± 0.09	0.14± 0.09	0.14 ± 0.09	0.14 ± 0.09	-
Clotrimazole	-	-	-	-	1 ± 0

4.4.2 Insecticidal activity

The insecticidal activities of *Fagaropsis angolensis*, *Melaleuca alternifolia* and *Callistemon citrinus* essential oil against termite (Isoptera) was investigated by using no-choice bioassay and Malathion as positive control (Appendex 5). The results obtained are given in (Table.4, 5, 6 and 7). It was found that the direct toxicity effect of essential oils decreased with the increasing time duration at 20 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h after oil treatment. At the same dose, gradually decreased effect of toxicity was observed over the time course of 10 min, 20 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h. The data shows that essential oils from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillari*s leaves were all active against insect termites. This difference of toxicity against termite may be the synergistic effect of active major component with minor components in the essential oils.

The wide difference in toxicity of essential oil of *Callistemon citrinus* and *Melaleuca armillaris* leaves were may be due to the presence of 1, 8-cineol as major constituents and chlorinated components, since the chlorinated components have high fumigation towards insects. The essential oil of *Callistemon citrinus* leaves studied from Nepal revealed anti-termicidal activity [83]. In insecticidal activity, the essential oil components may be able to act in a number of different ways, causing toxicity, slowing development, inhibiting feeding, and reducing fertility [12]. However,

there is no more report of termicidal activity of oils from *Melaleuca armillaris* leaves and *Fagaropsis angolensis* seed in the literature.

Table 4: The insecticidal activities of *Fagaropsis angolensis* seed essential oils

Concn (%)	***Fagaropsis angolensis* seed EO termiticidal activities per time in percent (%)**										
	5min	10min	20min	30min	1h	3h	6h	12h	24h	48h	72h
5	31.67 ± 2.35	100 ± 0									
2.5	24.99 ± 2.35	44.99 ± 2.35	100 ± 0								
1.25	0	18.33 ± 2.36	28.33 ± 2.36	51.66 ± 2.35	86.66 ± 4.71	100 ± 0					
0.625	0	8.33 ± 2.36	21.66 ± 2.35	29.99 ± 4.71	49.99 ± 4.71	69.99 ± 4.71	83.33 ± 4.70	94.99 ± 2.35	100 ± 0		
0.313	0	0	0	0	0	0	8.33 ± 2.36	13.33± 4.70	26.66 ± 4.71	100 ± 0	
0.16	0	0	0	0	0	0	0	8.33 ± 2.36	13.33± 4.70	66.66 ± 4.71	89.99 ±4.71
NC	0	0	0	0	0	0	0	0	0	13.33 ± 4.7	18.33 ±2.36

❖ Results are mortality in percent of mean ± SD, Concn = concentration

Table 5: The insecticidal activity of *Callistemon citrinus* leaves essential oils

Concn (%)	***Callistemon citrinus* leaves EO termiticidal activities per time in percent (%)**										
	5min	10min	20min	30min	1h	3h	6h	12h	24h	48h	72h
5	13.3± 4.70	31.7 ± 2.35	44.9 ± 2.35	51.7 ± 2.35	61.7 ± 2.12	100 ± 0					
2.5	0	18.3 ± 2.36	29.9 ± 4.71	31.7 ± 2.35	51.7 ± 2.35	100 ± 0					
1.25	0	0	0	28.3 ± 2.36	31.7 ± 2.35	39.9 ±4.71	41.7± 2.35	100 ± 0			
0.625	0	0	0	0	14.9± 2.35	21.7± 2.35	34.9± 2.35	54.9± 2.58	61.7 ± 2.12	69.9 ± 4.71	100 ± 0
0.313	0	0	0	0	0	8.3 ± 2.36	18.3 ± 2.36	41.7± 2.35	49.9 ± 4.71	54.9± 2.58	83.3 ± 4.70
0.16	0	0	0	0	0	0	11.7 ±2.35	21.7 ± 2.35	26.7 ± 4.71	34.9± 2.35	71.7 ±2.35

❖ Results are mortality in percent of mean ± SD, Concn = concentration

Table 6: The insecticidal activity of *Melaleuca armillaris* leaves essential oils

Concn (%)	***Melaleuca armillaris* leaves EO termiticidal activities per time in percent (%)**										
	5min	10min	20min	30min	1h	3h	6h	12h	24h	48h	72h
5	51.7 ± 2.35	100 ± 0									
2.5	0	51.7 ± 2.35	100 ± 0								
1.25	0	11.7 ±2.35	18.3 ± 2.36	28.3 ± 2.36	44.9 ± 2.35	100 ± 0					
0.625	0	0	0	0	39.9 ±4.71	51.7 ± 2.35	61.7 ± 2.12	71.7 ±2.35	91.6± 2.35	100 ± 0	
0.313	0	0	0	0	28.3 ± 2.36	49.9 ± 4.71	51.7 ± 2.35	69.9 ± 4.71	84.9± 2.35	86.6 ± 4.71	94.9 ± 2.35
0.16	0	0	0	0	18.3 ± 2.36	34.9± 2.35	44.9 ± 2.35	61.7 ± 2.12	71.7 ±2.35	84.9 ± 2.35	91.6 ± 2.35

❖ Results are mortality in percent of mean ± SD, Concn = concentration

Table 7: The insecticidal activities of positive control

Concn (%)	**Malathion termiticidal activity per min and time in percent (%)**										
	5min	10min	20min	30min	1h	3h	6h	12h	24h	48h	72h
5	61.7 ± 2.12	91.6 ± 2.35	100 ± 0								
2.5	34.9± 2.35	88.33 ±2.36	100 ± 0								
1.25	31.7 ± 2.35	84.9 ± 2.35	91.6 ± 2.35	100 ± 0							
0.625	28.3 ± 2.36	74.99± 2.35	88.33 ±2.36	100 ± 0							
0.313	18.3 ± 2.36	69.9 ± 4.71	84.9 ± 2.35	100 ± 0							
0.16	11.7 ±2.35	51.7± 2.35	81.66± 2.35	100 ± 0							

❖ Results are mortality in percent of mean ± SD, Concn = concentration

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The results of biological activities test indicate that all the tested essential oils have bacteriostatic and fungistatic properties. Based on the result the essential oil extracted from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves had remarkable antimicrobial and insecticidal activities against *E. coli, B. cereus, S. aureus, S. typhimurium, C. albicans and termites*. The essential oil extracted from *Fagaropsis angolensis* seed, *Callistemon citrinus* and *Melaleuca armillaris* leaves *showed 100 % mortality of termites for 5% essential oils concentration with short time of exposure from10min to 3 hours. The 100 % mortality of termites was observed for 5% essential oils with short time of exposure from10min to 3 hours. Therefore, the essential oils* had remarkable insecticidal activities against *termites*.

The GC-MS analysis, the most abundant components identified from *Fagaropsis angolensis* seed Bicyclo (3.1.0) hexane, 4-methylene-1-(1-methylethyl) (sabinene) (72.29%), from *Callistemon citrinus* leaves Eucalyptol (1, 8-cineol) (72.45%), from *Melaleuca armillaris* leaves Eucalyptol (1, 8-cineol) (80.08 %). The results support the idea of using essential oils as antimicrobial and as insecticide to control termite rather than using synthetic bactericides, fungicides and insecticides.

5.2 Recommendation

- Further studies are recommended to identify extra active components by using method other than hydro-distillation extraction
- Determining the effect of essential oils anticancer and antioxidant activities are recommended
- Further evaluation of antimicrobial activities of essential oil constituents' synergy mechanism that reveal mode action of constituents are recommended
- Further assessment of insecticidal activities of essential oils below 0.63% are recommended
- Determining the extra antimicrobial activities of essential oils above 50µl/ml concentration are recommended
- The two plants such as *Callistemon citrinus* and *Melaleuca armillaris* leaves activity to trap halogenated components for purpose of bioremediation are recommended

REFERENCES

[1] Rassem, H.; Nour, A.; R. M., Y. Techniques For Extraction of Essential Oils From Plants: A Review. *Aust. J. Basic Appl. Sci.*, **2016**, *10* (16), 117–127.

[2] Hamid,A.A., Aiyelaagbe, O. O., Usman, L. . Essential Oils: Its Medicinal and Pharmacological Uses. *Int. J. Curr. resear*, **2011**, *3* (2), 086–098.

[3] Zhang, C. Y.; Guo, M. Comparing Three Different Extraction Techniques on Essential Oil Profiles of Cultivated and Wild Lotus (Nelumbo Nucifera) Flower. *Life*, **2020**, *10* (9), 1–9.

[4] Naeem, A.; Abbas, T.; Ali, T. M.; Hasnain, A. Annals of Short Reports Essential Oils : Brief Background and Uses. **2018**, *1* (1).

[5] Bhavaniramya, S.; Vishnupriya, S.; Al-aboody, M. S. Grain & Oil Science and Technology Role of Essential Oils in Food Safety : Antimicrobial and Antioxidant Applications. *Grain Oil Sci. Technol.*, **2019**, *2* (2), 49–55.

[6] Noumi, E.; Snoussi, M.; Alreshidi, M. M.; Rekha, P.; Saptami, K.; Caputo, L.; Martino, L. De. Chemical and Biological Evaluation of Essential Oils from Cardamom Species. **2018**.

[7] Zhang, J.; An, M. I. N.; Wu, H.; Stanton, R. E. X.; Lemerle, D. Chemistry and Bioactivity of Eucalyptus Essential Oils Chemistry and Bioactivity of Eucalyptus Essential Oils. **2016**, No. April 2010.

[8] Paola Angelini, A. P. Some Biological Activities of Essential Oils. *Med. Aromat. Plants*, **2012**, *02* (05), 2–5.

[9] Adinew, B. GC-MS and FT-IR Analysis of Constituents of Essential Oil from Cinnamon Bark Growing in South-West of Ethiopia. **2014**, *1* (6), 22–31.

[10] Ramadan, T.; Zaher, A.; Amro, A.; Sultan, R. Chemical Composition and Biological Activity of Capetula and Shoots Essential Oils of Senecio Glaucus L. *J. Essent. Oil-Bearing Plants*, **2020**, *23* (1), 168–183.

[11] Jessen, M. Effects of Termites (Macrotermes) and Large Herbivores on Exotic and Native Tree Species in Lake Mburo National Park , Uganda. **2014**.

[12] Aleyrodidae, G. H.; Tia, E. V; Adima, A. A.; Niamké, S. L.; Jean, G. A.; Martin, T. Natural Product Communications Chemical Composition and Insecticidal Activity of Essential Oils of Two Aromatic Plants from Ivory Coast Against. **2011**, 2–7.

[13] Sales, T. A.; Das, M.; Cardoso, G.; Gustavo, L.; Guimarães, D. L.; Camargo, K. C.; Aparecida, D.; Selvati, D. C.; Brandão, R. M.; Souza, R. V.; et al. Essential Oils from the

Leaves and Flowers of Callistemon Viminalis : Chemical Characterization and Evaluation of the Insecticide and Antifungal Activities. **2017**, 2516–2529.

[14] Nakatsu, T.; Lupo, A. T.; Chinn, J. W.; Kang, R. K. L. Biological activity of essential oils and their constituents. **2000**, *21*, 571–631.

[15] Butnariu, M.; Sarac, I. Essential Oils from Plants. *J. Biotechnol. Biomed. Sci.*, **2018**, *1* (4), 35–43.

[16] Dixit, S.; Kelkar, S. H.; Part, P.; Name, P.; Lavender, S.; Flowers, O.; Orange, B.; Orange, S.; Balsam, T.; Plant, E.; et al. Essential oil extractions principles and practice. **2007**, No. August.

[17] Ahl, H. A. H. S.; Hikal, W. M.; Mahmoud, A. A. Journal of Chemical and Pharmaceutical Research , 2017 , 9 (8): 190-200 Essential Oils : Biosynthesis , Chemistry and Biological Functions. **2017**, *9* (8), 190–200.

[18] Rehman, R.; Hanif, M. A.; Mushtaq, Z.; Al-Sadi, A. M. Biosynthesis of Essential Oils in Aromatic Plants: A Review. *Food Rev. Int.*, **2016**, *32* (2), 117–160.

[19] Rehman, R.; Hanif, M. A.; Mushtaq, Z.; Mochona, B.; Qi, X. Natural Products Chemistry & Research Biosynthetic Factories of Essential Oils : The Aromatic Plants. *Nat. Prod. Chem. Res.*, **2016**, *4* (4).

[20] Tresvenzol, L. M. F.; Santos, P. A.; Ferri, P. H.; Sá, S. De; Fiuza, T. S. Chemical Composition of Essential Oils of Leaves, Flowers and Fruits of Hortia Oreadica. *Rev. Bras. Farmacogn.*, **2015**.

[21] Eslahi, H.; Fahimi, N.; Sardarian, A. R. Chemical Composition of Essential Oils. **2018**.

[22] Juliani, H. R. Physical and Chemical Properties , Composition , and Biological Activity of Essential Oils of Philippine Medicinal Plants. **2017**, *5* (2), 28–35.

[23] Review, A. C. Essential Oils ' Chemical Characterization and Investigation of Some Biological Activities : **2020**.

[24] Croteau, R.; Kutchan, T. M.; Lewis, N. G. Secondary Metabolites. **2000**, 1250–1318.

[25] Reddy, D. *Essential Oils Extracted from Medicinal Plants and Their Applications*; 2019.

[26] Stanojević, L.; Stanković, M.; Cakić, M.; Nikolić, V.; Nikolić, L.; Ilić, D.; Radulović, N. Uticaj Tehnike Hidrodestilacije Na Prinos, Sastav, Kinetiku i Antimikrobnu Aktivnost Etarskih Ulja Iz Cveta Lavandula Officinalis L. *Hem. Ind.*, **2011**, *65* (4), 455–463.

[27] CO2, J. R. and S. V. Facile methods for the extraction of essential oil from the plant. *Int.*

J. Pharm. Sci. Res., **2014**, *5* (4), 1107–1115.

[28] A. I. Chemical Technology. **2010**, *5* (1).

[29] Egza, T. F. A Review on Extraction , Isolation , Characterization and Some Biological Activities of Essential Oils from Various Plants. **2020**, *8* (1), 1692–1722.

[30] Mumtaz, W.; Mukhtar, H.; Anwar, F.; Nadeem, R. Extraction and Characterization of Essential Oil of Rosa Gruss-an-Teplitz. *Asian J. Chem.*, **2007**, *19* (2), 949–953.

[31] Mejri, J.; Aydi, A. Asian Journal of Green Chemistry Review Article Emerging Extraction Processes of Essential Oils : A Review. **2018**, *2*, 246–267. https://doi.org/10.22034/ajgc.2018.61443.

[32] Stratakos, A. C.; Koidis, A. *Methods for Extracting Essential Oils*; Elsevier Inc., 2016.

[33] Tongnuanchan, P.; Benjakul, S. Essential Oils : Extraction , Bioactivities , and Their Uses for Food Preservation. **2014**, *79* (7), 1231–1249.

[34] Buchbauer, K. H. C. B. and G. *Essential Oils; Science, Technology and Applications*; Ba¸ser, K. H. C., Ed.; 2010.

[35] Elshafie, H. S.; Camele, I. An Overview of the Biological Effects of Some Mediterranean Essential Oils on Human Health. *Biomed Res. Int.*, **2017**, *2017*.

[36] Marvin, C. M. *GC / MS A Practical User ' s Guide*, 2nd editio.; 2008.

[37] Sinica, D. C.; Kumar, A.; Kumar, A. Pelagia Research Library. **2014**, *5* (2), 91–94.

[38] Ibrar, M.; Rauf, A. Physicochemical Characterization of Essential and Fixed Oils of Skimmia Laureola and Zanthoxylum Armatum. **2012**, *1* (3), 51–58.

[39] Abera, A. Extraction and Physicochemical Analysis of Essential Oils in Lemongrass Leaves Grown In. **2020**, *9* (10), 638–641.

[40] Cordery, A.; Rao, A. P.; Ravishankar, S. Antimicrobial Activities of Essential Oils , Plant Extracts and Their Applications in Foods- A Review. **2018**, *7* (2), 76–89.

[41] Tanhaeian, A.; Sekhavati, M. H.; Moghaddam, M. Antimicrobial Activity of Some Plant Essential Oils and an Antimicrobial ‑ Peptide against Some Clinically Isolated Pathogens. *Chem. Biol. Technol. Agric.*, **2020**, 1–11.

[42] Li, Y.; Chemat, F. *Springer briefs in molecular science Essential Oils as Reagents in Green Chemistry*; 2014.

[43] E. J. European journal of. *Antimicrob. Appl. Differnet plant Essent. OILS*, **2017**, *4* (7), 740–750.

[44] Tanu, B.; Harpreet, K. Review Article. **2016**, *8* (6), 143–149.

[45] Hamid , ISSN : 0975-833X Research article essential oils : its medicinal and pharmacological uses **2011**, 86–98.

[46] Bachir, R. Antimicrobial Activity of Eucalyptus Globulus Oils Antimicrobial Activity of Eucalyptus Globulus Oils. **2017**,

[47] Celikel, N.; Kavas, G. Antimicrobial Properties of Some Essential Oils against Some Pathogenic Microorganisms. *Czech J. Food Sci.*, **2008**, *26* (3), 174–181.

[48] Shuaib, A.; Rohit, A.; Piyush, M. A Review Article on Essential Oils. **2016**, *4* (3), 237–240.

[49] Siddique, S. Essential Oils and Cosmetic Aromatherapy. *Trichology Cosmetol.*, **2017**, *1* (1), 7–8.

[50] Sarkic, A.; Stappen, I. Essential Oils and Their Single Compounds in Cosmetics **2018**, 1–21.

[51] Oliviu Vostinaru, S. C. H. and L. F.; Abstract. *Safety Profile of Essential Oils*; 2020. https://doi.org/DOI:

[52] Portoghese, P. S.; Waddell, W. J.; Wagner, B. M.; Hall, R. L.; Lucas-gavin, C.; Adams, T. B. A Procedure for the Safety Evaluation of Natural Flavor Complexes Used as Ingredients in Food : Essential Oils. 1–41.

[53] Balali, G. I.; Yar, D. D.; Gobe, V.; Dela, A.; Adjei-kusi, P. Microbial Contamination , an Increasing Threat to the Consumption of Fresh Fruits and Vegetables in Today ' s World. **2020**, *2020*.

[54] Mascellino, M. T. Prologue : Escherichia Coli , Listeria , and Salmonella. 1–7.

[55] Teplitski, M.; George, A.; Hochmuth, G. Salmonella and Pathogenic E . Coli in the Crop Production Environment : Potential Sources , Survival , Domestic and Wild Animals as Vectors of Human Pathogens Surveys of Microbiological Water Quality Source of Human Pathogens. **2015**, 1–3.

[56] Ávila-Quezada, G.; Sánchez, E.; Gardea-Béjar, A. A.; Acedo-Félix, E. Salmonella Spp. and Escherichia Coli: Survival and Growth in Plant Tissue. *New Zeal. J. Crop Hortic. Sci.*, **2010**, *38* (2), 47–55.

[57] Kumar, T. D. K.; Batra, H. S. M. H. V. Simultaneous Detection of Pathogenic B . Cereus , S . Aureus and L . Monocytogenes by Multiplex PCR. **2009**, No. September, 283–289.

[58] Bennett, S. D.; Walsh, K. A.; Gould, L. H. Foodborne Disease Outbreaks Caused by Bacillus Cereus , Clostridium Perfringens , and Staphylococcus Aureus — United States , 1998 – 2008. **2013**, *57*.

[59] Swidergall, M. Candida Albicans at Host Barrier Sites : Pattern Recognition Receptors and Beyond. **2019**, 1–14.

[60] Burillo, J.; Rota, C.; Carramin, J. J. In Vitro Antimicrobial Activity of Essential Oils from Aromatic Plants against Selected Foodborne Pathogens. **2004**, *67* (6), 1252–1256.

[61] Abdurrahman Ayvaz1a, Osman Sagdic2b, Salih Karaborklu3c, I. O.; 1Erciyes. Insecticidal Activity of the Essential Oils from Different Plants Against Three Insecticidal Activity of the Essential Oils from Different Plants against Three Stored-Product Insects. *f Insect Sci.*, **2010**, No. May 2014.

[62] Arena, J. S.; Laura, M.; Calvimonte, H.; Zygadlo, A. Fumigant and Repellent Activities of Different Essential Oils Alone and Combined against the Maize Weevil (Sitophilus Zeamais Motschulsky). **2017**, *20* (7), 249–253.

[63] Li, Y.; Yan, S.; Wang, J.; Li, L.; Zhang, J.; Wang, K. Insecticidal Activities and Chemical Composition of the Essential Oils of Ajania Nitida and Ajania Nematoloba from China. **2018**, *1577* (967), 1571–1577.

[64] Kim, J.; Jeong, I.; Lee, Y. S.; Lee, S. Insecticidal Activity of Cinnamon Essential Oils , Constituents , and (E) - Cinnamaldehyde Analogues against Metcalfa Pruinosa Say (Hemiptera : Flatidae) Nymphs and Adults. **2015**, *54* (4), 375–382.

[65] Khubeiz, M. J.; Mansour, G. Chemical Composition and Antimicrobial Activity of Essential Oil of Callistemon Viminalis from the South Syria. *Int. J. ChemTech Res.*, **2016**, *9* (06), 242–250.

[66] Bhandari, N. L.; Khadka, S.; Dhungana, B. R.; Bhandari, G. Study of Phyto- and Physicochemical Analysis , Antimicrobial and Antioxidant Activities of Essential Oil Extract of Callistemon Citrinus (Curtis) Skeel s Leaves. **2021**, *3* (2), 109–119.

[67] Pauliello, K. E.; Maria, D.; Tosta, S.; Filho, M. M.; Teixeira, M. A.; Rodrigues, A. Antihistaminic Action of Melaleuca Armillaris Ointment. **2019**, *13* (10), 236–241.

[68] Buldain, D.; Buchamer, A. V; Marchetti, M. L.; Aliverti, F.; Bandoni, A.; Mestorino, N. Combination of Cloxacillin and Essential Oil of Melaleuca Armillaris as an Alternative Against Staphylococcus Aureus. **2018**, 1–8.

[69] Buldain, D.; Castillo, L. G.; Buchamer, A. V. Melaleuca Armillaris Essential Oil in Combination With Rifaximin Against Staphylococcus Aureus Isolated of Dairy Cows. **2020**, 1–10.

[70] Kassa, B.; Misganaw, D. Antimalarial Activity of Fagaropsis Angolensis (Rutaceae) Crude Extracts and Solvent Fractions of Its Stem Bark Against Plasmodium Berghei in Mice. **2021**, No. January.

[71] Kuglerova, M.; Tesarova, H.; Grade, J. T.; Halamova, K.; Wanyana-, O.; Damme, P. Van; Kokoska, L. Antimicrobial and Antioxidative Effects of Ugandan Medicinal Barks. **2011**, *10* (18), 3628–3632.

[72] School of graduate studies A Survey of Nontimber Forest Products and Their Conservation Status in the Gimbo District , SNNPR , Southwest. **2008**,

[73] Modn, I. J.; Revs, R. International journal of modern research and reviews original article determination and its correlation of acid value in the various oil * A . Kumaresan , J . Jenisha , G . R . Aiswarya , B . Abina Department of Chemistry , St . Alphonsa College of Arts A. **2017**, No. 8, 1647–1649.

[74] Sahu, C.; Jain, V.; Mishra, P.; Prasad, K. N. Clinical and Laboratory Standards Institute versus European Committee for Antimicrobial Susceptibility Testing Guidelines for Interpretation of Carbapenem Antimicrobial Susceptibility Results for Escherichia Coli in Urinary Tract Infection (UTI). *J. Lab. Physicians*, **2018**, *10* (03), 289–293. https://doi.org/10.4103/jlp.jlp, 176_17.

[75] Haloui, T.; Farah, A.; Balouiri, M.; Chraibi, M.; Fadil, M.; Benbrahim, K. F.; Alaoui, A. B. Bacteriostatic and Bactericidal Profile of Leaves and Twigs Essential Oils of Moroccan Pistacia Lentiscus L . **2015**, *5* (May 2013), 50–53.

[76] Almeida, M. Antitermitic Activity of Plant Essential Oils and Their Major Constituentsagainst Termite Heterotermes Sulcatus (Isoptera : Rhinotermitidae). **2015**, No. January.

[77] Paramasivam, M.; Selvi, C. Laboratory Bioassay Methods to Assess the Insecticide Toxicity against Insect Pests-A Review. **2017**, *5* (3), 1441–1445.

[78] Godwin, A. O.; Adedayo, F. E.; Oluwayemisi, A. E. Physicochemical Properties of Essential Oils of Ginger (Zingiber Officinale), African Nutmeg (Monodora Myristica) and Ashanti Black Pepper (Piper Guineense). **2019**, *17* (4), 58–64.

[79] Aweke, N.; Yeshanew, S. Chemical Composition and Antibacterial Activity of Essential Oil of Callistemon Citrinus from Ethiopia. **2016**, *17* (1), 1–7.

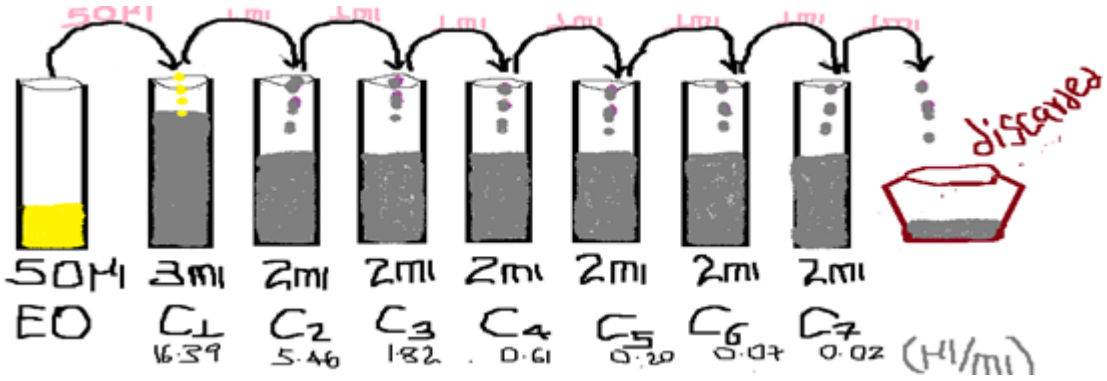
[80] Oyedeji, O. O.; Lawal, O. A.; Shode, F. O.; Oyedeji, A. O. Hemical Composition and Antibacterial Activity of the Essential Oils of Callistemon Citrinus and Callistemon Viminalis from South Africa. **2009**, No. June 2014.

[81] Amri, I.; Mancini, E.; Martino, L. De; Marandino, A.; Lamia, H. Chemical Composition and Biological Activities of the Essential Oils from Three Melaleuca Species Grown in Tunisia. **2012**, 16580–16591,

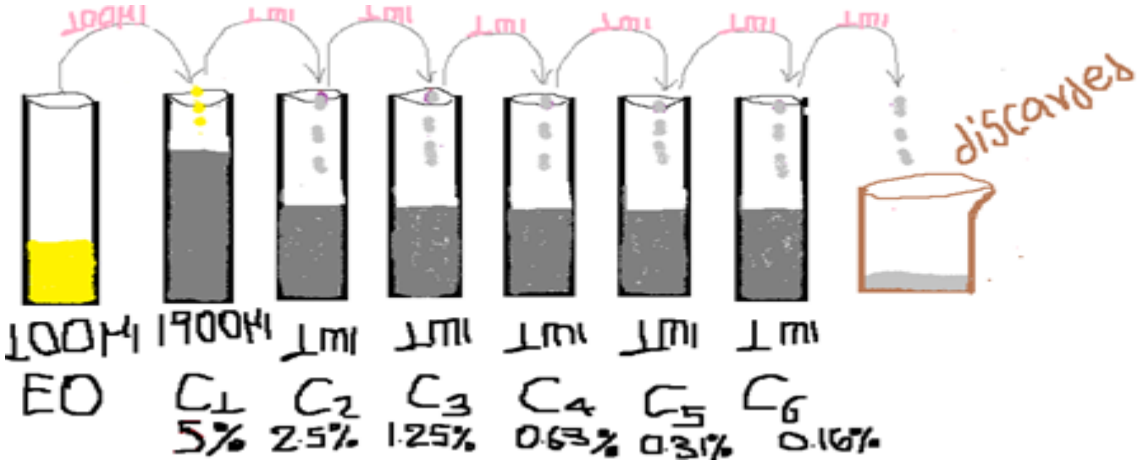
[82] Thi, N.; An, G.; Huong, L. T.; Satyal, P.; Tai, T. A.; Dai, D. N. And Chemical Compositions of Essential Oils from Four Species of Myrtaceae from Central Vietnam. **2020**.

[83] Samon Shrestha, Ambika Poudel, Prabodh Satyal, Noura S. Dosoky, Bhuwan K Chhetri, W. N. S. Chemical Composition and Biological Activity of the Leaf Essential Oil of Callistemon Citrinus from Nepal Samon. *Essent. oil Nat. Prod.*, **2015**, *3(1)* (05), 29–33.

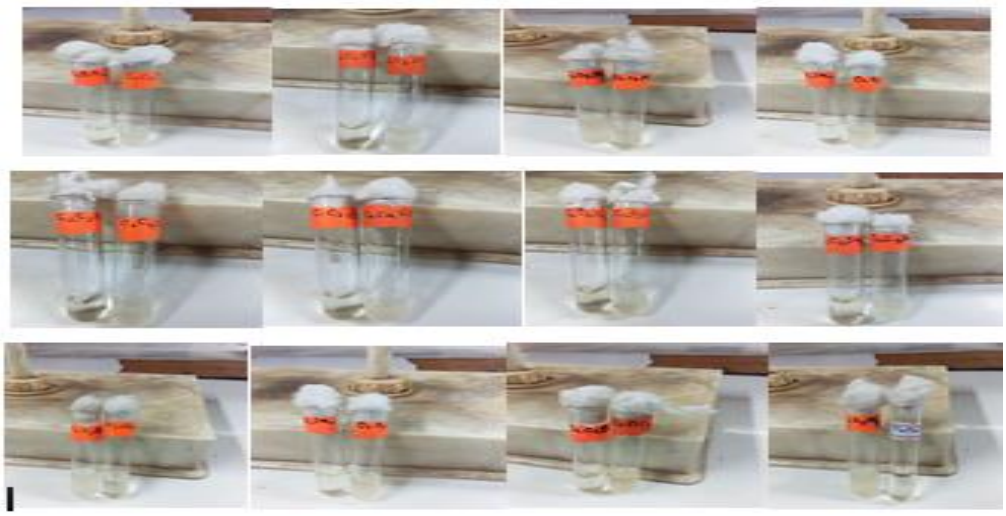
APPENDIXES



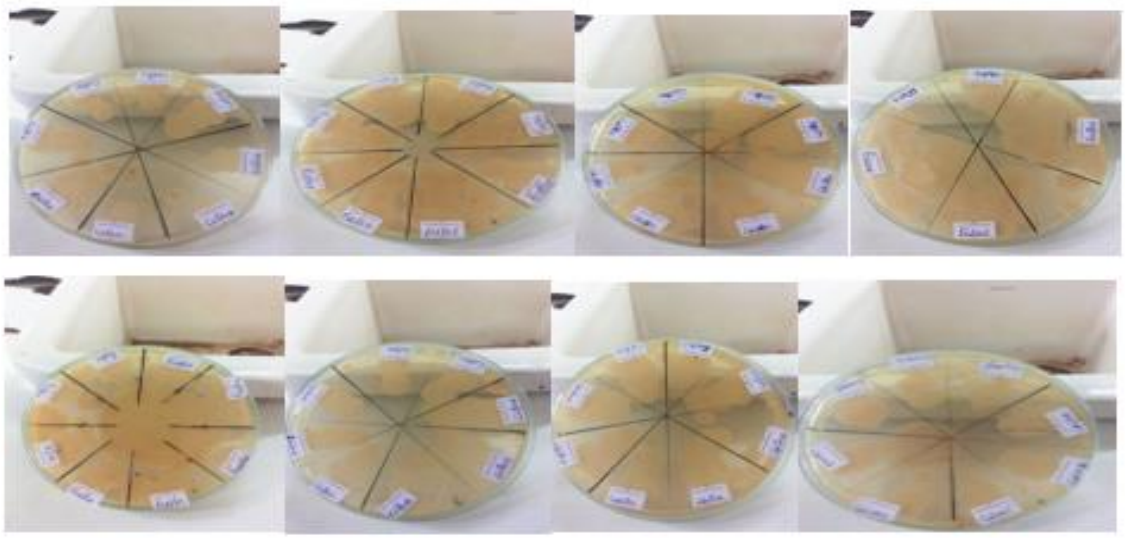
Appendix 1: Schematic diagram for three-fold serial dilution of sample for antimicrobial test



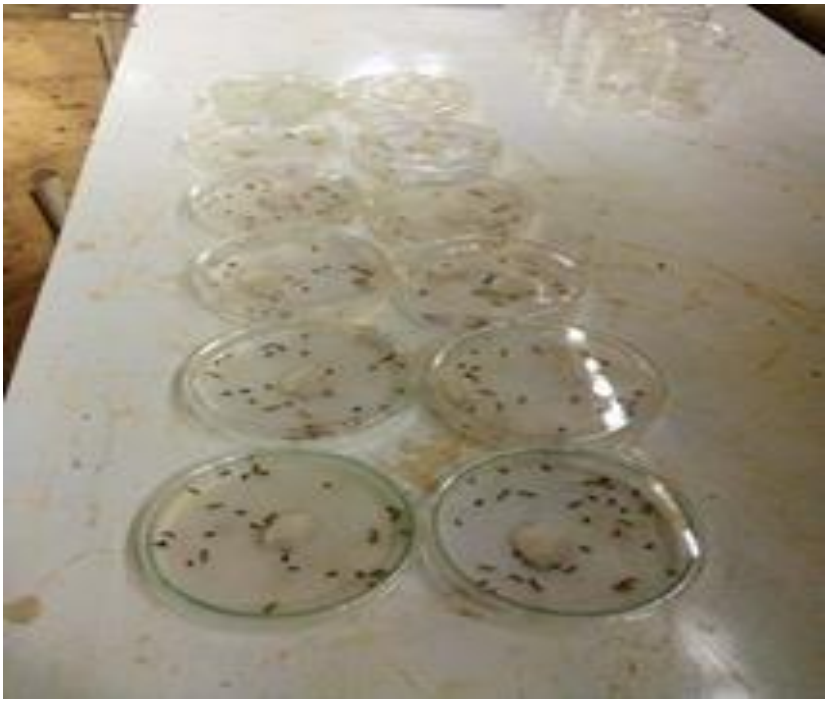
Appendex 2: Schematic diagram for two-fold serial dilution for insecticidal test



Appendix 3: Turbidity of test for MIC of EO



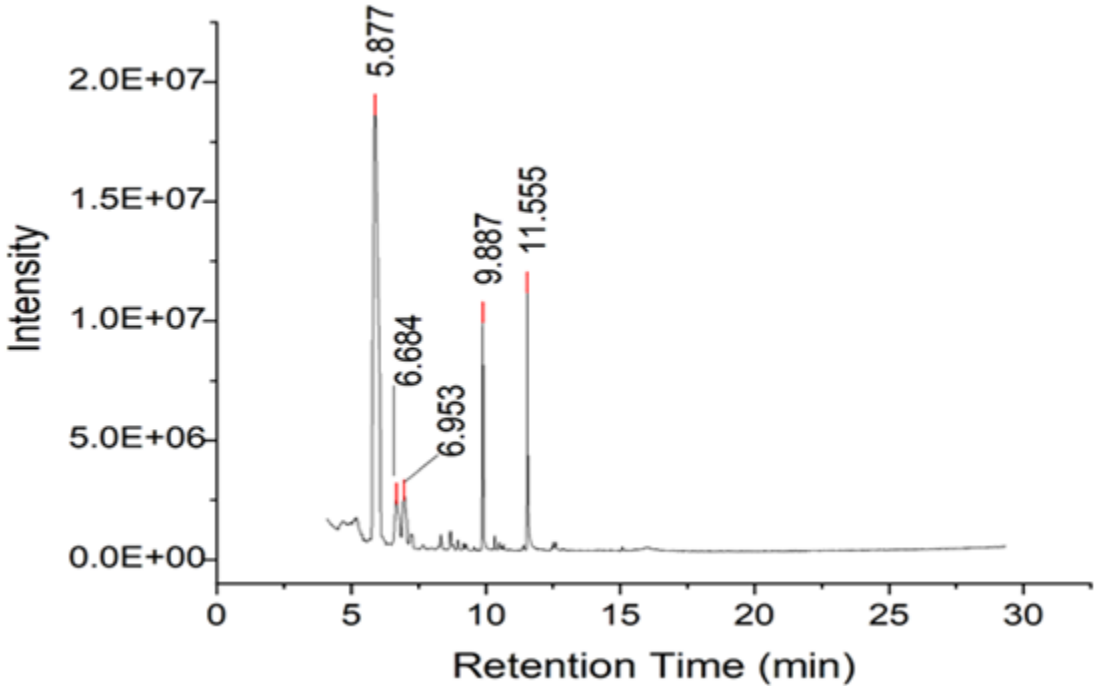
Appendix 4 : MBC and MFC test of essential oil



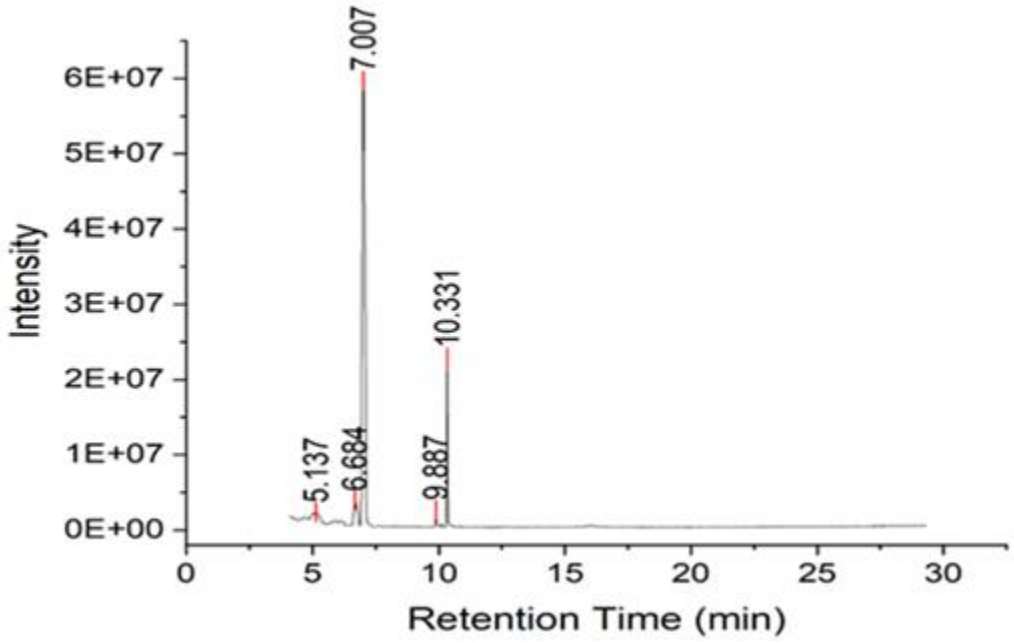
Appendix 5: Anti-termite bioassay

Path	File	Date Acquired	Sample	Misc.
D:\MassHunter\GCMS\1\5977\Sept 03, 2021\	01001010.D	Sept 03, 2021	*F.angolensis* seed	
PBM Apex				
Fri Sep 03 17:18:29 2021				

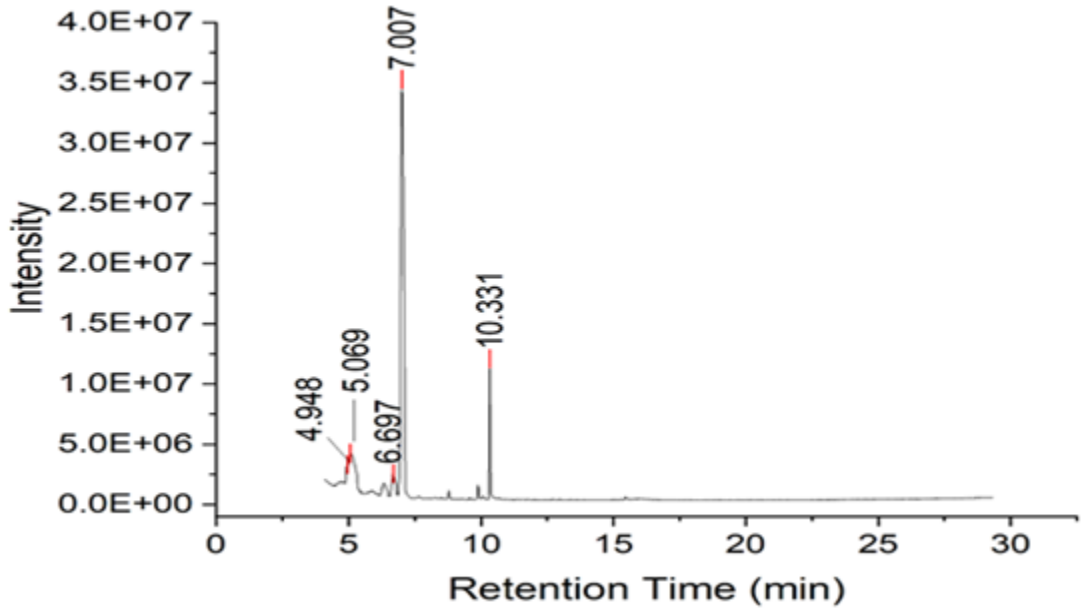
PK	RT	Area Pct.	Library/ID	Ref	CAS	Qual
1	5.88	72.29	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	16270	003387-41-5	96
2	6.68	6.42	1,4-Pentadiene	443	000591-93-5	22
3	6.96	5.99	p-Cymene	15143	000099-87-6	95
4	9.89	8.06	Terpinen-4-ol	27506	000562-74-3	93
5	11.56	7.23	2-Undecanone	39884	000112-12-9	94
Total	40.98	100				400



Path	File	Date Acquired	Sample	Misc.		
D:\MassHunter\GCMS\1\5977\Sept 03, 2021	**00801008.D**	**Sept 03, 2021**	***Melaleuca armillaris* leaves**			
PBM Apex						
Fri Sep 03 17:07:55 2021						
	RT	Area Pct.	Library/ID	Ref	CAS	Qual
1	5.143	4.826	Methylene chloride	1542	000075-09-2	60
2	6.6927	5.3483	D-Limonene	16046	005989-27-5	99
3	7.0188	80.0772	Eucalyptol	27458	000470-82-6	99
4	9.8949	1.0858	Terpinen-4-ol	27506	000562-74-3	60
5	10.3396	8.6627	Alpha –Terpineol	27523	000098-55-5	91
Total	39.09	100				409



Path		File	Sample	Misc.		
D:\MassHunter\GCMS\1\5977\Sept 03, 2021		**00901009.D**	***C.citrinus* leaves**			
PBM Apex						
Fri Sep 03 17:17:04 2021						
PK	RT	Area Pct.	Library/ID	Ref	CAS	Qual
1	4.9518	2.4158	Acetic acid, chloro-, ethyl ester	9932	000105-39-5	9
2	5.0779	13.6094	2-Chloroethanol	1054	000107-07-3	9
3	6.6968	4.7801	D-Limonene	16046	005989-27-5	99
4	7.0192	72.4549	Eucalyptol	27458	000470-82-6	99
5	10.3391	6.7397	Alpha -Terpineol	27523	000098-55-5	91
Total	34.08	99.99				307



Appendix 6: GC-MS results for essential oils