REPELLENCY AND ANTIMICROBIAL EFFECTS OF *PELARGONIUM CITROSUM* AND *ROSMARINUS OFFICINALIS* L PLANT EXTRACTS

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Repellency and Antimicrobial Effects of *Pelargonium citrosum* and *Rosmarinus officinalis* L Plant Extracts

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature……Date……

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This thesis has been submitted for examination with our approval as university supervisors.

Signature……Date……

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Signature……Date……

Prof. Mercy Githua, PhD

JKUAT, Kenya

DEDICATION

I dedicate this work to my wife Mrs. Irene Gitaari, children and siblings

ACKNOWLEDGEMENT

I am very grateful to the Almighty God for seeing me this far, giving me strength, good health and sound mind to accomplish this study. I recognize with great appreciation, my able and dedicated supervisors Prof. P. Kareru, and Prof. M. Githua for their tremendous guidance, mentorship and valuable support from the start of this work up to the end. They were always available for consultations and encouragement.

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ABBREVIATIONS AND ACRONYMS

ANOVA Analysis of Variance

DEET N,N-diethyl-*m*-toluamide

GC-MS Gas Chromatography Mass Spectrometry

ICIPE International Center of Insect Physiological and Ecology

LD_{50} Lethal dose

SD standard deviation

DMSO Dimethyl sulfoxide

ABSTRACT

Use of botanical environmentally friendly and biodegradable insect repellents as opposed to chemical insecticides is increasingly becoming important as an alternative method of insect control. Housefly (*Musca domestica* L.) has potential of transmitting pathogen which causes diseases such as parasitic worms, anthrax, tuberculosis, bacillary dysentery, cholera and typhoid. Human diseases can be treated and prevented by essential oils with biological properties which have been derived from aromatic plants. In this study antimicrobial activities and housefly repellence of *P. citrosum* and *R. officinalis* was evaluated separately and in products formulated from the extracts. Extraction of essential oils was by hydro-distillation. Condensed oil extracts were collected in n-hexane and insect behavioural response tested using adult houseflies (*Musca domestica* L.). *N,N*-diethyl-*m*-toluamide (DEET) acted as the positive control with acetone acting as the negative control. The bioactive oil was then analysed using GC-MS. The characteristic volatiles obtained from the two oils showed different compositions. *P. citrosum* oil comprised mainly of linalool, geraniol, m-camphorene, 2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha, geranylangelate while *R. officinalis* comprised mainly of α-Pinene, Eucalyptol, α-Terpinenol. Dose-response evaluations of these oils showed that *R. officinalis* oil (LD_{50} = 0.299 mg) was more repellent than that of *P. citrosum* (LD_{50} = 0.445 mg). The Disk diffusion method was used to carry out the antimicrobial activities of the *R. officinallis* and *P. citrosum*. The outcomes showed that the *P. citrosum* essential oils had antimicrobial activity against *Candida albicans, Escherichia coli* and *Bacillus subtilis* at a low concentration of 0.5 % v/v and that the activity was concentration dependent. *R. officinalis* essential oils, on the other hand, exhibited active antimicrobial properties against *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Pseudomonus aeruginosa*. The study showed that *P. citrosum* was more effective than gentamicin and nitrofuractoin drugs against *Staphylococcus aureus* at a higher concentration of 6 % v/v. Oil extracts from *R. officinalis* also illustrated similar trends and were similar to the positive controls against the tested microbes. These results provide scientific justification for traditional use of *R. officinalis* oil and *Pelargonium citrosum* essential oils for the control of housefly and other common insects in the household.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background of the study

Environmental pollution as a result of continued use of conventional pesticides is increasing as population increases in developing countries (Pavela, 2008). Botanical pesticides are better alternatives as they are environment friendly and biodegradable as opposed to chemical insecticides. They have repellent, insecticidal, antifeedant and insect growth regulator effects (Shooshtari *et al.,* 2013).

Musca domestica L. is a major problem to man in developing towns and industries (Abbas *et al.,* 2017). Housefly has the potential of transmitting more than a hundred pathogen causing diseases to human and animals such as ophthalmia, tuberculosis, anthrax, bacillary dysentery, typhoid and cholera (Rahuma *et al.,* 2005). The fly transmits pathogenic organism from, sewage, garbage, latrine and other filthy sources by mouth part through vomiting, faeces, and external body parts to foods (Zurek *et al.,* 2001). Control and management of housefly has relied upon using chemical pesticides for example organophosphates, pyrethroids and organochlorides. There are many drawbacks associated with synthetic pesticides including, of resistance development among insects (Ogbuewu *et al.,* 2011) environmental imbalances and destruction to non-target organism. Products in form of plant extracts and essential oil have sparked much attention as they have become effective alternatives to the artificial pesticides. Essential oils alters metabolic, biological and behaviour functions of the insect and with others known to affect insect reproduction, survival or even growth of the insects and vectors (Başer & Buchbauer, 2010).

The means by which essential oil compounds act is not completely understood, but provokes characteristics neurotoxic indications including hyperactivity, agitation, knock down and, paralysis (Onyambu *et al.,* 2015). Due to low rate of industrialization and increased poverty in rural areas, use of botanical pesticides has been practiced since ancient time (Hikal *et al.,*2017). The efficacy of these plants needs to be established or validated scientifically and their dosage established as well.

1.2 Literature review

1.2.1 Insect repellent compounds of plant origin

Housefly *Musca domestica* has shown different percentage repellence values when exposed to citral [Z, E]-3,7-dimethyl-2,6-octadienal] (1) and eugenol (4-allyl-2-methoxyphenol) (2) (Vartak *et al*., 1994). Pine essential oil (E.O), known for containing myrcene (3), p-cymene (4), γ-terpinene (5), and (±)-linalool (6) (Pitasawat *et al.*, 2003). A similar effect was observed when treated with citrus E.Os of which δ-limonene, α-pinene (7) and myrcene are the principal component (Oboh *et al.*, 2017), catnip, nepeta cataria (8) E.O (Scultz & Stoll, 2010). *Artemisia vulgaris* E. O. (Saeidi & Moharramipour, 2013).

Antifeedant activity against *M. domestica* was detected in the extract from *Plectranthus coesta* (Shubhangi *et al.*, 2014). Mordue & Nisbet (2000), reported that *M. domestica* flies were deterred by the bad taste of solution containing Neem *(Azadirachta indica)* essential oil. Figure 1.1 represents the various insect repellent compounds of plant origin.

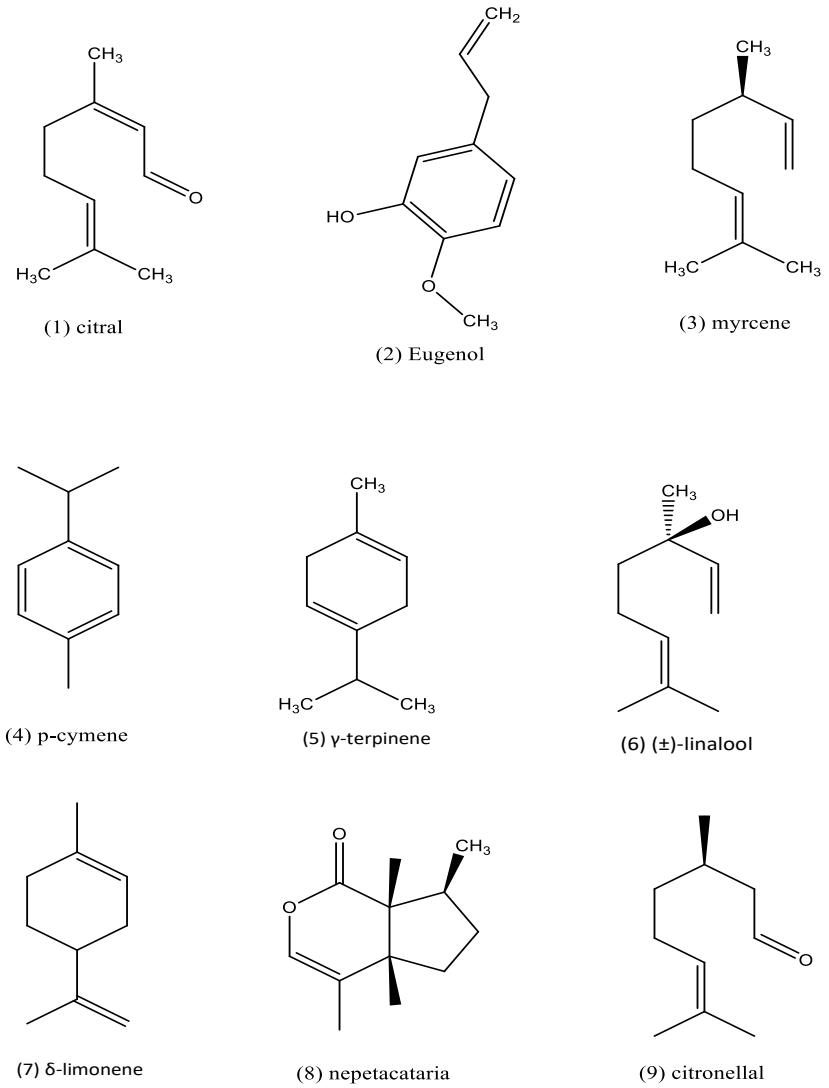


Figure 1.1: Insect repellent compounds of plant origin (Source: Jaramillo Ramirez *et al.*, 2012)

The essential oil of *Cymbopogon citrates* is regularly used as traditional medicine in many countries, it has been scattered in many tropical countries, such as Brazil and innate from Southeast Asia and India (Duarte *et al.,* 2015). There are numerous widespread usage for this plant, for example treatment for abdominal pains as well as having numerous pharmacological activities which include antifungal and anti-amoebic (Shubhangi *et al.,* 2014). Studies have found it to be useful in the control of insects like housefly (Herlekar *et al.,* 2014).

Lately, other studies discovered that essential oil in *C. citrates* and their key ingredients (1,8 cineole and citral) are vital pesticides and repellent against common

housefly, but the researches are fixated mostly in the immediate impacts after applying and not the long-time effect, (Jaramillo Ramirez *et al.*, 2012). Further, another study on effects of essential oils on housefly, insecticidal activities of the thirty-four essential oils, which had been extracted from plants were analysed and compared against *Musca domestica* where the essential oils in pogostemoncablin demonstrated to be the most effective at a lethal dose of 3µg/fly. After current application oil of *Nepeta cataria* and *Melliza officinalis* promising adulticidal activity on *M. domestica* with LD_{50} 24 and 23µg/insect respectively was shown (Pavela, 2008). These oils can also be investigated further for commercial preparation usage (Pavela, 2008). Another study on peel oil of navel orange and lemon grape fruit being tested for pesticidal qualities against *M. domestica* showed that oil from grape fruit peel and navel orange had more toxicity towards adult houseflies while oil from lemons was found to be more deadly to the larvae of houseflies (Shalaby & Allam 1998).

Presently bio-insecticide from plants origin has been progressively assessed in monitoring insects. Plants with bioactive organic chemicals in metabolite and plant extract forms usage locally in the preparation of herbal remedies cure diseases even before the initiation of conventional remedies in the developing countries (Ampofo, 2016). Alkaloids, flavonoids, limonoids, saponins, stubenes, sesquiterpenes, coumains and phenols of plant origin have been found to possess antifeedant effects and toxic growth regulating against a host of insect pests (Oyedokun *et al.*, 2011).

Citronellal (3,7-dimethyl-6-octenal) (9) and its natural compounds has been used to control the spread of flies and lice. It is commonly seen in aromatherapy candles, incense and in other commodities produced commercially. The *Cymbopogon nardus* L., a grass, which is native to Asia yields the Citronellal oil. More lately, tests have reinforced traditional stories about the repellent activities of the fruit of the osage orange tree or hedge apple, pomifera and muclura, catnip *Nepeta cataria* L. (Campbell, 2011). Much consideration has looked into one of the active constituents of the essential oil, nepetalactone. This is an iridoid monoterpenoid that looks as two (or more) isomers in catnip mainly as E,Z-neplatactone and Z,E-nepetalactone. In the study by Campbell (2011) it was found that both of the isomers repelled *Blattela germanica*, a German cockroach species, where E,Z-nepetalactone, even at a lower concentration was found to be more repellent.

Some of the repellent agents use unstable pyrethroid compounds, these products are a subsection of synthetic and artificial pyrethroids that change into a gaseous state easily. These compounds are distributed as spatial repellents rather than traditional adulticides. The tools are thought to form a vector-free zone using a single product, which can protect many people after applying it once (Salas, 2008). Repellence has been studied occasionally in unstable pyrethroids which include prallethrin, monofluthrin and transfluthirn (Debboun *et al.*, 2014).

1.2.2 Insects repellent plants to be investigated.

Based on insect repellent plants and literature information, the following plants (*Pelargonium citrosum* and *Rosmarinus officinalis* (Labiatae) were selected for this study.

1.2.2.1 *Pelargonium citrosum*

Pelargonium citrosum is a perennial sub-shrub with fragrant leaves that are reminiscent citronella. It is marketed as mosquito plant in Canada and the United States. Analysis of chemical components by the researchers discovered that combined essential oils from field grown citrosa and fresh greenhouse have, 10.4% citronellol, 6.8% linalool, 8.9% isomenthone, and 35.4±6.2% geraniol. The efficiency of the citrosa plant as a repellent agent on field populations of spring *Aedes* spp mosquitoes was looked into and compared to the formulation of 75% DEET (N,N-diethyl-*m*-toluamide). DEET showed more than ninety percent reduction of mosquitoes, biting subjects for up to eight hours after treatment. Figures 1.2 and 1.3 shows the major compounds found in *P. citrosum* and the structure of N,N-diethyl-*m*-toluamide (DEET), respectively.

Plate 1.1: *Pelargonium citrosum* leaves (a) and flowers (b) (Cooke, 2010)

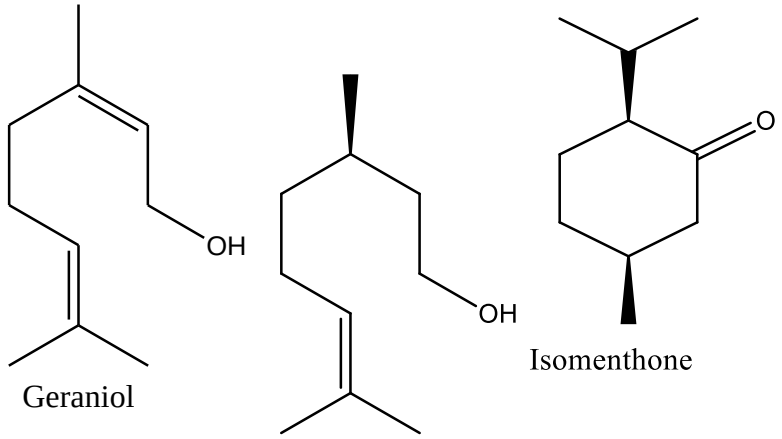


Figure 1.2: *P. citrosum* major compounds

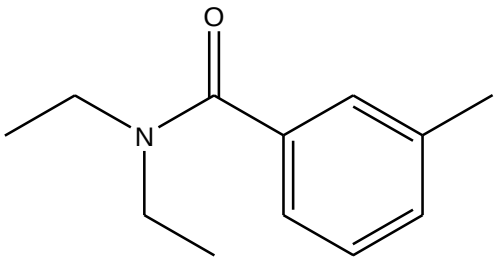


Figure 1.3: N, N-diethyl-m-toluamide (DEET)

1.2.2.2 *Rosmarinus officinalis*

Rosemary *Rosmarinus officinalis* is an evergreen woody persistent herb, with pink, white, blue or purple flowers and needle-like leaves. It is a native herb from Mediterranean and Asian regions. It is in the mint family (lamiaceal) which consists of many herbs. The upright forms can reach 1.5m (5ft) tall. After cultivation the flowing apices, twigs and leaves are extracted from the herb for use. Rosemary has been used for decorations in gardens where it may contain control of pests' capabilities. The leaves from the herb are used to flavour food such as roast meat and stubbing.

Rosmarinus officinalis holds a number of phytochemicals such as carnosol, camphor, ursolic acid, rosmarinic, betulinic, caffeic, and carnosic acids (Vallverdú-Queralt *et al.*, 2014). In folk medicine essentials oil and extracts from leaves and flowers are used with the belief they can be used to manage various ailments.(Vallverdú-Queralt *et al.*, 2014). In the middle age rosemary was associated with bridal ceremony then from this association, *R. officinalis* was thought to be a charm. It has long had a common reputation for enhancing memory (Vallverdú-Queralt *et al.*, 2014). Figures 1.4 and 1.5 represent the two major ant oxidative compounds found in *R. officinalis* and the other compounds that can be extracted from *R. officinalis* respectively.

Plate 1.2: Rosmarinus officinalis leaves (a) and flowers (b) source: (Torki et al., 2018)

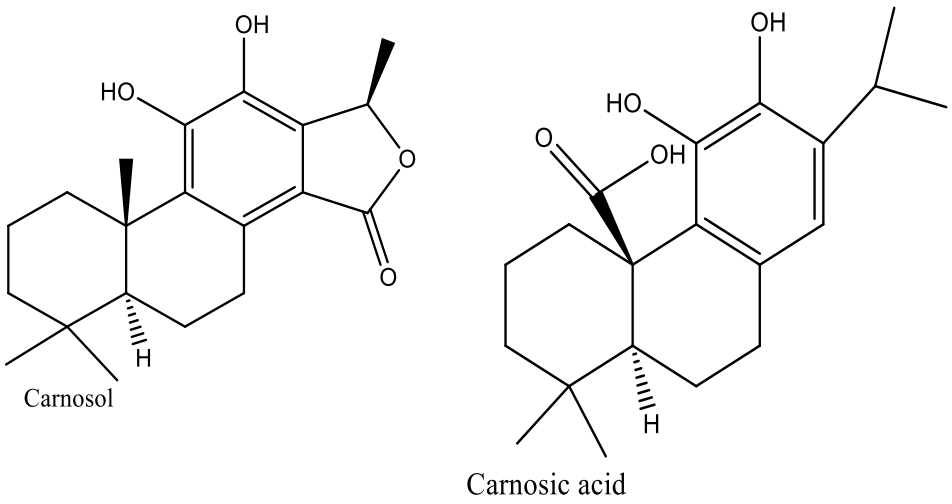


Figure 1.4: Two major antioxidative compounds from *R. officinalis*

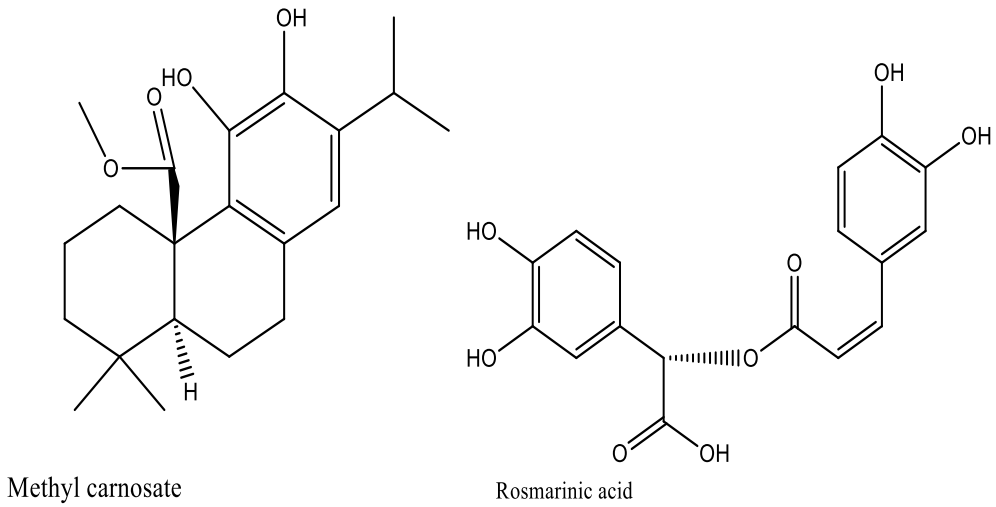


Figure 1.5: Other compounds from *R. officinalis*

1.2.3 Mechanism of action of phytochemicals

The antimicrobial compounds from plants prevent the growth of bacteria by using different techniques than those currently being used. This may have a critical clinical value in management of microbial strains that are resistant. They may interfere with the phospholipid bilayer of the cell membrane, which has a significance value of permeability, increasing loss of cellular constituents and restricting some metabolic processes and modulate gene expression by destruction or inactivation of genetic material. They also cause harm to enzymes dealing with synthesis of structural components and the production of cellular energy (Egamberdieva *et al.*, 2015)

Phenolic compounds are some of the most varied groups of secondary metabolites seen commonly in plants, which are edible. They are found in flowers as well as tea, wine, propolis and honey and in various stems, seeds, nuts vegetables and fruits. These compounds make-up common ingredients of the human diet. In the wild, these compounds provide resistance from predators and pathogens while protecting crops from pre-harvest seed germination and disease. In addition to this, they are involved in plant growth and reproduction (D Charles 2015).

There exist different types of polyphenols known as flavonoids, lignin and tannins. Flavonoids occur common and are found in many forms of vegetation consumed by humans. Dietary flavonoids are sought after for their beneficial properties in maintaining human health. Flavonoids are metal chelators, free radical scavengers and potent antioxidants; as they hinder lipid peroxidation and display various physiological activities, which include antimicrobial properties anti-arthritic, anti-hypersensitive, anti-carcinogenic, and anti-allergic and anti-inflammatory. Human ingestation of phenol-rich vegetables, fruits and beverages, has frequently been linked to cardiovascular disease risk reduction in epidemiological studies (Eva *et al.*, 2016.)

Polyphenols can be extracted by using a solvent like formic acid, methanol/formic acid, methanol, water, and hot water. Therefore, the total amounts of polyphenol detected from the same plant and their corresponding antimicrobial and antioxidant properties may vary widely, and this depends on the external conditions. Previous research has shown that phenolic compounds have antimicrobial action, which is connected to inactivation of cellular enzymes depending on the substance's rate of penetration into the plant cell or changes in membrane permeability.

Terpenoids and essential oils are also plant-derived antimicrobials. The oils are secondary metabolites with high-enriched compounds, which are based on the structure of isoprene. They are called terpenes. Compounds with additional oxygen elements are termed as terpenoids. These terpenes or terpenoids tend to work against protozoa, viruses and bacteria. Their mechanism of action is not fully known but speculated that it involves disruption of the membrane by compounds which are lipophilic (Du Fall & Solomon, 2011).

1.2.4 Microbial pathogens

1.2.4.1 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive bacterium belonging to the Staphylococcaceae family. It is a facultative anaerobic frequently found on the human skin and respiratory track. It is a non-spore and non-motile which forms bacteria that seen in grape-like clusters reproducing asexually by binary fusion and with large, yellow colonies (Sully *et al.,* 2014). It's a common cause of skin infections such as pimples, boils, and causes food poisoning. Infection by *S. aureus* can cause life-threatening infections like pneumonia and meningitis. *Staphylococcus aureus* are highly resistant to several conventional antibiotics thus limiting therapeutic options.

1.2.4.2 *Bacillus subtilis*

Bacillus subtilis is a common gram-positive eubacterial and considered the best-studied gram-positive bacterium and a model organism in the research of cell differentiation and bacterial chromosome replication. The word bacillus denotes the shape of the bacteria (rod-shaped) and subtilis denotes to be slender or slim. *B. subtilis* is commonly used as a probiotic preparation in the prevention and treatment of intestinal based disorders. It has been used in alternative medicine to produce antibiotics and fungicides for humans (Wayne *et al.,* 2000)

When subjected to stress, *B. subtilis* transforms into a spore. It then enters an inactive state, which allow the plant to withstand extreme environmental conditions. *B. subtilis* currently has the record for the longest survival on a NASA satellite, which is six years. *B. subtilis* is usually considered non-pathogenic although it has been implicated in cases related to food poisoning from poorly bakes products. The reason being that the spores are able to withstand the heat when food is being prepared. *B. subtilis* food poisoning is characterized by acute and rapid vomiting leading to diarrhoea (Yang *et al.,* 2011).

1.2.4.3 *Pseudomonas aeruginosa*

P. aeruginosa is a gram-negative, non-fermenting and motile organism, which belongs to the Pseudomonadaceae family. The organism is infectious and has a blue-green and

rod-shaped pigmented bacterium (Alhazmi, 2018). In 1960s, *P. aeruginosa* appeared as a critical human pathogen (Drake & Montie, 1988). By 1961, the organism's ability to cause both severe chronic and acute contaminations was documented (Alhazmi, 2018). Despite anti-pseudomonas activity of *P. aeruginosa,* it still is one of the most difficult and recalcitrant to treat.

The organism can tolerate temperatures as high as fifty degrees Celsius while being capable to grow under conditions which are anaerobic and aerobic (van Hartingsveldt & Stouthamer, 1974). Despite having large virulence factor numbers, *P. aeruginosa* is stubborn pathogen found in the hospital setting since it is unaffected by many antibacterial remedies. It can form resilient biofilms, both on the surfaces of medical equipment's and within the body (Moreau-Marquis *et al.*, 2008).

There are various clinical ailments linked to infections from *P. aeruginosa*. However, it is an opportunistic organism, infecting intravenous drug abusers, diabetic patients, long term catheters, neutropenic, leukemic transplants, CF and burns (van Hartingsveldt & Stouthamer 1974).

1.2.4.4 *Escherichia coli*

Escherichia coli is gram-negative rod-shaped bacilli that often resides in the large intestine and is and are naturally excreted from the body in faeces. *E. coli's* common site is the urinary track and causes ninety percent of simple Urinary Tract Infections (UTIs) (Woodward, 2015). Infections caused by *E. coli* bacteria can cause septic shock, which manifests itself with hypothermia and hypertension or fever and hypertension. In extreme cases of the infection, it causes complications by leading to coma, acute respiratory distress syndrome, hepatic failure, uremia, and in extreme cases, death. It is a systemic reaction to lipopolysaccharides or endotoxin (cytokines) that happens within the bacteria that can cause death and circulated intravascular coagulation (Woodward, 2015).

1.2.4.5 *Candida albicans*

Candida albicans is a fungal pathogen, which is opportunistic and exists as a harmless commensal in the genitourinary and gastrointestinal areas in about seventy-five

percent of women and seventy percent in humans. (Watamoto *et al*., 2011). However, it turns out to be opportunistic pathogen in people with compromised immune systems or even in people in good health. Candidiasis is commonly used to refer to infections caused by the *C. albicans* fungal pathogen. Due to the improvement and advancement in healthcare systems worldwide, patients with compromised immune systems as well as the elderly has increased significantly. It has been observed that the Candida species constitutes one of the four common causes of cardiovascular and blood infections found in hospitals in the United States (Mokaddas & Khan 2007).

1.2.5 The house fly *(Musca domestica* L.*)*

1.2.5.1 House fly life cycle

The most momentous features of the common housefly are its active productiveness. This productiveness is promoted by common supply of prerequisites enabling rapid larvae growth and nutrition (Scott *et al.*, 2014). Egg-laying female house flies get attracted to conducive areas for laying their eggs with decomposing organic matter being the most common of places (Deonier, 1938). In a span of three to four days, the female housefly lays up to five hundred eggs, which are in lots of between seventy-five and one hundred and twenty eggs placed in crevices or cracks. After breeding, the flies lay eggs on decaying matter, which should be less than seventy-two hours in age, which makes it a perfect environment since it is not too dry or too wet to hinder the eggs' hatching. (Miller & Thatcher, 1974). A full metamorphosis is followed in the fly's lifecycle. This is a four-step process, which starts with mating and laying of eggs. The second stage is the larvae stage. In the third step in the metamorphosis, the larvae changes into the pupae which transform into adult flies in the last stage (Chown & Gaston, 2010).

House fly eggs are creamy white in colour and one to two millimetres long with a banana shape and hatch between six and twelve hours (Miller *et al.,* 1974). Different weather conditions may delay this hatching process up to eight days with cold weather prolonging the hatching period (Axtell & Arends, 1990). To start the hatching, the dorsal concave of the egg detaches itself and lifts up. Temperatures that are below fifteen degrees and above forty are not favourable for the survival of the egg. After

hatching, the larvae feed on material which has decayed found in the environment (Miller *et al.,* 1974).

This larval stage marks the birth of these houseflies, which then changes into the pupa and then adult form. These larvae have no legs developing in instars, the three stages of larvae development (Hussein *et al.,* 2017)

In the beginning of the larvae developmental stage, the maggots are one to one point five millimetres in length. These maggots have bodies divided into eleven segments creamy white in colour. Furthermore, they have spindle shaped bodies and small pointed heads (Scott *et al.,* 2014). The small heads carry sets of mouth hooks, black pincer like structures, that are positioned underneath the heads (Hussein *et al.,* 2017). These worms reach lengths of between ten to twenty millimetres (Hafez, 2009). In weather conditions that are warm, this larvae stage can be shortened to three days. However, weather conditions that are unfavourable and cold, stage will take up to eight weeks before maturity is reached (Freund, 1968). The larvae move to drier and cooler areas right before the onset of the pupation stage.

The housefly pupae are immobile unlike pupae of other *Diptera* species which can move such as in mosquito pupae (Scott *et al.,* 2014). In this stage, the pupae changes to its adult form. Normally, the maggots will feed continuously until they are ready for the next stage, where they settle in a single area until the adult form is reached. Inside the pupae's hard shell, imaginal discs are formed to aid in the development of broken larvae tissue into adult fly organs such as the thorax and the abdomen. After the development is finished, the adult fly frees itself from the cocoon using a structure located on its head (Miller *et al.,* 1974).

The metamorphosis of a housefly depending with warm temperature can be as short seven to ten days. In places where there are winter conditions the metamorphosis to adult life span can last between four to twelve weeks (Hafez, 2009).

1.2.5.2 Diseases vectored by house flies

Flies are a difficult problem to control as they develop in massive numbers found under pens for chickens. The management and control of these numbers is crucial to human

comfort and health despite that these flies do not bite. Significant damage associated with houseflies is their irritation and the indirect damage from pathogens carried by these organisms. Pathogens such as nematodes, protozoa, fungi, bacteria and viruses are connected with houseflies. These pathogens get collected by flies from sewage, garbage and other filth course, which are moved on their mouth, Later, by vomiting, and defecating they contaminate animal food and external parts of humans (Lindsay & Scudder, 1956)

There is big concern on houseflies' movement from human and animal faeces to human food often eaten while still uncooked. In addition, some of these dangerous pathogens can be stored in the alimentary canal or in mouthparts or alimentary canal for some time before it is transmitted when flies regurgitate or defecate. Open latrines cause serious problems when located near slaughterhouses, hospitals and outdoor food markets. Pathogens normally carried by house flies are Chlamydia, Enterococcus, Escherichia, Campylobacter, Shigella and Salmonella (Eilenberg *et al*., 2015). These flies are associated with outbreaks of shigellosis and diarrhoea, but also are mixed up with the spread of parasitic worms, ophthalmia, anthrax, tuberculosis, typhoid fever and food poisoning (Capinera, 2008)

1.3 Statement of problem

The short-term conventional methods for the control of houseflies is the use of synthetic pesticides. Nonetheless, the prevalent and enormous application of chemical insecticides commonly produce risks in the development of pest resistance and leaving residue destructive to human and its environment (Ming & Daeschel, 1993). Thus, there is need to develop a novel, safe, effective & cheap bio-insecticide for housefly control leading to healthy population & improved economy.

1.4 Justification of the study

There is increased rate of pathogenic transmitted diseases by housefly, which include cholera, typhoid, conjunctivitis, dysentery, gastroenteritis, salmonellosis & tuberculosis. The use of non-biodegradable chemical pesticides leads to deleterious effects on beneficial insects and humans, development of tolerance, excessive pesticide residues, soil, air, and water pollution. Botanical pesticides on the other hand

have smooth degradation pathways in nature after application and there is less pollution to the environment; as the botanical insecticides have many insecticidal ingredients with special modes of action, it is difficult for the pests to develop pesticide resistance. They generally have features of strong selectivity, low toxicity to human and livestock and relatively low development and use costs. However, local community uses few insect repellent plants. Their effectiveness and dosage remain scientifically unestablished. There is therefore a need to evaluate the repellency of oil extracted from the leaves of *Pelargonium citrosum* and *Rosmarinus officinalis* against the housefly. Compounds in the selected bioactive plants are non-toxic, biodegradable and have high repellent efficiency.

1.5 Hypothesis

The compounds in the selected bioactive plants are non-toxic, biodegradable and have high repellent efficiency.

1.6 Objectives

1.6.1 General objective

To evaluate the housefly repellency and antimicrobial activities of *Pelargonium citrosum* and *Rosmarinus officinalis* and their formulated products.

1.6.2 Specific objectives

i. To extract and characterize compounds from the *P. citrosum and R. officinalis* L.

ii. To assess the antimicrobial and repellence activity of the plant extracts.

iii. To formulate insect repellent detergent from the active plants extracts.

iv. To formulate insect repellent paint from the complexed active plants extracts.

v. To test the activity and stability of the insect repellent products at different temperatures.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Sampling

2.2 Plant materials

The leaves of *Pelargonium citrosum* and *Rosmarinus officinalis* were collected from Githunguri in Kiambu County based on their reported ethnobotanical information as insect repellents. Githunguri is in the Coordinates: 1.0586°S 36.7779°E. A taxonomist was engaged in the field for the identification of the plants and voucher specimens of the plants collected were deposited at the East African Herbarium in the National Museum of Kenya. Figure 2.1 represents the map of Githunguri, Kiambu County.

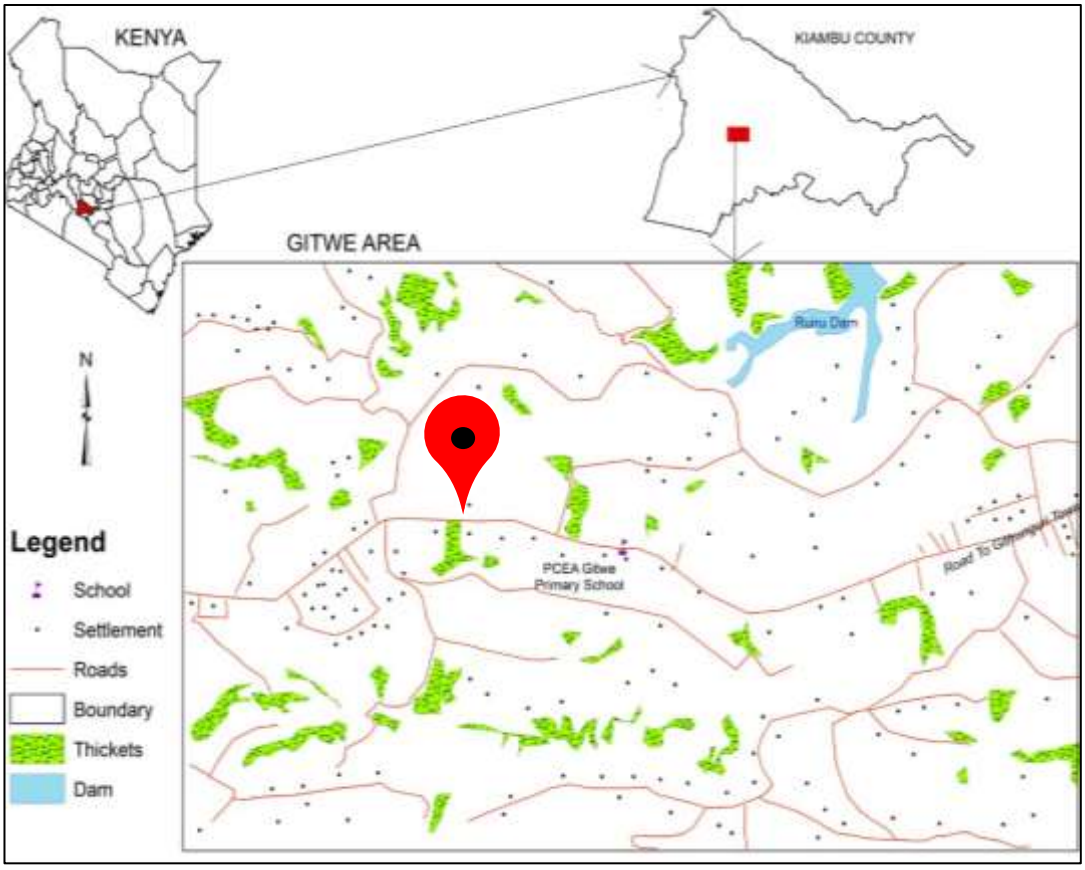


Figure 2.1: Map of Githunguri Kiambu County

2.3 Insects

Two weeks old houseflies were obtained from International Centre of Insect Physiological and Ecology (ICIPE) in Kenya.

2.4 Extraction

10kg of *Pelargonium citrosum* and 5 kg *Rosmarinus officinalis* was collected. The fresh plant materials were washed with running tap water and divided into smaller pieces to increase surface area. After washing, the samples were air dried at room temperature then pulverized with an in-house mechanical blender. Extraction of essential oils from the ground plant samples were done through hydro-distillation using Clevenger apparatus. The condensed extract (oil) was collected in n-hexane and filtered using Whatman® grade 1 filter paper, which contained anhydrous Sodium Sulphate to remove any amounts of water. The removal of Hexane was done by distillation at sixty degrees Celsius (^{o}C) using 'Contes' Short Path distillation. The collected oil was weighed into smaller amber covered vials and stored at 4^{o} C ready for repellency tests.

2.5 GC-MS analysis of the repellent oil

The bioactive oil was analyzed using gas chromatography coupled with a mass spectrometer detector (GC-MS).The sample (oil) was diluted with dichloromethane, and an aliquot of 0.5 µL injected into GC-MS under the following conditions: Temperature ramp: 60^{o} C (2 min); at 8^{o} C/min to 250^{o} C (3 min); Column: DB-XLB (standard non-polar); Injection mode: Split, 200:1; Mass range: 40 – 470 u; Source temperature: 200^{o} C; Interface temperature: 250^{o} C; Carrier gas: He, 99.999% purity; Flow rate: 1 ml/min; and Pressure of 8 kPa. The fragmentation patterns of MS-chromatograms was proposed.

2.6 Repellency bioassays

2.6.1 Short term repellency bioassays

The behavioral response of the insects to the repellent test oil was determined using adult houseflies (*Musca domestica* L.). This provides a measure of contact irritancy

that is more appropriate for using to control pests who invade structures. Test solutions of various concentrations of extract oil and *N,N*-diethyl-*m*-toluamide (DEET) were prepared consisting of 5.0, 3.0, 1.0, 0.5, 0.3 and 0.1 % (v/v) in acetone (Schultz *et al.*, 2004). DEET was used as the positive control and acetone as the negative control.

One millilitre of test solution or solvent was applied to one-half of 12.5cm-diameter round filter paper with an area of 61cm^2 and then left to dry. A solvent-only half-piece of filter paper was put in the remaining one-half of the 15cm plastic Petri dish after the solvent had evaporated. At the time of commencement, a single insect was introduced through a hole on the center of the petri dish lid and then shielded using a masking tape. The time the insect spent on control and treated filter paper out of 5 min (300 s) was recorded using two stopwatches. Ten replications for each concentration was performed, and the percentage repellence calculated using the formula: [(Time on Untreated – Time on Treated)/300] × 100%.

A random-number table was used to determine the location of the treated filter paper in each replicate.

2.6.2 Extended repellency bioassay

Test solutions were made comprising of 5, 3, 1, 0.5, 0.3 and 0.1% concentrations by volume in acetone for the steam distillate oil and DEET. One-half of a 12.5cm filter paper with surface area of 61 cm^2 was treated with 1 ml of test solution and left to dry (30s) before being put in a 15cm petri dish. For control, the other half of the filter paper was treated with solvent (acetone) only. One adult housefly was placed in each petri dish and enclosed by a mesh, to eliminate any fumigation effects and to permit the spread of the repellent under conducive laboratory conditions. The position of the insect (presence on the untreated or treated filter paper) was logged in at seven time-points after commencement: 15 min, 30 min, 1 h, 2h, 4h, 6h, and 24h. Each specific preparation was replicated ten times.

2.6.3 Probit analysis

Dose-response data were put to a probit analysis and simple regression by using the percent repellence values got from the experiments that had been replicated and the development of a regression model was done based on the following equation;

$$\text{Probit}[\Pi(\text{dose } 1)] = \beta_0 + \beta_1 x + \epsilon,$$

Where β_0 the coefficient of the model representing y-intercept is, β_1 is the coefficient of the model representing dose1, *x* is the various concentrations of essential oils. Dose1 is the Log_{10} (dose), ϵ is the error term (residual term) representing the difference between the actual observed value and that predicted by the model variable, *x* is the dose of the essential oil and Π is the repellency probability.

2.7 Formulation and test of housefly repellent products

2.7.1 Preparation of housefly repellent detergent

Considering the probit analysis results obtained and the calculated values of LC_{50} and LC_{75}, the housefly repellent detergent was prepared by mixing the plant extract with 5% glycerine and the extract was then incorporated into the pre-prepared detergent. Glycerine was used as an emulsifier in this procedure. The total active components in the detergent was two percent (v/v) and all the ingredients were mixed using a mechanical stirrer for one hour. Lastly, the mixture was transferred into plastic vessels and the repellency test performed on the product.

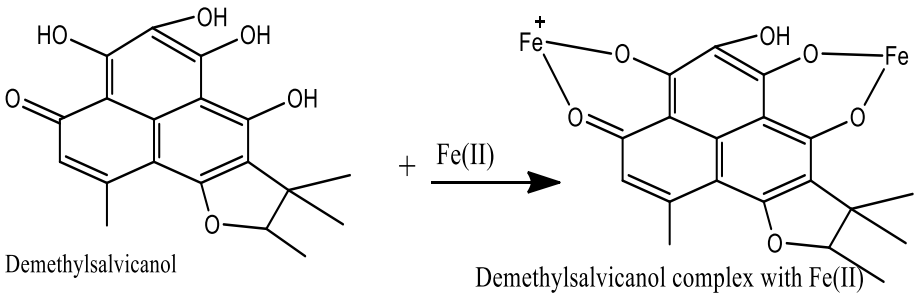
Table 2.1: Formulation of repellent detergent with 2% extract of *P. citrosum* and *R. officinalis*

Percentage (in Kg) %	Raw material name
0.250	Sodium hydroxide (NaOH)
0.250	Simet (Sodium metasilicate)
0.250	Carboxyl methyl cellulose (C.M.C)
5.000	Industrial salt
5.000	Ungarol (Sodium Laureth Sulphate)
2.500	Ufacid (Linear Alkyl Benzene Sulphonic Acid)
10.000	20% plant extract + 10% glycerine + 70% water
76.75	Water
Total = 100Kg	

Sg (specific gravity) =1.00, pH = 8.5, viscosity = 90kU

2.7.2 Preparation of the housefly repellent paint

Considering the probit analysis results obtained and the calculated values of LC_{50} and LC_{75} from the iron complexed plant extract, the housefly repellent paint was prepared by dissolving the Iron complexed plant extract in ethyl acetate. Example of complexed compound with iron (ii) salt.



Demethylsalvicanol

Demethylsalvicanol complex with Fe(II)

Glycerine 5%, was then added and then incorporated into the pre-prepared paint. Glycerine was used as an emulsifier in this procedure. The total active ingredients in the paint was 1% (v/v) and a mechanical stirrer was used to stir the compounds for one hour. Lastly, the mixture was poured into plastic containers and the repellency test performed on the product.

Table 2.2: Formulation of repellent paint with 1% complexed extract of *P. citrosum* and *R. officinalis*

Percentage (in Kg) %	Raw material name
16.000	Water (for dispersion of the pigments)
0.400	Acticide Ep paste
0.400	Mergal k14
0.100	Sodium benzoate
0.200	Calgon
0.200	Dispex N40
0.300	Tergitol /NPG
0.300	Troykyd D720
0.500	Bermacol 481 (tylose)
14.000	TiO_2R
5.000	China clay kaosal
29.000	Whiting 10
5.000	Talc.300mesh
0.300	Ammonia 25%
18.000	SA/VA (Emulsion resin)
5.000	5% complexed extract + 5% glycerine + 90% water
4.350	Water (for adjusting viscosity)
1.000	Texanal/ucar filner
Total = 100Kg	

Sg (specific gravity) =1.5, viscosity= 135-140kU, pH= 8.5

2.7.3 Repellency effects of detergent and paint made from plant extracts

The laboratory test was done by dissolving the repellent product in distilled water then applied on a bench with a rotten meat on top. Adult houseflies were released and the time of contact between the housefly and the bench recorded for eight hours. Another bench was cleaned using the normal detergent and used as control for this experiment.

The field analysis was conducted in three restaurants around Juja area in Kiambu county. The housefly repellent detergent was used to clean the floor, walls and tables of one room and another room cleaned using the normal detergent. An observation was done for eight hours in both the rooms for the presence of the housefly. The housefly repellent paint was also tested by applying on the walls and observation was done for four months for the presence of the housefly. The normal emulsion paint was used as control for this experiment.

2.8 Antimicrobial activity of plant extracts *R. officinalis* and *P. citrosum*

2.8.1 Microorganisms

The following microorganisms were used: *Pseudomonus aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans***.**

2.8.2 Antimicrobial assay

The microorganisms used in this work were: gram-negative bacteria (*Pseudomonus aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25992), gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*) and fungi (*Candida albicans* ATC 10231). Nutrient Agar plates were used for the yeast and bacteria cultures. Seeding the culture medium under germ-free environments was made with a decontaminated platinum loop followed by incubation.

Disk diffusion method was used to investigate the antimicrobial activity. Different concentrations of the effective plant extracts (0.1, 0.2, 0.5, 2.0, 3.0, 4.0, 5.0 and 6.0% v/v) were organized separately by dissolving in Dimethyl sulfoxide (DMSO), decontaminated through the use of Millipore filters and then loaded their specific amounts over filter paper discs that are sterile (7 mm in diameter). Mueller-Hilton agar was poured into sterilized Petri dishes and seeded with bacterial suspensions of pathogenic strains. The loaded filter paper discs with distinct concentrations of the plant extract were put on the top of the Mueller-Hilton agar plates. These plates were then put in a refrigerator, cooled to five degrees Celsius (º C) for two hours, and then incubated at thirty-seven degrees for twenty-four hours. This allows diffusion between agar and the sample. Following this process, growth zones with inhibition characteristics were measured using a Vernier calliper and logged against plant extracts with effective concentrations.

The antimicrobial properties were tested in comparison with reference to substances that are antibacterial (or positive controls), used as microcapsules for antibiograms: Norfloxacin (NX), Ofloxacin (OF), Ceftriaxone (CTR), Sulphamethoxazole (SX), Amoxylclar (AMC), Nitrofuractoin (NIT), Nalidixic acid (NA) and Gentamicin (GEN)

2.9 Complexing of plant active extracts

2.9.1 Complexing using Iron

The synthesis of Iron complex was done by adding 0.01 M Ferrous Chloride and the *R. officinalis* extract in 2:5 proportion in a clean sterilized flask. The reacting mixture was placed in a magnetic hot stirrer at 50-60^{o}C for an hour. The change of colour from pale green to brown signified oxidation of the ions. The key influences, pH was kept between 7.5 to 8.5 for three to four hours, with the temperature between 50-60^{0}C throughout the experiment. The solution was put in a centrifuge with 350 rpm for ten minutes and the supernatant was put away. The resulting pellet was then washed with distilled water and was centrifuged again to remove any impurities. Complexing of *P. citrosum* extract was also done in the same way.

2.9.2 Complexing using Manganese salt

An aqueous solution of Manganese acetate (1ml) was made for the complexing using manganese salt. The whole process of the reaction used double distilled water. Temperature and pH were constantly monitored to achieve more accurate results. Reduction of manganese ions was done by adding fresh manganese solution (10 ml) was mixed with 10ml plants extracts in a beaker. The mixture was stirred constantly to allow complete metal ion oxidation. The resulting mixture was put in the magnetic hot stirrer at 50-60^{o}C for an hour. Metal ion oxidation was signified with changing of colour from pale green top pale yellow. Then freshly prepared plant extract (*R. officinalis* and *P. citrosum* separately) was added and the above solution mixture for stability with stirring continuing for an hour. Color of the solution changed from yellow to yellowish brown gradually with a permanent reddish-brown color in the end to indicate the complex has finished stabilizing. The main factors; the temperature was at 50-60^{0}C and pH maintained at 7.5 to 8.5 for 3 to 4 hours throughout the experiment. The solution was placed in a centrifuge and further washing to get a complex with high purity values. The supernatant was transferred and kept in oven until it was completely dry.

2.10 Characterization of the complexes

2.10.1 UV-Visible spectra analysis

The UV-Visible absorption spectra of the complexes were measured on a Shimadzu UV-Vis 530A spectrophotometer in the range of 200-800nm. The absorption peaks of the complexes and those of the pure extracts were compared and the shift in the absorption peaks were recorded (Iniya Udhaya *et al.*,2015).

2.10.2 FT-IR analysis

FT-IR spectrum in the range 4000 to 400cm^{-1} at a resolution of 4cm^{-1} using Perkin-Elmer spectrometer was used to detect the functional groups in the complex. The sample was mixed with KBr. Thin sample discs were prepared by pressing with the disc-preparing machine and placed in Fourier Transform Infra-Red (FTIR) for the analysis of the complex (John & Shri, 2014).

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 GC-MS analysis of the repellent oil

Figure 3.1 shows the GC-MS chromatogram of oils extracted from *R. officinalis* and figure 3.2 the chromatogram of oils extracted from *P. citrosium*. The tables 3.1 and 3.2 shows the class of compound and the retention time of the extracted compounds respectively.

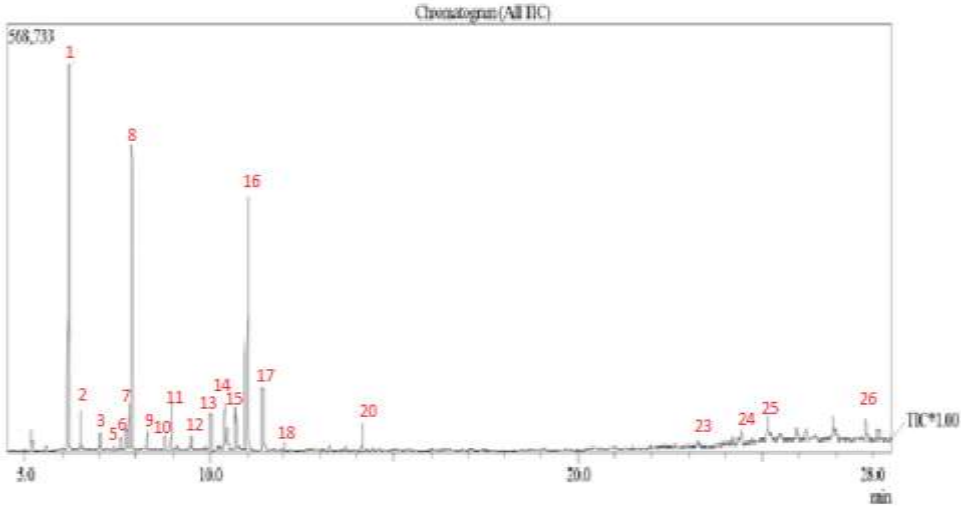


Figure 3.1: GC-MS profile of R. officinalis

1=α-Pinene, 2=Camphene, 3=β-pinene, 4=β-myrcene, 5= α-terpinene, 6=β-cymene, 7= D-Limonene, 8=Eucalyptol, 9=γ-terpiinene 10= Isoterpinolene, 11=linalools, 12=2-Pinen-7-one, 13= Camphor, 14=Borneol, 15=Terpinen-4-ol, 16=α-Terpinenol, 17=2-Pinen-4-one, 18=Cis-Geraniol, 19=2-Camphanol acetate, 20=Geraniol acetate, 23=Ferruginol, 24=Isocarnosol, 25=α-pentyl-4-oxa-5, beta-androstane3-17-dione, 26= Demethylsalvicanol. The spectra are attached in appendix 1.

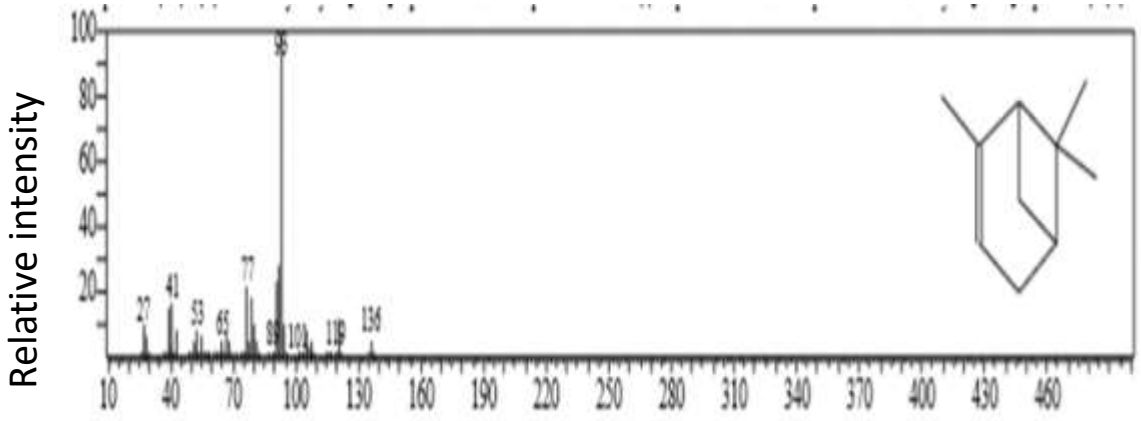


Figure 3.2: MS profiles of α-pipene from *R. officinalis* compounds

pipene MS-fragmentation

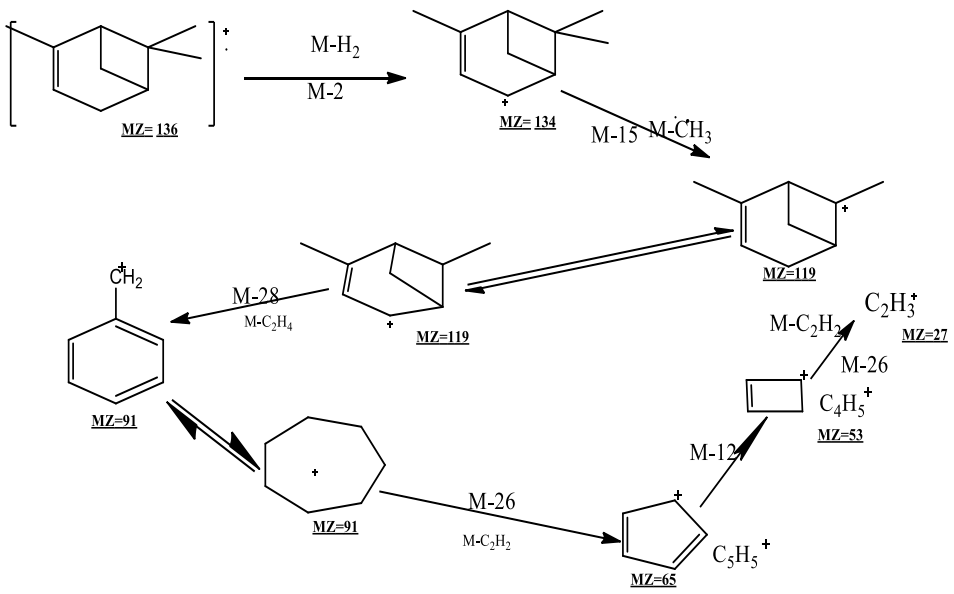


Figure 3.3: Fragmentation pattern of pinene compounds

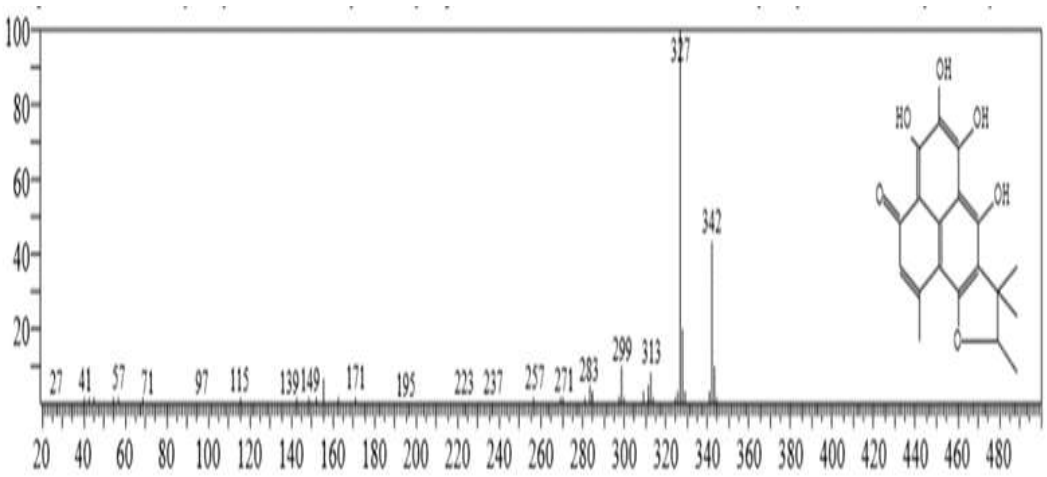


Figure 3.4: MS profiles of Demethylsavicanol from *R. officinalis*

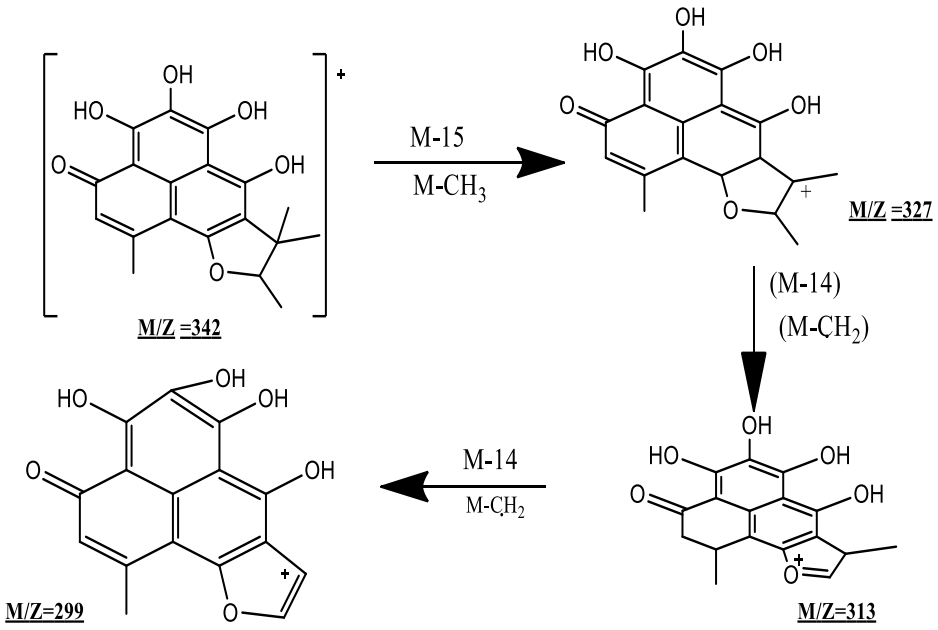


Figure 3.5: Fragmentation pattern of Demethylsalvicanol

Table 3.1: Retention time and the class of compound extracted compounds

No.	Compound	M/Z Ratio	Retention time	Peak height %	Class of compound
1	α-PInene	136	6.160	21.42	Monoterpene
2	Camphene	136	6.483	1.93	Monoterpene
3	β-pinene	136	6.97	0.71	Monoterpene
4	β-myrcene	136	7.025	0.72	Monoterpene
5	α-terpinene	136	7.595	0.44	Monoterpene
6	β-cymene	134	7.750	0.95	Monoterpene
7	D-Limonene	136	7.820	2.38	Monoterpene
8	Eucalyptol	154	7.915	19.28	Monoterpenoid
9	γ-terpiinene	136	8.300	1.13	Monoterpene
10	Isoterpinolene	136	8.775	0.59	Monoterpene
11	Linalools	154	8.960	2.60	Monoterpenoid
12	2-Pinen-7-one	150	9.500	0.82	Monoterpenoid
13	Camphor	152	10.015	2.11	Monoterpenoid
14	Borneol	154	10.400	2.79	Monoterpenoid
15	Terpinen-4-ol	154	10.470	1.21	Monoterpenoid
16	α-Terpinenol	154	10.710	18.14	Monoterpenoid
17	2-Pinen-4-one	150	11.000	4.12	Monoterpenoid
18	Cis-Geraniol	154	11.750	2.54	Terpenoid
19	2-Camphanol acetate	196	12.095	0.56	Terpenoid
20	Geraniol acetate	196	13.240	0.23	Terpenoid
23	Ferruginol	286	24.430	1.48	Terpenoid
24	Isocarnosol	330	25.190	2.81	Terpenoid
25	α-pentyl-4-oxa-5,beta-androstane3-17-dione	360	26.185	2.18	Steroid
26	Demethylsalvicanol	318	26.905	1.88	Phenol

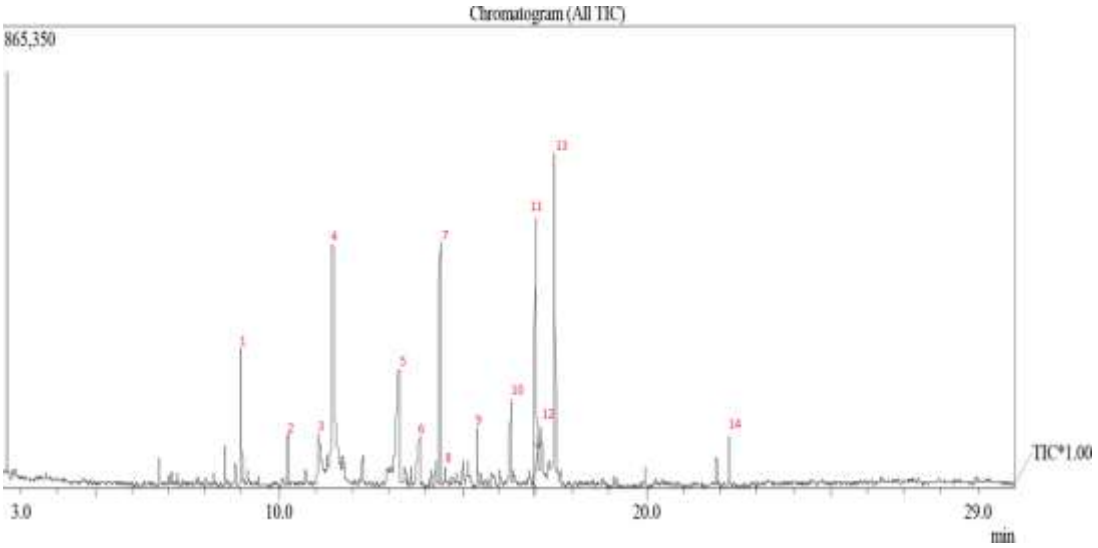


Figure 3.6: GC-MS profile of *P. citrosum*

1=linalool, 2=2-isopropyl-5-5methylcyclohexanone (p-menthone), 3=Trans-farnesol, 4=Geraniol, 5=Decanoic acid, 6=8-methyl-6-nonenoic acid, 7=m-Camphorene, 8=1S,2R,5S-Guia-6,9-diene, 9=Delta-cadinene, 10=2-phenylethyltiglate, 11=2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha, 12=5-Azulenemethanol, 13=Geranylangelate, 14=Geranylacetate. The spectra are attached in appendix 2.

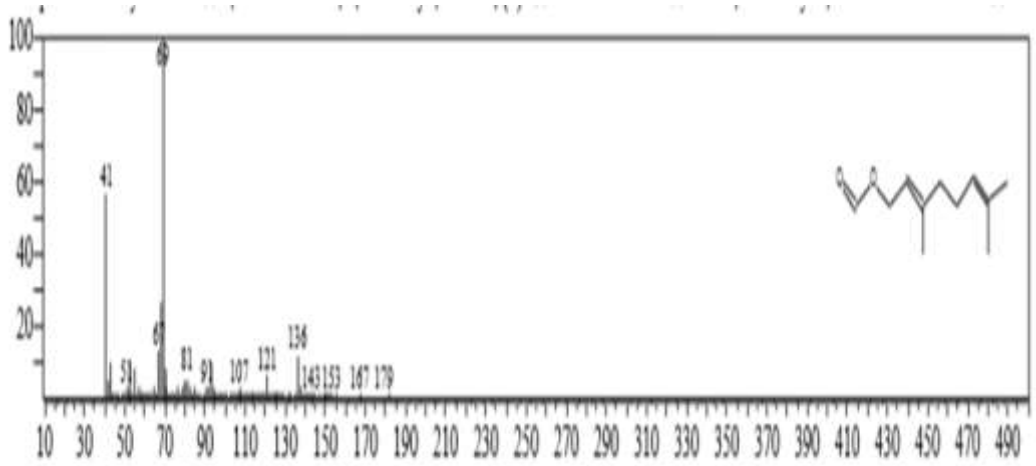


Figure 3.7: MS profiles of geraniol from *P. citrosum* compounds

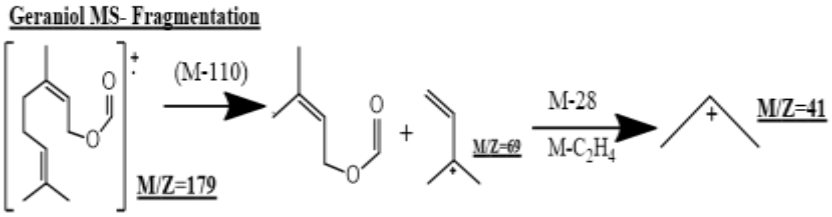


Figure 3.8: Fragmentation pattern of geraniol

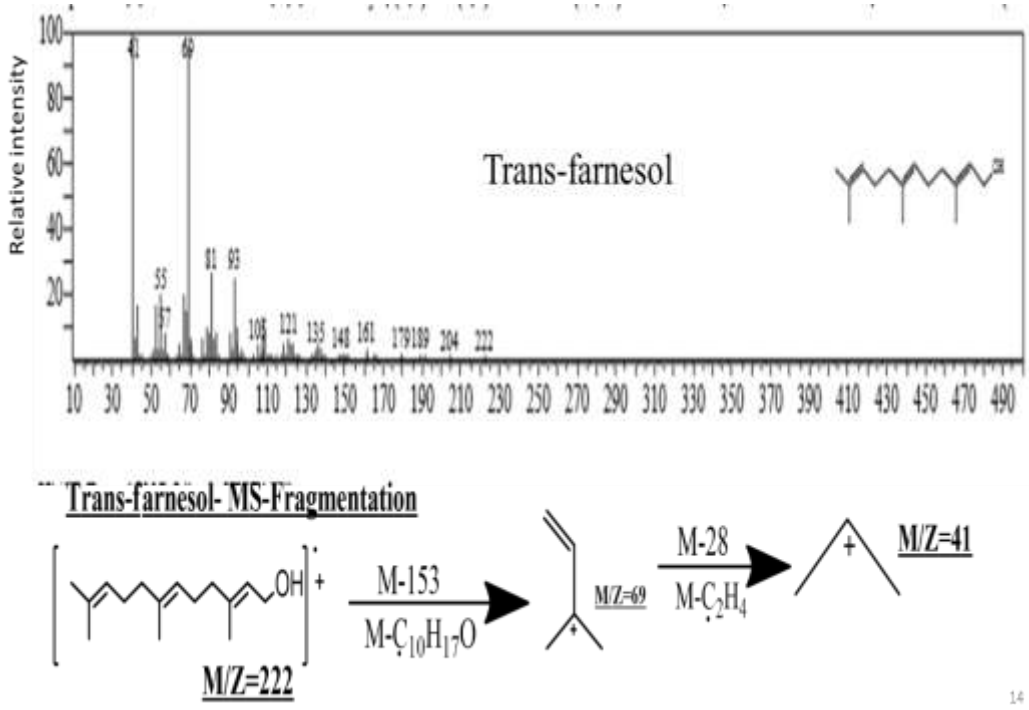


Figure 3.9: MS profiles of trans-tarnesol from *P. citrosum* compounds

Table 3.2: Retention time and the class of compound extracted compounds

No.	Compound	M/Z Ratio	Retention time	Peak height (%)	Class of compound
1	Linalool	154	8.975	6.57	Monoterpenoid
2	2-isopropyl-5-5methylcyclohexanone (p-menthone)	154	10.245	1.97	Monoterpenoid
3	Trans-farnesol	222	11.060	2.15	Terpenoid
4	Geraniol	154	11.470	16.36	Terpenoid
5	Decanoic acid	172	13.255	12.78	Fatty acid
6	8-methyl-6-nonenoic acid	170	13.830	5.17	Fatty acid
7	m-Camphorene	272	14.260	10.98	Diterpene
8	1S,2R,5S-Guia-6,9-diene	204	14.375	2.95	Sesquiterpene
9	Delta-cadinene	204	15.400	5.17	Sesquiterpene
10	2-phenylethyltiglate	204	16.29	5.58	Terpenoid
11	2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha	222	16.9	14.33	Sesquiterpene
12	5-Azulenemethanol	264	17.130	2.96	Terpenoid
13	Geranylangelate	167	17.505	13.9	Terpenoid
14	Geranylacetate	196	22.235	2.45	Terpenoid

GC-MS analysis of *R. officinalis* revealed various compounds. α-pinene, eucalyptol, α-terpinenol, 2-pinen-4-one and linalool for instance found in *R. officinalis* are toxic to eggs and larvae of insects (Scott *et al.*, 2014). Linalool, geraniol, capric acid, m-camphorene, delta-cadinene, 2-phenylethyltiglate and geranylangelate found in *P. citrosum* has demonstrated repellence against arthropod pests.

Essential oils affect basic behavioural, biochemical, and metabolic biochemical functions in arthropods. Essential oils are either absorbed, ingested or inhaled by these insects. The speedy actions against some pests is an indication of a neurotoxic mode of action and there is evidence which support the interference of GABA-gated chloride or neuromodulator octopamine (Campbell, 2011).

3.2 Repellent activity

Table 3.3 below represents the repellent activity of *R. officinalis* and *P. citrosum* while figure 3.3 shows the correlation between the repellent activities of the plant extracts and the controls

Table 3.3: Repellent activity of *R. officinalis* and *P. citrosum*

Percentage(v/v)	**Percentage (%) repellency**		
Concentration **(%v/v)**	*P. citrosum*	*R. officinalis*	DEET
5	87.9±2.0	80.7±7.5	Knock down
3	82.5±12.5	79.1±6.8	Knock down
2	78.6±6.5	73.9±3.4	96.9±1.4
1	49.0±3.8	62.3±7.3	85.2±2.6
0.5	36.1±2.6	59.7±3.8	80.2±3.0
0.3	25.4±3.2	48.4±5.3	74.0±2.0
0.2	19.4±2.3	39.5±2.1	65.3±2.5
0.1	16.9±1.6	32.9±1.3	43.2±5.8
0.05	10.2±0.8	28.3±1.8	42.7±0.6
0.025	2.5±0.4	26.1±0.2	39.2±1.2

Table 3.4: Test for correlation between the repellent activities of the *R. officinalis* and *P. citrosum* extracts

P. citrosum (X)	*R. officinalis (Y)*	X^2	Y^2	XY
88	81	7744	6561	7128
83	79	6889	6241	6557
79	74	6241	5476	5846
49	62	2401	3844	3038
36	60	1296	3600	2160
25	48	625	2304	1200
19	40	361	1600	760
17	33	289	1089	561
10	28	100	784	280
3	26	9	676	78
409	531	25955	32175	27608
40.9	53.1			
∑x=409	∑y=531	$\sum X^2$= 25955	$\sum Y^2$=32175	∑XY=27608
$\bar{X}$=40.9	$\bar{Y}$=53.1			

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[\,n\sum x^2 - (\sum x)^2\,][\,n\sum y^2 - (\sum y)^2\,]}}$$

$$\frac{10(27608)-(409)(531)}{\sqrt{(259550-167281)(321750-281961)}}$$

$$r \quad \frac{58901}{60591.18} = 0.9721$$

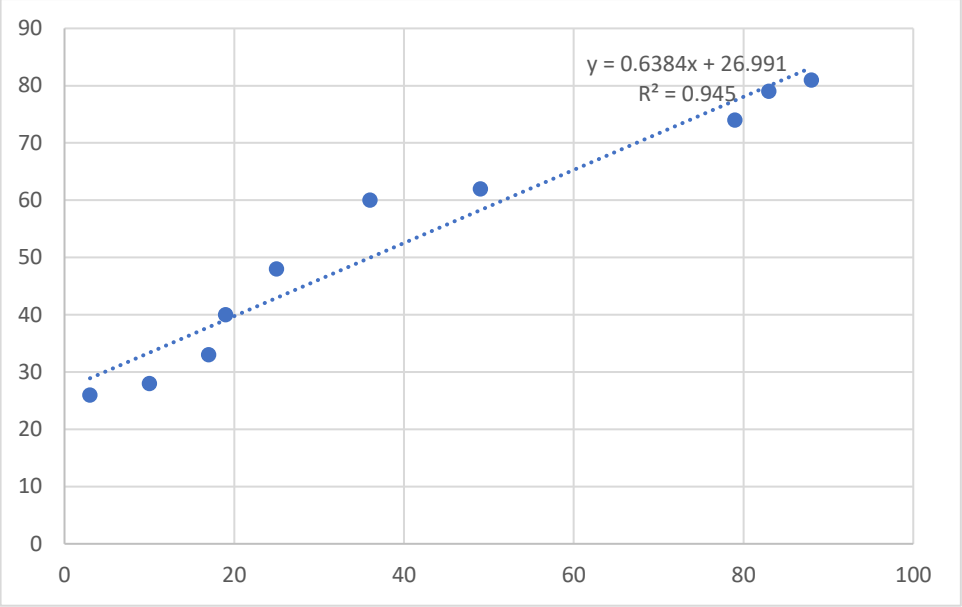


Figure 3.10: Correlation between the repellent activities of the R. officinalis(y) and P. citrosum(x) extracts

The conclusion is that a significant correlation does exist. The r values are closer to 1, i.e. as the straight-line relationship is strong.

T-test

$$t = \frac{(x_1 - x_2)}{\sqrt{\frac{(s_1)^2}{n_1} + \frac{(s_2)^2}{n_2}}}$$

$$t = \frac{53.1 - 40.9}{\sqrt{\frac{442.1}{10} + \frac{1025.2111}{10}}}$$

$$t = 1.0071609$$

t calculated is 1.0071609 while t table value is 1.734. t-calc < t-tab then the results are not significant at the 5% level hence the null hypothesis is accepted.

Pooled Standard Deviation: 27.0861

Pooled DF: 18

95% Confidence Interval for the Difference (-13.2488, 37.6488)

Test Statistic t= 1.0072

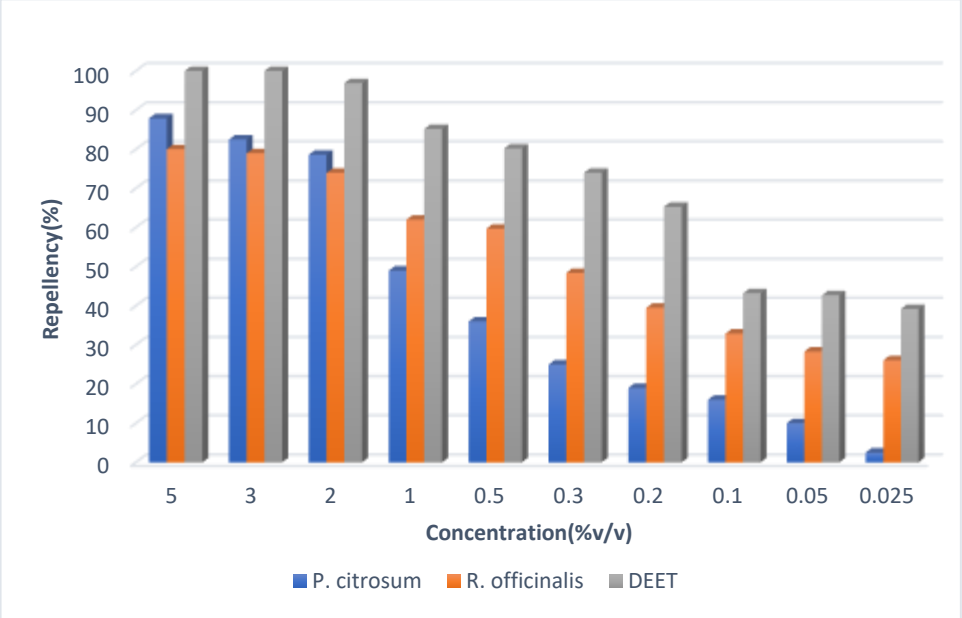


Figure 3.11: The correlation between the repellent activities of the plant extracts and the standard

The essential oil of *R. officinalis* was found to be considerably more effective in insect repellence than that of *P. citrosum* at low corresponding doses, however *P. citrosum* showed more repellence against the housefly at higher doses. In both the essential oils of *P. citrosum* and *R. officinalis,* there was substantial association between dose and repellence (Pearson Correlation, $\alpha = 0.01$).

Comprehensive repellence studies with *R. officinalis* and *P. citrosum* oils at ten doses established higher repellence properties of the former against *Musca domestica L.* Interestingly, its repellent effectiveness is relatable to that of commonly used repellent DEET at 0.1 mg dose (with essential oil of *R. officinalis* producing a repellent effect of 32.9 ± 1.3% compared with that of DEET, 43.2 ± 5.8%).

Relative to controls, 2% DEET provide > 90% repellence after 8 hours and >70% repellence for the two plant extracts in this study.

3.2.1 Probit analysis results

Lethal dose at 50% and 75% concentration is presented in table 3.5

Table 3.5: Lethal dose at 50% and 75% concentration

Sample	LD_{50} (mg)	LD_{75} (mg)
R. officinalis	0.299	2.487
P. citrosum	0.445	1.820

Key: LD_{50}-lethal dose at 50% repellency; LD_{75}- lethal dose at 75% repellency.

Development of model of bioassay data of the two essential oils allowed approximations of LD_{50} and LD_{75} (Table 3.4). *R. officinalis* was found to be more effective at LD_{50} compared to *P. citrosum*, this was however not the case at $LD_{75,}$ *P. citrosum* exhibited higher repellency at high concentration (LC_{75}). The high repellency of *R. officinalis* at lower concentration can be attributed to the easy mobility of the 26 compounds leading to more evaporation hence effective repellency. Higher concentrations of *R. officinalis* lead to more hydrogen bonding between the compounds hence reducing their ability to vaporize.

Previous work has been done on Thyme oil and have shown it to have repellent properties on the varroa mite (Salzer & Furia, 1977). Determination of larvicidal productivity for thymol was lowest doses LD_{50} 32.9 and 14.2mg/L for the third and fourth instars respectively of *Culex quinquefasciatus*. According to Pavela (2008c), oil form the clover leaf displayed decent repellency at a concentration of 0.005mg/cm^2 against *Culex pipiens.* From this, several essential oils presented insecticidal properties against various pests so that there is an increased interest in developing insecticides that are plant based.

From the data illustrated on plant instability, each species appears to have its own unique chemical structure with little likeness. In summary the results of this study, further reinforces the view that *R. officinalis* and *P. citrosum* are potential sources of

insect repellants. The results provide scientific justification for traditional use of raw products of these plants in controlling housefly.

3.3 Antimicrobial assay

The antimicrobial activity (zone of inhibition) of *Rosmarinus officinalis* extracts against *Candida albicans, Bacillus subtilis, Pseudomonus aeruginosa, Staphylococcus aureus,* and *Escherichia coli* is presented in table 3.6.

Table 3.6: Antimicrobial activity (zone of inhibition in mm) of *Rosmarinus officinalis* extracts

Conc (%v/v)	PA. Ave	EC. Ave	SA. Ave	BS. Ave	CA. Ave
6	13.0 ± 1.0	13.3 ± 1.5	10.7 ± 1.5	13.0 ± 1.0	11.7 ± 0.6
5	11.7 ± 1.2	11.7 ± 0.6	9.7 ± 0.6	11.0 ± 1.0	9.7 ± 1.2
4	11.7 ± 0.6	10.0 ±1.0	8.3 ±0.6	9.3 ±0.6	8.3 ±0.6
3	10.3 ± 0.6	9.7 ±1.2	7.3 ±0.6	8.7 ± 0.6	9.0 ±1.0
2	8.7 ± 0.6	8.7 ±0.6	7.0 ±0.0	7.7 ±0.6	7.3 ±0.6
0.5	7.3 ± 0.6	7.3 ±0.6	-	7.0 ±0.0	7.0 ±0.0
0.2	7.0 ±0.0	7.0 ±0.0	-	7.0 ±0.0	7.0 ±0.0
0.1	7.0±0.0	7.0±0.0	-	7.0±0.0	7.0±0.0
DMSO	7.0 ±0.0	7.0 ±0.0	-	7.0 ±0.0	7.0 ±0.0

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis, CA: Candida albicans* and DMSO: Dimethyl sulfoxide

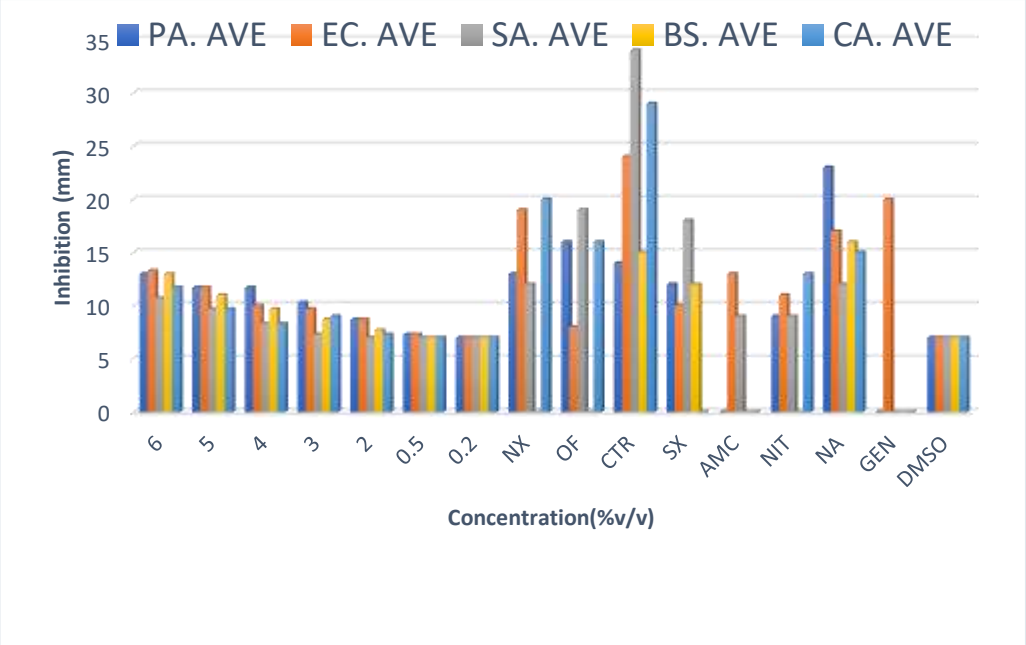


Figure 3.12: The antimicrobial activity of Rosmarinus officinalis oil extracts and the controls

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis,* and *CA: Candida albicans.* For antibiograms: Norfloxacin (NX), Ofloxacin (OF), Ceftriaxone (CTR), Sulphamethoxazole (SX), Amoxylclar (AMC), Nitrofuractoin (NIT), Nalidixic acid (NA), Gentamicin (GEN) and DMSO: Dimethyl sulfoxide

R. officinalis had the highest zone of inhibition in *Escherichia coli* at concentration of 6%. The lowest zone of inhibition was in *Staphylococcus aureus.* Lowest effective concentration was 0.5% for *Pseudomonus aeruginosa* and *Escherichia coli* at 7.3 ± 0.6 while in *Staphylococcus aureus* it was 3% and 2% for *Bacillus subtilis* and *Candida albicans.*

The antimicrobial activity of *P. citrosum* against *Candida albicans, Bacillus aureus, Escherichia coli* and *Pseudomonus aeruginosa* is presented in Table 3.6.

Table 3.7: Antimicrobial activity (zone of inhibition in mm) of *P. citrosum* oils

Concentration (%v/v)	PA. Ave	EC. Ave	SA. Ave	BS. Ave	CA. Ave
6	12.3 ±2.1	15.0 ±1.0	10.0 ±1.0	13.0 ±2.0	13.7 ±1.5
5	11.3 ±0.6	12.3 ±1.2	9.7 ±1.2	13.0 ±1.0	11.0 ±2.0
4	9.7 ±0.6	11.3 ±2.1	8.7 ±0.6	12.0 ±1.0	10.7 ±1.5
3	8.7 ±0.6	9.7 ±1.5	7.0 ±0.0	10.0 ±1.0	9.3 ±1.5
2	8.0 ±1.0	8.3 ±0.6	-	8.3 ±0.6	8.3 ±1.2
0.5	7.3 ±0.6	7.7 ±0.6	-	7.7 ±0.6	7.0 ±0.0
0.2	7.0 ±0.0	7.0 ±0.0	-	7.0 ±0.0	7.0 ±0.0
DMSO	7.0 ±0.0	7.0 ±0.0	-	7.0 ±0.0	7.0 ±0.0

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis, CA: Candida albicans* and DMSO: Dimethyl sulfoxide

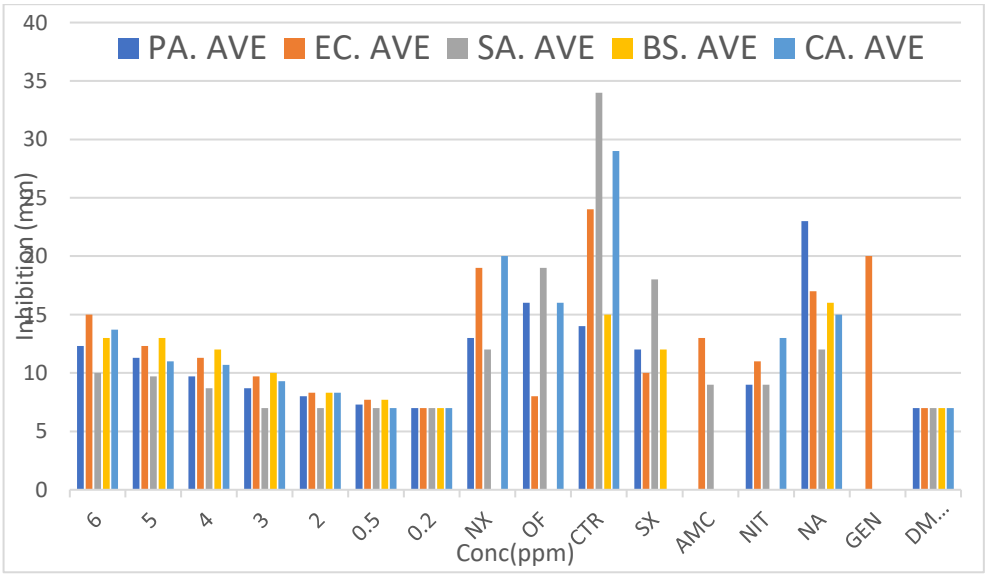


Figure 3.13: The antimicrobial activity of P. citrosum oil extracts and the controls

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis,* and *CA: Candida albicans*. For antibiograms:

Norfloxacin (NX), Ofloxacin (OF), Ceftriaxone (CTR), Sulphamethoxazole (SX), Amoxylclar (AMC), Nitrofuractoin (NIT), Nalidixic acid (NA), Gentamicin (GEN) and DMSO: Dimethyl sulfoxide

At 6%, zone of inhibition in *Escherichia coli* was 15.0 ±1.0 while it was least at *Staphylococcus aureus* 10.0 ±1.0. Similar trend as *R. officinalis* was observed in *P. citrosum* where lowest effective concentration was 0.5% in *Pseudomonus aeruginosa, Escherichia coli* and *Bacillus subtilis*, 2% in *Candida albicans* and 3% in *Staphylococcus aureus.*

Comparing the antimicrobial activities of the extracts with the oils, this study found that at 6% concentration the inhibition zone of *P. citrosum* against *Staphylococcus aureus* was found to be more effective than Nitrofuractoin and Gentamicin drugs. The antimicrobial activity of *Pelargonium citrosum* against *Bacillus subtilis* was found to be more effective than Sulphamethoxazole, Norfloxacin, Amoxylclar, and Ofloxacin at concentrations higher than 5%. *R. officinalis* also demonstrated similar trends as the standards against various microbes, for instance, at 6% concentration the zone of inhibition of *R. officinalis* against *Escherichia coli* was 15.0 ±1.0 slightly above that of Norfloxacin, Sulphamethoxazole, Nitrofuractoin and Nalidixic acid.

These findings align with those by other researchers. The antibacterial properties from extracts of *R. officinalis* against Listeria strains which was measured in another study disclosed that extracts from *R. officinalis* have many potential of antilisterial activity against all of the eleven strains which were tested (Hammer, Carson & Riley, 1999). According to (Moreno *et al.*, 2006) *R. officinalis* plants are rich sources of phenolic compounds with high antimicrobial and antioxidative properties.

3.4 Characterization of metal-oil complexes

3.4.1 Characterization using the FTIR

Figures 3.7-3.10 illustrate the absorption spectra of the essential oil samples gotten from the complexed extracts of *R. officinalis* and *P. citrosum* measured in the wavelength range 400-4000 cm^{-1}, with a resolution of 4 cm^{-1}. The spectrum of the uncomplexed extracts for each sample is represented in figures 3.11 and 3.12. The

region 1400- 400 cm^{-1} (fingerprint) of the IR spectrum contains absorption bands that characterize the entire molecular structure by vibrations of the spectrum: combing and deformation of harmonic bands that cannot usually be attributed to normal vibrations.

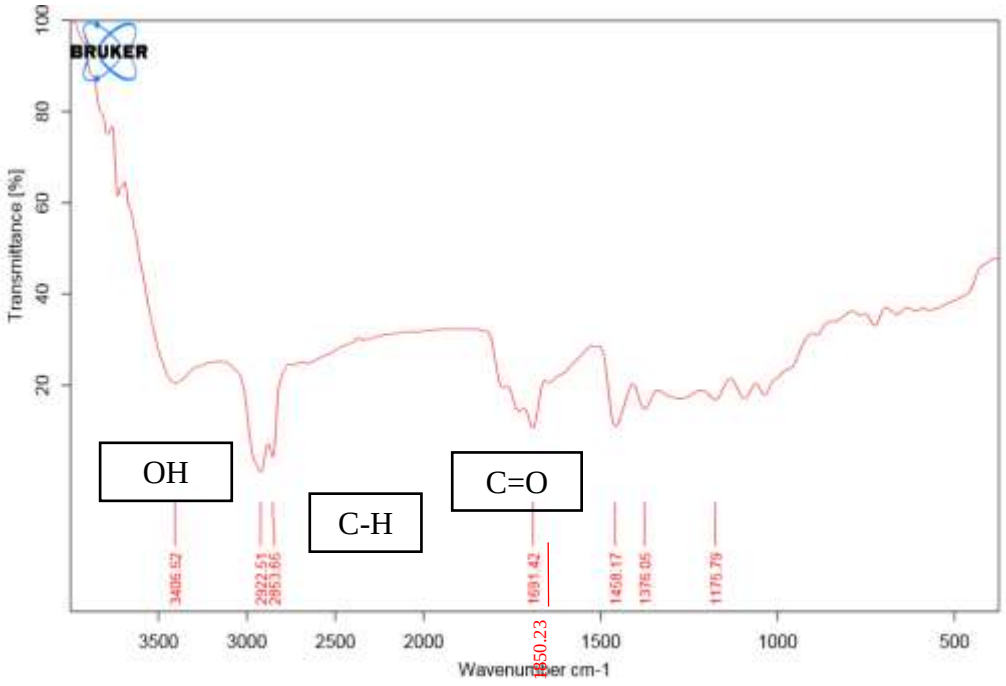


Figure 3.14: FTIR spectrum of *R. officinalis* plant extracts complexed with Mn

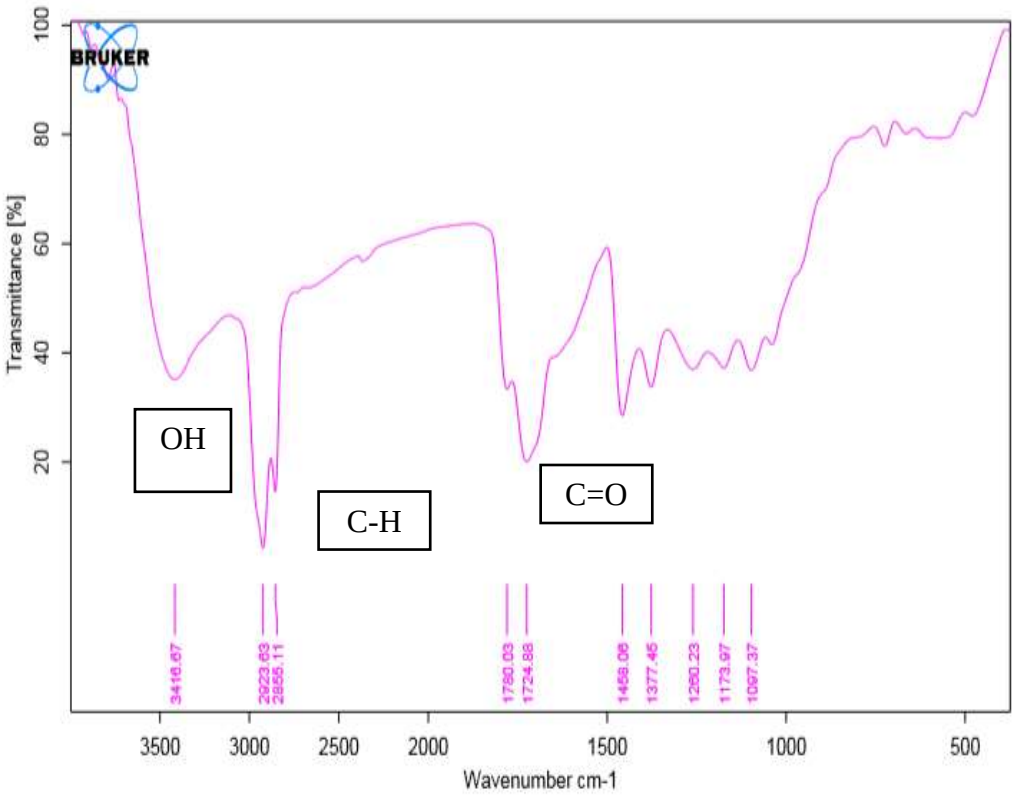


Figure 3.15: FTIR spectrum of R. officinalis plant extract complexed with Iron

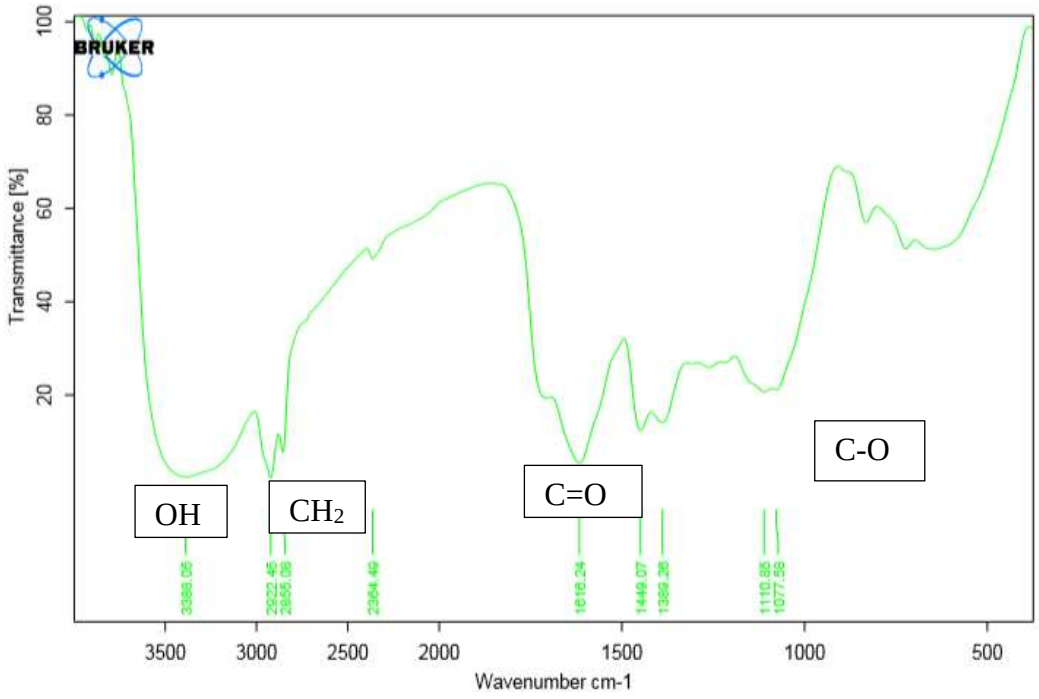


Figure 3.16: FTIR spectrum of *P. citrosum* complexed with Manganese

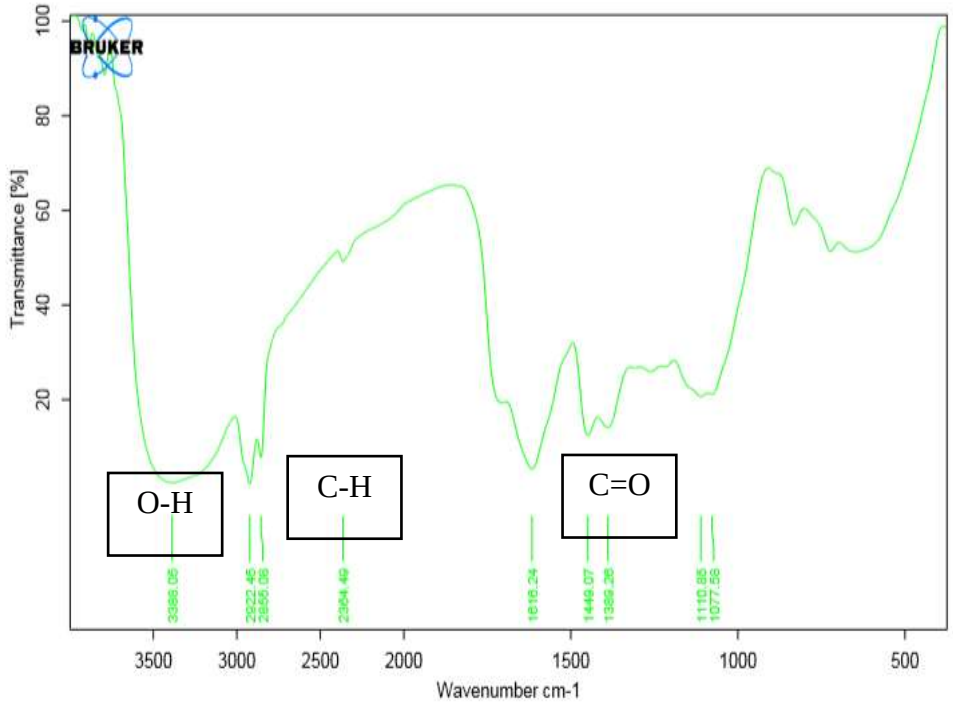


Figure 3.17: FTIR spectrum of *P. citrosum* complexed with Iron

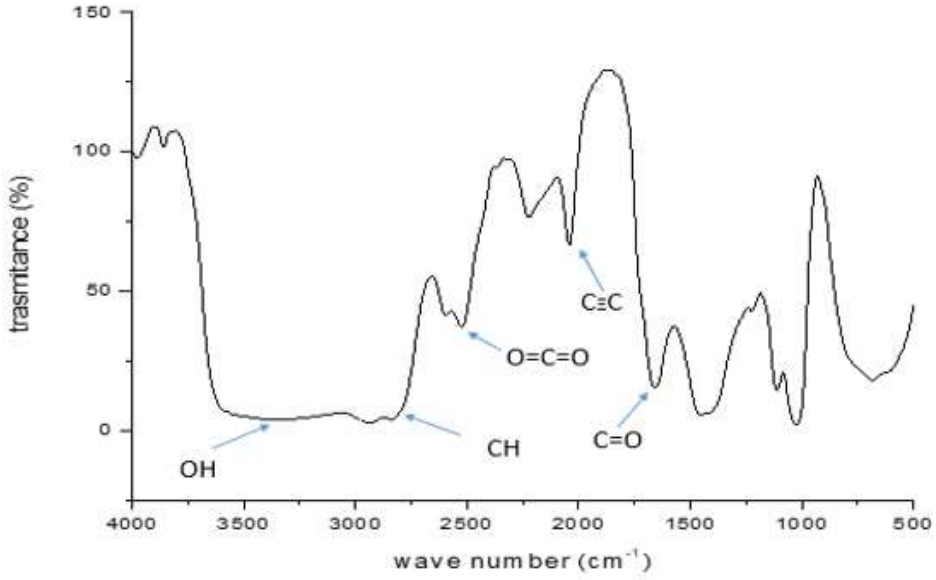


Figure 3.18: FTIR spectrum of *R. officinalis* plant extract

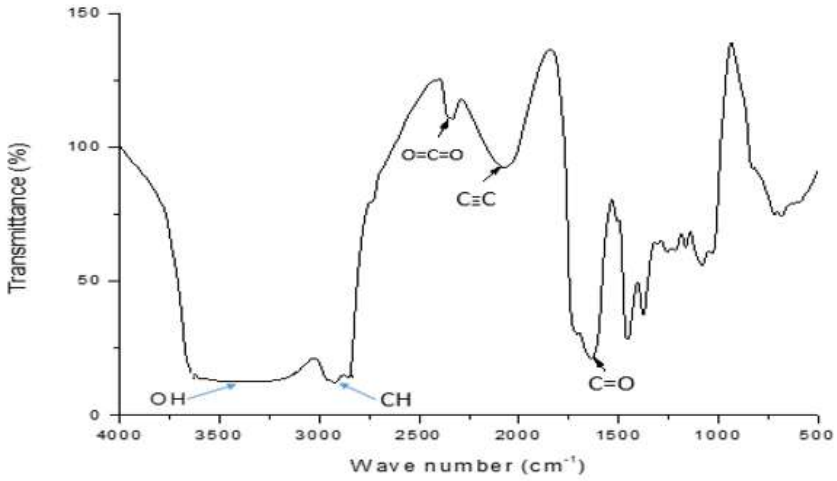


Figure 3.19: FTIR spectrum of *P. citrosum* plant extract

Some of the bands observed in the complexed extract were different in intensity compared to those of the uncomplexed extract. Bands located at around 1850 -3403cm^{-1} corresponding to carbonyl, and the hydroxyl groups respectively present in the complexed extracts were at slightly different locations from those of the uncomplexed extract where the main functional groups observed were C=O at 1710cm^{-1} and broad O-H spectrum at around 3500cm^{-1}. The shifting of the carbonyl group in the complexed extract could be due to the formation of the metal carbonyl while the sharpening of the OH peak at around 3400cm^{-1} is attributed to the loss of the carboxylic acid hydrogen bonding. From complexed and non-complexed extracts, the following peaks (CH 3-O-) 2820–2810 corresponds to Methoxy, C-H stretch, 1150–1050 Alkyl-substituted ether, C-O stretch, 1140–1070 Cyclic ethers, large rings, C-O stretch and 1270–1230 Aromatic ethers, aryl -O stretch

3.4.2 Characterization using the UV/VIS

Figures 3.13 and 3.14 shows the absorption spectra of the samples of essential oil obtained from *R. officinalis* and *P. citrosum* respectively measured in the wavelength range 800-200nm. The observed absorption wavelength of the complexed extracts for *R. officinalis* enhancement of the peaks intensities at 320nm and disappearance of the peak at 430 nm.

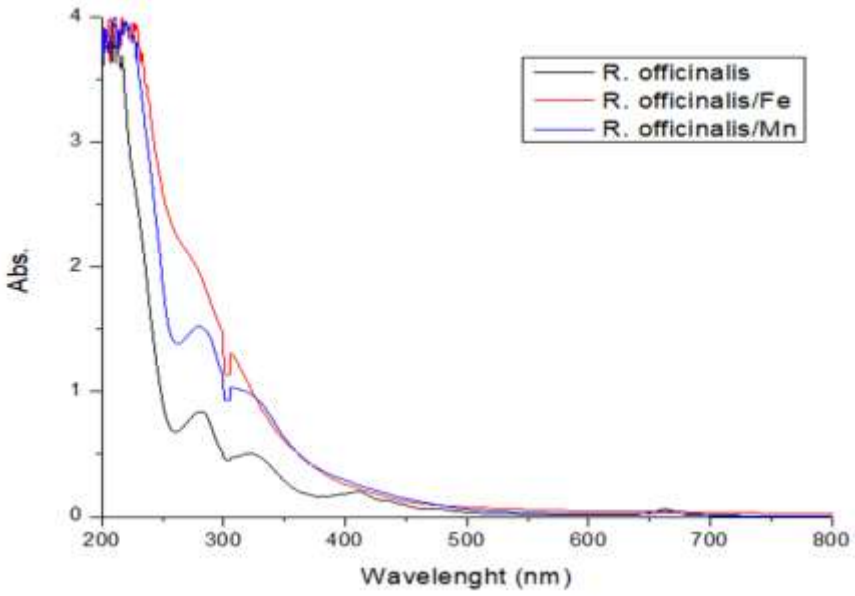


Figure 3.20: UV/VIS spectrum of *R. officinalis* oil extract and the various complexes

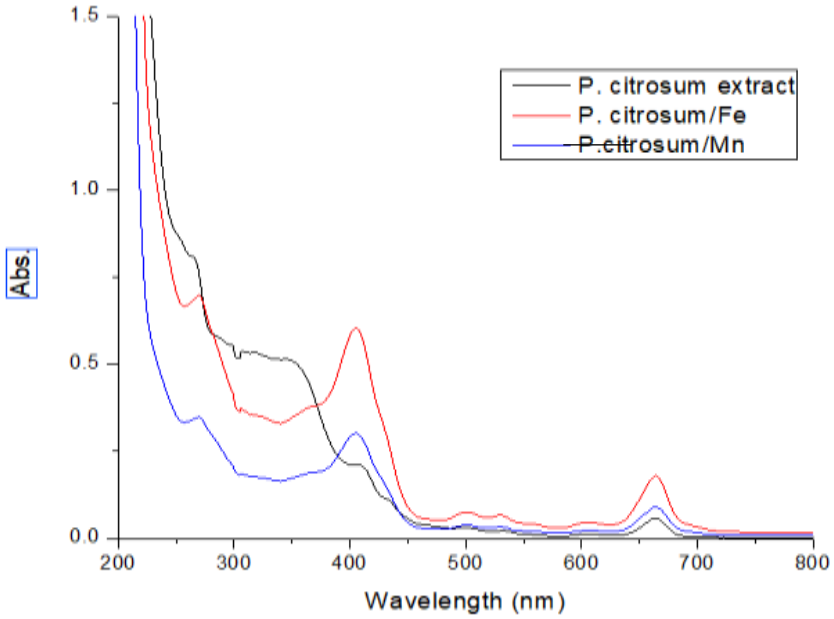


Figure 3.21: UV/VIS spectrum of *P. citrosum* oil extract and the various complexes

The observed absorption wavelength of the complexed extracts indicated a shift towards the shorter wavelength as compared to the uncomplexed extracts. These shifting is attributed to the reduction of conjugation as a result of the reaction between the metal ions and the lone pair and pi electrons in the compounds present in the extract. For *P. citrosum complex* there is enhancement of the peak intensities at approximately 400 and 670nm.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATION

4.1 Conclusions

i. The plant oil extracts had *Musca domestica* repellence and antimicrobial effects
ii. Metal complexes (Fe and Mn) with the plant extract had antimicrobial and insect repellence effects
iii. Detergents and paints formulated with hydro-distilled oils had insect repellence effects
iv. We finally conclude that the developed plant based complex essential oil system were thermodynamically stable owing to its improved bioavailability and biocompatibility formulated complex, can be used in various biomedical application including drug as well as disease transmitting housefly vector control.

4.2 Recommendations for further studies

i. Further studies are required to examine the integration of the plant extracts into suitable paint and detergent preparations and assess any changes that are chemically brought about by these incorporations.
ii. To blend *R. officinalis* plant extract *P. citrosum* extract to find out whether activity is enhanced.
iii. To analyse repellence activity of *R. officinalis* and *P. citrosum* plants propagated in different agro ecological zones against *M. domestica*
iv. Full field trials need to be done to rule out any possible variances in repellent properties from the overlapping of repellence range of the treatment with that of the behaviour and control *M. domestica* when they are constrained.

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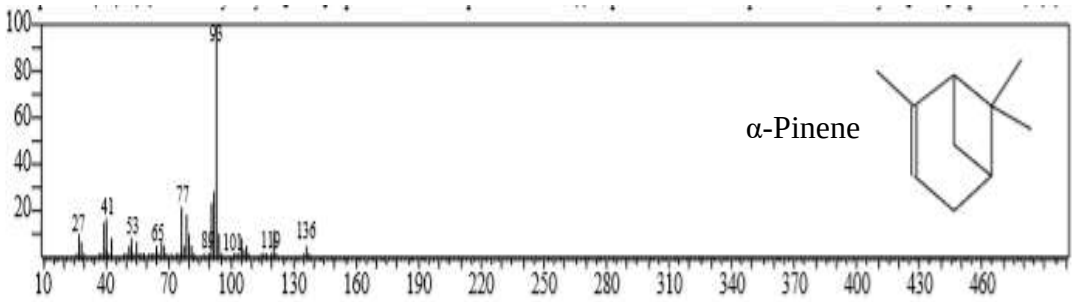
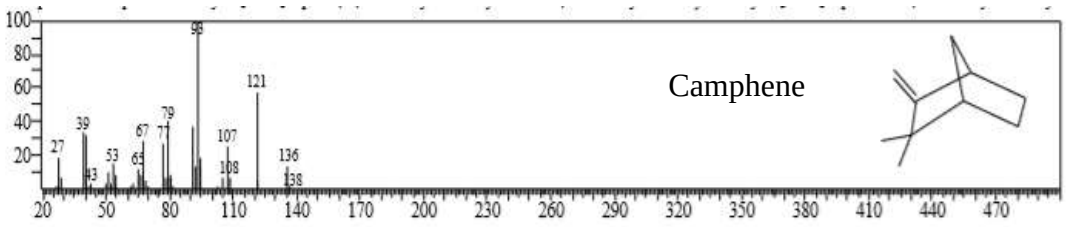
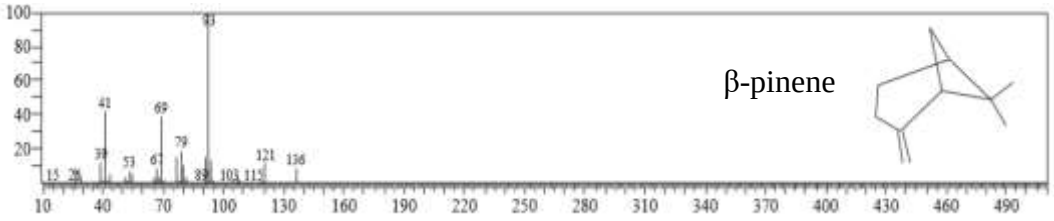
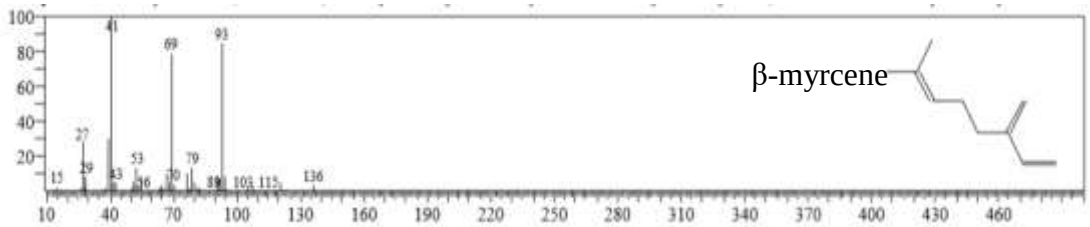
APPENDICES

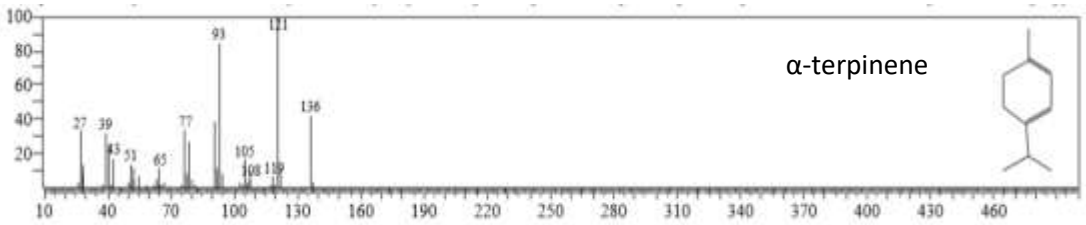
Appendix I: GC-MS profiles of essential oils from R. officinalis

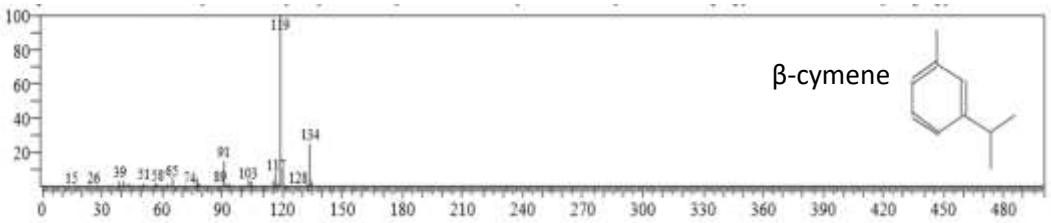








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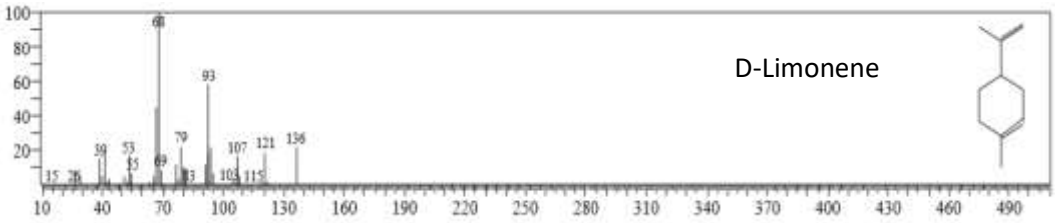
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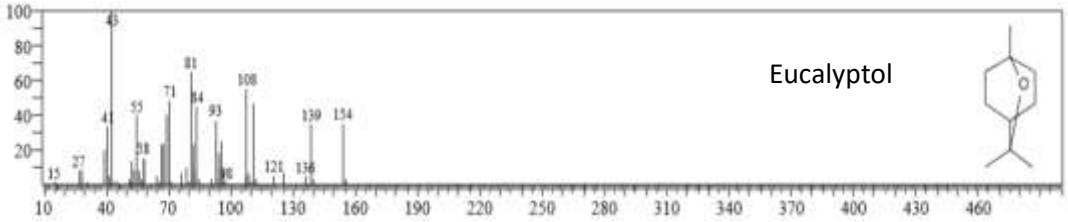
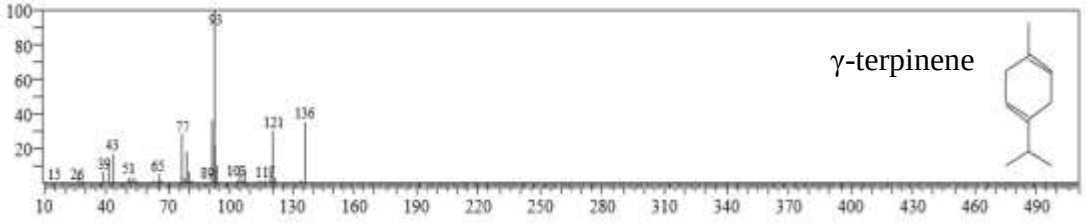

Eucalyptol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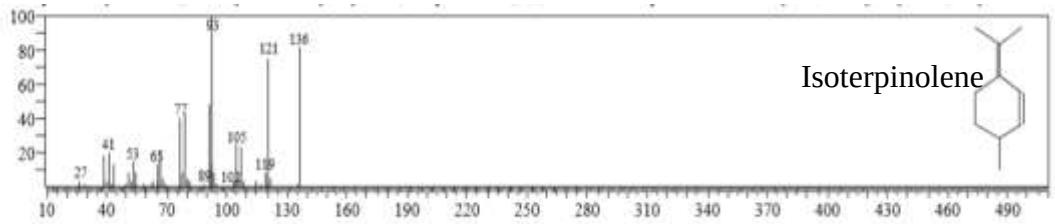
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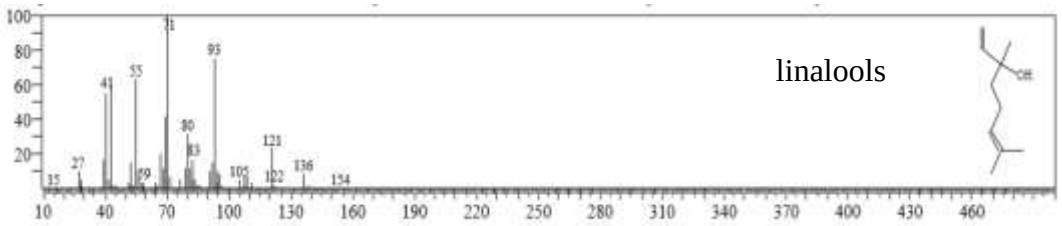
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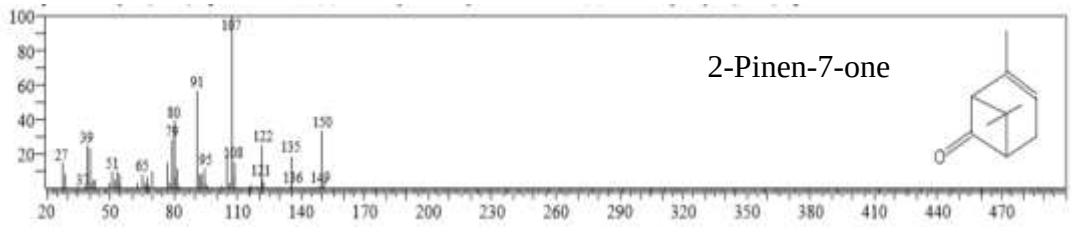
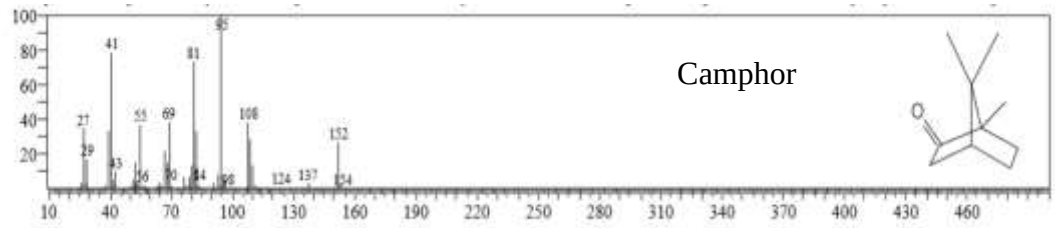
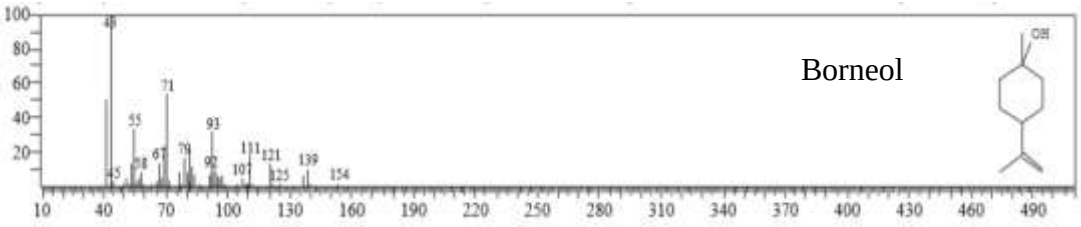

2-Pinen-7-one


Camphor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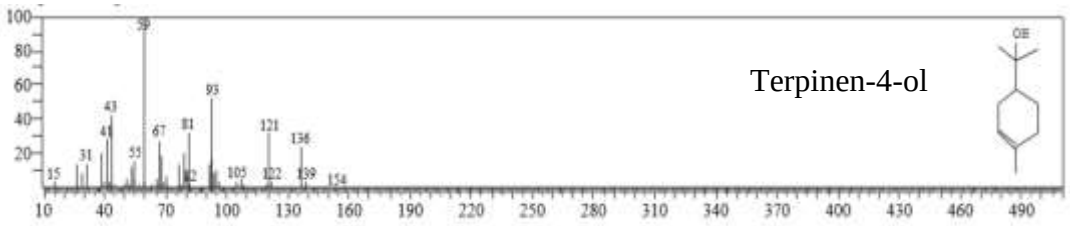
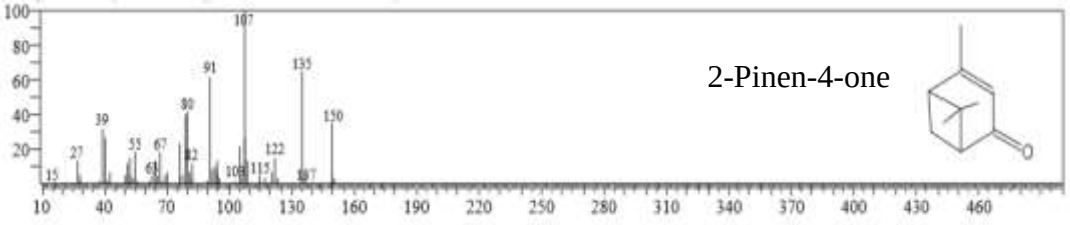

Terpinen-4-ol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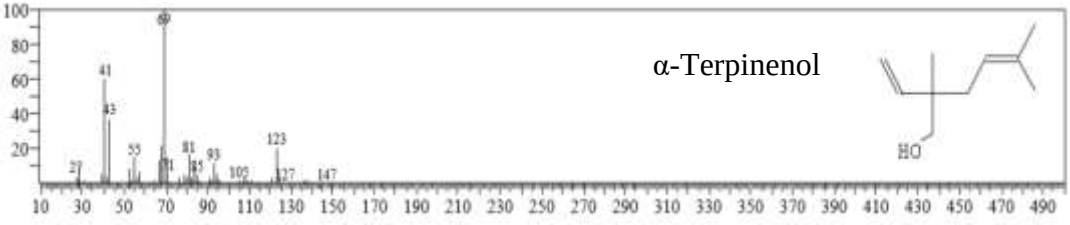
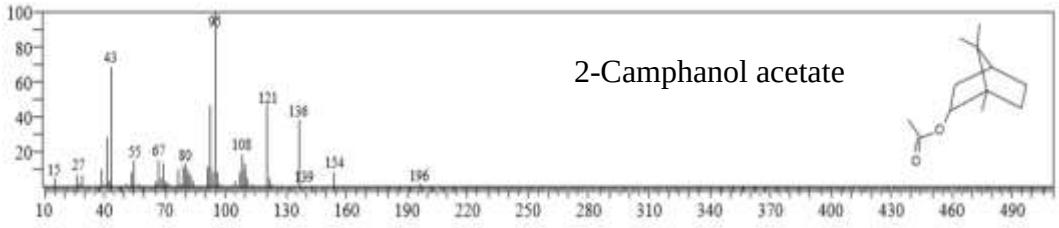

α-Terpinenol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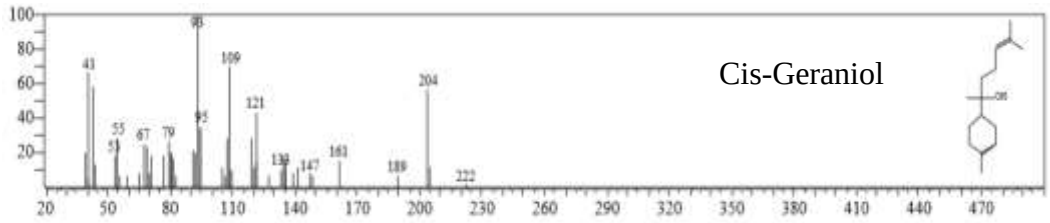
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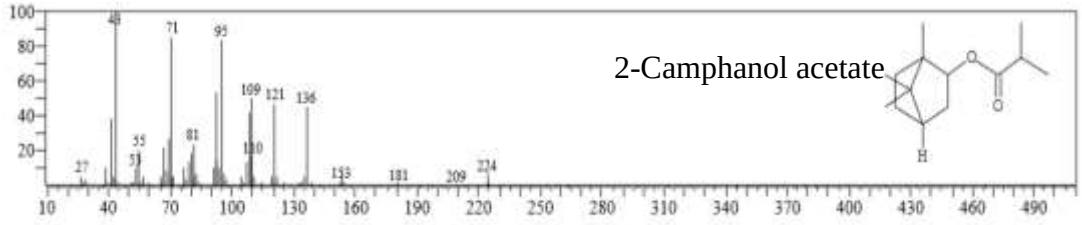
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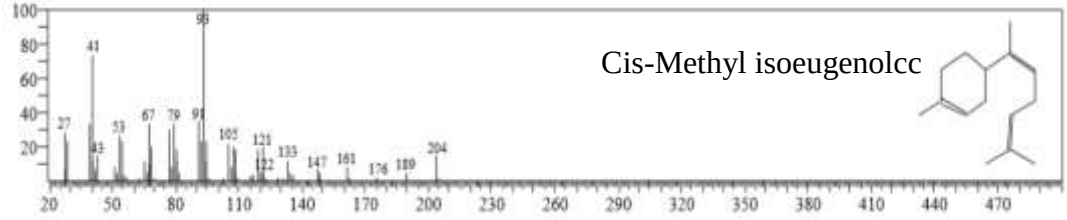
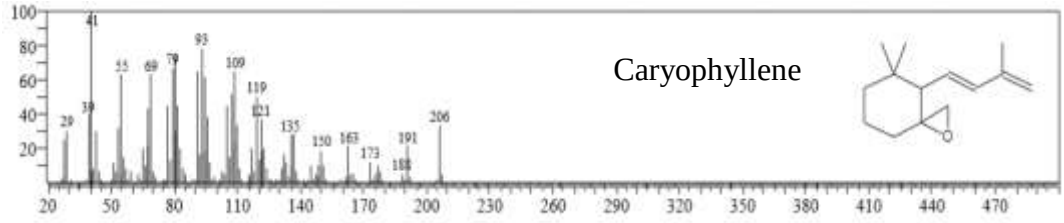

Cis-Methyl isoeugenolcc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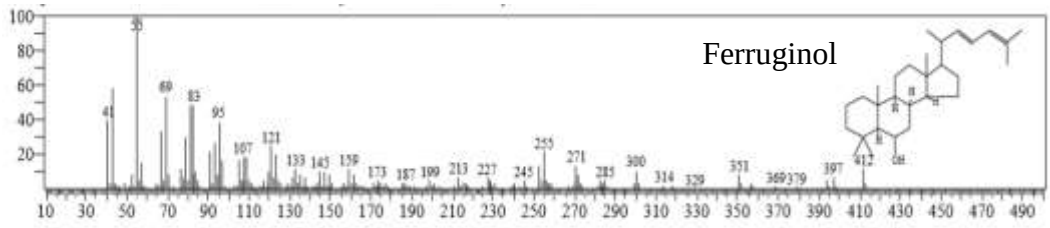
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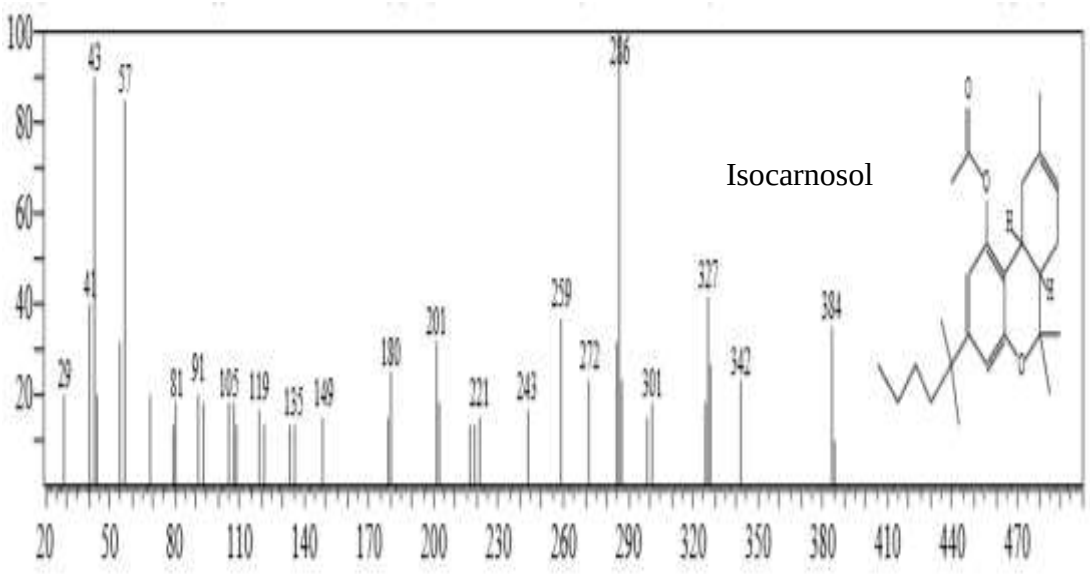
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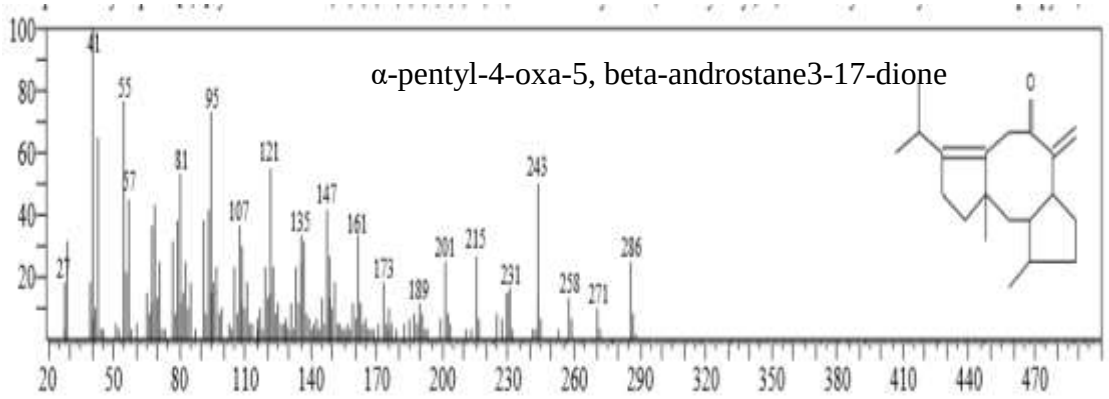
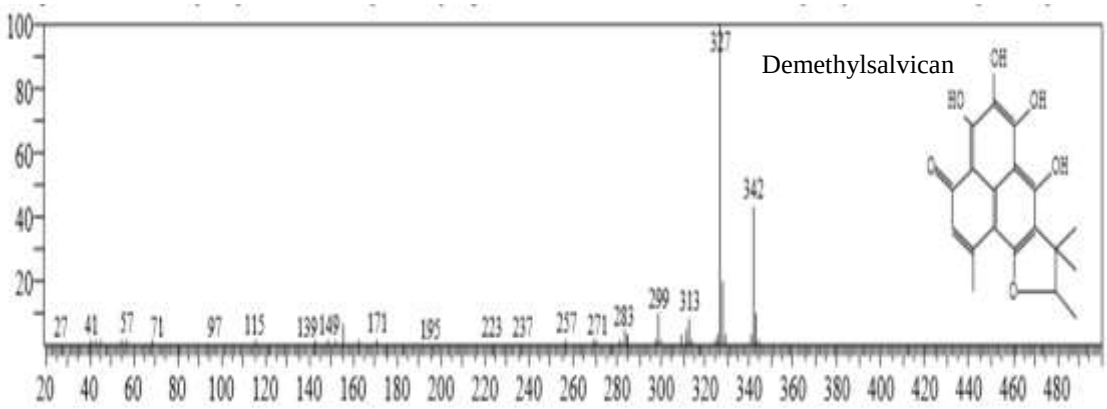
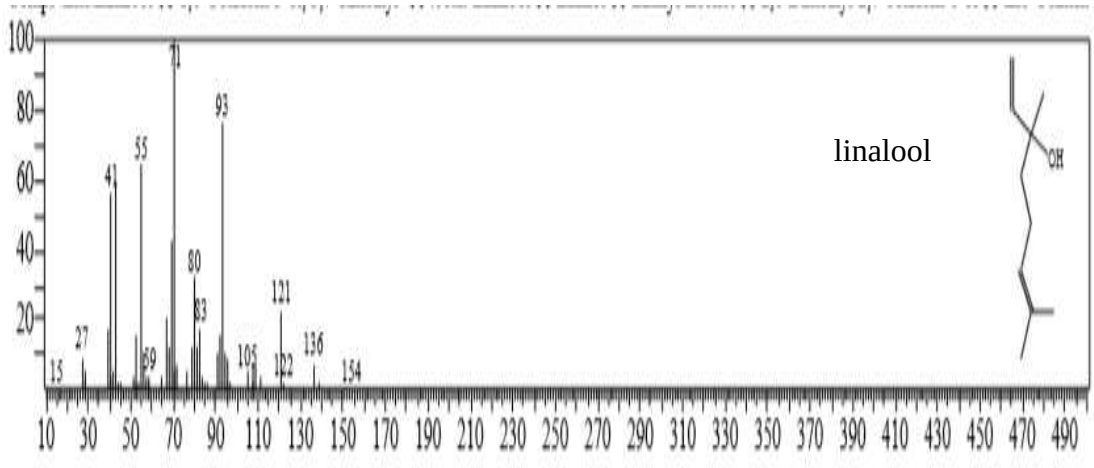
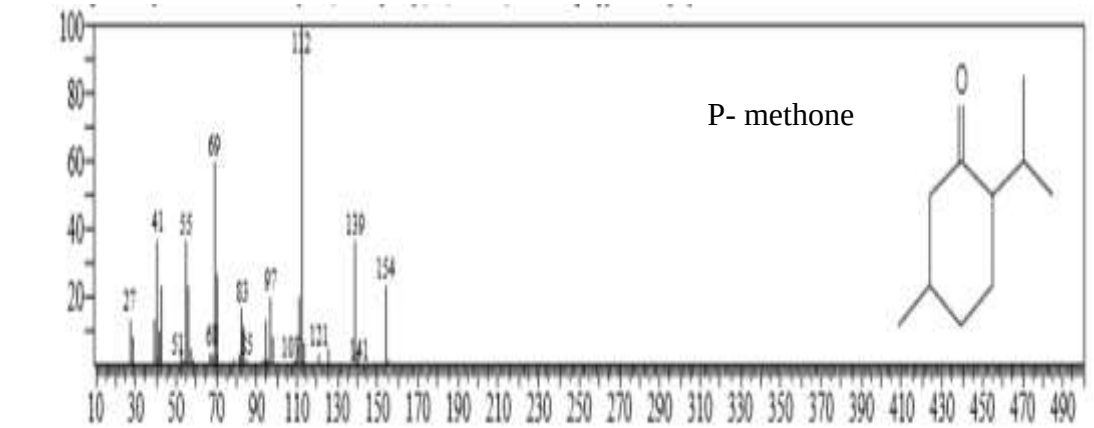
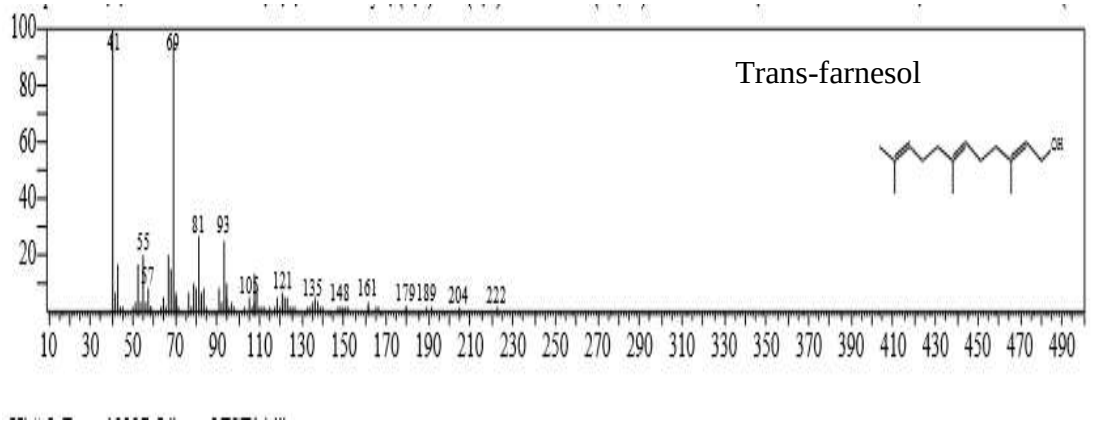

α-pentyl-4-oxa-5, beta-androstane3-17-dione

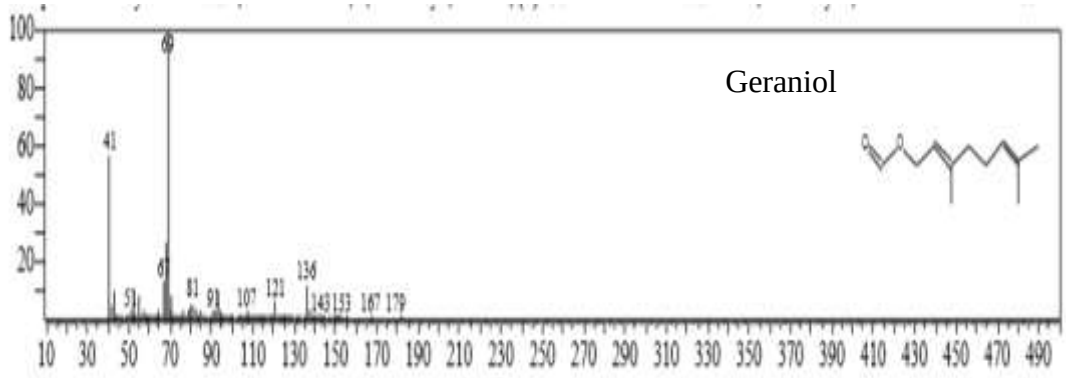

Demethylsalvican

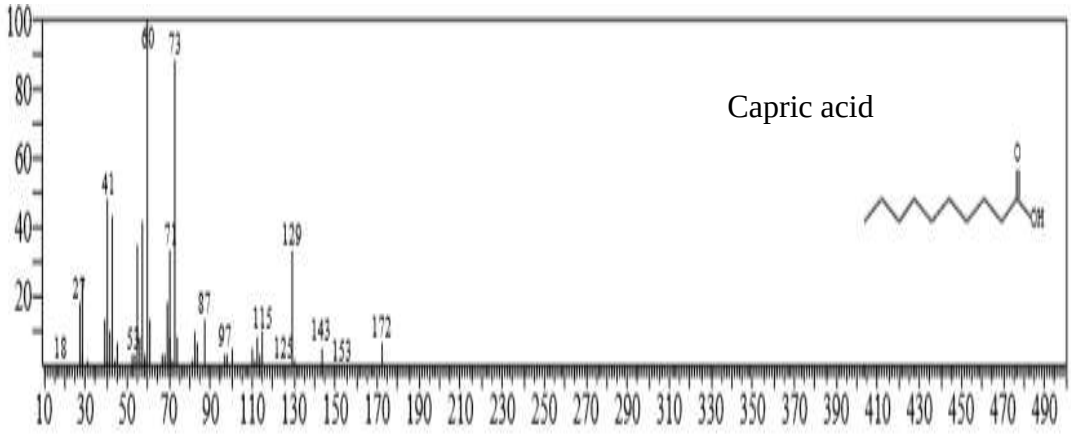
Appendix A: GC-MS profile of *P. citrosum*

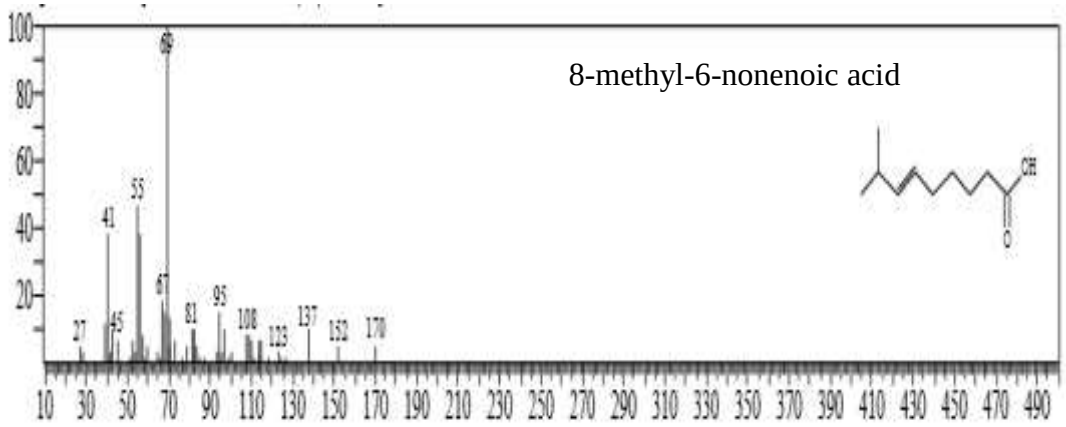


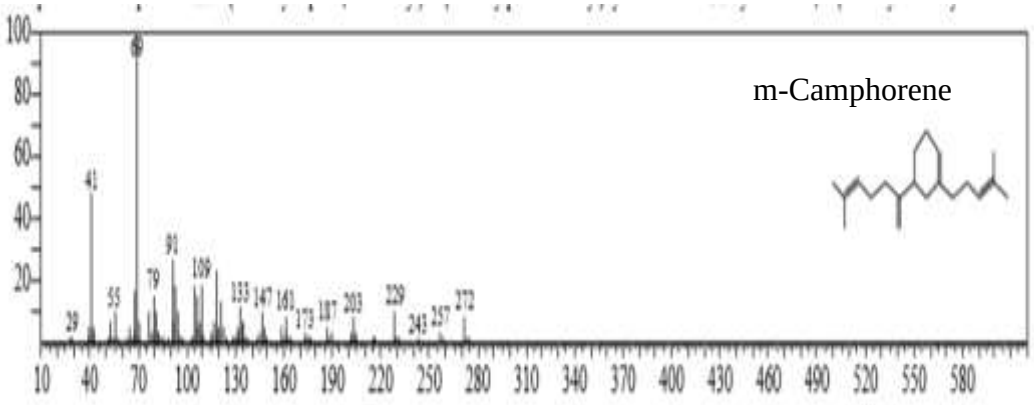


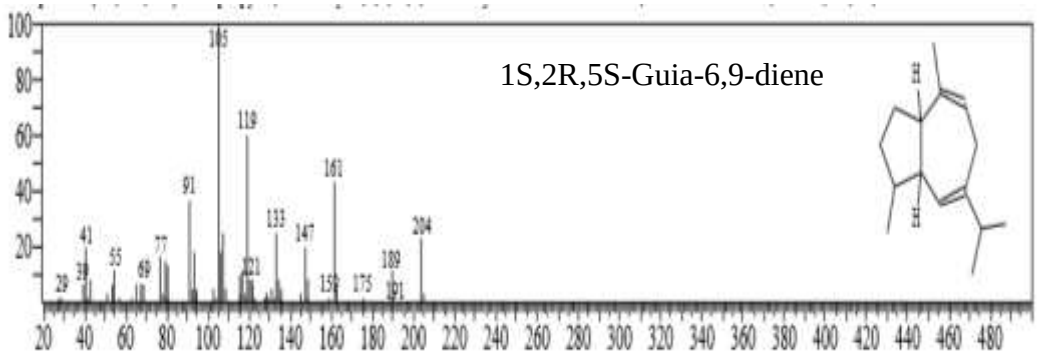



Geraniol


Capric acid


8-methyl-6-nonenoic acid


m-Camphorene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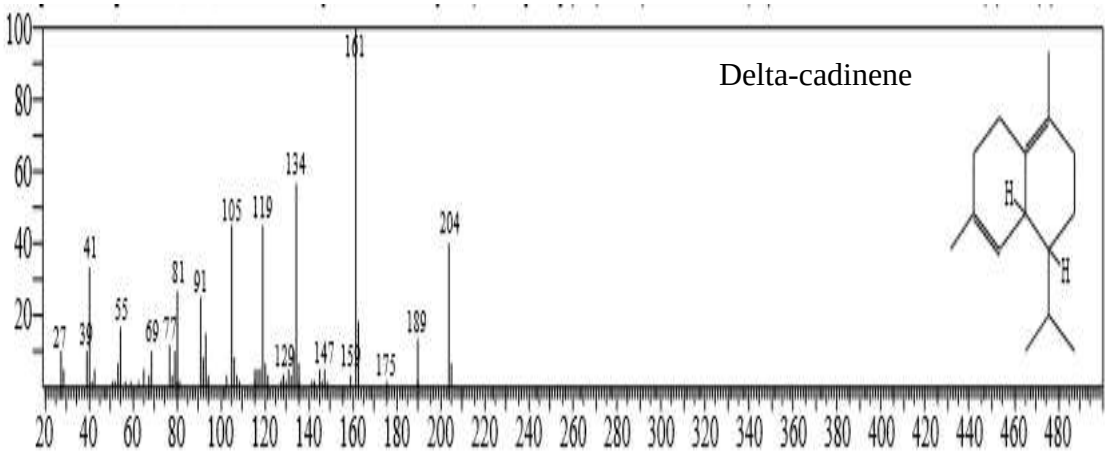
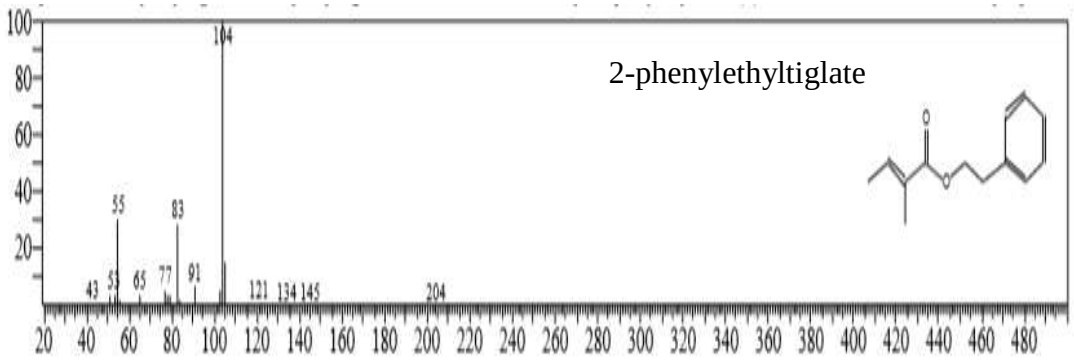

Delta-cadinene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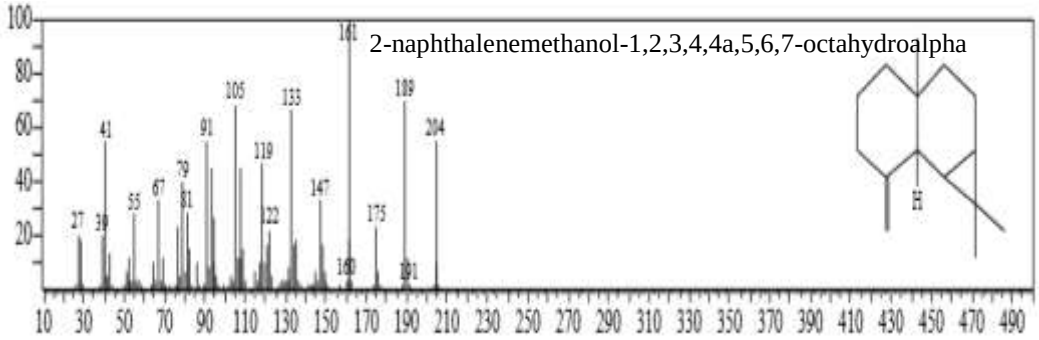
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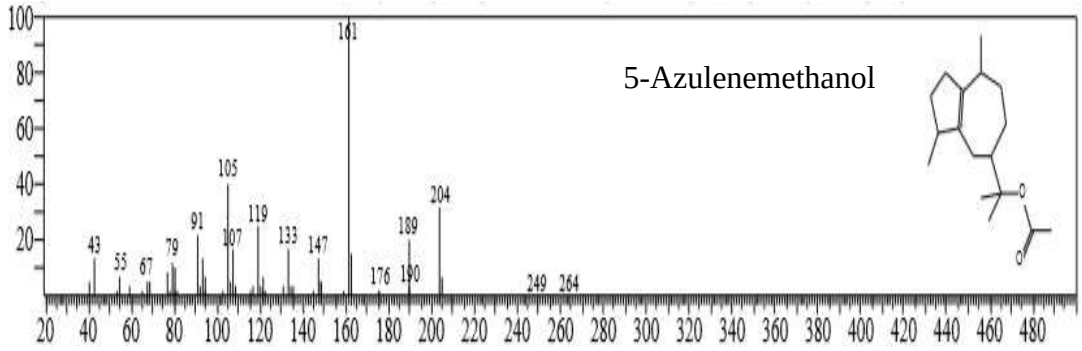
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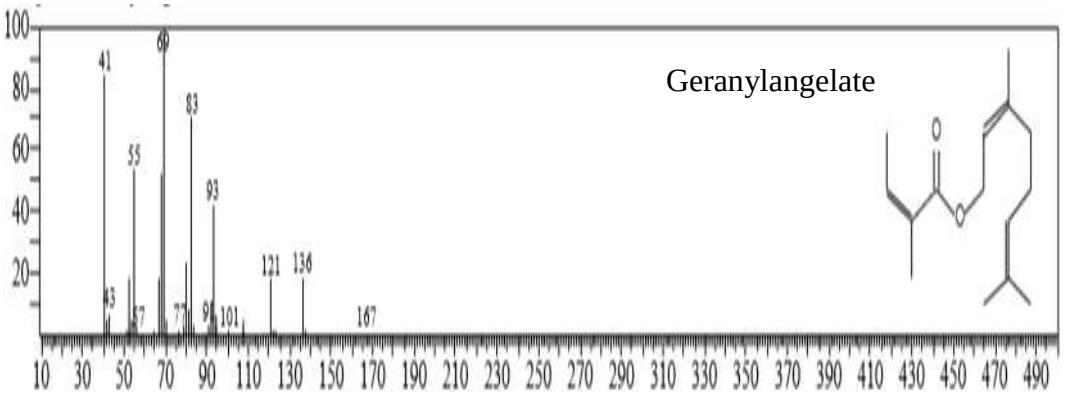
Geranylangelate
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77
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101
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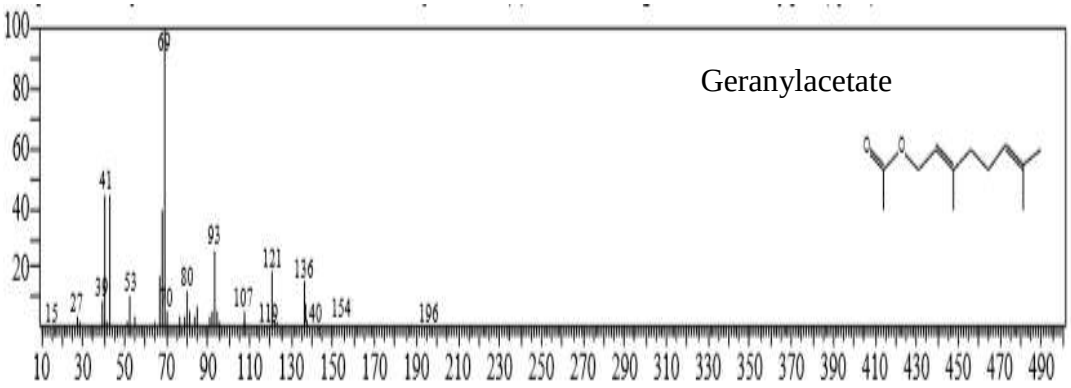
Geranylacetate
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10 30 50 70 90 110 130 150 170 190 210 230 250 270 290 310 330 350 370 390 410 430 450 470 490

Appendix II: Publications

Chemical Science International Journal

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ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

Repellency Effects of *Pelargonium citrosum* and *Rosmarinus officinalis* Essential Oils against Housefly, *Musca domestica* L. (Diptera: Muscidae)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Use of botanical environmentally friendly and biodegradable insect repellants as opposed to chemical insecticides is increasingly becoming important as an alternative method of insect control. Housefly (*Musca domestica* L.) has potential of transmitting pathogen causing diseases such as typhoid, cholera, bacillary dysentery, tuberculosis, anthrax, ophthalmia, and parasitic worms. The aim of this study was to evaluate the repellency of oil extracted from the leaves of *Pelargonium citrosum* and *Rosmarinus officinalis*. Extraction of essential oils was by hydro-distillation. Condensed oil extracts were collected in n-hexane and insect behavioral response tested using adult houseflies (*Musca domestica* L.). *N, N*-diethyl-*m*-toluamide (DEET) was used as the positive control and acetone as the negative control. The bioactive oil was then analyzed using GC-MS. The characteristic volatiles obtained from the two plants showed different compositions. *P. citrosum* oil comprised mainly of Linalool, Geraniol, m-Camphorene, 2-naphthalenemethanol-1,2,3,4,4a,5,6,7-octahydroalpha, Geranylangelate while *R. officinalis* comprised mainly of α-Pinene, , Eucalyptol, α-Terpinenol. Dose-response evaluations of these oils showed that *R. officinalis* oil was more repellent (LD_{50} = 0.299 mg) than that of *P. citrosum*

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Antimicrobial Potential of *Pelargonium citrosum* and *Rosmarinus officinalis* Essential Oils

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Essential oils derived from aromatic plants have exhibited biological properties and can be used to prevent and treat human diseases. The goal of this work was to investigate the antibacterial and antifungal potential of the essential oils extracted from R. *officinalis and P. citrosum* against Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonus aeruginosa, Escherichia coli*) and fungus (*Candida albicans*). The antimicrobial activities of the *R. officinalis and P. citrosum* essential oils were carried out using the Disk diffusion method. The results indicated that the essential oils from *P. citrosum* had antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* and *Candida albicans* at a low concentration of 0.5 % v/v and that the activity was concentration dependent. Essential oil from R. *officinalis* on the other hand showed effective antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Pseudomonus aeruginosa* and *Candida albicans. P. citrosum* was found to be more effective than Nitrofuractoin and Gentamicin drugs against *Staphylococcus aureus* at a higher concentration of 6% v/v. *R. officinalis* oil extracts also demonstrated similar trends and were comparable to the positive controls against the tested microbes. It was therefore concluded that *R. officinalis and P. citrosum* plant extracts were effective against the tested antimicrobial agents and have potential to be used against the tested microbes.

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Formulation of Paint and Detergent with *Pelargonium citrosum* & *Rosmarinus officinalis* Extracts as *Musca domestica* Repellent Agent

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Use of botanical environmentally friendly and biodegradable insect repellants as opposed to chemical insecticides is increasingly becoming important as an alternative method of insect control. Housefly (*Musca domestica* L.) has potential of transmitting pathogen causing diseases such as typhoid, cholera, bacillary dysentery, tuberculosis, anthrax, ophthalmia, and parasitic worms. Essential oils derived from aromatic plants have exhibited biological properties and can be used to prevent and treat human diseases. The aim of this study was to formulate the housefly repellent paint and detergent. Extraction of essential oils was by hydro-distillation. Condensed oil extracts were collected in n-hexane and insect behavioral response tested using adult houseflies (*Musca domestica* L.). *N, N*-diethyl-*m*-toluamide (DEET) was used as the positive control and acetone as

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