CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Chemical analysis of the essential oil of selected African plants

MASTER'S THESIS

Prague 2024

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Declaration

I hereby declare that I have done this thesis entitled “Chemical Analysis of Essential Oils of Selected African Plants” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

Acknowledgement

To the maker of all things, I am enternally grateful, for the gift of life, knowledge, and tenacity to remain focused despite all odds. All adorations to you almighty God.

To my supervisor, Doc. lng. Klara Urbanova, Ph.D. thank you for your time and effort despite the frequent disturbances, thank you for your tutelage and guidance, may your wisdom never wane.

To all my lecturers who have seen me through this program, thank you for your contributions.

My lovely mum, Mrs. Janet Ogundele, of course, without you and your guidance I will not have come this far. Your place in my heart is so special.

ABSTRACT

Plant species, climate, and the origin of the source all affect the chemical makeup of essential oils. According to this theory, every alteration to the structures of essential oils results in distinct behaviours. However, the chemical makeup of three specific African plants' essential oils has been investigated in this study. *Jatropha gossypifolia*, *Cymbopogon citratus*, and *Ocimum gratissimum* are the three plants that were researched. The essential oils from the plant samples were extracted using the Soxhlet and hydrodistillation techniques, and the extracts were analysed using gas chromatography with mass detection technique. Some of the major compounds detected in the essential oils were β-Myrcene, Geranial, Thymol, γ-Terpinene and Phytol. This study showed that the choice of extraction method influences the profile of the volatile compound obtained; for instance, it was observed that hydrodistillation favored β-Myrcene in *Cymbopogon citratus*, while Soxhlet extraction favored Geranial. Thymol was consistently abundant in *Ocimum gratissimum* regardless of the extraction method. The compounds such as Myrcene, Geranial, Thymol, Caryophyllene and Phytol exhibited various therapeutic properties, including antibacterial, analgesic, antioxidant, and anti-inflammatory effects. The study concludes that the complexity of essential oil composition is influenced by extraction methods and understanding this variation will be helpful for optimizing the therapeutic potential and commercial applications of essential oils derived from medicinal plants.

Keywords: hydrodistillation, Soxhlet extraction, GC/MS, *Jatropha gossypifolia*, *Cymbopogon citratus*, *Ocimum gratissimum*

Table of contents

List of Tables

Page

List of Figures

Page

List of abbreviations

EO – Essential oil

GC/MS – Gas chromatography/Mass spectrometry

Lit – Literature

Calc – Calculated

NIST – National Institute of Standards and Technology

P/N – Peak Number

HD – Hyrdodistillation

SFME – Solvent-free microwave extraction

UAE – Ultrasound-assisted extraction

MHG – Microwave hydro diffusion and gravity

SFE – Supercritical fluid extraction

MAHD – Microwave-Assisted Hydrodistillation

USDA – United States Department of Agriculture

LEO – Lemongrass essential oil

FAO – Food and Agriculture Organisation

GMP – Good manufacturing practice

HACCP – Hazard analysis and critical control point

GRAS – Generally Recognised As Safe

FDA – The Food and Drug Administration

1. Introduction and Literature Review

1.1 Introduction

In Africa, numerous plants are used for domestic use as medicament. Several of these plants are still unexplored and some that have not been sufficiently explored need to be investigated through research for use as new drugs or foods. The research on these plants brings improvement to flavor industries, personal care, and the pharmaceutical industrial. In a case study of food quality, the consumers now favour food that is easy to prepare, of high-quality, safe, natural, and minimally processed, but with a longer shelf life. By using food preservation technology, foodstuffs can be preserved for longer periods of time while still retaining their original nutritional and sensory qualities (Sauceda 2011; Dave & Ghaly 2011; Sivakumar & Bautista-Banos 2014). The food industry has long utilised artificial preservatives, with antimicrobial preservatives being the most common. However, recent research suggests that consuming chemical additives may increase the risk of allergies, intoxications, cancer, and other degenerative disorders (Sauceda 2011; Aminzare et al. 2016). Customers devalue them as a result, which drives up the urge to hunt for alternatives (Laranjo et al. 2017).

"Natural conservation" is one of the options that have received more attention. It refers to the use of naturally occurring antimicrobial preservatives found in plants, animals, or microorganisms, particularly those that are derived from extracts of different plant species and plant parts that are used as flavouring agents in some foods (Hayek et al. 2013; Gyawali & Ibrahim, 2014; Aminzare et al. 2016; Macwan et al. 2016). Foods are treated with antimicrobials to inhibit microbial growth - food safety - and natural deterioration processes - food preservation (Tajkarimi et al. 2010). Extracting, purifying, stabilising, and incorporating these antimicrobials into food items without compromising their sensory quality and safety is challenging (Sauceda 2011). The Food and Drug Administration (FDA) classifies plant-based goods that yield essential oils oleoresins and natural extracts, as well as their distillates as GRAS (Generally Recognised As Safe) products when they contain antibacterial compounds of natural origin (Sauceda 2011).

Because aromatic plant extracts and essential oils can inhibit the growth of harmful bacteria, the food sector has recently demonstrated a great deal of interest in them (Burt 2004; Campos et al. 2016). This may be because of their potent biological activity,

which has been shown to outweigh that of some synthetic antioxidants—many of which have been linked to cancer (Suhaj 2006; Ogueke et al. 2018). As a result, they must be replaced with strong, more effective, and naturally occurring antioxidants derived from plants. Thus, research on the antioxidant properties of spices, herbs, and medicinal plants is ongoing (Ogueke et al. 2018). Aromatic and medicinal plants play a major role in several businesses, including the cosmetic, pharmaceutical, and fragrance sectors (Swamy et al. 2016a). Although they make up a sizable portion of the natural flora, 80 % of people on the planet employ traditional medicinal and aromatic plant-based treatments to treat a range of illnesses. Approximately 1500 species are recognised for their flavour and scent, and over 9000 native plants have been identified and documented for their therapeutic qualities (Swamy et al. 2016a; Arumugam et al. 2016).

The majority of essential oils are volatile, complex substances with overpowering scents. In numerous sections of the plant, aromatic plants synthesise them as secondary metabolites (Laranjo, 2017). According to several studies (Cui et al. 2015; Sonker et al. 2015; Beatovic et al. 2015), a number of essential oils have antibacterial and antifungal properties. Additionally, they have been shown to have antioxidant qualities (Beatovic et al. 2015) and, when given to some human cancer cell lines, to have cancer-suppressive action (Adaramoye et al. 2011; Kuete et al. 2015; Sado et al. 2015).

Plant species, climate, and place of origin all affect the chemical makeup of essential oils. According to this theory, every alteration to the structures of essential oils results in distinct behaviours. More than 250 different types of essential oils are marketed annually on the international market, driven by consumers' increased interest in natural components and their concerns about potentially dangerous synthetic chemicals (Reyes-Jurado et al. 2014; Swamy et al. 2016a; Swamy et al. 2016b). These products must be removed from the plant matrix in order to be used. For this, a variety of techniques can be applied, including emerging techniques, solvent extraction, distillation, and expression (Reyes-Jurado et al. 2014). Environmental concerns have led to the development of new separation methods that use less energy and emit less CO_2. Supercritical fluid extraction, microwave assisted extraction, and ultrasonic assisted extraction are new techniques for extracting essential oils. Knowing the essential oils' chemical makeup is crucial after extraction because it determines the antibacterial activity of the oils. Although there are other techniques for analysing essential oils, gas

chromatography (GC) has been said to be the most appropriate approach (Reyes-Jurado et al. 2014; Adams, 2017). These days, mass spectrometry and gas chromatography are combined to enhance the isolation of various essential oil components.

Jatropha gossypifolia (bellyache bush or black physic nut), *Cymbopogon citratus* (lemongrass), and *Ocimum gratissimum* (scent leaf) are plants that are widely known to be medicinal and some used, especially *Ocimum gratissimum* as food in some parts of Western African. In the case of *Cymbopogon citratus*, it possesses many pharmacological characteristics, which include antibacterial, anti-amoebic, anti-filarial, anti-diarrheal, anti-inflammatory, antifungal, antimalarial, anti-mycobacterial, anti-mutagenicity, hypoglycemic, antioxidant, and neurobehavioral effects, all have been demonstrated by scientific studies to be present in *Cymbopogon citratus* oil (Ekpenyong et al. 2015; Ajayi et al. 2016; Chukwuocha et al. 2016; Aly 2021). Multiple scientific reports say that *Ocimum gratissimum* has potential antioxidant, antimicrobial (Joshi, 2013), anti-inflammatory (Ajayi et al. 2014), anthelmintic (Aderibigbe and Idowu, 2020), antimutagenic (Gontijo et al. 2014), antidiarrhoeal, anticancer and antidiabetic (Ashokkumar et al. 2020) activities. While the extracts of *Jatropha* gossypifolia was known to have shown pharmacological activities such anti-inflammatory (Yerramsetty et al. 2013), anti-fertility (Sachin et al. 2012), anti- schistosomicidal, analgesic (Panda et al. 2009), antimicrobial (Seth & Sarin 2010), antipyretic, purgative (Murugalakshmi et al. 2014), anti-coagulating (Parvathi et al. 2012), neuropharmacological and anti-diarrheal (Apurba at al. 2013) anticholinestrate (Pratap & Singh 2013) activities.

1.1.1 Statement/research problem

In actuality, a great deal of research has undoubtedly been done on the extraction of essential oils and their chemical constituents. Nevertheless, little study has been done to compare essential oils derived from various plants in greater detail with regard to their chemical components, the extraction process adopted and their location or centre of origin. This is necessary to demonstrate the chemical component comparison of each plant and provide sound advice for maintaining high-quality standard.

1.2 Food quality

Humans have a greater basic requirement for food than for clothing and shelter. It sufficiently supports the body's development, upkeep, healing, and reproduction. Foods containing vital elements, including proteins, lipids, carbs, vitamins, and minerals, come from plants and animals. Food typically passes through a variety of metabolic processes after ingestion, which ultimately results in the creation of energy, the preservation of life, and/or the promotion of growth (Rajput et al. 2021).

The most widely used method in laboratories for both fresh and processed foods is food analysis, which uses a standardised form. These analytical techniques are employed to yield data regarding a broad range of dietary features, such as composition, structure, physicochemical and phytochemical properties, and sensory aspects. This knowledge is essential to our ability to provide consistently safe, nutrient-dense, and appealing foods at a reasonable cost and to enable customers to make educated diet decisions. It also helps us to understand the elements that impact the attributes of foods (Rajput et al. 2021).

The standards known as quality control work to keep food goods at a level that is acceptable to customers. To maintain nutrient-dense food, a variety of physical, chemical, microbiological, nutritional, and sensory factors are employed. These quality parameters are dependent on certain characteristics, such as sensory characteristics, which include flavour, colour, fragrance, taste, and texture; quantitative characteristics, which include the percentage of protein, sugar, fibre, and so forth; and hidden characteristics, which include peroxides, free fatty acids, and enzymes (Fazaeli et al. 2012; Vasanthan 2021; Rajput et al. 2021). Even if there are numerous qualities, not all of them need to be taken into account for every product at every given time. Determining the proportional importance of an element in relation to the overall quality of the product is crucial at all times. A product's quality attribute is determined by its composition, anticipated deteriorative responses, packaging, necessary shelf life, and target consumer demographic (Rajput et al. 2021).

Protecting the consumer is the most crucial component and ultimate aim of food quality control. The related acts affecting marketing, production, labelling, use of food additives, dietary supplements, enforcement of good manufacturing practice (GMP), hazard analysis and critical control point (HACCP), federal laws and regulations,

factory inspections, and import/export inspections are covered by food laws and regulations to ensure standardisation of these procedures (Rajput et al. 2021; Vasanthan 2021).

1.3 Quality parameters

Several parameters are assessed using various techniques to guarantee the appropriate quality of diverse food products. These parameters include: Physicochemical and rheological parameters; phytochemical parameters; Packaging materials (Table 1 and 2).

Table 1: Physicochemical and rheological parameters for quality of selected food products (Rajput et al. 2021).

Parameter name	Instruments and chemicals used	Products
Admixture	Visual observation	Cereals, pulses
Bellier turbidity temperature	Visual	Oils
Bulk density	Calibrated graduated cylinder	Cereals, fruits, and vegetables and other products
Colour on Lovibond scale	Lovibond Tintometer	Oil, fat
Crude fibre	Chemical	Most of the fruits and vegetables and cereal products
Fat or oil	Chemical and Soxhlet method	Most of the food products, animal feeds
Insect infestation	Visual Observation	Cereals, pulses

Table 2: Phytochemical parameters for quality of selected food products (Rajput et al. 2021).

Parameter name	Instruments and chemicals used	Products
Anthocyanins	Chemical and spectrophotometer	Red colour-rich fruits and vegetables and other food products
Antioxidant activity	Chemical and spectrophotometer	Most of the fruits and vegetable products
Ascorbic acid	Chemical and titration method	Most of the fruits and vegetable products
Lycopene	Chemical and spectrophotometer	Coloured fruits and vegetables and other products
Total carotenoids and β-carotene	Chemical and spectrophotometer	Yellow colour rich food products
Total phenols	Chemical and spectrophotometer	Most of the fruits and vegetable products

1.4 Essential oils

Guillen et al. (2012) investigated the prioritization of chemicals in the aquatic environment based on risk assessment, it was discovered that the current antibacterial agents are mainly synthetic chemicals with many disadvantages. These disadvantages include carcinogenicity, acute toxicity, teratogenicity, etc., and can also lead to the pollution of the environment. All these disadvantages have led to a wide disapproval of synthetic antimicrobial agents, however, it has also made scientists divert their focus to natural compounds to be incorporated in foods (Qin 2021). One of the important products of agriculture based industry is the essential oils of plant origin. They are used commonly as flavouring agents in food products, perfumeries, cosmetics, drinks and pharmaceuticals (Burt 2004; Teixeira et al. 2013 and Raut & Karuppayil, 2014). According to FAO (2005) it was revealed that essential oils are grouped into a unique four sectors, which include flavor industries, personal care, pharmaceutical and industrial (Figure 1).

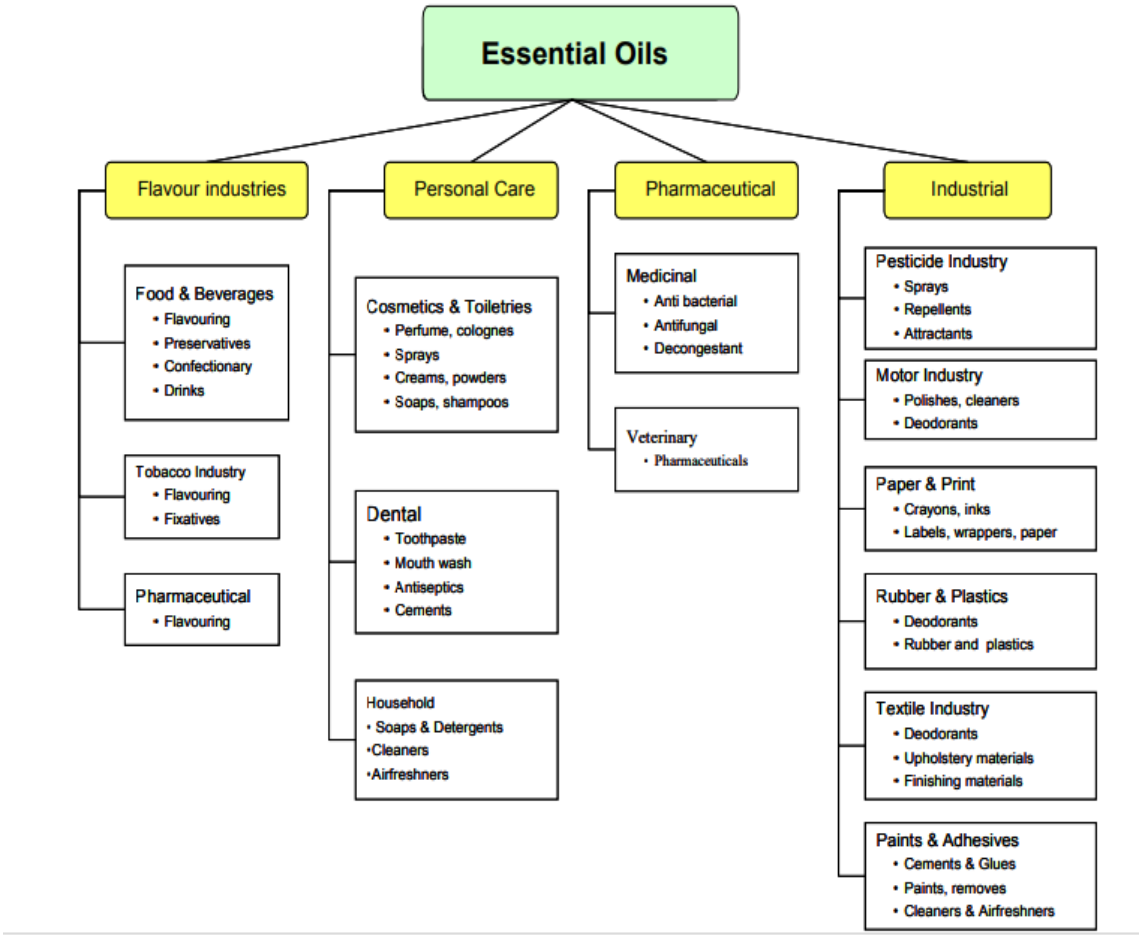

Figure 1: Industries and product categories that use essential oils (FAO, 2005)

Essential oils are not limited to oils; however, they are aromatic compounds that are usually not water soluble. They are generally known to have a pleasant smell and sometimes have a very unique taste. In Burt (2004) and Ogueke et al. (2018) researches, it was discovered that herbs and spices commonly used in food have provided a large number of essential oils with antimicrobial activity. They are also known to be complex aromatic chemicals rich in phenols, aldehydes, ketones, fatty acids and esters (Moghaddam & Mehdizadeh 2017). Research also shows that essential oils can be extracted from leaves, bark, stems, roots, fruits and flowers as in the case of cinnamon, geranium, ginger, jasmine, citronella and bergamot (Ali et al. 2015). Baser and Demirci (2007) and Raut and Karuppayil (2014) discovered that essential oils are known to be stored in oil ducts, resin ducts, glands or trichomes (glandular hairs) of the plants. Essential oils are mostly complex mixtures of low molecular weight – which is usually less than 500 daltons – compounds extracted by the following processes: distillation,

hydrodistillation or solvent extraction (Raut & Karuppayil 2014). However, steam distillation is a preferred method for the extraction of essential oil, on the commercial scale.

Essential oils are known to have antioxidant, anti-cancer, anti-inflammatory and antiseptic agents, which can successfully inhibit the growth of bacteria and fungi. They are known to stop the growth of cells as well as the production of toxic bacterial metabolites. No doubt the numerous characteristics of essential oils make them a suitable natural additive in comparison with the antibiotics (Patra & Yu 2012). Essential oils are known to be more effective in killing gram-positive bacteria than gram-negative bacteria. According to Nazzaro et al. (2013), hydrophobic molecules could easily penetrate the cell wall of gram-positive bacteria than the complex gram-negative bacteria. The study revealed that citron essential oils were effective on gram-positive *Listeria* than gram-negative *Escherichia coli* and *Salmonella*. Essential oil's antimicrobial activity is determined by its chemical composition, which involves a series of reactions that take place in the entire cell (Raut & Karuppayil 2014).

It has also been discovered that the effectiveness of essential oils could be reduced by some factors such as fat content, protein, pH, water activity and enzymes of the food (Burt 2004). Friedly et al. (2009) study revealed that low pH at 6.55 can bring about an increase in the solubility and stability of essential oil and, therefore, enhance the activity of antibacterial against *Listeria*. Increasing the salt content to 0.01 % and reducing the temperature to room temperature can also boost the activity of essential oils.

It was discovered that essential oils are hydrophobic in nature and can separate the lipids of bacterial cell membranes and mitochondria, and make bacterial cells more permeable in the process. Although it is known that essential oils could have considerable antimicrobial effects, however, the smell at the level that is bactericidal could be unacceptable (Winska et al. 2019).

Friedly et al. (2009) suggested the use of essential oils in multiple hurdle technique; this simply means the combination of several essential oils gotten from different sources to act as a single antimicrobial agent in other to bring about a decrease in the microbial loads. To prove its potency with other essential oils, it had been discovered that it can work with organic acids and salts; example includes sodium (Nehme 2021). Hirshified

et al. (2003) discovered that organic acids can aid in the destabilization of the pH cells and change the osmotic pressure to improve the essential oil's antibacterial properties.

1.4.1 Essential oils as antibacterial agents

Raut and Karuppayil (2014) in their review paper 'A status review on the medicinal properties of essential oils', stated that plant molecules are well-known for their antimicrobial characteristics. Essential oils, especially from plants, have been revealed to showcase broad spectrum inhibitory activities against various Gram-positive and Gram-negative bacterial pathogens (Lang & Buchbauer 2012; Teixeira et al. 2013; Raut & Karuppayil 2014). However, the effectiveness of the antibacterial activity varies from one essential oil to another as well as with different bacteria. For instance, research from Hammer and Carson (2011) revealed that manuka oil (*Leptospermum scoparium*), sandalwood (*Santalum album*) and vetiver (*Chrysopogon zizanioides*) oils, are effectively active against Gram-positive bacteria, but show no activity against Gram negative bacteria. *Pseudomonas aeruginosa,* in comparison to others, is known to exhibit tolerance to inhibition by plant essential oils.

The most active antimicrobial essential oils are generally sourced from lemon grass, cinnamon, rosewood, bay, thyme, tea-tree, clove, lemon-myrtle and oregano. They are known to be active at concentrations less than 1 % vol/vol, that is, they exhibit minimum growth inhibitory concentrations of less than 1 % (Hammer & Carson 2011). Lemongrass, oregano, bay, thyme and clove inhibit *Escherichia coli*'s growth at concentrations of 0.06, 0.05, 0.02, 0.05 and 0.04 %, respectively. Rosemary, lemongrass, thyme, bay oils, peppermint and clove all have the potential to inhibit *Staphylococcus aureus* at concentrations of less than or equal to 0.05 %, while eucalyptus oils and basil prevents it at 1 % concentration (Hammer & Carson 2011). It is also widely known that essential oils with phenolic and aldehydes exhibit more effective antibacterial efficacies than other essential oils (Raut & Karuppayil 2014).

The mode of action of essential oils primarily, is to destabilize the membrane. The lipophilic nature of the essential oils allows them easy access through the cell wall and cell membrane. Also, essential oils interactions and their components with polysaccharides, fatty acids and phospholipids make the membranes of the bacteria more permeable, to bring about the death of the cell because there will be loss of ions

and cellular contents (Saad et al. 2013). In the same vein, loss of viability can also be as a result of interference in proton pump activity, loss of membrane integrity and leakage of cellular contents (Di Pasqua et al. 2007; Qin 2021). In Qin (2021) research, it was discovered that other essential oils' mechanisms of action include the denaturation of cytoplasmic proteins and the inactivation of cellular enzymes, leading to bacterial cell death.

1.4.2 Antifungal activities of essential oils

Pathogenic fungi are eukaryotes, meaning they share molecular and cellular characteristics with their hosts. As a result, mushrooms are difficult to attack (Routh et al. 2011). Numerous immunocompromised people are affected by opportunistic fungal pathogens, such as Aspergillus, Candida, and Cryptococcus species, which are well-known for their harmful effects. For effective antifungal chemotherapy, there aren't many medication choices (Kathiravan et al. 2012). The prevention and treatment of fungal infections are complicated by the emergence of drug-resistant strains, biofilm infections linked to devices, and adverse drug reactions from already prescription medications. As a result, invasive fungal infections have extremely high rates of morbidity and death (Sardi et al. 2013). It has been discovered that a variety of plant and human pathogenic fungi, including yeasts, are sensitive to essential oils. The target organisms and the oil under test affect how effective the inhibition is. For instance, three Apiaceae family members, with minimum growth inhibitory concentrations of 0.25 %, 0.5 %, and 1 %, respectively, exhibit varied anti-*Candida albicans* efficacy with a pattern of coriander > anise > fennel. According to Irkin and Korukluoglu (2009), *Cymbopogon sp*. generally demonstrates encouraging properties against pathogenic yeast. The essential oils that showed the greatest promise against *Candida albicans* were cinnamon, lemongrass, Japanese mint, ginger grass, geranium, and clove oil. Between 0.01 and 0.15 % are the effective concentrations (Devkatte et al. 2005; Hammer & Carson 2011). Essential oils high in phenylpropanoids, such as eugenol, and monocyclic sesquiterpene alcohols, like bisabolol, can effectively suppress the growth of dermatophytes and their spore development (Bajpai et al. 2009; Maxia et al. 2009; Pragadheesh et al. 2013). Essential oils derived from plants inhibit the growth and generation of aflatoxin in moulds such as *Aspergillus flavus* (Kumar et al. 2010; Lang &

Buchbauer 2012). One of the best oils for combating filamentous fungus is lemongrass oil, which has active concentrations between 0.006 and 0.03 %. *Aspergillus niger*, *Aspergillus flavus*, *Penicillium verrucosum*, and *Penicillium chrysogenum* are inhibited by orange, lemon, mandarin, and grapefruit oils at concentrations of less than 1 % (Viuda-Martos et al. 2008)

Terpenoid-rich essential oils suppressed drug-sensitive and drug-resistant pathogenic yeasts, including the main human pathogen, *Candida albicans* (Zore et al. 2011). It is crucial to know how well essential oils and their constituents work against *Candida albicans* drug-resistant biofilms. According to Raut et al. (2013), these actions might be mediated by blocking the membrane ergosterol and signalling pathways that go from yeast to the formation of hyphae. Additionally, essential oils have the ability to block the cell cycle in *C. albicans*. For instance, it has been shown that the main components of Geranium, tea tree, and eucalyptus oils—Citral, Citronellol, Geraniol, and Geranyl acetate—block *Candida albicans* during the S phase of the cell cycle (Zore et al. 2011). According to Rao et al. (2010), Eugenol, Thymol, and Carvacrol also have an impact on Ca^{2+} and H^{+} hemostasis, which results in ion loss and *Saccharomyces cerevisiae* suppression. Fungal viability is lost due to abnormalities in membrane fluidity, which causes cytoplasmic contents to seep out. For instance, the presence of tea tree oil inhibits the respiratory chain activity and membrane permeability of *Candida albicans* cells, ultimately leading to the death of the cells. Treatment with essential oils causes permeabilization of the mitochondrial membrane, which results in necrosis and apoptosis and, ultimately, cell death. Additionally, some components of essential oils may disrupt the yeasts' TOR signalling system, causing the cells to become less viable (Rao et al. 2010). When *Phytophthora infestans* is treated with plant essential oils, SEM and TEM examination reveal modifications of the plasma membrane, cytoplasm, and nucleus (Soylu et al. 2006).

1.4.3 Essential oils as antioxidants

Cellular macromolecules are harmed by oxidative stress, which is brought on by the production of free radicals and reactive oxygen species (Raut & Karuppayil 2014). Numerous health issues, including ageing, arteriosclerosis, cancer, Alzheimer's, Parkinson's, diabetes, and asthma have all been linked to oxidative damage (Edris

2007). Various antioxidants maintain the equilibrium of free radicals within cells. Important antioxidant effects are demonstrated by flavonoids, terpenoids, and phenolic constituents of essential oils (Cavar et al. 2012; Sanchez-Vioque et al. 2013). Oils from *Tagetes filifolia, Bacopa monnierii, Curcuma longa*, and *Origanum majorana*, for instance, show strong antioxidant properties (Maheshwari et al. 2006). Potential antioxidant or free radical scavenging action is present in the essential oils of *Salvia cryptantha, S. multicaulis, Achillea millefolium, M. alternifolia, Ocimum sp*., and *Mentha sp*. (Gulluce et al. 2007; Hussain et al. 2008). Thymol and carvacrol, two major constituents of Thymus and Origanum essential oils, have been demonstrated to have potent antioxidant properties (Miguel 2010). According to Romeilah et al. (2010), *Coriandrum sativum, Allium sativum, Allium cepa, Cuminum cyminum*, and *Petroselinum sativum* also have strong free radical scavenging properties. Overall, clove > cinnamon > nutmeg > basil > oregano > thyme is the order of efficacy among the essential oils with good radical-scavenging and antioxidant characteristics (Tomaino et al. 2005; Raut & Karuppayil 2014).

1.4.4 Anti-inflammatory activities

Ocimum sanctum essential oil has long been known to have anti-inflammatory properties (Raut & Karuppayil 2014). *Mentha sp., Eucalyptus sp., Baphia nitida*, and *Lavandula angustifolia* are additional plant essential oils that have anti-inflammatory properties (Gulluce et al. 2007). Essential oils like eucalyptus, rosemary, lavender, pine, clove, and myrrh may be able to prevent inflammation (Barbieri Xavier et al. 2013). Rigid oxygen species are formed during the oxidative burst of an inflammatory response. The effective scavenging of free radicals is one of the many processes linked to the anti-inflammatory properties of essential oils (Miguel 2010). Potential anti-inflammatory properties can be found in the essential oils of Aloe-vera (*Aloe barbadensis*), bergamot (*Citrus aurantium*), cinnamon leaf (*Cinnamomum zeylanicum*), lavender (*Lavandula officinalis*), thyme (*Thymus vulgaris*), and ylang-ylang (*Cananga odorata*). According to Miguel (2010), these mechanisms include repression of pro-inflammatory genes, inhibition of lipoxygenase, inhibition of COX-2 enzyme, inhibition of leukotriene synthesis, inhibition of pro-inflammatory cytokines, interleukin-1β (IL-1β), and tumour necrosis factor-α (TNF-α).

1.4.5 Use of essential oils in food industry

1.4.5.1 Meat and meat products

Research has shown that the use of essential oils improved both the safety of food and shelf-life of products of meat. These were reported majorly in beef, chicken, lamb or rabbit fresh meat (Karabagias et al. 2011; Ballester-Coasta et al. 2013). The use of thyme and cinnamon essential oils in ham has been reported to significantly decrease the *Listeria monocytogenes* population (Dussault et al. 2014). The shelf-life of mortadella has been extended with the use of rosemary/thyme essential oils (Viuda-Martos et al. 2010a), while with the use of oregano essential oil, the shelf-life of bologna sausages was extended (Viuda-Martos et al. 2010b). It was reported that through the antibacterial effect of garlic and oregano essential oils against *Salmonella spp., Listeria monocytogenes* and *Staphylococcus aureus*, dry-cured sausages were safely improved (Garcia-Dez et al. 2016).

1.4.5.2 Cheeses

The antimicrobial activity of the different essential oils in dairy products can be determined by several factors, which include the chemical composition of these products, the concentration in which the oils are used and the microorganisms that are targeted to be increased or terminated.

In the comparison of the anti-microbial effect of clove, cinnamon, bay and thyme essential oils in different concentrations (0.1 %, 0.5 % and 1 %) in low-fat and full-fat soft cheese, Smith-Palmer et al. (2001) discovered that 1 % was the most effective concentration for all essential oils. The anti-*Listeria monocytogenes* effect was more significant in the low-fat cheese; however, clove essential oil at 1 % was more effective in the two types of cheese. This essential oil, at the same concentration, was more effective against *Salmonella enteritidis* in full-fat cheese than in low-fat cheese. The population of *Salmonella enteritidis*, when used at a concentration of 0.5 %, was recovered in the low-fat cheese, but not in full-fat cheese. In fact, fat plays a protective role of the bacterial cells over antimicrobial agents. However, the protein content is higher in low-fat cheeses and can contribute to the reduction of the activity of the

essential oils due to the formation of complexes between the phenolic compounds of the essential oils and the proteins of these products (Laranjo 2017).

Essential oils can increase the shelf-life of dairy products, not limited to the elimination of unwanted microorganisms, but also significantly reducing the degree of chemical deterioration during storage and marketing periods (Laranjo 2017).

1.4.5.3 Fruits

Essential oils of numerous plant species have been researched, because of their antibacterial capabilities, being useful in the control of fungus first of all in vitro and later on in vivo in vegetables and fruits. Numerous studies have shown how effective essential oils are in controlling certain ailments. Several researches have examined the function of essential oils and their application in the active packing of fruits and other foods. (Sivakumar & Bautista-Banos 2014; Gyawali & Ibrahim 2014; Guerra-Rosas 2017; Munhuweyi et al. 2017; Ricardo-Rodrigues et al. 2017; Waithaka et al. 2017). Lately, fresh-cut fruits and vegetables—also known as minimally processed products—have been given a longer shelf life through the creative application of essential oils' potential antimicrobial effect (Patrignani et al. 2015). One such approach is the addition of essential oils to edible films (Maria et al. 2012). Greater interest in minimally processed items that are "easy to eat" is being displayed by increasingly informed customers, who also want to make sure that these goods are natural, clean label, and "friendly" to the environment.

1.5 Chemical composition of essential oils

Plant species, climate, and provenance all affect the chemical makeup of essential oils. According to this theory, every alteration to the structures of essential oils results in distinct behaviours. It has also been demonstrated that the content of essential oils differs noticeably between different portions of the same plant (Martinez et al. 2006; Pragadheesh et al. 2013; Tranchida et al. 2013; Lermen et al. 2015; Pragadheesh et al. 2015; Eslahi et al. 2017). Essential oils are complex matrices with many compounds with multiple functional groups and structures due to diverse procedures and circumstances that affect them. (Table 3)

Table 3: Selected main components of essential oils (Source Raut and Karuppayil, 2014)

Essential oil	Plant part	Botanical source	Main components	% of total
Angelica	Roots	*Angelica archangelica L.*	α-Pinene	24.7
			δ-3-Carene	10.5
			α-Phellandrenetmyrcene	10.8
			Limonene	12.9
			β-Phellandrene	10.4
			p-Cymene	7.7
Bergamot	Fruits	*Citrus bergamia*	β-Pinene	7.7
		Risso et poit	Limonene+ β-Phallandrene	39.4
			γ –Terpinene	8.6
			Linalool	11.1
			Linaly acetate	28.0
Cinnamon	Inner bark	*Cinnamomun*	(*E*)-Cinnamldehyde	77.1
		Zeylancium Blu.	Eugenol	7.2
			p-Cymene	6.1
			Linalool	72.0
Dill (Indian)	Seeds	*AnethumsowaRoxb*	Limonene	50.9
			Trans-Dihydrocarvone	10.4
			Carvone	20.3
			Dillapiole	36.6
Eucalyptus	Leaves	*Eucalyptus Citriodora*	Citronellal	72.8
			Citronellol	14.5
Ginger	Roots	*Zingiber officinal*	Camphene	14.1
		Rosc.	Neral	4.9
			Geranial+ bornyl acetate	8.1

			β-Bisabolene	22.1
			ar-Curcumene	14.5
			β-Eudesmol	5.4
Jumpier	Berries	*Juniperus commuunis L*	α-Pinene	33.7
			Sabinene	27.6
			Myrcene	5.5
Orange	Peel	*Citrus sinensis L. Osbeck*	Limonene	91.5
Pepper	Fruits	*Piper nigrum L.*	α-Pinene	9.0
			β-Pinene	10.4
			Sabinene	19.4
			δ -3-Carene	5.4
			Limonene	17.5
			β –Caryophyllene	14.7
Rosemary	Whole plant	*Rosemarius officinalis L.*	α-Pinene	7.4
			β-Pinene	5.0
			1,8-Cinessential oille	43.6
			Camphor	12.3
Tea tree	Branches	*Melaleuca alternifolia L.*	α-Terpinene	10.4
			1,8-Cinessential oille	5.1
			Terpinene-4-ol	40.1
			γ-Terpinene	23.0

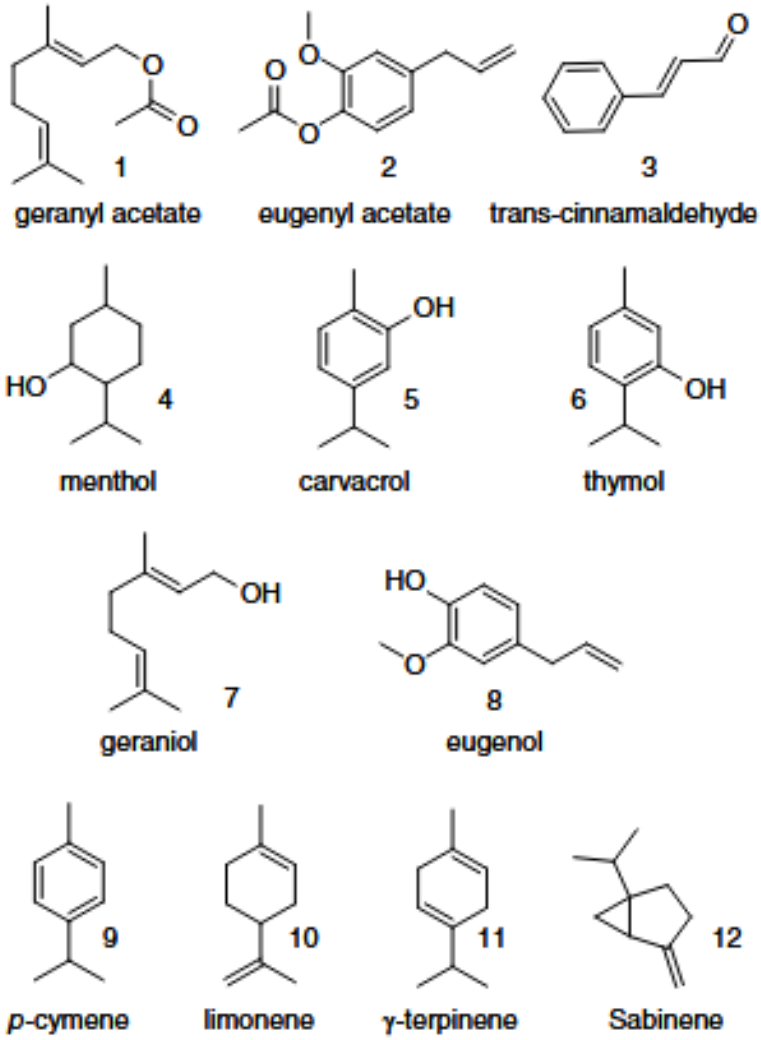

Figure 2: Chemical structures of important compounds of essential oils (Eslahi et al. 2017)

According to Eslahi et al. (2017), essential oils are made up of 20–60 identified components, the primary components of which can make up anywhere from traces to about 85 % of the essential oil. These primary elements typically dictate the biological characteristics of the essential oils (Pavela 2015).

The two primary categories of essential oils are (i) aromatic and aliphatic chemicals and (ii) hydrocarbon terpenes (isoprenes) and terpenoids (isoprenoids). Sesquiterpenes and monoterpenes are the two basic types of terpenes. Furthermore, there are hemiterpenes, diterpenes, triterpenes, and tetraterpenes. Terpenoids are biochemically altered terpenes that have had a methyl group moved or removed, as well as oxygen molecules added by

enzymes. A terpenoid is a terpene that contains oxygen. However, phenols, alcohols, ketones, aldehydes, acids, esters, and ethers are examples of the oxygenated family of terpenes known as isoprenoids, or terpenoids (Bakkali et al. 2008). According to El Asbahani et al. (2015), terpenoids are made up of both oxygenated monoterpens and sesquiterpenes or sesquiterpenoids.

The production of nearly all terpenes involves the condensation of branching five-carbon (isoprene units) molecules. Isoprene units (5-carbon-5-base (C5)) combine to generate terpenes, a class of secondary metabolites. The fundamental building blocks of terpenes are isoprene units (2-methyl-1,3-butadiene), which are connected in a head-to-tail fashion (Eslahi et al. 2017).

Various isoprene unit combinations give rise to distinct groups of terpenes with varying structural and functional properties (Rubio et al. 2013). A variety of aliphatic hydrocarbons, including low molecular weights like ramified, linear, saturated, and unsaturated ones, lactones, alcohols, acids, acyclic esters or aldehydes, coumarins, compounds containing nitrogen and sulphur, and homologues of phenylpropanoids, can be found in monoterpenes, sesquiterpenos, and diterpenes (Eslahi et al. 2017).

1.5.1 Terpenes and terpenoids

The head-to-tail paradigm was followed when combining isoprene units (5-carbon-base (C5)) or (2‑methyl‑1,3-butadiene) to create terpenes as secondary metabolites. The different categories of terpenes are physically and functionally derived from different isoprene combinations. The number of connected isoprene units is represented by the structural formula $(C_5H_8)n$, which determines the classification of which is based on the number of isoprene units. For instance, two isoprene units produce monoterpenes, which are two C5 groups with the molecular formula $C_{10}H_{16}$. Different compounds are produced via other conjugations, including alternate hemiterpenes (C5) and sesquiterpenes (C15), as well as diterpenes (C20), triterpenes (C30), and tetraterpenes (C40). The main terpene groups found in spices and herbs are monoterpenes, diterpenes, and sesquiterpenes. These compounds have been shown to exhibit biological activities, including antimicrobial actions on several pathogens, including Candida species (Alves et al. 2013; Rubio et al. 2013; Eslahi et al. 2017).

In nature, terpenes are extensively distributed. They are readily available in large quantities and are reasonably priced components. Because they attract pollinators and strengthen their antifungal defences, monoterpenes in plants play an ecologically detrimental role in preventing herbivores from feasting on them. Mammals use them to stabilise cell membranes, control enzymatic processes, and maintain metabolic pathways. Terpenes may also be helpful for chlorinated media in real-world applications such degreasing metal, cleaning electronic components and cables, and cleaning aviation parts. Researchers are also looking into the industrial use of monoterpenes as alternatives to chlorofluorocarbons that deplete the ozone layer (Eslahi et al. 2017).

The most diverse structures, known as monoterpenes, include a variety of organic functional groups and monocyclic, bicyclic, and acyclic components. These include hydrocarbons like Camphene, p-Cymene, and Myrcene, as well as alcohols like Menthol, Linalool. A variety of other functional groups have been added, including ethers like 1,8-Cinessential oille and Menthofurane, peroxides like Ascaridole, Phenols like Thymol and Carvacrol, and ketones like Camphor, Carvone, and Pulegone—as well as esters like Citronellyl acetate, Linalyl acetate, and Menthyl. These chemicals are found in a wide variety of plants, including wormwood, sweet basil, celery, parsley, ylang-ylang, orange, rosemary, angelica, bay leaves, thyme, celery, and mint (Pavela 2015).

Sesquiterpenes continue to form once three isoprene units (C15) have been formed, as was covered in the preceding paragraphs. An extensive variety of structures are produced as a result of the chain extension increasing the amount of cyclizations. Sesquiterpenes share the same structure as monoterpenes: β-Bisabolene, β-Caryophyllene, Azulene, Cadinenes, Logifolene, Elemenes, Curcumenes, Zingiberene, and Farnesenes are examples of hydrocarbons; Alcohols: Farnesol, Patchoulol, Carotol, β-Nerolidol, β-Santalol, Cedrol, Bisabol, and Viridiflorol; Ketones: Longipinan 2,7-Dione, Turmerones, Germacrone, cis-β Vetinone, and Nootkatone; Humulene and Caryophyllene oxides are examples of epoxides. Lemon, bergamot, mint, angelica, coriander, celery, eucalyptus, caraway, citronella, juniper, geranium, lavander, lavandin, mandarin, lemongrass, peppermint, orange, pine, petitgrain, sage, rosemary, and thyme are a few examples of plants that contain these chemicals (Bakkali et al., 2008; Eslahi et al. 2017).

Scientists can explore the typical orders of Cl0 (mono), C15 (sesqui), C20 (di), C30 (tri), C40 (tetra), and C > 40 (poly) terpenoids in these types by using the fundamental five-carbon units formed in the same way as sequentially assembling terpenes. It is common to observe them in plants as combinations of molecules that contain various sorts of five-carbon groups (Eslahi et al. 2017).

Commonly referred to as "lower terpenoids," C10 and C15 compounds are also known as "essential oils" when found in combination. However, the term "higher terpenoids" refers to both molecules with 20 carbons or more. In the most recent category, nonvolatile terpenoids (diterpenoids or triterpenoids) that mix to become volatile ones (monoterpenoids and/or sesquiterpenoids) are referred to as "resin."

Table 4: Terpene nomenclature for isoprenes (Eslahi et al. 2017)

Carbon atoms	Isoprene units	Nomenclature
40	8	Tetraterpenes
30	6	Triterpenes
25	5	Sesterterpenes
20	4	Diterpenes
15	3	Sesquiterpenes
10	2	Monoterpenes

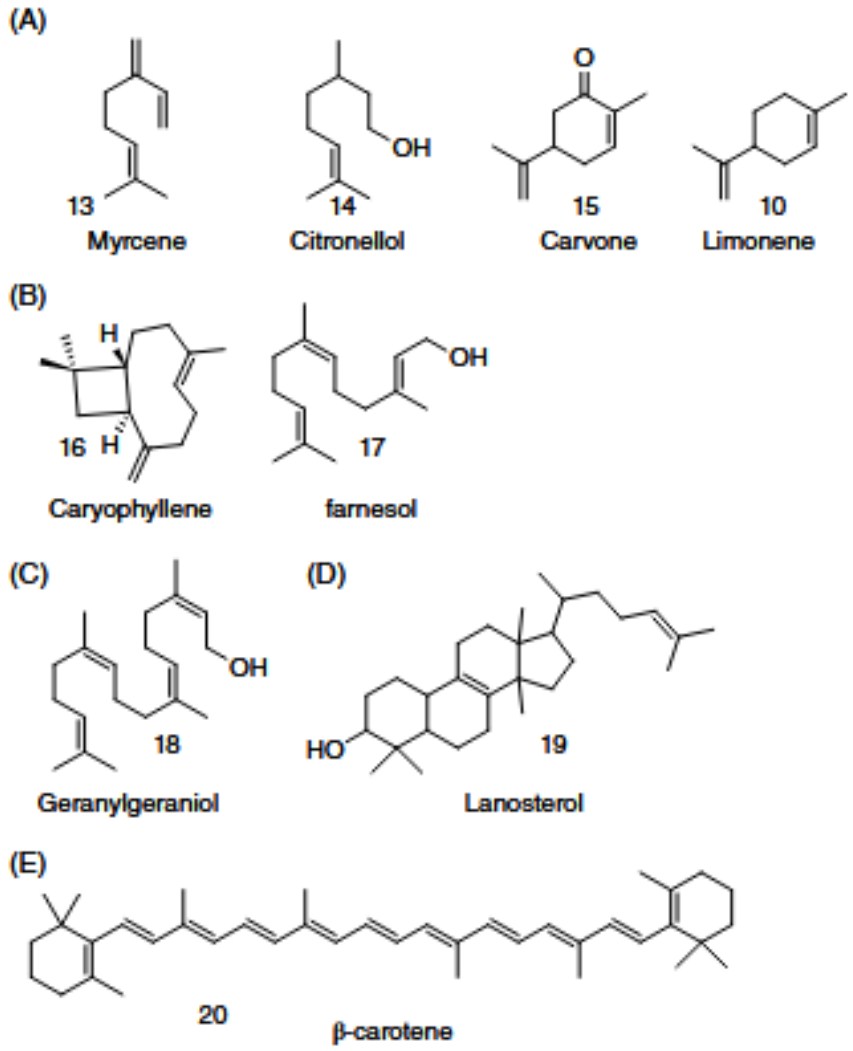

Figure 3: Structures of selected typical terpenes. (A) Monoterpenes: 13 = myrcene, 14 = Citronellol, 15 = carvone, 10 = limonene; (B) sesquiterpenes: 16 = caryophyllene, 17 = farnesol; (C) diterpene; 18 = granylgeraniol; (D) triterpene: 19 = lanosterol; (E) tetraterpene: 20 = β-carotene. (Eslahi et al. 2017)

1.5.2 Aromatic compounds

In general, terpenes occur more frequently than aromatic chemicals. Anise, nutmeg, clove, parsley, fennel, star anise, sassafras, tarragon, and several other botanical groupings (Lamiaceae, Apiaceae, Rutaceae, Myrtaceae) are among the numerous plant sources that contain these chemicals (Pavela 2015). Like phenylpropane, these substances are often found in smaller amounts than terpenes. Terpenes and phenylpropanic derivatives are typically formed by different biochemical pathways in

plants, but in certain instances, they can be synthesised by the same pathway (Bakkali et al. 2008; Eslahi et al. 2017).

Typically, plants are used for the biosynthesis of phenylpropanoids and terpene derivatives; however, in certain instances, this process may coexist alongside the primary technique (Bakkali et al. 2008; Pavela 2015; Eslahi et al. 2017)

The applications of aromatic compounds are similar to those of sesquiterpenoids and monoterpenoids. Figure 4.3 lists the following: methoxy compounds (methyleugenol, elemicine, estragole, anethole), phenols (chavicol, eugenol), alcohols (cinnamic alcohol), aldehydes (cynnamaldehyde), and methylene dioxy derivatives (safrole, myristine, apiole). (Eslahi et al. 2017)

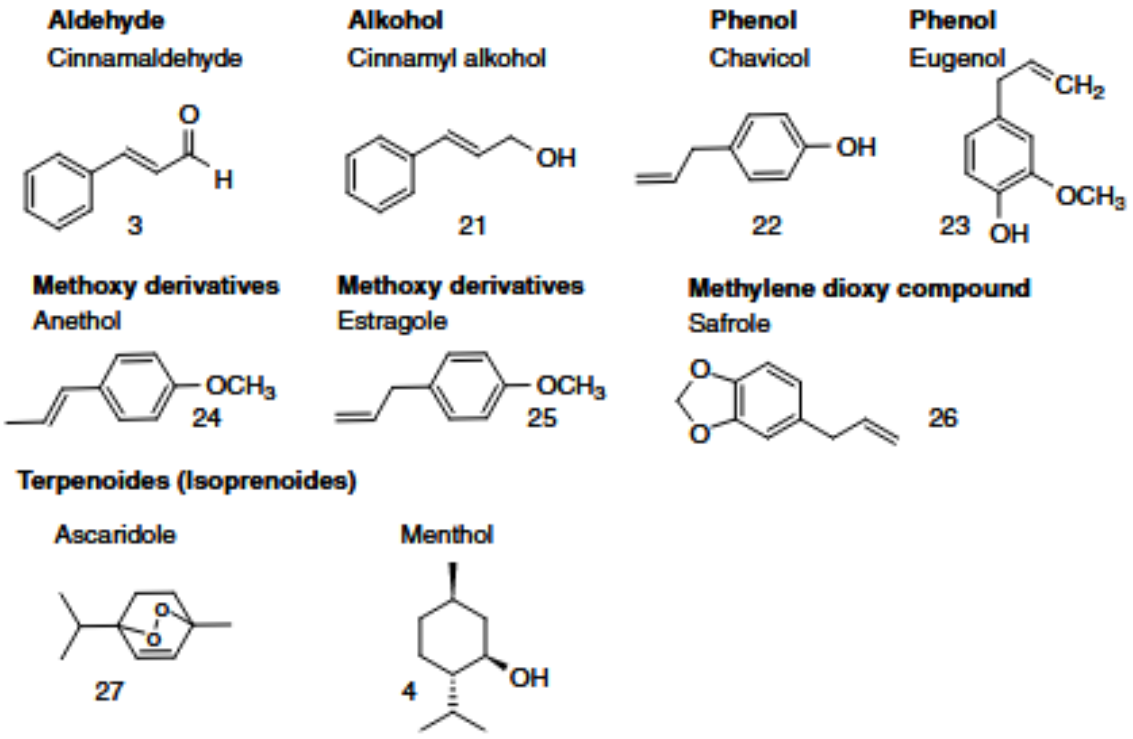

Figure 4: The structure of important aromatic compounds (Eslahi et al. 2017)

1.6 *Jatropha gossypifolia* (Euphorbiaceae)

The Euphorbiaceae shrub *Jatropha gossypifolia* is used as a traditional medicine to treat cancer, diabetes, and skin conditions (Oskoueian et al. 2011). It is commonly known as bellyache bush or black physic nut. Fresh leaf aqueous extract is used in Nigerian traditional medicine to treat oral cancer and stop nose and skin bleeding. The stem is

also used as a toothbrush for teeth that are in good condition (Kayode & Omatoyinbo 2009). In India, leaves are used to treat and prevent a number of illnesses, such as eczema, diarrhoea, dysentery, and itching (Seth & Sarin 2010). In Trinidad and Tobago, decoction of *Jatropha gossypifolia* was found to be effective in treating wounds, easing pain, and curing snatch sores (Okoh et al. 2016). Phytochemical investigations have revealed the presence of alkaloid compounds, flavonoids, and phenolics in various *Jatropha gossypifolia* sections (Seth & Sarin 2010). Phytol, Germacrene, and Linalool were listed by Aboaba et al. (2015) as some of the leaf volatile oil constituents of *Jatropha gossypifolia*.

1.6.1 Chemical analysis of the essential oil of *Jatropha gossypifolia*

According to Reis et al. (2013), the major chemical compounds found in *Jatropha gossypifolia* using Soxhlet apparatus as a means of extraction are α-Terpineol(0.22 %), Geraniol (0.25 %), α- Copaene (4.52 %), β-Caryophyllene (1.49 %), *p*-Cresol, 2,6-di-tert-Butyl-(10.18 %), Cadinene, Nerolidol<(E)> (0.73 %), Humulane-1,6-dien-3-ol (21.28 %), Muurolol <epi-α-> (3.62 %), Cadinol<α-> (2.36 %), Hexadecanoic acid, Ethyl ester (0.43 %), Phytol (15.58 %), Linolenic acid, ethyl ester (1.55 %). In Aboaba et al. (2015) research using the hydrodistillation extraction method, they reported major constituents such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpenes, aromatic compounds, fatty acids and non-terpenes. While the compounds detected were Methylcyclohexane, Toluene, Dimethylfulvene, Ethylbenzene,m-Xylene, α-Phellandrene, m-Cymene, Linalool, n-Nonanal, β-Cyclocitral, Cycloisosativene, α-Copaene, β-Caryophyllene, α-Cedrene, (E)-β-Ionone, α-Muurolene, δ-Cadinene, Germacrene, Globulol, Tetradecanal, Acorenone, epi-α-Cadinol, Cubenol, α-Cadinol, Z,Z,Z-7,10,13-Hexadecatrienal, Hexahydrofarnesylacetone, (E)-Phytol, and (Z)-9,17-Octadecadienal.

1.6.2 Essential oil of *J. gossypipifolia* as an antimicrobial agent

Research has shown that, in addition to the traditional uses of *Jatropha gossypifolia* leaves and stems, the essential oil contains potent bioactive phytochemicals that hold

promise as novel antimicrobial agents, a substitute for synthetic antioxidants, and potential applications as food preservatives (Okoh et al. 2016). In Okoh and colleagues (2016) research, they discovered that the essential oil extracted from the leaves and stems of *Jatropha gossypifolia* strongly exhibited inhibitory activity against *Escherichia coli, Enterococcus faecium, and Staphylococcus aureus*. However, further discoveries indicated that the stem essential oils were more active than the leaf essential oils.

1.7 *Cymbopogon citratus* (Poaceae)

A tropical perennial with aromatic leaves; lemongrass is a member of the genus *Cymbopogon* and family Graminae (Poaceae). The Greek terms "kymbe" (boat) and "pogon" (beard), which indicate a floral spike arrangement, are the source of the name *Cymbopogon* (Shah et al. 2011). It is commonly known as lemongrass. According to New Directions Aromatics (2017), Suryamanshi et al. (2016), Chantal *et al*. (2012), and other sources, the herb is still grown and sold today throughout subregions of Africa, Asia, North America, South America, Australia, and Oceania. *Cymbopogon citratus* (West Indian lemongrass), *Cymbopogon flexuosus* (East Indian lemongrass), and *Cymbopogon pendulus* (Jammu lemongrass) are among the approximately 55–80 species of lemongrass that have previously been identified (Chowdury et al. 2015; Lawal et al. 2017). Only two of the identified species—*C. citratus* and *C. flexuosus*—have commercial significance as cultivated plants (Avoseh et al. 2015). Directorate Plant Production (2012) states that *Cymbopogon citratus* is a perennial herb with a lemon aroma that grows quickly and averages a height of around one metre. With distinctive bluish-green leaves, it typically doesn't set seed. As the plants grow, they generate a large number of bulbous stems that increase the clump diameter.

1.7.1 Chemical analysis of the essential oil of *Cymbopogon citratus*

The major components in most lemongrass essential oil include Neral, Isoneral, Geranial, Isogeranial, Geraniol, Geranyl acetate, Citronellal, Citronellol, Germacrene-D, and Elemol. And this makes up about 60–80 % of LEO (Adbulazeez et al. 2016; Mukarram et al. 2021). However, according to Tajidin et al. (2012), they discovered that the maturity stage of lemongrass essential oil determines the chemical composition

using the hydrodistillation method. For instance, they discovered that 14-hexadiene,5-methyl-3-(1-methylidene)-, Myrcenol, 3,6,6-Trimethyl-cyclohex-2-enol, (-)-Isopinocampheol, trans-(-)-Carveol, Oxiranmethanol,3-methyl-3(4-mathyl-3-pentenyl), Bicyclopentylone, β-Maalinene, γ-Muurolene, α-Amorphene, Valencene and Viridiflorol were only detected 5.5 months after planting, but were not detected 6.5 and 7.5 months after planting. Hence, the maturity stage of lemongrass has a huge role to play in its chemical composition.

1.7.2 Essential oil of *Cymbopogon citratus*

Lemongrass plants yield essential oil and can thrive for several growing seasons. Numerous phytochemical substances, including Phenol, Citral, Geranial, terpenoids, benzenoids, and other nitrogenous compounds, are present in it and aid in the plant's metabolic process. The predominant constituent of lemongrass, Citral, gives the leaves and oil of the plant their distinctive aromatic flavours reminiscent of lemons (Okpo & Edeh 2023). About 1-2 % of the essential oil in dry lemongrass leaves is present. The pharmaceutical, cosmetic, food processing, and perfumery industries have all identified uses for lemongrass extracts in the industry. Many pharmacological characteristics, including antibacterial, anti-amoebic, anti-filarial, anti-diarrheal, anti-inflammatory, antifungal, antimalarial, anti-mycobacterial, anti-mutagenicity, hypoglycemic, antioxidant, and neurobehavioral effects, have been demonstrated by scientific studies to be present in *Cymbopogon citratus* oil (Ekpenyong et al. 2015; Ajayi et al. 2016; Chukwuocha et al. 2016; Aly 2021).

1.8 *Ocimum gratissimum* L. (Labiatae)

Ocimum gratissimum is a type of basil that grows wild in Hawaii and is locally known as scent leaf, smell leaf, clove basil, African basil, and African basil (USDA 2019). It is naturalised in Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, Bolivia, and Madagascar. It is native to Africa, Madagascar, southern Asia, and the Bismarck Archipelago. *Ocimum* leaves are commonly referred to as smell leaves because they have a pleasant minty scent and are used as a spice in cuisine and soup preparation (Akinjogunola et al. 2009). Nigeria is home to a large cultivation of *Ocimum*

gratissimum, which accounts for its abundance there. Numerous bioactive substances, such as tannins, saponins, alkaloids, glycosides, phenols, and flavonoids, are found in *Ocimum gratissimum* leaves (Jumare 2018). These bioactive substances, sometimes referred to as phytochemicals, have been linked to a number of health advantages, including the ability to reduce cholesterol, be antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant (Udochukwu et al. 2015; Alexander 2016; Kin et al. 2018).

1.8.1 Chemical analysis of the essential oil of *Ocimum gratissimum*

According to Ashokkumar et al. (2020), using the hydrodistillation as an extraction method it was revealed that *Ocimum gratissimum*' essential oil constitutes the following chemical composition. α-Thujene, Sabinene, β-Myrcene, β-Ocimene, γ –Terpinene, Linalool, β-Terpineol, cis-Verbenol, Eugenol, Copaene, Methyleugenol, Caryophyllene, Humulene, γ-Muurolene, Germacrene, γ-Elemene, Isoledene and Caryophyllene oxide. However, the lemongrass researched here was sourced from South western Ghats, India.

1.8.2 Essential oil of *Ocimum gratissimum* as an antibacterial agent

Amengialue and colleagues (2013), in their research, reaffirm the essential oil of *Ocimum gratissimum* as an antibacterial agent, however, it was discovered that the efficacy of the essential oil varies from one bacterial to another. For instance, in their research the activity of the essential oil against *Staphylococcus aureus* was higher than that of the other bacteria that was investigated. The other bacteria that were investigated include: *Shigella flexineri, Salmonella enteritidis, Escherichia coli.*

1.9 Essential oils extraction methods

The production and use of essential oils and perfumes worldwide are rising at an extremely rapid pace. Thus, improving the overall yield and quality of essential oil requires advanced production technologies. Many extraction techniques are used to extract essential oils from plant source materials (Rassem, 2016). The various extraction

processes are divided into two categories: Conventional methods and innovative methods for extracting essential oils.

Conventional Methods: Numerous techniques exist for the behaviour of essential oil extraction. In many regions of the world, the timid technologies employed in the preparation of essential oils are still misused despite their great relevance. Hydrodistillation (HD), Steam distillation (SD), Solvent extraction, Enfleurage, Cohobation, and Maceration are the roughly traditional and generally used methods.

1.9.1 Hydrodistillation (HD)

The conventional technique for extracting essential oils is hydrodistillation. One of the simplest and oldest techniques being employed in the extraction of essential oil is water or hydrodistillation (Ranjitha & Vijiyalakshimi 2014). The process of hydrodistillation is typically employed to separate essential oils from aromatic and therapeutic plants. Hydrodistillation (HD) is the traditional method for extracting essential oils. In hydrodistillation, a mixture of water or another solvent and plant materials is heated to evaporate the essential oils, which are then liquefied in a condenser. In order to collect the condensate and separate essential oils from water, respectively, the setup also includes a condenser and a decanter. Isotropic distillation serves as the foundation for the extraction process. In actuality, water or other solvent and oil molecules are extracted at atmospheric pressure and during the heating process (Rassem et al. 2016).

The French Pharmacopoeia developed a kind of steam distillation called hydro-distillation (HD) specifically for the extraction of essential oils from dried plants and the laboratory quality control of essential oils. Hydrodistillation comes in three flavours: water immersion, direct vapour injection, and water immersion plus vapour injection. Both large and small enterprises can use this international method. Depending on the plant material being treated, the distillation time varies. Long-term distillation adds undesirable high boiling point chemicals and oxidation products but yields very little essential oil (Rassem et al. 2016)

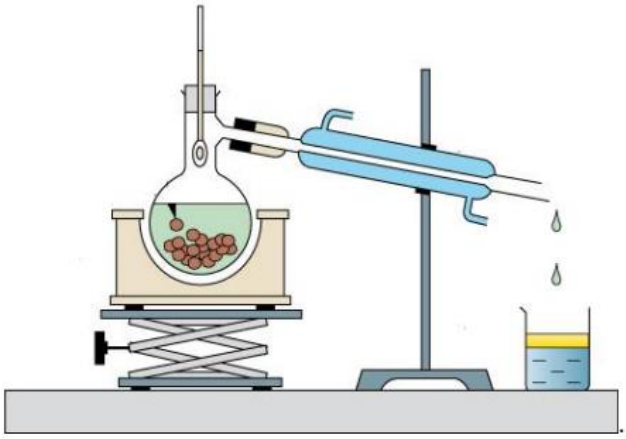

Figure 5: The schematic subsidize apparatus for hydrodistillation (Rassem et al. 2016)

1.9.2 Steam distillation

One kind of distillation (a separation or extraction procedure) for a plant that is temperature-sensitive, such as naturally occurring aromatic chemicals, is steam distillation. Vacuity distillation has rendered this once-common laboratory technique for organic compound purification outdated. In some industrial sectors, steam distillation is still significant (Fahlbusch et al. 2003). One of the oldest and officially recognised techniques for separating essential oils from plant materials is steam distillation. Without being macerated in water, the plant materials charged in the alembic are exposed to the steam. From the bottom to the top of the alembic, the injected steam travels through the plants. One technique that uses steam to pass through the substance is steam distillation (Rassem et al. 2016).

The raw materials pores are broken up and the essential oil is released by the action of this steam. The desired essential oil and vapour are produced by the system. The essential oil is then extracted when this vapour is further condensed (Rai & Suresh 2004). The idea behind this method is that the volatile components, whose boiling points range from 150 to 300 °C, can evaporate at a temperature that is similar to that of water if the combined vapour pressure reaches the ambient pressure at roughly 100 °C. In addition, this method can also be used under pressure, depending on how tough it is to extract the essential oils (Mu'azu et al. 2009; Rassem et al. 2016).

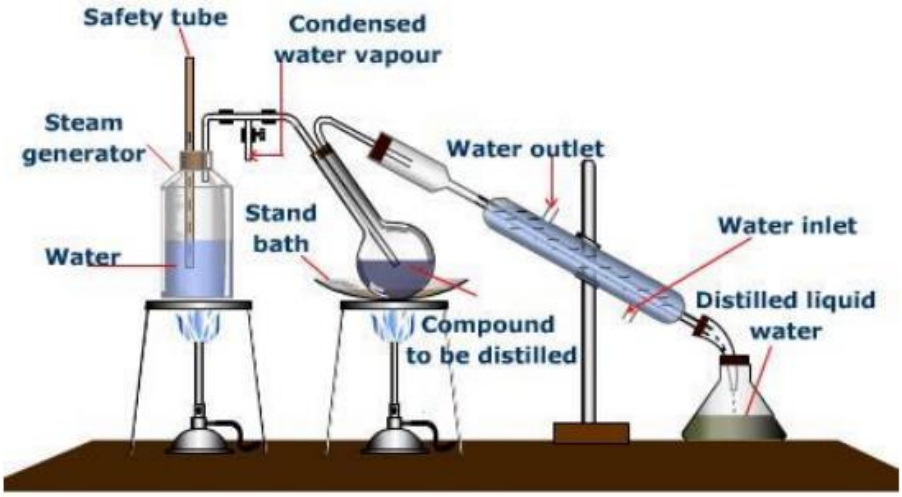

Figure 6: The schematic subsidize apparatus for Steam distillation (Rassem et al. 2016)

1.9.3 Solvent extraction

A substance can be separated by solvent extraction, sometimes referred to as liquid-liquid extraction or partitioning, depending on how soluble each of its constituent parts is. This is accomplished by combining two non-mixing liquids, such as water and an organic solvent. An extracting unit is loaded with perforated trays of essential oil plant material and repeatedly rinsed with the solvent in the solvent-extraction process of recovering essential oils. Solvent extraction is utilized to prepare vegetable oil, biodiesel, and fragrances. On sensitive plants, solvent extraction is employed to provide larger yields of essential oils at a cheaper cost (Rassem et al. 2016). The method of sample preparation used in plant material analysis is the most common. Because the procedure is constrained by the solubility of the compounds in the particular solvent utilised, the type of additional heat provided determines the quality and amount of the extracted mixture. Despite the method's relative simplicity and efficiency, it has several drawbacks, including a lengthy extraction period, a comparatively high solvent consumption, and frequently inadequate repeatability (Dawidowicz et al. 2008).

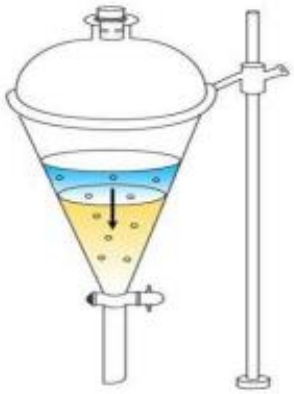

Figure 7: Solvent Extraction (Rassem et al. 2016).

1.9.4 Soxhlet extraction

Franz von Soxhlet created the Soxhlet extractor, a piece of scientific equipment, in 1879. In 1879, Soxhlet and his colleagues' original purpose was to remove a lipid from a solid substance. When the target molecule has a restricted solubility in a solvent and the impurity is insoluble in that solvent, a Soxhlet extraction is typically utilised. While effectively recycling a small amount of solvent to dissolve a larger amount of material, it permits unmonitored and unregulated operation. By dissolving one or more chemicals from a solid into a refluxing liquid phase, Soxhlet extraction uses solid-liquid contact. The solid matrix is put in a cavity in a traditional Soxhlet device, and the extracting liquid phase is progressively added to the cavity by condensation of vapours from a distillation flask. A syphon draws the cavity's contents back into the distillation flask when the liquid reaches a predetermined level, transferring the extracted analytes into the bulk liquid. Until nearly all extraction is accomplished, this process is repeated (Rassem et al. 2016).

The application of Soxhlet extraction has various benefits. The most crucial ones are that new solvent parts are frequently introduced to the sample. This process improves the extraction of the analyte from the matrix and keeps the solvent from being saturated with extractable material. Furthermore, the system's temperature is nearly at the solvent's boiling point. The system's extraction kinetics is accelerated by this increased energy in the form of heat. The sample is diluted in huge quantities of solvent during Soxhlet extraction, which can take hours or days to complete. Additionally, losses from

thermal degradation and volatilization have been documented as a result of heating the distillation flask (Rassem et al. 2016).

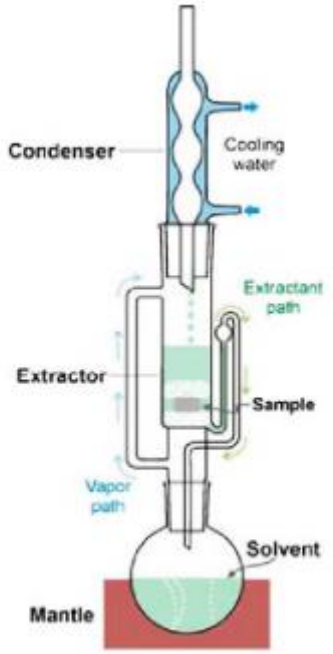

Figure 8: Soxhlet extraction setup (Rassem et al. 2016)

1.9.5 Cold pressing method

Theoretically, the term "cold pressed" refers to expeller-pressed oil that is subjected to low pressure and temperature. One of the greatest techniques for extracting essential oils is the cold-pressed process. Most essential oils and carrier oils are processed using this method. By using this method, the final oil is guaranteed to be 100 % pure and to maintain all of the plant's characteristics. This is a mechanical extraction technique that minimises heat generation during raw material batching. The scarification method is another name for the cold-pressed technique. The cold-pressed process is mostly used to extract essential oils from flowers, seeds, plants, and citrus fruits like lemon and tangerine. Scrubbing is done to remove the oil-containing outer layer from the plants during this phase. The entire plant is then compressed in order to extract the material from the pulp and the essential oil from the pouches. Centrifugation is used to separate the essential oil from the material once it rises to the surface (Rassem et al. 2016)

1.9.6 Innovative techniques of essential oils extraction (non-traditional): The thermolability of essential oil components—which are subject to chemical changes (hydrolyse, isomerization, and oxidation) as a result of high applied temperatures—is one of the drawbacks of traditional methods. As a result, the quality of extracted essential oils is severely compromised, especially if the extraction process is prolonged. It is crucial that the natural proportion and chemical makeup of essential oils be preserved during the extraction process. The development of essential oil extraction processes has continued uninterrupted because the modern industrial production has made economy, competitiveness, eco-friendliness, sustainability, high efficiency, and good quality its watchword. In actuality, there are other methods besides traditional ones for extracting essential oils. In order to obtain natural extracts that are on par with or superior to those obtained using official methods, new techniques that adhere to green extraction concepts and principles have been continuously developing in recent years. Additionally, shorter extraction durations, less energy, less solvent use, and lower CO_2 emissions are required of new extraction procedures (Rassem et al. 2016).

The most popular techniques for extracting essential oils on a commercial basis are the traditional methods, which have been discussed. Though they may not be widely used for the commercial production of essential oils, new techniques that have been developed as a result of technological advancements are thought to be valuable in certain situations (Rassem et al. 2016). Examples of these situations include the extraction of essential oils for micro-analysis or the production of expensive essential oils in their natural state without altering any of their thermosensitive components. The following are a few non-traditional techniques: solvent-free microwave extraction (SFME), ultrasound-assisted extraction (UAE), microwave hydro diffusion and gravity (MHG), supercritical fluid extraction (SFE), and microwave-Assisted Hydrodistillation (MAHD).

1.10 Gas chromatography/ mass spectrometry (GC/MS)

According to De Vos et al. (2007), GC/MS is used to identify and quantify a wide range of volatile and non-volatile metabolites, primarily those involved in primary metabolism. These include organic and amino acids, sugars, sugar alcohols, and phosphorylated intermediates (found in the polar fraction of extracts), as well as

lipophilic compounds like fatty acids and sterols (found in the polar fraction). Due to their low molecular weight, most volatile chemicals are frequently found in the 30-400 m/z mass range.

In the process of identifying certain high molecular weight metabolites, mass scanning may encompass a broad range of 30-600 m/z. Given the standardisation techniques utilising the Kovats index and retention index, as well as the fact that GC became a prominent technique about sixty years ago, it is not surprising that there are reputable databases for identifying the mass spectrum produced from GC/MS. Chemical ionisation in either a positive or negative mode or electron impact can be used to carry out fragmentation in GC/MS.

Generally speaking, GC/MS studies provide stronger ionisation energy than LC/MS studies do. This results in a large number of fragment ions, but they can still be easily matched to compounds in the National Institute of Standards and Technology database using deconvolution techniques. One crucial thing to keep in mind is that sugars in seaweeds are frequently reduced and per acetylated when analysed by GC/MS. However, because all sugars are reduced to their respective sugar alcohols and react with acetic anhydride to give volatile sugar derivatives that can be analysed using GC/MS, this method does not allow for the differentiation of mannose and mannitol (Van Hal et al. 2014).

GC/MS is the most crucial technique for characterising components in EOs study. Certain molecules, like the several varieties of monoterpenes, have the same chemical formula but distinct structural kinds. A greater number of isomers may have relatively comparable mass spectra. Once the component elution time is mastered and integrated with MS, the data quality rises and gains immense value. It has long been standard practice to combine IR spectroscopy with mass spectrometry (IR/MS) with GC/MS as a useful method for the identification and separation of volatile chemicals (Wilkins 1994).

2.11 Summary

Clearly the researches reviewed gives insights into the significance of food quality and its standards, especially the role of chemical analysis. It also gives detailed information about essential oils, their importance, especially in the aspect of chemical compounds identified and their extraction methods. The latter part of this chapter gives the precise beneficial information about the plant materials used as a source of essential oil to carry

out this research. To sum it all, this literature review has given a lot of background knowledge to how the research will be carried out and to clearly define the focus of this thesis.

2. Aims and Objectives

2.1 Aim of the study

The aim of this study was the extraction and subsequent chemical analysis of essential oils of selected African plants.

1.3.2 Objectives of the study

The objectives of the study are:

1. Extraction of essential oils using two different extraction techniques and subsequent chemical analysis using gas chromatography with mass detection. Specifically, the following plants, *Jatropha gossypifolia*, *Cymbopogon citratus*, and *Ocimum gratissimum* were used. They were selected based on their practical use and also according to their availability and usability in African countries especially in Nigeria.
2. Comparison of the chemical composition of essential oil extracted using hydrodistillation and Soxhlet solvent extraction and subsequent evaluation of both methods used.

3. Materials and Method

3.1 Collection of Plant materials

The plant material used came from Nigeria, West Africa, and included leaves from *Jatropha gossypifolia*, *Cymbopogon citratus*, and *Ocimum gratissimum*. The leaves were adequately air-dried for five days at room temperature.

Figure 9: *Ocimum gratissimum* (Scent leaf), *Cymbopogon citratus* (lemongrass), and *Jatropha gossypifolia* (Bellyache bush) samples (from left to right). (Source: Author of the thesis)

3.2 Preparation and extraction of Essential oils

The dried leaves of the plants were weighed and crushed into small pieces. Subsequently, they were subjected to two different processes of essential oil extraction, namely Soxhlet extraction and hydrodistillation.

3.3 Hydrodistillation method

Samples of dried *Jatropha gossypifolia*, *Cymbopogon citratus*, and *Ocimum gratissimum* were prepared. 50 grams were picked from each sample, and then the samples were separately put into 1 l of distilled water in the glass flask. The sample was

then distilled using a Clevenger distillation apparatus. The process lasted four hours. After, the obtained essential oil was transferred to a 2 ml vial. Essential oil samples were diluted with n-Hexane (Merck, Darmstadt, Germany) to a concentration of 20 µl/ml for further analysis.

Figure 10: Hydrodistillation extraction of essential oil from the plant samples. (Source: Author of the thesis)

3.4 Soxhlet method

The extractions were done by using a Solvent Autoextractor (Velp Scientifica SER 158 Series Automatic Solvent Extractor, Italy). A modified AOAC 2003.06 extraction method was used. 7 g of the dried sample was placed in an extraction thimble with 130 ml of n-Hexane. After extraction, the solvent was evaporated to dryness at room temperature. The sample was then diluted with n-Hexane to a concentration of 20 µl/ml.

3.5 Characterization of essential oils by Gas Chromatography-Mass Spectrometry (GC/MS)

The samples were analysed using the gas chromatograph system Agilent GC-7890B coupled to a single quadrupole mass selective detector (MSD) Agilent MSD-5977B

(Agilent Technologies, Santa Clara, CA, USA). For the separation of components, HP-5MS column (30 m × 0.25 mm, 0.25 µm, Agilent 19091s-433) was used. The samples were diluted in n-Hexane (Merck, Darmstadt, Germany) to the concentration of 20 µg/mL, and 1 µL of the solution was injected in split mode (1:20) into the inlet (250 °C), carrier gas He (1 mL/min) was used. The temperature of the oven was adjusted to 60 °C for 1 min., then to 240 °C at a rate of 3 °C/min for 5 min. The National Institute of Standards and Technology Library ver. 2.2.f (NIST, USA), along with the genuine standards and literature, was consulted in order to compare the retention indices (RI) and spectra of the constituents (Adams 2007). MassHunter Workstation Software Qualitative Analysis B.07.00 was used to process the data. Through electronic integration, the peak area was discovered. Based on a comparison of the observed chemicals' mass spectra with those in the NIST/EPA/NIH version 2.2 collection, the substances were identified. By matching the measured RI with the National Institute of Standards and Technology (NIST, USA) database, the identity was verified. The retention durations of the n-alkanes series, which range from C7 to C40 (Sigma-Aldrich, Prague, CZ), were used to compute the RI.

4. Results

4.1 Compounds of *Cymbopogon citratus* EO

The volatile compounds identified in *Cymbopogon citratus* (lemongrass) as affected by the extraction method are presented in Table 5. A total of 37 volatile compounds were identified in *Cymbopogon citratus* EO, 37 were identified in hydrodistillation extraction method samples representing 95.38 % of essential oil and 28 volatile compounds were identified in Soxhlet extraction method samples representing 84.07 % of essential oil. The study found β-Myrcene to be the most abundant in hydrodistillation with 26.9 % and it recorded 13.42 % in Soxhlet. However, Geranial was found to be the most abundant in Soxhlet, with 23.68 % while 16.69 % was recorded in hydrodistillation. Other volatile compounds found to be relatively abundant in hydrodistillation method include Linalool (4.82 %), Rosefuran epoxide (6.34 %), β-Citral (12.19 %) and Thymol (3.49 %). Volatile compounds found to be relatively abundant in Soxhlet include Isogeranial (3.71 %), β-Citral (12.62 %) and 6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cyclohexa-1,3-dienecarbaldehyde (4.27 %).

Other volatile compounds such as 6-Methyl-5-heptene-2-one, m-Cymene, β-Ocimene, 2-Octen-1-ol, Linalool oxide, 3-Methyl-2-(2-methyl-2-butenyl)-furan, 7-Methyl-3-methyleneoct-6-enal, Isoneral, Isogeranial, 3,7-Dimethyl-6-nonenal, 3-Methyl-3-(4-methyl-3-pentenyl)-2-oxiranecarbaldehyde, Citronellol, Undecan-2-one, Geranyl formate, Megastigma-4,6,8-triene, Geranyl acetate, Isocaryophyllene, α-Bergamotene, β-Farnesene, β-Eudesmene, Tridecan-2-one, Caryophyllene oxide, Selin-6-en-4α-ol, Pogostole, Pentadecan-2-one, Geranyl caproate, Hexahydrofarnesyl acetone, Hexadecan-1-ol, Geranyl caprylate, and 6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cyclohexa-1,3-dienecarbaldehyde identified in hydrodistillation were found in little proportions. However 6-Methyl-5-heptene-2-one, m-Cymene, β-Ocimene, Linalool oxide, 3-Methyl-2-(2-methyl-2-butenyl)-furan, β-Eudesmene, Pentadecan-2-one, Geranyl caproate and Hexadecan-1-ol were absent in the *Cymbopogon citratus* essential oil extracted through Soxhlet method.

Table 5: Chemical composition of *Cymbopogon citratus* essential oil

P/N	Compound name	Retention index		Hydro (%)	Soxhlet (%)
		Calc.	Lit.		
1	6-Methyl-5-heptene-2-one	988	988	0.94	0.00
2	β-Myrcene	993	992	26.9	13.42
3	m-Cymene	1028	1023	0.11	0.00
4	β-Ocimene	1038	1032	0.41	0.00
5	2-Octen-1-ol	1055	1060	0.25	0.28
6	Linalool oxide	1076	1065	0.16	0.00
7	3-Methyl-2-(2-methyl-2-butenyl)-furan	1100	1093	2.02	0.00
8	Linalool	1107	1106	4.82	1.63
9	7-Methyl-3-methylene-oct-6-enal	1149	1147	0.77	2.49
10	Isoneral	1170	1165	1.81	2.85
11	Rosefuran epoxide	1182	1177	6.34	2.12
12	Isogeranial	1188	1185	2.61	3.71
13	3,7-dimethyl-6-nonenal	1201	*	0.24	0.39
14	3-Methyl-3-(4-methyl-3-pentenyl)-2-oxiranecarbaldehyde	1229	1236	0.29	2.06
15	Citronellol	1243	1240	0.84	2.82
16	β-Citral	1254	1249	12.19	12.62
17	Lemonol	1275	1267	6.31	2.59
18	Geranial	1288	1279	16.69	23.68
19	Undecan-2-one	1295	1291	1.19	0.6
20	Geranyl formate	1305	1305	0.2	0.15
21	Thymol	1318	1306	3.49	0.59
22	Megastigma-4,6,8-triene	1375	1373	0.16	0.2
23	Geranyl acetate	1388	1386	1.46	1.21
24	Isocaryophyllene	1425	1425	0.27	0.32
25	α-Bergamotene	1439	1439	0.54	0.6
26	β-Farnesene	1458	1457	0.22	0.28
27	β-Eudesmene	1491	1490	0.11	0.00
28	Tridecan-2-one	1499	1498	1.22	1.23
29	Caryophyllene oxide	1590	1589	0.66	0.56

30	Selin-6-en-4α-ol	1628	1636	0.52	0.39
31	Pogostole	1669	1655	0.15	0.47
32	Pentadecan-1-one	1700	1699	0.14	0.00
33	Geranyl caproate	1755	1755	0.11	0.00
34	Hexahydrofarnesyl acetone	1846	1846	0.14	0.32
35	Hexadecan-1-ol	1884	1884	0.16	0.00
36	Geranyl caprylate	1951	1953	0.54	2.22
37	6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cyclohexa-1,3-dienecarbaldehyde	2117	2113	0.4	4.27
	Total			**95.38**	**84.07**

*Indicates that the retention index of the compound is not available in the literature for comparison.

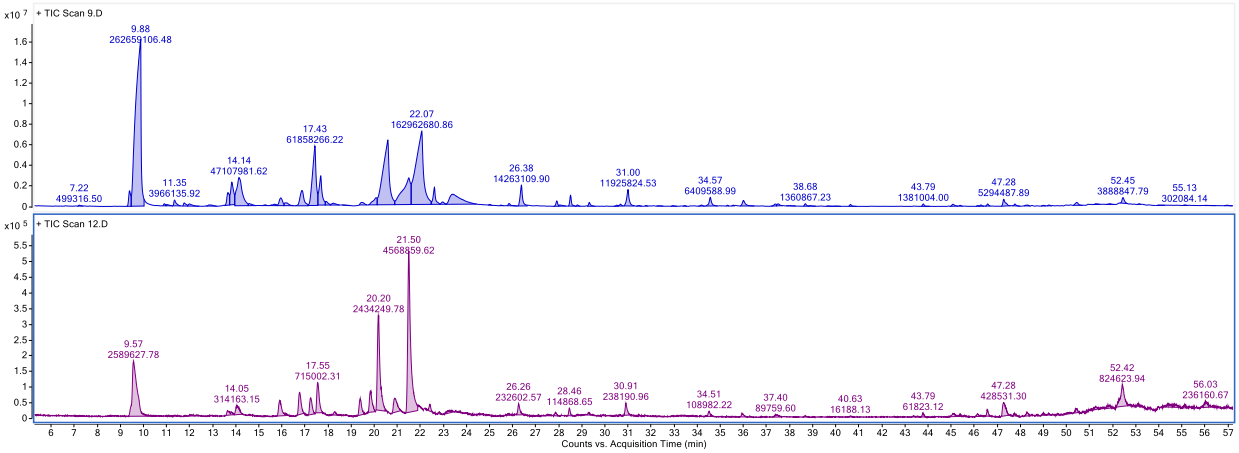

Figure 11: Chromatograms of *Cymbopogon citratus* essential oil obtained by hydrodistillation and Soxhlet extraction (from top to bottom).

4.2 Compounds of *Ocimum gratissimum* EO

Table 6 presents the volatile compounds of essential oil identified in *Ocimum gratissimum* as affected by the extraction method. This study identifies a total of 41 volatile compounds in *Ocimum gratissimum* EO, 35 volatile compounds were identified in the hydrodistillation extraction method sample representing 98.95 % of essential oil and 39 volatile compounds were identified in the Soxhlet hydrodistillation extraction method sample representing 95.94 % essential oil. The study identified α-Thujene (3.32 %), γ-Terpinene (10.58 %), Thymol (49.83 %), Caryophyllene (6.66 %) and β-Eudesmene (6.15 %) as abundant volatile compound in hydrodistillation extraction method while α-Thujene (5.04 %), α-Pinene (3.67 %), γ-Terpinene (17.12 %), Thymol (27.98 %), Caryophyllene (8.53 %), β-Eudesmene (9 %) and α-Selinene (3.03 %) were identified as abundant volatile compound in Soxhlet extraction method.

Camphene, Sabinene, β-Myrcene, 3-Carene, α-Terpinene, Crithmene, Sabinene hydrate, 4-Thujanol, β-Terpineol, (*E*)-Sabinene hydrate, 1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-one, Terpinen-4-ol, Verbenone, Thymol methyl ether, p-Menth-2-en-1,4-diol, γ-Cadinene, β-Elemen, (*E*)-α-Bergamotene, Humulene, Germacrene D, α-Panasinsen, Hexa-hydro-farnesol, Caryophyllene oxide, Humulene epoxide II, Neointermedeol, Aromadendrene oxide II, (1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol, Dihydro-3-oxo-β-ionol, 6,10,14-Trimethylpentadecan-2-one, Androst-4-en-9,11-epoxy-3,17-dione and Phytol were identified in little proportion in the Soxhlet extraction method sample. However β-Myrcene, Crithmene, 1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-one, p-Menth-2-en-1,4-diol, 6,10,14-Trimethylpentadecan-2-one and Phytol were absent in the volatile compounds identified in hyrodistillation extraction method samples.

Table 6: Chemical composition of *Ocimum gratissimum* essential oil

P/N	Compound name	Retention index		Hydro. (%)	Soxhlet (%)
		Calc.	Lit.		
1	α-Thujene	927	931	3.32	5.04
2	α-Pinene	934	939	2.8	3.67
3	Camphene	949	949	0.24	0.23
4	Sabinene	974	973	0.23	0.49
5	β-Myrcene	978	992	0.00	1.05
6	Sabinene	978	978	0.85	0.00
7	β-Myrcene	992	992	1.17	1.69
8	3-Carene	1012	1011	0.17	0.2
9	α-Terpinene	1019	1018	1.2	1.12
10	γ-Terpinene	1031	1030	10.58	17.12
11	Crithmene	1060	1060	0.00	2.34
12	γ-Terpinene	1062	1062	2.67	0.00
13	Sabinene hydrate	1072	1072	0.66	2.1
14	4-Thujanol	1094	1096	0.24	0.15
15	β-Terpineol	1105	1121	0.28	0.48
16	(*E*)-Sabinene hydrate	1115	1104	0.16	0.03
17	1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-one	1177	1174	0.00	0.2
18	Terpinen-4-ol	1183	1183	0.8	0.32
19	Verbenone	1197	1204	0.2	0.28
20	Thymol methyl ether	1238	1235	0.64	0.6
21	p-Menth-2-en-1,4-diol	1290	1273	0.00	0.2
22	Thymol	1318	1306	49.83	27.98
23	γ-Cadinene	1379	1367	0.25	0.4
24	β-Elemen	1397	1397	0.36	0.51
25	Caryophyllene	1431	1430	6.66	8.53
26	(*E*)-α-Bergamotene	1438	1438	0.88	1.32
27	Humulene	1458	1460	0.98	1.11
28	Germacrene D	1489	1489	0.96	1.53
29	β-Eudesmene	1494	1496	6.15	9.00

30	α-Selinene	1505	1505	2.02	3.03
31	α-Panasinsen	1525	1527	0.54	0.79
32	Hexa-hydro-farnesol	1528	1563	0.31	0.31
33	Caryophyllene oxide	1593	1593	2.52	1.8
34	Humulene epoxide II	1616	1614	0.21	0.08
35	Neointermedeol	1665	1662	0.25	0.23
36	Aromadendrene oxide II	1684	1678	0.16	0.1
37	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1696	1695	0.11	0.13
38	Dihydro-3-oxo-β-ionol	1731	1726	0.44	0.36
39	6,10,14-Trimethylpentadecan-2-one	1846	1847	0.00	0.12
40	Androst-4-en-9,11-epoxy-3,17-dione	2008	*	0.11	0.13
41	Phytol	2117	2118	0.00	1.17
	Total			**98.95**	**95.94**

*Indicates that the retention index of the compound is not available in the literature for comparison.

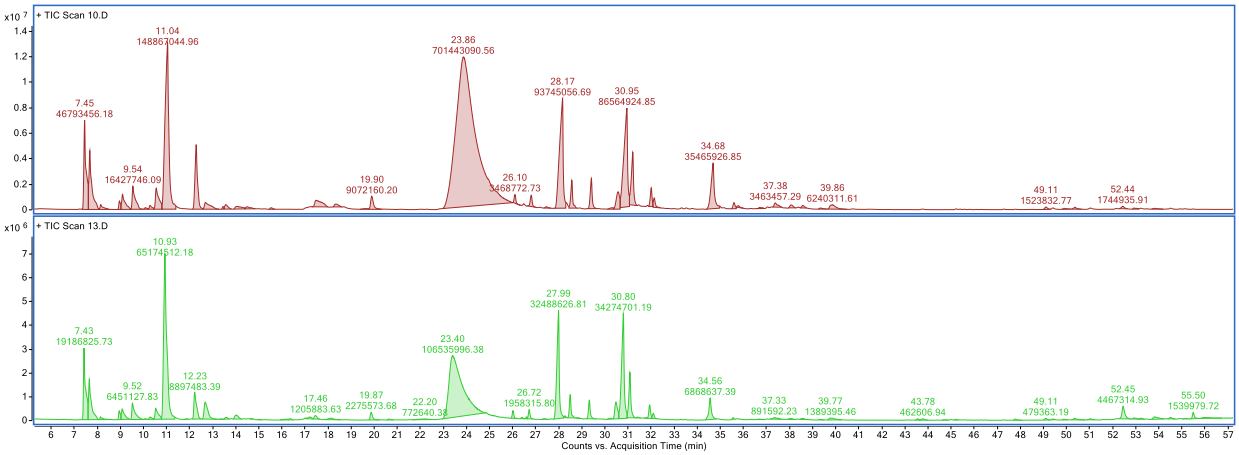

Figure 12: Chromatograms of *Ocimum gratissimum* essential oil obtained by Soxhlet extraction and hydrodistillation (from top to bottom).

4.3 Compounds of *Jatropha gossypifolia* EO

Table 7 presents the volatile compounds of essential oil identified in *Jatropha gossypifolia* as affected by the extraction method. This research identifies a total of 50 volatile compounds in *Jatropha gossypifolia* EO, 45 volatile compounds were identified in hydrodistillation extraction method sample representing 91.93 % essential oil and 36 volatile compounds were identified in Soxhlet extraction method sample representing 83.89 % essential oil. This research identifies Thymol (46.94 %), 6,10-Dimethyl-5,9-undecadien-2-one (6.05 %), Eremophilene (7.13 %) and Phytol (5.7 %) as the abundant volatile compound in hydrodistillation extraction method sample. Thymol (11.06 %), Hexahydrofarnesyl acetone (4.43 %) and Phytol (39.52 %) were identified to be abundant in the Soxhlet extraction method sample.

Furthermore in the hydrodistillation extraction method sample, 2-Hexenal, Sulcatone, Ethylhexanol, 4-Methylundec-1-ene, Linalool oxide, β-Terpineol, 2,6-Dimethyldecane, L-4-terpineneol, Dodec-1-ene, 4,7-Dimethylundecane, 2-Methyl-2-nonen-4-one, Trimethyl-1-cyclohexene-1-carbaldehyde, 4,8-Dimethylnonanol, 2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde, 2-Butyloctan-1-ol, Tridec-1-ene, 1,3,3-Trimethyl-2-vinyl-1-cyclohexene, Caryophyllene, γ-Muurolene, α-Bergamotene, 6,10-Dimethyl-5,9-undecadien-2-one, Pentadec-1-ene, Methyldodecan-11-ol, β-Selinene, Selina-3,7(11)-diene, σ-Cadinene, Nerolidol, Caryophyllene oxide, Humulene epoxide II, Cedrol, Heptadec-1-ene, 2-Hexyl-cinnamaldehyde, Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-β]furan, Galaxolide, Hexadecan-1-ol, Hexadecanoic acid, methyl ester, Dibutyl phthalate, 3,7,11,16-Tetramethyl-hexadeca-2,6,10,14-tetraen-1-ol, 2(3H)-Furanone, 5-Dodecyldihydro- and tert-Hexadecanethiol were identified in few proportion in hydrodistillation extraction method sample. However, 2-Hexenal, Sulcatone, Dodec-1-ene, 2-Methyl-2-nonen-4-one, Trimethyl-1-cyclohexene-1-carbaldehyde, 2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde, Selina-3,7(11)-diene, σ-Cadinene, Nerolidol, Humulene epoxide II, Cedrol, 2-Hexyl-cinnamaldehyde, Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-β]furan and Galaxolide volatile compounds were absent in the Soxhlet extraction method sample.

Table 7: Chemical composition of *Jatropha gossypifolia* essential oil

P/N	Compound name	Retention index		Hydro (%)	Soxhlet (%)
		Calc.	Lit.		
1	2-Hexenal	856	854	0.53	0.00
2	Sulcatone	988	988	0.32	0.00
3	Decane	1000	1000	0.00	2.33
4	Ethylhexanol	1033	1034	0.17	0.54
5	4-Methylundec-1-ene	1075	1085	0.6	0.86
6	Linalool oxide	1092	1093	0.08	0.77
7	β-Terpineol	1105	1121	0.43	1.52
8	2,6-Dimethyldecane	1117	1121	1.11	1.74
9	2-Methyl-undecane	1155	1164	0.00	1.72
10	2,3-Dimethyldecane	1159	1157	0.00	0.4
11	3-Methylundecane	1164	1171	0.00	0.43
12	L-4-Terpineneol	1171	1175	0.33	0.43
13	Dodec-1-ene	1194	1192	0.1	0.00
14	4,7-Dimethylundecane	1200	1207	0.2	0.48
15	2-Methyl-2-nonen-4-one	1219	1215	0.13	0.00
16	Trimethyl-1-cyclohexene-1-carbaldehyde	1225	1224	0.17	0.00
17	4,8-Dimethylnonanol	1248	1230	0.11	0.49
18	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	1262	1261	0.11	0.00
19	2-Butyloctan-1-ol	1276	1277	0.15	0.43
20	Tridec-1-ene	1301	1293	0.13	0.49
21	Thymol	1319	1306	46.94	11.06
22	1,3,3-Trimethyl-2-vinyl-1-cyclohexene	1382	*	0.5	0.81
23	Caryophyllene	1424	1423	1.59	1.04
24	γ-Muurolene	1429	1444	0.39	0.73
25	α-Bergamotene	1439	1433	0.28	0.62
26	6,10-Dimethyl-5,9-undecadien-2-one	1458	1456	6.05	0.66
27	Pentadec-1-ene	1480	1492	0.65	0.59
28	Methyldodecan-11-ol	1487	*	0.49	0.78
29	Eremophilene	1494	1500	7.13	1.93
30	β-Selinene	1501	1509	1.35	0.49

31	Selina-3,7(11)-diene	1524	1524	0.25	0.00
32	σ-Cadinene	1535	1541	0.37	0.00
33	Nerolidol	1570	1570	0.28	0.00
34	Caryophyllene oxide	1592	1593	2.18	0.63
35	Humulene epoxide II	1618	1616	0.13	0.00
36	Cedrol	1628	1619	0.19	0.00
37	Heptadec-1-ene	1672	1673	2.92	0.62
38	2-Hexyl-cinnamaldehyde	1756	1749	0.29	0.00
39	Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-β]furan	1766	1766	0.22	0.00
40	Hexahydrofarnesyl acetone	1848	1848	3.23	4.43
41	Galaxolide	1860	1851	0.66	0.00
42	Hexadecan-1-ol	1886	1883	0.16	1.48
43	Farnesyl acetone	1924	1924	3.59	1.13
44	Hexadecanoic acid, methyl ester	1928	1928	0.22	1.18
45	Dibutyl phthalate	1970	1970	0.25	0.4
46	3,7,11,16-tetramethyl-Hexadeca-2,6,10,14-tetraen-1-ol	2035	*	0.56	0.52
47	2(3H)-Furanone, 5-dodecyldihydro-	2103	2104	0.13	0.51
48	Phytol	2121	2122	5.7	39.52
49	tert-Hexadecanethiol	2204	*	0.56	0.75
50	4,8,12,16-Tetramethylheptadecan-4-olide	2361	2364	0.00	1.38
	Total			**91.93**	**83.89**

*Indicates that the retention index of the compound is not available in the literature for comparison.

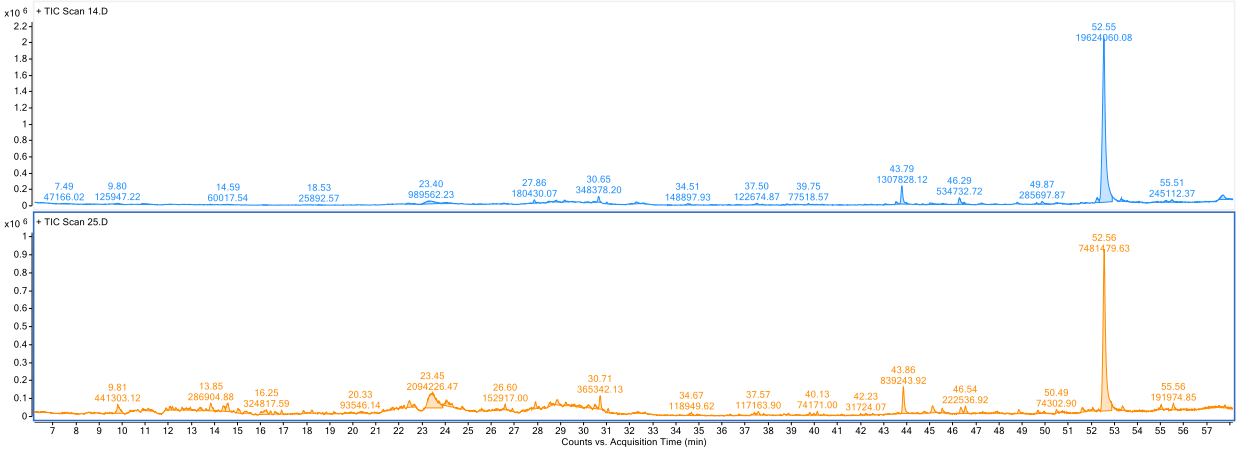

Figure 13: Chromatograms of *Jatropha gossypifolia* essential oil obtained by Soxhlet extraction and hydrodistillation (from top to bottom).

5. Discussion

In this research it was observed that there were major differences between some volatile compounds identified when compared with other journals that researched on the same plant. For instance, comparing some of the volatile constituents identified in lemongrass essential oil in the study of Plata-Rueda et al. (2020), it was reported that Neral had 24.6 % as against the β-Citral (Neral) that had 12.19 % and 2.62 % for the two extraction methods adopted for this study. Furthermore, their research reported 12.3 % of Geranial; however, in this research 16.69 % and 23.68 % were reported as impacted by the two extraction methods for the same volatile compound in the same plant. Moreso, in their research they reported 1.89 % of Caryoophyllene oxide, while 0.66 % and 0.56 % were reported in this research. These might have been as a result of different factors, as stated by Dung et al. (2021), who reported that the quality of essential oils is determined by their method of composition, which is very susceptible to soil types, environment, and weather. The temperature, harvest season, and other factors might also change how an essential oil smells. The composition and functionality of essential oils are directly impacted by the discovery that harvesting plant sources in the early morning or late afternoon is crucial for maintaining the quality of essential oils.

This study shows that the most abundant compound identified in *Cymbopogon citratus* using the hydrodistillation extraction method is β-Myrcene while Geranial was noted for Soxhlet extraction method. This implies that hydrodistillation is the most suitable extraction method when β-Myrcene is desired, while Soxhlet method should be adopted when Geranial is desired in higher quantity. According to Kiani et al. (2022) Myrcene is an antibacterial and analgesic compound found in lemongrass. Geranial, which is also named as α-Citral has been researched to exhibit antibacterial activity (Shah et al. 2011). Linalool and Thymol, also identified in a significant quantity using the hydrodistillation extraction, have been researched to optimize the effect of the available drugs against pathogens that cause diarrhea disease (Aelenei 2016). β-Citral, which is also named as Neral elicits an antibacterial action (Shah et al. 2011). Thymol that has also been majorly identified using the hydrodistillation extraction method, has been used majorly in the treatment of the upper respiratory system as expectorant, antiviral, antiseptic, anti-inflammatory and antibacterial agent (Kowalczyk et al. 2020).

In this study the most abundant volatile compound identified in *Ocimum gratissimum* using both the hydrodistillation and Soxhlet extraction method is Thymol. The Thymol extracted from *Ocimum gratissimum* has been researched to be used for the management of cough and tuberculosis in Nigeria due to its antimycobacterial action (Okhale et al. 2015). Another major component that was identified both in hydrodistillation and Soxhlet extraction method is γ-Terpinene. γ-Terpinene is responsible for the lemon odour of *Ocimum gratissimum,* it has a strong antioxidant activity. γ-Terpinene is widely used in pharmaceutical, soaps, cosmetics, food, perfumes and flavours industries (National Center for Biotechnology Information 2024). Caryophyllene that was also majorly identified in *Ocimum gratissimum* using both hydrodistillation and Soxhlet extraction methods and has been reported by researchers for many biological activities such as anti-inflammatory, gastroprotective, anxiolytic, antibacterial, anti-oxidant, antiprotective and antiaging potentials (Pant et al. 2014, Cheng et al. 2014; Paula-Freire et al. 2014). β-Eudesmene, which was also majorly identified in both the hydrodistillation and Soxhlet extraction method, has been reported by You et al. (2017) to have a repellant effect on *T. castaneum.* Singh et al. 2009 also noted in their research that α-Selinene is an important constituent for perfumery and thus commercially important.

In this research, Thymol has been discovered to be the highest volatile constituent using the hydrodistillation extraction method in *Jatropha gossypifolia*, while Phytol was the highest volatile constituent using the Soxhlet extraction method. The volatile compound Thymol identified has been reported to contain antimicrobial, anticancer, and antioxidant potentials (Kang et al. 2016; Miladi et al. 2016). It was reported by Kasrati et al. (2014) that Thymol has been part of the basic compounds specific to certain genera of the Lamiaceae family. Phytol, which has been noted to be significantly present using both extraction methods – hydrodistillation and Soxhlet extraction– has been reported to be a diterpenoid alcohol, which demonstrates an antioxidant effect in vivo. It has also been noted to eliminate hydroxyl and nitric oxide radicals and, at the same time, prevent formations of lipid peroxides as measured by thiobituric acid reactive substances (de Menezes et al. 2013). Eremophilene identified has also been reported by Utegenova et al. (2018) to have antibacterial potential. Hexahydrofarnesyl acetone was noted to have anti-nociceptive, anti-inflammation and antibacterial activities (Avoseh et al. 2021; WeI et al. 2016).

In this study, it was discovered that the two extraction processes of the three plants - *Cymbopogon cirtratus, Ocimum gratissimum* and *Jatropha gossypifolia* - were subjected to have an impact on the number of volatile compounds that were identified by the GC/MS analysis. The differences observed were not the same; however, they were in close gaps. For instance, in *Cymbopogon cirtratus,* the compounds identified in the hydrodistillation extraction method were more than those identified in the Soxhlet extraction method. This was also the case for *Jatropha gossypifolia.* However, in the case of *Ocimum gratissimum,* more volatile compounds were identified in the Soxhlet extraction method when compared with the hydrodistillation extraction method. Msaada et al. (2012) study using coriander fruit for their research reported that the extraction process has an impact on the authentic composition and quality. This was also the case for Njoku et al. (2022) in their research; they discovered that yield, chemical composition and quality of *Drosera regia* essential oil were affected by different extraction methods.

6. Conclusions

This research highlights significant variations in the volatile compounds identified in essential oils extracted from three different plants, namely *Cymbopogon citratus*, *Ocimum gratissimum*, and *Jatropha gossypifolia*, using two different extraction methods: hydrodistillation and Soxhlet extraction. The findings have been categorically stated below.

1. Variability in Constituents: There are notable differences in the composition of volatile compounds between studies, indicating that factors such as extraction method, environmental conditions, and plant variability play crucial roles.

2. Extraction Method Impact: The choice of extraction method influences the profile of volatile compounds obtained. For example, hydrodistillation favored β-Myrcene in *Cymbopogon citratus*, while Soxhlet extraction favored Geranial. Thymol was consistently abundant in *Ocimum gratissimum* regardless of the extraction method.

3. Therapeutic Potential: Identified compounds such as Myrcene, Geranial, Thymol, Caryophyllene, and Phytol exhibit various therapeutic properties, including antibacterial, analgesic, antioxidant, and anti-inflammatory effects. This underscores the pharmacological significance of these plants and their essential oils.

4. Extraction Process Influence: The extraction process significantly impacts the number and composition of volatile compounds identified. This finding aligns with previous research highlighting the importance of extraction methods in determining the quality and composition of essential oils.

In conclusion, this research emphasizes on the complexity of essential oil composition influenced by extraction methods and environmental factors. Understanding these variations is crucial for optimizing the therapeutic potential and commercial applications of essential oils derived from medicinal plants. Further studies exploring additional extraction techniques and considering broader environmental factors could enhance our understanding of volatile compound composition and its implications for various applications, including pharmaceuticals, cosmetics, and aromatherapy.

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