University of Shendi

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Investigation of Oils from Some Sudanese Medicinal Herbs

A Thesis Submitted in Fulfillment of the Requirements for the M.Sc. Degree in Chemistry

By

Asrar Adil Algray Ahmed

(B.Sc. Chemistry and Zoology)

Supervisor: Prof. Mohamed Abdel Karim Mohamed

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استهلال

بسم الله الرحمن الرحيم

قال تعالى:

(وَالْأَرْضَ مَدَدْنَاهَا وَأَلْقَيْنَا فِيهَا رَوَاسِيَ وَأَنْبَتْنَا فِيهَا مِنْ كُلِّ شَيْءٍ مَوْزُونٍ ﴿19﴾وَجَعَلْنَا لَكُمْ فِيهَا مَعَايِشَ وَمَنْ لَسْتُمْ لَهُ بِرَازِقِينَ ﴿20﴾ وَإِنْ مِنْ شَيْءٍ إِلَّا عِنْدَنَا خَزَائِنُهُ وَمَا نُنَزِّلُهُ إِلَّا بِقَدَرٍ مَعْلُومٍ ﴿21﴾)

صدق الله العظيم

سورة الحجر

DEDICATION

To my:

Lovely parents

Brother and sisters

Acknowledgements

Thank to **Almighty Allah** for giving me health to complete this work successfully. I am greatly indebted to Prof. Dr. Mohamed Abdel Karim, for his keen interest, supervision, encouragement, support, and guidance throughout this study. I would like to express my gratitude to Dr. Faroug Bakheit Elsonni,for his support and encouragement. I am grateful to Dr. Hassan Alamein, for his kind help.Imust not forget to thank Dr. Atif Babker for his great help. Thanks to my family for their continual support. Also my thanks extend to laboratory staff of the Medicinal and Aromatic Plants Research Institute, and to the Ministry of Agriculture and Forestry- Sudan for all facilities.

Abstract

The essential oils from *Cymbopogon citratus, Brassica nigra* and *Cymbopogon nervatus* were analyzed by GC-MS. In addition, these oils were investigated for antimicrobial activity.

GC-MS revealed that the dominant constituents in *Cymbopogon citrates* oil are; 2,6-octadienal, 3,7-dimethyl (E) (43.20%), 2,6-octadienal,3,7-dimethyl (Z) (31.36%) and 5, hepten-2-one, 6-methyl (3.87%). The major constituents in the oil of *Brassica nigra* were; 13-docosenoic acid, methyl ester (35.65%), 9,12-octadecadienoic acid (Z,Z) , methyl ester (18.20%), 11-eicosenoic acid, methyl ester (12.82%), 9-Z-octadecenoic acid, methyl ester (8.90%), 9,12,15-octadecatrienoic acid, methyl ester (8.60%), hexadecanoic acid methyl ester (3.83%), 15-tetracosenoic acid, methyl ester (3.77) and Cis-11-eicosenoic acid, methyl ester (2%). Most dominant constituents in the oil of *Cymbopogon nervatus* are;P-mentha-1(7),8-dien-2-ol (24.27%), trans-p-mentha-1(7),8-dien-2-ol (21.07%), trans-p-mentha-2,8-dienol (13.28%), carveol (10.43%), cis-p-mentha-2,8-dien-1-ol (9.43%), [1,1-bicyclopentyl]-2-one (5.65%), (-)-carvone (5.27%) and D-limonene (2.41%). In the disc diffusion bioassay, *Cymbopogon citrates* oil showed excellent antibacterial activity against all test bacteria at 100 mg/ml but did it not exhibit any anticandidal activity. It also showed excellent activity against *S. aureus* and *P. aeruginosa* at 50 and 25mg/ml. Significant antimicrobial activity was observed for *Cymbopogon nervatus* oil at 100 mg /ml for all test organisms. *Brassica nigra* oil showed excellent antimicrobial activity at 100 mg/ml against all test organisms except for *S.*

aureus. It also exhibited significant activity against *B. subtilis* and *E. coli* at 50 and 25 mg/ml.

المستخلص

الزيوت الطيارة لحشيشة الليمون ، الخردل الأسود و النال تم تحليلها بواسطة الكروموتوغرافيا الغازية السائلة – طيف الكتلة. بالإضافة إلى ذلك ، تم فحص هذه الزيوت كمضادات بكتريا.

الكروموتوغرافيا الغازية السائلة – طيف الكتلة بينت أن زيت حشيشة الليمون يحويالمركبات الرئيسة التالية :

2,6-octadienal, 3,7-dimethyl (E) (43.20%), 2,6-octadienal,3,7-dimethyl (Z) (31.36%) and 5, hepten-2-one, 6-methyl (3.87%).

بينما زيت الخردل يحوى المكونات الرئيسة التالية:

13-Docosenoic acid, methyl ester (35.65%), 9,12-Octadecadienoic acid (Z,Z) methyl ester (18.20%), 11-Eicosenoic acid, methyl ester (12.82%), 9-Z-Octadecenoic acid, methyl ester (8.90%), 9,12,15-Octadecatrienoic acid, methyl ester (8.60%), Hexadecanoic acid methyl ester (3.83%), 15-tetracosenoic acid, methyl ester (3.77) and Cis-11-eicosenoic acid, methyl ester (2%).

اما زيت النال فهو يتكون من المكونات الرئيسة التالية:

P-Mentha-1(7),8-dien-2-ol (24.27%), Trans-p-mentha-1(7),8-dien-2-ol (21.07%), Trans-p-mentha-2,8-dienol (13.28%), Carveol (10.43%), Cis-p-mentha-2,8-dien-1-ol (9.43%), [1,1-Bicyclopentyl]-2-one (5.65%), (-)-carvone (5.27%) and D-Limonene (2.41%).

اختبرت الزيوت كمضادات بكتريا حيث اعطت حشيشة الليمون فعالية ممتازة ضد *S.aureus and P. aerugenosa* عند تركيز 100mg/ml و لكن لم يعطي اي فعاليه ضد *Candida albicans*. اما زيت النال فقد اعطى فعالية عالية ضد جميع الميكروبات عند تركيز 100mg/ml. واعطى الخردل فعالية ممتازة عند تركيز100mg/mlضد جميع الميكروبات ما عدا*S. Aureus* , كذلك اعطى فعالية عالية ضد *B. subtilis and E.coli* عند تركيز 25mg/ml and 50mg/ml.

Table of Contents	
Contents	Page

List of tables	
	page

List of Figures	
	page

Introduction

1.1. *Cymbopogon citratus*

1.1.1. Nomenclature

Cymbopogon citratus is commonly known as lemongrass. (Akande, *et al.*, 2011).

1.1.2. Classification

Kingdom: *Plantae.*

Subkingdom: *Tracheobionta.*

Super division: *Spermatophyta.*

Division: *Magnoliophyta.*

Class: *Liliopsida.*

Subclass: *Commelinidae.*

Order: *Cyperales.*

Family: *Poaceae.*

Genus: *Cymbopogon Spreng.*

Species: *Cymbopogon citraues.*

1.1.3. Plant description and distribution

Lemongrass is a perennial grass plant widely distributed worldwide and most especially in tropical and subtropical countries (Francisco, *et al.*, 2011). Several reports have linked its origin to Asia (Indochina, Indonesia and Malaysia), Africa and the Americas. The plant could grow up to 6 inch high and its bulblike stems consist of terete and glabrous linearly venated sheathed leaves with narrow base and acute apex. The leaf height is about 100 cm in length and 2

cm in width. When squeezed, the leaves usually produce yellow or amber colored (Adejuwon and Esther, 2007).

1.1.4. Chemical constituents

The chemical composition of the essential oil of *Cymbopogon citratus* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered. Lemon grass contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. The essential oil consists of mainly citral a volatile oil with strong lemon fragrance. Citral is a mixture of two stereoisomeric monoterpene aldehydes; the trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%) and is used in manufacture of perfumes, colored soaps and synthesis of vitamin A (Shah, *et al.,* 2011). It consists of luteolin and its 6-C and 7-O –glycosides, isoorientin 2'-O-rhamnoside (Matouschek and Stahl, 1991) and the flavonoids: quercetin, kaempferol and apiginin from aerial parts (Miean and Mohamed, 2001). The phenolic compounds elimicin, catecol, chlorogenic acid, caffeic acid and hydroquinone were isolated from plant (Faruq, 1994).

1.1.5. Uses of *Cymbopogon citratus*

Cymbopogon citratus has a wide range of therapeutic, nutritional, and cosmetic uses. Its aqueous extract is commonly used as an aromatic drink while the whole plant is well incorporated into traditional food for its lemon flavour. It also enjoyed wide

application in folk medicine (Figueirinha, *et al.*, 2008), scientific research has found some potentially toxic substance in this species. Hepatotoxic and nephrotoxic effects in mice treated with fluid extracts of *C. citratus* (30% and 80%) were observed (Guerra, *et al.* 2000), indicating the necessity of more detailed research on its cytotoxicity (Negrelle and Gomes 2007). Traditionally, the plant is used as an antimicrobial, antioxidant, and anti-inflammatory, hypoglycemic, insect repellant, cardio protective, anti-carcinogenic, and antipyretic (Akande, *et al*., 2011).

Recent evidence indicates that infusions prepared from dry or fresh leaves of *Cymbopogon citratus* are extensively used in traditional medicine in many parts of the world, including Cuba, Brazil, India, and Indonesia, as a diuretic for treatment of hypertension and associated cardiovascular disorders, in bladder disorders, including inflammatory conditions of the urinary tract, and for treatment of renal stones and urine retention; it is also used in treatment of gout. Nutritionally, the used in traditional cuisines, baked food and confections. Cosmetically, it is essential oil is used in fragrances, soaps, detergents, and body creams (Mirghani, *et al.*, 2012).

1.1.6. Anti- microbial activity of *Cymbopogon citratus*

a- Anti-bacterial potential

Anti-bacterial activity in extracts of plant materials has been elucidated from various sources in recent times with promising results. This characteristic has also been investigated in the volatile

oil portion of the aqueous extract of lemon grass. Among the major bioactive compounds identified in the oil were α-citral (geranial) and β-citral (neral) components. These components demonstrate their antibacterial activity by inhibiting the growth of both Gram positive and Gram negative bacteria. However the third component myrcene possess no antibacterial activity individually but do enhance activity when combined with others. Chromatographic fraction of essential oil in agar plate was active on *Bacillus subtilis*, *Escherichia coli*, *Staphylococus aureus* and *Salmonella paratyphi*, *Shigella flexneri*(Melo, *et al.*, 2001).

b- Anti-fungal activity

The action of essential oils extracted from lemon grass decoction against both pathogenic and edible fungi is of immense contribution as investigated by researchers. Lemon grass oil showed a promising prospect among several essential oils by inhibiting the growth of fungi cells which are implicated in secreting mycotoxins during storage of grains and other food products (Fandohan, *et al.*, 2008; Nguefacka, *et al.*, 2012). The synergistic effects of oil fractions showed both synergistic and antagonistic effects among different portion of characterized oils (Viana, *et al*., 2000; Nguefacka, *et al.*, 2012). Essential oil fraction of lemon tea has been reported to exhibit anti-fungal effects against filamentous fungi of different classes thereby showing its broad spectrum of activity against both disease causing and non pathogenic fungi. Similarly, the oil is

capable of inactivating disease causing yeast cells (*Candida* spp.) by inhibiting their growth (Dharmendra, *et al.*, 2001).

1.1.7. Anti-oxidant properties of *Cymbopogon citratus*

Researchers have identified antioxidant potentials of lemongrass extracts and documented their abilities to reduce reactive oxygen species. Such mechanism include inhibition of lipoperoxidation and decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sharma and Bhat, 2009). Infusions and decoctions prepared from lemongrass showed anti-oxidant properties by scavenging superoxide anion, inhibiting lipoperoxidation and decolorizing DPPH. These effects are higher in infusion than decoction (Cheel, *et al*., 2005). Similarly, lemongrass infusion exhibited stronger antioxidant activities in relation to other extracts (methanolic, 80% aqueous ethanol and decoction). Further studies revealed that tannin and flavonoid fractions of oil-free infusion extract are most active anti-oxidative agents compared to phenolic acids fraction (Figueirinha, *et al.*, 2008). Aqueous ethanol extract was reported to exhibit antioxidant properties by decreasing reactive oxygen species production and lipid peroxidation, as well as, increasing superoxide dismutase activity and glutathione formation (Tiwari, *et al.*, 2010). Recently, essential oil of lemongrass was also reported to show antioxidant property by DPPH scavenging test. The results showed that both leaves and stalk extracts possess radical scavenging ability in a dose dependent manner (Mirghani, *et al*., 2012).

1.1.8. Anti-Inflammatory activity

Anti-Inflammatory Activity of *Cymbopogon citratus* leaf infusion was studied and used for the treatment of inflammatory diseases, in particular of the gastrointestinal tract (Figueirinha, *et al*., 2010). Citral and other monoterpenes from lemongrass, exhibits *in-vivo* anti-inflammatory activity using carrageenan induced paw edema and peritonitis in model rat. Paw edema was reportedly reduced by application of citral (100 and 200 mg/kg body weight) and peritonitis was also reduced (Quintans-Júnior, *et al*., 2011).

1.1.9. Anti-diarrhoeal activity

In practice, the whole stalk and the leaf of lemongrass are boiled and the decoction is drunk to relieve the diarrhea (Tangpu and Yadav, 2006).

1.1.10. Anti-hepatotoxic activity

The aqueous leaf extracts of *Cymbopogon citratus* showed anti-hepatotoxic action against cisplatin, induced hepatic toxicity in rats. Hence the extracts have the potential to be used for the management of hepatopathies and as a therapeutic adjuvant in cisplatin toxicity (Arhoghro, *et al.*, 2013).

1.2. *Brassica nigra*

Brassica nigra commonly called is black mustard (Anand, *et al.*, 2009).

1.2.1. Classification:

Kingdom: Plantae.

Subkingdom: Tracheobionta.

Super division: Spermatophyta.

Division: Magnoliophyta.

Class: Liliopsida.

Subclass: Dilleniidae.

Order: Capparales.

Family: Brassicaceae.

Genus: Brassica L.

Species: Brassica nigra (L).

1.2.2. Plant description and distribution

The plant is found in some parts of the world like southern Mediterranean region of Europe, south Asia, Canada, India, Ethiopia, North America, German, Ukraine, Myanmar, Russian, New Zealand (Zemede, 1995; Sokman, 1999). *Brassica nigra* originated in the Middle East. The plant can grow from two to eight feet tall, with racemes of small yellow flowers. The leaves are covered with small hairs. Stem base, half way branched, quite erect, bluish lower part. Alternate, stalked basal bluish green leaves. Its seeds grow in long, slender pods. Each pod contains 10 – 12 brown or black seeds (Gomezde, *et al.*, 1998).

1.2.3. Chemical constituents

Mustard seeds contain omega-3 fatty acids, essential oils, the minerals selenium, phosphorus, manganese, magnesium, iron, calcium, zinc, vitamins A, B-complex, and C, dietary fiber, protein, and phytonutrients (Nielsen and Rios, 2000). The proximate analysis of *Brassica nigra* seeds shows that it contains saponins (12.82%), alkaloids (20.58%), flavonoids (6.57%), glycosides (20.01%), reducing sugar (5.56%), phlobatanins (15.05%) and volatile oil (25.13%) moisture (4.16%), ash (5.14%), crude fat (30.30%), crude fibre (0.30%), crude protein (24.70%) and carbohydrate (35.40%). Crude fibre has the least value, while carbohydrate has the highest value. The carbohydrate content 35.40% of the seeds is lower than that of *Chromolaena odoratum* 45.70%. The ash content indicates the presence of mineral elements in the seeds. The value (5.14%) is higher compared to 1.80% in sweet potato leaves but lower than that of *chromolaena odoratum* 7.88% (Uzama, *et al.,* 2013).

1.2.4. Uses of *Brassica nigra*

Brassica nigra is widely used in ethnomedicine to treat several non-communicable diseases, chronic diseases and other degenerative disorders (Velisek, *et al.,* 1995). *Brassica nigra* seeds are used as a spice. They have also been used to treat rheumatism. The seed oil is used for common cold and arthritis. The seed is also used for relieving water retention (edema) by increasing urine production and increasing appetite (Gomezde, *et al.,* 1998).The seeds have significant amount of fatty oil, which is used as cooking

oil. Ground seeds of the plant are mixed with honey and used as cough suppressant. It is also used to treat respiratory infections (Zahra, *et al.*, 2012). The oil extracted from the seeds is very effective as antibacterial. Oil of *Brassica nigra* seeds is known to cleanse the blood and treat skin diseases because of its high sulphur content and have been used as preservatives in pickles and salads(Aiyaa, 2012).

1.2.5. Anti- microbial activity of*brassica nigra*

a- Anti a bacterial potential

The methanol extract of *Brassica nigra* was effective against *Pseudomonas aeruginosa*, *E. coli* and *Bacillus amyloliquifaciens* and offered inhibition zone of 10mm, 9mm, and 11mm respectively. *Lactococcus lactis* was found resistant to methanol extracts of *Brassica nigra*. On the other hand acetone extract of *Brassica nigra* recorded zone of inhibition of 8mm against *Lactococcus lactis*, 11mm against *E. coli* and 14mm against *Bacillus amyloliquifaciens*. *Pseudomonas aeruginosa* was found resistant to acetone extract of *Brassica nigra*. Negative control disc (containing only methanol and acetone) showed no zone against any tested bacteria. All the positive controls showed antibacterial activity against tested bacteria (Seemal, *et al*., 2013).

b- Anti-fungal activity

The essential oil of *brassica nigra* was inhibited the growth of *Aspergillus-niger, Aspergillus ochraceus* and *Penicillium citrinum* (Mejia, *et al.*, 2015)

1.2.6. Anti-oxidant properties of *Brassica nigra*

The plant is a source of α-tocopherol which is known to protect cells against oxidative damage triggered by free radicals (Yusif, *et al.*, 2007). Aqueous extract of *Brassica nigra* is claimed to inhibit lipid peroxidation on human erythrocyte membranes (Sujatha, 1995). Experimental researches suggest the existence of flavonoids with antioxidant effects in the hydro-alcoholic *Brassica nigra* seed extract (Oliver, *et al.*, 1990). The seed also consists of vitamin A that is a potent antioxidant. So, vitamin A is able to prevent the kindling and convulsion, and is also able to inhibit the challenge of dose-induced tonic seizure (Sayyah, *et al.*, 2005), one of the mechanism's actions of the plant that could be related to it. Free radicals are involved in pathogenesis of many diseases such as epilepsy. The important effect of free radicals is membrane lipid peroxidation and tissue injury by which results in cell membrane destruction and its dysfunction. Normally, biological effects of free radicals in the body is controlled by a lot of antioxidants such as vitamins A, C and E, glutathione and also via anti-oxidant enzymes like glutathione reductase (GR), glutathione peroxidase (GP), and catalase (Ilhan, *et al.*, 2006 ; Sudha and Rao, 2001).

1.2.7. Anti-inflammatory activity

With respect to the findings of *Brassicanigra* extracts on inflammatory models, it can be mentioned that the ethanolic extracts have a significant effect on acute inflammation as shown in the carrageenan-induced paw oedema model but not on subacute

inflammation as shown in the Cotton-pellet granuloma model (Gupta, *et al.*, 2010).

1.2.8. Cytotoxic activityof *Brassica nigra*

The *Brassica nigra* extracts were tested for cytotoxic activity against five cancer cell lines. *Brassica nigra* extracts showed a potent cell growth inhibition activity on all tested cancer cell lines. Cytotoxicity screening models provide important preliminary data to help selecting plant extract with potential antineoplastic properties for future work (Cardellina, *et al.*, 1990).

1.2.9. The antiepileptic activity of *Brassica nigra*

The antiepileptic activity of methanolic extract of *Brassica nigra* seeds was investigated on maximal electroshock induced seizures (MES) in mice. It was found that the extract (200 and 400 mg/kg, orally), significantly prolonged the onset of tonic seizures and reduced the duration of incidence of seizures (Uppala, *et al.*, 2013).

The anti-epileptic effect of the methanolic extract of *Brassica nigra* seeds (75, 150 and 300 mg/Kg; ip) was evaluated in pentylentetrazole (PTZ) - induced kindling in mice. The methanolic extract of *Brassica nigra* seed reduced the intensity and duration of seizure. In addition, the *Brassica nigra* extract increased the SOD and NO levels and decreased the MDA level in the brain tissues (Kiasalari, *et al.*, 2013).

1.3. *Cymbopogon nervatus*

Common names of this plant are Nal or Naal.

1.3.1. Classification

Kingdom: *Plantae.*

Subkingdom: *Tracheobionta.*

Super division: *Spermatophyta.*

Division: *Magnoliophyta.*

Class: *Liliopsida.*

Subclass: *Commelinidae.*

Order: *Cyperales.*

Family: *Poaceae.*

Genus: *Cymbopogon* Spreng.

Species: *Cymbopogon nervatus* (Hochst.) Chiov.

1.3.2. Plant description and distribution

Africa: north-east tropical, Asia-temperate: Arabia, Asia tropical: Indo-China (Clayton, *et al.,* 2006), *Cymbopogon nervatus* (Hochst) Chiov, represents one of the predominant wild grasses in the central Sudan, east central Sudan as well as western Sudan. Rarely perennial grass, up to 1.5 mhigh, usually around 1.0 m.First,erect glabrous, tufted annual, leaves broadly ovate – lanceolate, acute at the apex, slightly narrowed at the base, glabrous, not keeled, 0.4 cm long. Leaf sheath loose, glabrous andlight glaucous (Braun *et al.,* 1991).

Culm glabrous, nods glabrous, sometime purplish. Broadened semi – amplexcaule at the base, glabrous, base covered with mealy

white excrete. Leaf sheath tight in lower, more or less loose in upper parts, glabrous, striate, covered more or less mealy white excrete, Ligules membranous. Inflorescences narrow, more or less dense, spathes lanceolate, keeled, glabrous, spatheoles narrowly lanceolate, glabrous, fertile spike lets sessile, lanceolate, awned, glumes bordered with green nerves and brown oil marks on each side, keels whitish and wringed, the upper pedicel led spike let is male, the lower nature. Seeds spindle – shaped, glabrous, brown, 2.0mm (Braun, *et al.,* 1991).

1.3.3. Chemical constituents

Generally essential oil content of *Cymbopogonnervatus* inflorescence in Sudan vary from 0.8 – 1.5% (Anand, 2010). the main constituents of the essential oil, which represented about (1.3%) of the dried inflorescence, were: cis-p-mentha-1(7), 8-dien-2-ol (25.2%), trans-p-mentha-1(7)-8-dien-2-ol (22.9%), 2-(1- methyl-propyl)-cyclopentanone (11.3%), trans-carveol (9.6%) and trans-p-2,8-mentha-dien-1-ol (8.4%) (Abushama, *et al*.,2013).

1.3.4. Uses of *Cymbopogon nervatus*

In Sudanese traditional medicine, the inflorescence of *Cymbopogon nervatus*is used as decoction to treat kidney pains and urethritis (El-Kamali, *et al.,* 2005). Traditionally the leaves are used to treat indigestion and also as a carminative and tonic (El-Kamali and El-Khalifa, 1999).

1.3.5. Anti- microbial activity of *Cymbopogon nervatus*

a-Anti-bacterial potential

The Antibacteriala ctivity of the leaves essential oil of *Cymbopogon nervatus* was tested against six standard bacteria. The leaves essential oil of *Cymbopogon nervatus* showed high activity against all organisms tested (Ahmed, *et al.*, 2015). *Cymbopogon nervatus* inflorescence essential oil has demonstrated antibacterial activities against Shigella dysenteriae and Klebsiella pneumonia (El-Kamali, *et al.*, 2005). The oil of Cymbopogon nervatus showed high activity against both Gram positive *Staphylococcus aureus* and *Bacillus subtilis*, and against Gram negative *Escherichia coli* and *Pseudomonas aeruginosa* (Abushama, *et al.*, 2013).

b-Anti-fungal activity

The oil of *Cymbopogon nervatus* showed high activity against *C. cyminum* and *O.basilicum* (Nwosu and Okafor, 1995). The essential oil *C. nervatus* showed its maximum antifungal effect on *A. niger* and *C.albicans* (Abushama *et al.*, 2013). This oil reduced spore germination in *A. flavus*, *A. fumigatus*, *A. alternata*, *P. citrinum* and *T. harzianum* (Mahanta, *et al.*, 2007).

1.3.6. Anti-oxidant properties of*Cymbopogon nervatus*

In the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, essential oil from inflorescence of *C. nervatus*showed moderate activity with SC50 value of 23.8 µl/ml (Hellali, *et al.*, 2016).

1.3.7. Spasmolytic activity of essential oil of *Cymbopogon nervatus*

Spasmolytic activity against spontaneous contractions essential oil (10- 200 μg/ml) dose-dependently relaxed spontaneous contractions of isolated ileum and in concentration of 200 μg/ml exhibited 88.43% of maximal relaxant effect of atropine (6,4 μM) (Devi, *et al.*, 2011).

1.4. Essential Oils

Essential oils are volatile oils distilled from plant materials and represent the typical flavour and aroma (the essence) of a particular plant essential oils are also known as volatile oils, ethereal oils or simply as the oil of the plant from which they were extracted (Agarwal, 1996). An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils (Somesh, *et al*., 2015).

Essential oils are natural products that plants produce for their own needs other than nutrition (i.e. protection or attraction). In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants (Harrewijn, *et al.*, 2001).

Essential oils are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colorless at room temperature. They have a characteristic odor, and usually liquid at room temperature and have a density less than

unity, with the exception of a few cases (cinnamon, sassafras, and vetiver). They have a refractive index and a very high optical activity. These volatile oils contained in herbs are responsible for different scents that plants emit. They are widely used in the cosmetics industry, perfumery, and also aromatherapy (Burt, 2004).

They can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and therefore extracted from these parts, where they are stored in secretary cells, cavities, canals, epidemic cells or glandular trichomes (Bakkali, et al. 2008). Oil is essential in the sense that it contains the essence of the plant's fragrance the characteristic fragrance of the plant from which it is derived. The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism (Albert, *et al*., 1980).

The extracted oil content of plant material is low, typically 1 to 3% of the plant weight. They are thus low-volume, very high value products. This makes them attractive crops for remote smallholders where high transport costs prevent the transport of lower value cash crops. The quality of the oil obtained from a particular species will be influenced by where it is grown and how it has been processed. Essential oils have been used for over 5000 years for a variety of different purposes, including personal care (i.e., perfumes and cosmetics), foods, home care, repellents for humans and animals (livestock and domestic animals), and health-

promoting agents for the treatment of various diseases (Kumar and Tripathi, 2011).

Despite differences in chemical composition of essential oils obtained from different plants with diverse preparation methods, their main constituents belong to the same chemical classes, such as mono- and sesquiterpenes, aldehydes, ketones, ethers and esters, alcohols and hydrocarbons. The presence of these compounds determines both chemico-physical properties (i.e., liquid at room temperature, soluble in organic solvents and insoluble in water) and biological properties such as antibacterial, antifungal, antioxidant, spasmolytic, carminative, hepatoprotective, and analgesic activities (Grbovic, *et al.*, 2010).

1.4.1. Chemistry of essential oils

Extracted oils are complex mixtures of sometimes hundreds of chemical compounds. Pure essential oils can be essentially classified into two groups (Oprean, *et al*. 1998)

a-Volatile fraction

Essential oil constituting of90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

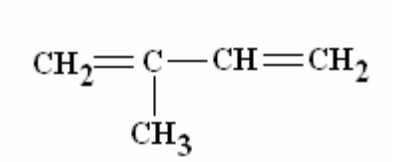
b-Nonvolatile residue

It comprises 1–10% of the oil containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoid.

Most of these compounds can be grouped into a few major classes but there are also many components of essential oils that bear little resemblance to these classes. In the overview of important and characteristic components given below, compounds are classified into four major groups: aliphatic compounds, terpenes and terpene derivatives, benzene derivatives and miscellaneous compounds (Tezel and Hortacsu, 2006)

i- Hydrocarbons

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic hydrocarbon found in plants is isoprene having the following structure.

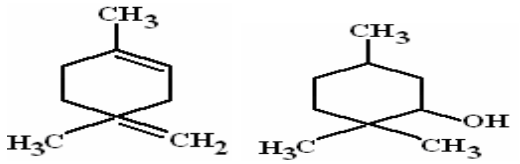


ii- Terpenes

The terpenoids are the most important group of natural products as essential oils are concerned. Generally have names ending in "ene." For examples: limonene, pinene, piperene, camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal (Baser and Demirci 2007). Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes, referring back to isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes (Oprean, *et al.,* 2001).

a-Monoterpenes [$C_{10}H_{16}$]

Monoterpenes are analgesic, bactericidal, expectorant, and stimulant. monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C_{10}), but some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids (Kumar and Tripathi, 2011). Monoterpenes can be cyclic molecules (menthol, monocyclic; camphor, bicyclic; pinenes (α and β) – pine genera as well (Tezel, *et al*., 2000).



Limonene Menthol

b-Sesquiterpenes

Sesquiterpenoids contain 15 carbon atoms and this result in their having lower volatilities and hence higher boiling points than monoterpenoids (Baser and Demiric, 2007). Sesquiterpenes are anti-inflammatory, antiseptic, analgesic, and antiallergic. Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic.

c-Diterpenes

Diterpenes are antifungal, expectorant, hormonal balancers, hypotensive. Diterpenes are made up of four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils.

Diterpenes occur in all plant families and consist of compounds having a C_{20} skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones such as gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. Diterpenes have limited therapeutical importance and are used in certain sedatives (Kumar and Tripathi, 2011).

iii- Alcohols

Alcohols are antiseptic, antiviral, bactericidal and germicidal. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use.

iv- Aldehydes

Aldehydes are antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal, disinfectant and sedative. Medicinally, essential oils containing aldehydes are effective in treating *candida* and other fungal infections.

v- Acids

Acids are anti-inflammatory. Organic acids in their free state are generally found in very small quantities within essential oils. Plant acids act as components or buffer systems to control acidity (Oprean, *et al.*, 2001).

vi- Esters

Esters are formed through the reaction of alcohols with acids.Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system (Virendra and Diwaker, 2006).

vii- Ketones

Ketones are anticatarrhal, cell proliferant, expectorant, and vulnerary. Ketones often are found in plants that are used for upper respiratory complaints because they assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic.

viii- Lactones

Lactones are known to be particularly effective for anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action then ketones (Kumar and Tripathi, 2011).

1.4.2. Application of essential oil

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world

production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential oils are obtained from plant raw material by several extraction methods (Wang and Weller, 2006).

***i*-As aromtherapy**

Aromatherapy is a form of alternative medicine that uses volatile plant materials, known as essential oils, and other aromatic compounds for the purpose of altering a person's mood, cognitive function or health. Science has discovered that our sense of smell plays a significant role in our overall health. Essential oils have been used in medicine because of their medicinal properties, for example some oils have antiseptic properties. In addition, many have an uplifting effect on the mind, though different essential oils have different properties. When essential oil is inhaled it goes directly from olfactory system to limbic system of the brain. Brain responds to the particular scent affecting our emotions and chemical balance. Essential oils also are absorbed by the skin and carried throughout the body via the circulatory system to reach all internal organs. We can be benefited by choosing carefully the desired and suitable oils which can promote overall health. Benefits depend upon the unique nature of each person’s response to an aromatic stimulus (Yatri, et al., 2011).

ii-As antiseptic

The antiseptic properties of essential oil make them active against wide range of bacteria as on antibiotic resistant strains. In addition to this they are also used against fungi and yeasts. The most common sources of essential oils used as antiseptics are: *cnnamon*, *thyme*, *clover*, *eucalyptus*, *culinsavory*, and *lavender*. Citral, geraniol, linalool and thymol are much more potent than phenol (Manthey,2004).

iii- Expectorants and diuretics

When the essential oils are used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains. Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilatation and in consequence bring about a diuretic effect.

iv- Spasmolytic and sedative

Essential oils from the Umbellifereae family, Mentha species and verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia (Rapisararda,1999).

v- Antimicrobial activity

The antimicrobial properties of essential oils and of their constituents have been considered and the mechanism of action has been studied in detail (Lambert, *et al.*, 2001). An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable (Sikkema, *et al.*, 1994). This can then cause leakage of ions and other cellular molecules (Gustafson, *et al.*, 1998; Cox, *et al.*, 2000). Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death (Denyer and Hugo, 1991).

Essential oils and/or their constituents can have a single target or multiple targets of their activity. For instance, trans-cinnamaldehyde can inhibit the growth of *Escherichia coli* and *Salmonella typhimirium* without disintegrating the outer membrane (OM) or depleting intracellular ATP. Similar to thymol and carvacrol, trans-cinnamaldehyde likely gains access to the periplasm and deeper portions of the cell (Farag, *et al.*, 1989). Carvone is also ineffective against the OM and does not affect the cellular ATP pool (Cosentino, *et al.*, 2002). It has been reported that Essential oils containing mainly aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol were characterized by the highest antibacterial activity, followed by essential oils containing terpene alcohols. Other essential oils, containing ketones or esters,

such as myrcene, thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive (Dorman and Deans, 2000).

Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, have important antibacterial activities (Lambert, *et al.*, 2001; Dorman and Deans, 2000). These compounds are responsible for the disruption of the cytoplasmic membrane, the driving force of protons, electron flow, active transport, and also coagulation of cell contents (Sikkema, *et al.*, 1994; Denyer and Hugo, 1991; Pauli, 2001). The chemical structure of essential oils affects their mode of action concerning their antibacterial activity. The importance of the presence of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, was confirmed (Fabian, *et al.*, 2006).

However, the relative position of the phenolic hydroxyl group on the ring does not appear to influence the intensity of the antibacterial activity. The thymol action against *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol, for example (Lambert, *et al.*, 2001; Ultee, *et al.*, 2002). However, carvacrol and thymol act differently against Gram-positive and Gram-negative species. Thymol, eugenol, and carvacrol have an antimicrobial effect against a broad spectrum of bacteria: *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica*, *Clostridium jejuni*,

Lactobacillus sake, *Staphylococcus aureus*, and *Helicobacter pyroli* (Marino, *et al.*, 1999; Senatore, *et al.*, 2000).

Other families of compounds also have valuable antibacterial properties: certain alcohols, aldehydes, and ketones, monoterpene (geraniol, linalol, menthol, terpineol, thujanol, myrcenol, citroneliai, neral, thujone, camphor, carvone, etc.), phenylpropanes (cinnamaldehyde), and monoterpenes (terpinene, p-cymene). Among these compounds, carvacrol is the most active. Known to be non-toxic, it is used as a preservative and food flavoring in drinks, sweets, and other preparations. It is Important to mention that essential oils are more active against Gram-positive than Gram-negative bacteria. The latter are less susceptible to the action of essential oils with the outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide film (Canillac and Mourey, 2001).

Furthermore, the antibacterial activity of essential oils related to their chemical composition, the proportions of volatile molecules, and their interactions. An additive effect is observed when the combination is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less important when they are tested together than when used individually (Gill, *et al.,* 2002). A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects (Reichling, *et al.*, 2009). Some studies have shown that the use of the whole essential oil provides an effect which is

greater than that of the major components used together (Burt, 2004). This suggests that minor components are essential for activity and may have a synergistic effect. It has been reported additive and synergistic effects of the combinations of 1,8-cineole and aromadendrene against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistantent erococci (VRE) and *Enterococcus faecalis* by using checkerboard and time-kill assays, respectively (Edris, 2007).

The combined effects of plant volatile oils and benzoic acid derivatives against *L. monocytogenes* and *S. enteritidis* are considered as synergistic (Braga, *et al.*, 2006). Increased antifungal effects were caused by combinations (1:5, 1:7, and 1:9) of essential oils of *S. aromaticum* (clove) and Rosmarinus officinal is against *C. albicans* (Aruoma, 1998). Moreover, Lambert et al. reported that, combined, carvacrol and thymol showed additive effects against *S. aureus* and P. *aeruginosa* by using half-fold dilutions within the Bioscreen plat. Two hypotheses have been proposed to explain synergistic effects of cinnamaldehyde/thymol or cinnamaldehyde/carvacrol against *S. typhimurium*: proving, on one hand, that thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell, and, on the other hand, that thymol or carvacrol could increase the number, size, or duration of the existence of the pores created by the binding of cinnamaldehyde to proteins in the cell membrane (Kamatou and Viljoen, 2010).

These facts justify a synergistic effect achieved when these two components are used in combination. Mechanisms of interaction that produced antagonistic effects were less studied (Maruyama, *et al.*, 2005). In addition, essential oils have also revealed to be effective on the inhibition of growth and reduction in numbers of the more serious food borne pathogens, such as *Salmonella spp*., *E. coli* O157:H7, and *Listeria monocytogenes* (Braga, *et al.*, 2006).

vi- Antiviral activity

The complex mixture of essential oils usually shows a higher antiviral activity than individual compounds (due probably to synergism phenomena); with exception of β-caryophyllene which is the most famous antiviral compounds found in many different essential oils from different plant families. Different mechanisms of antiviral activity of different essential oils and their constituents seem to be present. The antiviral activity of the essential oil is principally due to direct virucidal effects (by denaturing viral structural proteins or glycoproteins). Proposed mechanisms suggest that essential oils interfere with the virus envelope by inhibiting specific processes in the viral replication cycle or by masking viral components, which are necessary for adsorption or entry into host cells, thus, they prevent the cell-to-cell virus diffusion (Saddi, *et al.*, 2007).

vii- Antioxidant Activity

Numerous studies have demonstrated the antioxidant properties of essential oils. The antioxidant potential of an essential oil depends on its composition. It is well established that phenolics and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties (Koh, *et al.*, 2002). Most of the essential oils are dominated by oxygenated monoterpenes such as alcohols (*Achillea filipendulina*), aldehydes (*Galagania fragrantissima*), ketones (*Anethum graveolens*, *Artemisia rutifolia*, *Hyssopus seravschanicus*, *Mentha longifolia*, and *Ziziphora clinopodioides*), and esters (*Salvia sclarea*). *Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons, whereas phenolic terpenoids, such as thymol or carvacrol, characterize *Origanum tyttanthum* and *mentha longifolia* essential oils, which would explain why both plants exhibited generally the strongest antioxidant activity. Thymol and carvacrol, which are predominant in *Origanum tyttanthum*, are also responsible for the antioxidant activity of several other essential oils, such as *Mentha longifolia* and *Thymus serpyllus* (Caldefie-Chézet, *et al.*, 2004).

The essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties (Aruoma, 1998). Thymol and carvacrol are the most active compounds. Their activity is related to their phenolic structure. These phenolic compounds have redox properties and,

thus, play an important role in neutralizing free radicals and also in peroxide decomposition (Burt, 2004). The antioxidant activity of essential oils is also due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1, 8-cneoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: terpinene, -terpinene and terpinolene (Aruoma, 1998).

In fact, diseases may result from cellular damage caused by free radicals (Kamatou and Viljoen, 2010). Essential oils have shown their action as hepatoprotective agents in ageing polyunsaturated fatty acids mammals and it has been proved that they possess a beneficial impact upon the PUFAs, in particular the long chain C_{20} and C_{22} acids (Caldefie-Chezet, *et al.*, 2006). Moreover, essential oils being able to scavenge free radicals may also play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and immune system decline (Hart, *et al.*, 2000).

Sharififar et al. evaluated the antioxidant activity of *Zataria multiflora* Boiss (Lamiaceae) essential oil in rats. Antioxidant activity was measured by the test of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition and inhibition of lipid peroxidation by measuring the index of thiobarbituric acid reactive substances (TBARs). Three doses of 100, 200, and 400 µL/kg were administered to animals by intra gastric intubation (i.g) route for 10 days. The blood was collected in eleventh day through direct puncture and the liver was rapidly excised. The histopathology

studies of the animals were compared to animals in butylated hydroxyl toluene (BHT) group. The authors reported that all *Zataria multiflora* oils (ZMO) tested doses were able to scavenge DPPH radical ($p < 0.05$). Moreover, ZMO decreased TBARs in a dose-dependent manner. No alteration in liver function test LFT enzymes or changes in histopathology of the liver was considered in ZMO treated groups. The results indicated that ZMO might be used in human health and food industry (Sharififar *et al.*, 2011).

viii- Anti-Inflammatory Activity

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self cells) and to remove dead or damaged host cells. The inflammatory response induces an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines, such as interleukins and tumor necrosis factor (TNF). It also stimulates the activity of several enzymes (oxygenases, nitric oxide synthases, peroxidases, etc.), as well as the arachidonic acid metabolism. Recently, essential oils have been used in clinical settings to treat inflammatory diseases, such as rheumatism, allergies, or arthritis (Maruyama, *et al.*, 2005). *Melaleuca alternifolia* essential oil was reported to have a considerable anti-inflammatory. This activity is correlated with its major compound: -terpineol activity (Koh, *et al.*, 2002; Caldefie-Chezet, *et al.*, 2004 and Hart, *et al.*, 2000).

The active compounds act by inhibiting the release of histamine or reducing the production of inflammation mediators. Geranium essential oil is another example. Linalool and linalyl acetate showing anti-inflammatory activity on oedema of paw-induced mouse carrageenan (Maruyama, *et al.,* 2005).Yoon et al. reported that the oils of *Torreya nucifera Siebold* et Zucc. oil, which mainly consists of limonene, δ-3-carene, and α-pinene, has an inhibitory effect on COX-2, thus inducing a significant inhibitory effect on prostaglandin (PGE2) production. Furthermore, 1,8-cineole, present in many essential oils, was reported as an inhibitor of leukotrienes (LTB4) and PGE2, biogenerated both from pathways of arachidonic acid metabolism. The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. Essential oils, therefore, represent a new option in the treatment of inflammatory diseases (Sharififar, *et al.,* 2011).

ix- Cancer Chemoprotective Activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researchers for their potential activity against cancer. They and their volatile constituents are target for the discovery of new anticancer natural products (Edris, 2007). Essential oils would act in the prevention of cancer, as well as at its removal. It is well known that certain foods, such as garlic and turmeric, are

good sources of anticancer agents (Pyun and Shin, 2006). Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer, Diallylsulfide, diallyldisulfide, and diallyltrisulfide are examples (Milner, 2001; Milner, 2006).

x- Cytotoxicity

Due to their complex chemical composition, essential oils have no specific cellular ligands (Carson and Riley, 1995). As lipophilic mixtures, they are able to cross the cell membrane and degrade the layers of polysaccharides, phospholipids and fatty acids, and permeabilize. This cytotoxicity appears to include such membrane damage. (Sylvestre, *et al.,* 2007; Di Pasqua, *et al.,* 2006).

xi- Allelopathic Activity

According to the International Allelopathy Society (IAS), allelopathy was defined in 1996 as "The science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural and biological systems". Allelopathic interactions derive from the production of secondary metabolites. The secondary metabolites are synthesized for a wide range defense by plant and microorganisms. The secondary metabolites involved are called allelochemicals (Moon, *et al.,* 2006). Volatile oils and their constituents are being explored for weed and pest management, and are viewed as an important source of lead molecules in agriculture (Priestley, *et al.,* 2006).

Bioactive terpenoids constitute an important part of the defensive mechanisms of a large number of organisms and represent a fairly untapped source of active compounds of potential use both in the agricultural field (Rim and Jee, 2006). In fact, a large number of highly phytotoxic allelochemicals are derived from the terpenoid pathway and the phytotoxicity of essential oils has been investigated (Macías, *et al.*, 2006; Angelini, *et al.*, 2003). The allelopathic activity of *Melaleuca alternifolia* (Maiden and Betche) Cheel (tea tree) essential oil was investigated by Angelini et al., against *Trichoderma harzianum*, which is a fungal contaminant that causes extensive losses in the cultivation of Pleurotus species (Macias, *et al.*, 2006).

This essential oil has, in vitro, an allelopathic ability to control *Trichoderma harzianum*. The antifungal activity of *M. alternifolia* essential oil and antagonist activities between Pleurotus species against three *T. Harzianum* strains were studied in dual-culture experiments done with different concentrations. Santos et al. reported that leaves' and rhizomes' essential oils caused a decrease in dry matter. They also reported a reduction of shoot length in lettuce seedlings. Evaluating the effect of these essential oils on the germination and vigor of the lettuce seedlings, they noticed a reduction of these parameters and concluded that rhizomes' oil caused a greater reduction in all of the variables than the oil from the leaves. *Portulaca oleracea* seeds' germination and growth were

significantly decreased by the treatment with rosemary EO (Dudai, *et al.*, 1999).

The results of the research of Saad and Abdelgaleil, 2014 revealed a correlation between essential oils chemical composition and their effects on germination and seedling growth. It was reported that the most active compounds belonged to the groups of ketones and alcohols and were followed by the group of aldehydes and phenols (Astani, *et al.*, 2010). Moreover, Kotan, et al. suggested that, in general, a potentphytotoxic activity of plant essential oils is correlated to a high amount of oxygenated monoterpenes. Almost all the effective oils had high percentages of oxygenated monoterpenes and this was in agreement with previous work of de Almeida, *et al.* and Vokou, *et al.* Dudai, et al. who reported that monoterpenes act on seeds at very low levels. In particular, among the lamiaceae family, many species release phytotoxic monoterpenes that hinder the development of herbaceous species, including pinene, limonene, p-Cymene, and 1, 8-cineole (Angelini, *et al.*, 2003).

xii- Repellent and Insecticidal Activity

Essential oils constitute a rich bank of structurally-diverse compounds with a variety of insecticidal and repellent mechanisms. Numerous studies have demonstrated that these compounds, as well as their parent blends, possess biological activity capable of eliciting adverse effects in arthropod pests. Several factors affecting the commercialization of plant essential oil extracts as repellents include regulatory requirements, intellectual property value, biological

activity, product performance, and product quality (Ahmed and Eapen, 1986).

The toxic effect of essential oils was not only suitable for granary insects but also for flying insects: Gaultheria (Ericaceae) and Eucalyptus (Myrtaceae) oils exhibited very high killing power on insects, such as the rice weevil *Sitophilus oryzae*, the beetles *Callosobruchus chinensis* (Coleoptera: Bruchidae) and *S. paniceum*, and also on *M. domestica* (Mateeva and Karov, 1983). Actually, the activities of essential oils on species are manifold. *Mentha lavandula* (Lamiaceae), or *Pinus* (Pinaceae) essential oils were noted for their toxicity against *Myzus persicae* (Homoptera: Aphidida*e*) and the greenhouse white fly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), as well as the Colorado beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and the pear bug *Stephanitis pyri* (Hymenoptera: Stephanidae) (Hamraoui and Regnault-Roger, 1995).

Commonly, essential oils can be inhaled, ingested, or skin-absorbed by insects. The fumigant toxicity of essential oils and their main components, the volatile monoterpenes, has been described (Regnault-Roger and Hamraoui, 1995). Insects were also very sensitive to topical applications *Sitophilus zea-mais* (Coleoptera: Curculionidae), *Tribolium castaneum* and *Prostephanus truncatus* (Coleoptera: Bostrychidae) reacted to citrus (Rutacae) essential oils. *Pediculus capitis* (Anoplura: Pediculidae), *Anopheles funestus* (Diptera:Culicidae), *Cimex lectularius* (Hemiptera: Cimicidae), and

Periplaneta orientalis (Dictyoptera: Blattidae)were killed by contact with *Eucalyptus saligna* (Myrtaceae) oil within 2 to 30 min. Essential oils belonging to plants in the *citronella genus* (Poaceae) are commonly used as ingredients of plant-based mosquito repellents, mainly *Cymbopogon nardus*, which is sold in Europeand North America in commercial preparations (Maia and Moore, 2001).

1.5. Extraction of essential oils

There are a number of methods for essential oils isolation, e.g. hydrodistillation (HD), steam distillation (SD) and organic solvent extraction (Presti, *et al.*, 2005). Traditional methods for the extraction of essential oils from medicinal plants are known to be SD and HD. It is known that these methods suffer from some disadvantages including losses of volatile compounds, long extraction times and energy intensive. However, these are simplest methods of extraction and their equipments are often more available than novel methods of extraction like microwave-assisted hydrodistillation (MAHD) and ohmic-assisted hydrodistillation (OAHD) which are using microwave and ohmic energy as the heating source, respectively (Stashenko, *et al.*, 2004, Gavahian, *et al.*, 2011).

1.5.1.Conventional Extraction

1.5.1.1. Hydrodistillation (HD)

Hydrodistillation is a traditional method for removal of essential oils. Water or hydrodistillation is one of the oldest and easiest methods being used for the extraction of essential oils.

Hydrodistillation normally used for isolation essential oils from the aromatic and medicinal plant. The conventional method for the extraction of essential oils is hydrodistillation (HD), in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser. The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively (Ranjitha and Vijiyalakshmi, 2014).

The principle of extraction is based on the isotropic distillation. Hydro-distillation (HD) is a variant of steam distillation, which is bespoke by the French Pharmacopoeia for the extraction of essential oils from dried plants. There are three types of hydrodistillation: with water immersion, with direct vapor injection and with water immersion and vapor injection. It is a multilateral process that can be utilized for large or small industries. The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of essential oil, but does add unwanted high boiling point compounds and oxidation products (Meyer-Warnod, *et al*., 1984).

1.5.1.2. Steam Distillation

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation is still important in certain

industrial sectors. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plant from the base of the alembic to the top (Rai and Suresh, 2004).

Steam distillation is a method where steam flows through the material. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water. Furthermore, this technique can be also carried out under pressure depending on the essential oils extraction difficulty (Fahlbusch, *et al*., 2003).

1.5.1.3. Solvent extraction

Solvent extraction, also known as liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example, water and an organic solvent. In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. Solvent extraction is used in the processing of

perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost (Chrissie, *et al*., 1996).

The quality and quantity of extracted mixture are determined by the type of extra heat applied because of the method is limited by the compound solubility in the specific solvent used. Although the method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility (Dawidowicz, *et al*., 2008).

1.5.1.4. Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase (Harwood, *et al.*, 1989).

In a conventional soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back

into the distillation flask, thus carrying the extracted analytes into the bulk liquid. This procedure is repeated until virtually complete extraction is achieved. There are several advantages of Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix (Schantz, *et al*., 1998).

Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed (Luque de Castro and García-Ayuso, 1998).

1.5.1.5. Cold Pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification

method. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils. In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation (Arnould, *et al*., 1981).

1.5.1.6. Super Critical CO_2 Extraction

Supercritical CO_2 extraction (S CO_2) involves carbon dioxide heated to 87 degrees F and pumped through the plant material at around 8,000 psi, under these conditions; the carbon dioxide is likened to a 'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the esential oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance (Singh, *et al.*, 2003).

1.5.2. Green Extraction with Innovative Techniques

Since economy, competitiveness, eco-friendly, sustainability, high efficiency and good quality become keywords of the modern industrial production; the development of essential oils' extraction

techniques has never been interrupted. Strictly speaking, conventional techniques are not the only way for the extraction of essential oils. Novel techniques abided by green extraction concept and principles have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of official methods while reducing operation units, energy consumption, CO_2 emission and harmful co-extracts in specific cases. The principles of green extraction can be generalized as the discovery and the design of extraction processes which could reduce the energy consumption, allow the use of alternative solvents and renewable innovatory plant resources so as to eliminate petroleum-based solvents and ensure safe and high quality extracts or products (Chemat, 2012).

1.5.2.1. Turbo Distillation

This technique is developed to reduce energy and water consumption during boiling and cooling in hydro-distillation. The turbo extraction allows a considerable agitation and mixing with a shearing and destructive effect on plant materials so as to shorten distillation time by a factor of 2 or 3. Furthermore, it is an alternative for extraction of essential oils from spices or woods, which are relatively difficult to distill.Besides, an eco-evaporator prototype could be added with aspect of the recovery and the reuse of the transferred energy during condensation for heating water into steam (Chemat, 2010).

1.5.2.*2.* Ultrasound-Assisted Extraction

With the aim of higher extraction yields and lower energy consumption, ultrasound assisted extraction has developed to improve the efficiency and reduce the extraction time in the meanwhile. The collapse of cavitation bubbles generated during ultrasonication gives rise to micro-jets to destroy essential oils' glands so as to facilitate the mass transfer and the release of plant essential oils. This cavitation effect is strongly dependent to the operating parameters (e.g. ultrasonic frequency and intensity, temperature, treatment time, etc.) which are crucial in an efficient design and operation of sono-reactors.

(UAE) Extraction showed less thermal degradation with a high quality and a good flavor (Porto, *et al.*, 2009; Asfaw, *et al.*, 2005). However, the choice of sonotrode should be careful as the metallic contamination which may accelerate oxidation and subsequently reduce essential oils' stability. This technique has already proved its potency to scale up, which shows 44 % of increment on extraction yield of essential oils from Japanese citrus compared to the traditional methods (Mason, *et al.*, 2011).

1.5.2.3. Microwave-Assisted Extraction

Microwave is a non-contact heat source which can achieve a more effective and selective heating. With the help of microwave, distillation can now be completed in minutes instead of hours with various advantages that are in line with the green chemistry and extraction principles. In this method, plant materials are extracted in

a microwave reactor with or without organic solvents or water under different conditions depending on the experimental protocol (Craveiro, *et al.*, 1989).

The first Microwave-Assisted Extraction (MAE) of essential oils was proposed as compressed air microwave distillation (CAMD). Based on the principle of steam distillation, the compressed air is continuously injected into the extractor where vegetable matrices are immersed in water and heated by microwave. The water and essential oils are condensed and separated outside the microwave reactor. The CAMD can be completed in just 5 min and there is no difference in quantitative and qualitative results between extracts of CAMD and 90 min conventional extraction using steam distillation. In order to obtain high quality essential oils, vacuum microwave hydro-distillation (VMHD) was designed to avoid hydrolysis (Mengal, *et al.*, 1993).

Fresh plant materials are exposed to microwave irradiation so as to release the extracts; reducing the pressure to 100–200 mbar enables evaporation of the azeotropic water-oil mixture at a temperature lower than 100 °C. This operation can be repeated in a stepwise way with a constant microwave power, which is contingent on the desired yield. The VMHD, which is 5–10 times faster than classic HD, showed comparable yield and composition to HD extracts. The essential oils have organoleptic properties very close to the origin natural materials. Moreover, the occurrence of thermal degradation reduces because of the low extraction temperature.

Beyond that, in fact, there exist a couple of modern techniques assisted by microwave such as microwave turbo hydrodistillation and simultaneous microwave distillation, which are impressive for short treatment time and less solvent used (Ferhat, *et al.*, 2007; Périno-Issartier, *et al.*, 2010).

On account of growing concern for the impact of petroleum-based solvents on the environment and the human body, several greener processes without solvent have sprung up in the last decade. Solvent-free microwave extraction (SFME) was developed with considerable success in consistent with the same principles as MAE. Apart from the benefits mentioned before, the SFME simplifies the manipulation and cleaning procedures so as to reduce labor, pollution and handling costs (Li,*et al.*, 2013).

The SFME apparatus allows the internal heating of the in situ water within plant materials, which distends the plant cells, thus leads to the rupture of oleiferous glands. A cooling system outside the microwave oven allows the continuous condensation of the evaporated water-oil mixture at atmospheric pressure. The excessive water is refluxed to the reactor in order to maintain the appropriate humidity ofplant materials. It is interesting to note that the easy-controlled operating parameters need to be optimized for maximization of the yield and final quality. The potential of using SFME at laboratory and industrial scale has been proved on familiar plant materials with a considerable efficiency compared to conventional techniques (Filly, *et al.*, 2014).

Inspired by SFME, a number of its derivatives have emerged, which offer significant advantages like shorter extraction time, higher efficiency, cleaner feature, similar or better sensory property under optimized conditions (Michel, et al., 2011). In 2008, anovel, green technique namely microwave hydro-diffusion and gravity (MHG). This technique is a microwave-induced hydro-diffusion of plant material at atmospheric pressure, which all extracts including essential oils and water drop out of the microwave reactor under gravity into a continuous condensation system through a perforated Pyrex support. It is worth mentioning that the MHG is neither a modified MAE that uses organic solvents, nor an improved HD that are high energy and water consumption, nor a SFME which evaporates the essential oils with the in situ water only. In addition, MHG derivates such as vacuum MHG and microwave dry-diffusion and gravity (MDG) has developed later with the consideration of energy saving, purity of end-products and post-treatment of wastewater (Farhat, *et al.*, 2010; Zill-e-Huma, *et al.*, 2011).

1.5.2.4. Instantaneous Controlled Pressure Drop Technology

The DIC process is a direct extraction-separation technique, which is not like the molecular diffusion in conventional techniques. It allows volatile compounds to be removed by both evaporation for a short time at high temperature (180 °C) and high pressure (10 bars) and auto-vaporization from alveolated plant structures resulting from multi-cycle instantaneous pressure drop (Rezzoug, *et al.*, 2005; Besombes, *et al.*, 2010).

This solvent-free process presents a significant improvement whether in efficiency or in energy consumption and a very short heating time in each DIC cycle eliminate the thermal degradation. Moreover, the DIC obtained the same or even higher yield of Essential oils with a higher quality than conventional methods regarding to their more oxygenated compounds and lower sesquiterpene hydrocarbons. In addition, heating time and cycle number in particular, have an influence on the extraction efficiency of DIC for all aromatic herbs and spices (Allaf, *et al.*, 2013).

1.6. Gas Chromatography-Mass Spectrometry

Gas chromatography is a technique for separating volatile substance by percolating a gas stream over a stationary phase. This depends upon the adsorptive properties of the column packing to separate sample (Joulain, 1994). Gas chromatography (GC) is a method used to help identify a mixture of compounds by separating compounds according to each compound's retention time. The potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known (Vekey, 2001).

Gas chromatography (GC) is a widely applied technique in many branches of science andtechnology. For over half a century, GC has played a fundamental role in determining howmany components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds isambiguous and reduced, and requires a

spectroscopic detection system. The most used, is themass spectrometric detector (MSD), which allows obtaining the "fingerprint "of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elementalcomposition, if a high resolution mass spectrometer is used, functional groups present, and,in some cases, the geometry and spatial isomerism of the molecule (Stashenko and Martínez, 2014).

1.6.1. Principle

The mass spectrometer is a universal detector for gas chromatographs since any compound that can pass through a gas chromatograph is converted into ions in mass spectrometer. At the same time, the highly specific nature of mass spectrum makes the mass spectrometer a very specific gas chromatographic detector. Gas chromatography is an ideal separator, whereas mass spectrometry is excellent for identification(Willard, *et al.,* 1988). The aim of an interfacing arrangement is to operate both a gas chromatograph and a mass spectrometer without degrading the performance of either instrument. The problem is compatibility. One incompatibility problem is the difference in pressure required for the operation of a gas chromatograph and the mass spectrometer. Whereas the former operates at high pressures, the latter is designed to run under high vacuum. An associated problem is the presence of much carrier gas and little sample in the effluent from the gas chromatograph. If the gas chromatograph is using packed column the flow of carrier gas may be in excess of 30ml/min, which would

collapse the vacuum of the mass spectrometer. Therefore carrier gas must be substantially removed and various designs have to be developed (Sahil, *et al*., 2011).

1.6.2. Importance of gas chromatography

The two main chromatographic techniques used in modern analytical chemistry are Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC).HPLC uses a liquid mobile phase to transport the sample components (analytes) through the column, which is packed with a solid stationary phase material.HPLC was first proposed by the Russian botanist Mikhail Tswett who first used the term 'Chromatography' (Latin for 'colored drawing') in 1906, to describe the separation that occurred when solutions of plant pigments were passed through columns of calcium carbonate or alumina ,using petroleum ether.In contrast, gas chromatography uses a gaseous mobile phase to transport sample components through either packed columns or hollow capillary columns containing a polymeric liquid stationary phase. In most cases, GC columns have smaller internal diameter and are longer than HPLC columns. GC has developed into a sophisticated technique since the pioneering work of Martin and James in1951 , and is capable of separating very complex mixtures of volatile analytes (Berger, 1996).

Aim of the study

This study was aimed to:

- Extraction of the essential oils from three medical herbs, *Cymbopogon citratus, Brassica nigra and Cymbopogon nervatus.*
- Analysis of essential oils by GCMS.
- Screening the antimicrobial activity of essential oils.

Material and Methods

2.1. Materials

2.1.1. Plant material

- *Cymbopogon citrates*: was collected from Ministry of Agriculture and Forestry farm -Sudan.
- *Cymbopogon nervatus*: was collected from Medicinal and Aromatic Plants Research Institute (MAPRI).
- *Brassica nigra*: was collected from the local market-Omdurman -Sudan.

2.1.1.2. Bacterial microorganisms

- *Bacillus subtilis* NCTC 8236 (Gram + ve bacteria)
- *Staphylococcus aureus* ATCC 25923(Gram +ve Bacteria)
- Escherichia coli ATCC 25922(Gram -ve bacteria)
- *Pseudomonas aeruginosa* ATCC 27853 (Gram -ve bacteria)

2.1.1.3.Fungal microorganisms

- *Candida albicans* ATCC7596

2.2. Methods

2.2. 1. Extraction of essential oils

Cymbopogon citratus and *Cymbopogon nervatus* oils were extracted by steam distillation. Whole plant of *Cymbopogon citratus* and inflorescence of *Cymbopogon nervatus* were put separately into distillation apparatus over water, then the water was heated, the steam passed through the herb, vaporizing the volatile compounds. The vapors flowed through a coil, where they were condensed back to liquid, which is then collected in the

receiving vessel. At the end of the distillation process essential oils were separated from water depending on the difference in their density by using laboratory separating funnel. The oils were collected in dark pure bottle and had been ready to tests.The essential oil was isolated from mature seeds of *Brassica nigra* by hydro distillation.

2.2.2. GC-MS analysis

The qualitative and quantitative analysis of the sample was done by using GM/MS technique model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, with capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The samplewas injected by using split mode, helium as the carrier gas passed with flow rate 1.61ml/min, the temperature program was started from 60^0Cwith rate 10^0C/min reaching 300^0C as final temperature degree, the injection port temperature was 300^0C, the ion source temperature was 200^0C and the interface temperature was 250^0C.The sample was analyzed by using scan mode in the range of m/z 50-550 charges to ratio .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library ,the National Institute of Standards and Technology (NIST). , results were recorded.

Table 2.1: Chromatographic conditions

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

2. 2.3. Antimicrobial test

2.2.3. 1. Bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^{8}- 10^{9} C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The

plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.2.3.2.2. Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspended were stored in the refrigerator until used.

2.2.3.3. Testing of antimicrobial susceptibility

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the

inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Results and Discussion

3.1.GC-MS analysis of *Cymbopogon citratus* essential oil

Theessential oil of *Cymbopogon citratus* was analyzed by GC-MS. Fifty seven components appeared in total ion chromatogram (Fig.3.1). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in table (3.1).

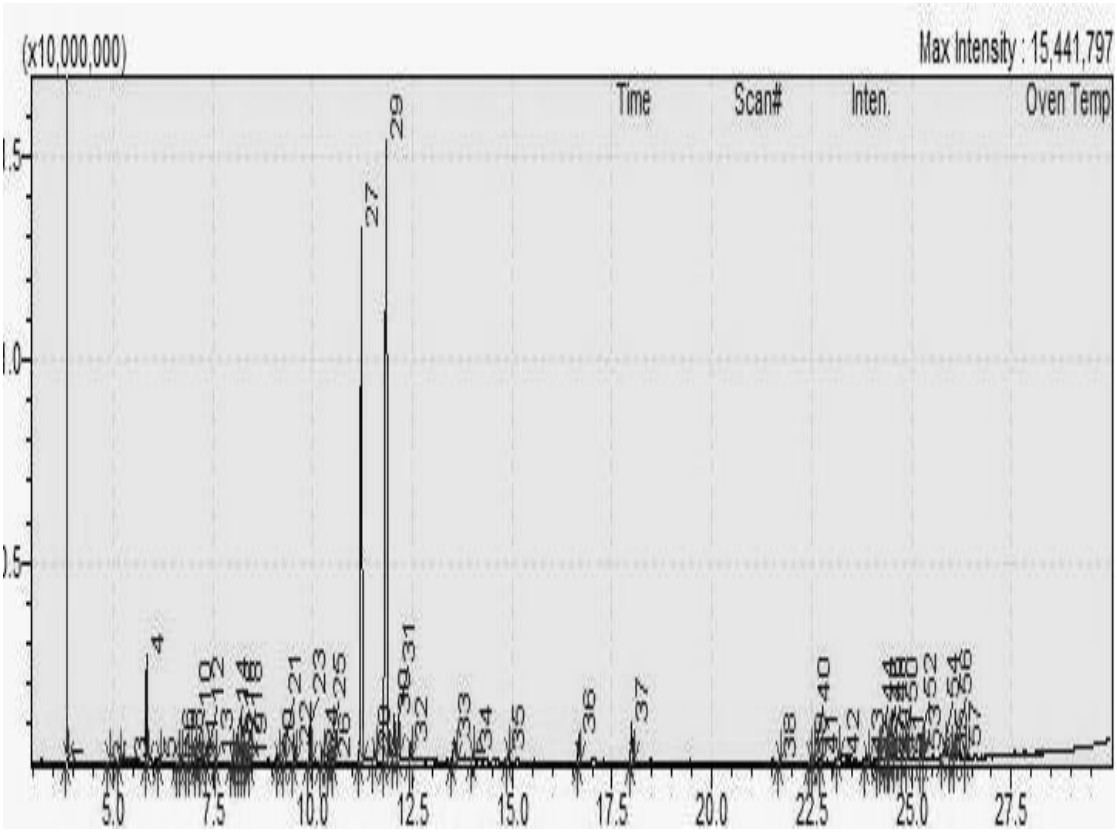


Fig.3.1: Total ion Cromatograms of *Cymbopogon citratus* oil

Table 3.1: Constituents of *Cymbopogon citratus* essential oil

Peak#	R.Time	Area	Area%	Name
1	3.847	43153	0.04	4-Heptanone
2	4.886	45854	0.04	.alpha.-Pinene
3	5.173	95965	0.08	Camphene
4	5.843	4662825	3.87	5-Hepten-2-one, 6-methyl-
5	6.155	128056	0.11	Octanal
6	6.618	65421	0.05	6-Octen-1-yn-3-ol, 3,7-dimethyl-
7	6.710	113342	0.09	D-Limonene
8	6.850	38681	0.03	trans-.beta.-Ocimene
9	6.933	31912	0.03	Acetic acid, cyclohexyl ester
10	7.069	41165	0.03	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-
11	7.203	117239	0.10	Cyclopropane, 2-(1,1-dimethyl-2-propenyl)
12	7.362	131234	0.11	Cyclohexane, (1,1-dimethylpropyl)-
13	7.582	378664	0.31	4-Nonanone
14	7.963	54361	0.05	.alpha.-Methyl-.alpha.-[4-methyl-3-penteny
15	8.105	89793	0.07	Ascaridole epoxide
16	8.174	988876	0.82	1,6-Octadien-3-ol, 3,7-dimethyl-
17	8.252	95764	0.08	Nonanal
18	8.338	233438	0.19	Carane, 4,5-epoxy-, trans
19	8.435	216588	0.18	di-t-Butylacetylene
20	9.132	419064	0.35	1,5-Heptadiene, 3,3-dimethyl-, (E)-
21	9.295	566455	0.47	Cyclopentane, 1-methyl-1-(2-methyl-2-prop
22	9.547	689909	0.57	cis-Verbenol
23	9.930	1932095	1.60	3-Cyclohexene-1-carboxaldehyde, 2,4,6-tri
24	10.237	1010247	0.84	Cyclopentanol, 1,2-dimethyl-3-(1-methylet
25	10.404	355914	0.30	Decanal
26	10.529	219237	0.18	Carveol
27	11.230	37801662	31.36	2,6-Octadienal, 3,7-dimethyl-, (Z)-
28	11.550	484671	0.40	2-Cyclohexen-1-one, 3-methyl-6-(1-methyle
29	11.850	52069929	43.20	2,6-Octadienal, 3,7-dimethyl-, (E)-
30	12.042	2095496	1.74	2-Furanmethanol, 5-ethenyltetrahydro-.alp
31	12.153	1982494	1.64	1,3-Propanediol, 2,2-diethyl-
32	12.455	744966	0.62	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-
33	13.556	2131724	1.77	Geranic acid
34	14.017	456007	0.38	Geranyl acetate
35	14.907	551763	0.46	Caryophyllene
36	16.665	1517211	1.26	.gamma.-Muurolene
37	17.989	2148279	1.78	Caryophyllene oxide
38	21.710	95384	0.08	2-Pentadecanone, 6,10,14-trimethyl-
39	22.458	57564	0.05	Ethyl geranate
40	22.538	111400	0.09	Cyclopropanecarboxaldehyde, 2-methyl-2-
41	22.750	278479	0.23	Formic acid, 3,7,11-trimethyl-1,6,10-dodec
42	23.179	387004	0.32	(1R,2R,3S,5R)-(-)-2,3-Pinanediol
43	23.874	307220	0.25	2-Bornanol, 2-methyl-
44	24.163	422956	0.35	(1R,4R)-p-Mentha-2,8-diene, 1-hydroperox
45	24.228	95060	0.08	2-Methylisoborneol
46	24.362	174988	0.15	endo-Borneol
47	24.409	159556	0.13	Bicyclo[5.2.0]nonane, 4,8,8-trimethyl-2-me
48	24.455	645362	0.54	Bicyclo[2.2.2]oct-2-ene, 1,2,3,6-tetramethyl
49	24.582	204673	0.17	7-Octylidenebicyclo{4.1.0}heptane
50	24.762	225163	0.19	1-Cyclobutanol, 1-methyl-2-(2,2-dimethyl-(
51	24.896	262622	0.22	Carveol, phenylcarbaminate(ester)
52	25.224	630283	0.52	Neric acid
53	25.306	424086	0.35	2-Buten-1-one, 1-(2,2,5a-trimethylperhydr
54	25.793	567911	0.47	Phytol
55	25.973	181991	0.15	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E
56	26.076	186026	0.15	5-Hydroxymethyl-1,1,4a-trimethyl-6-methy
57	26.341	363208	0.30	Myrtanol, 2-mercapto-
		120530390	100.00	

The following major constituents were detected by GC-MS

2,6-octadienal,3,7-dimethyl,(E) (43.20%)

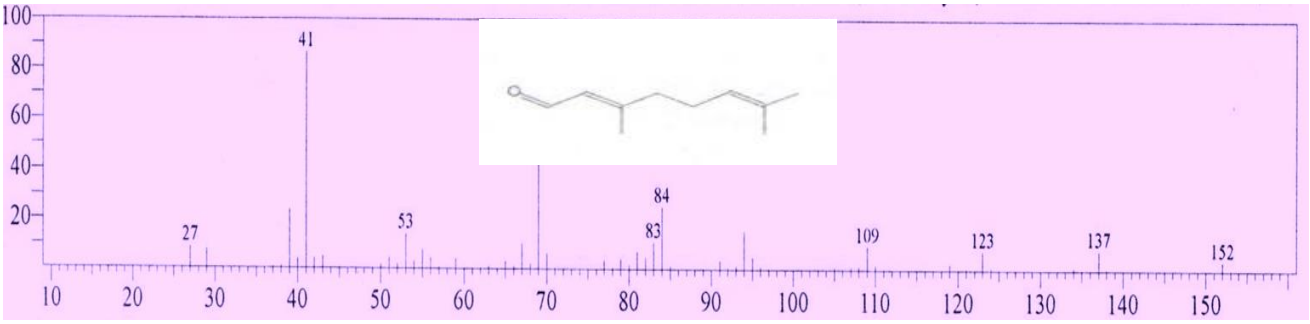


Fig.3.2: Mass spectrum of 2,6-octadienal,3,7-dimethyl,(E)

The EI mass spectrum of 2,6-octadienal,3,7-dimethyl,(E) is shown in Fig. 3.2. The peak at m/z 152, which appeared at R.T. 11.850 in total ion chromatogram, corresponds to $M^{+}[C_{10}H_{16}O]^{+}$ .The peak at m/z137 corresponds to loss of a methyl function.

2,6-octadienal,3,7-dimethyl,(Z) (31.36%)

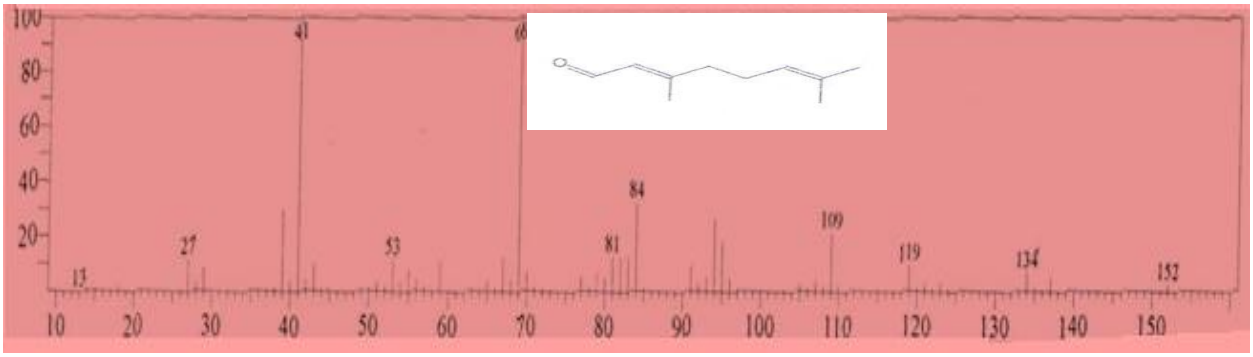


Fig.3.3: Mass spectrum of 2,6-octadienal,3,7-dimethyl,(z)

The EI mass spectrum of 2,6-octadienal,3,7-dimethyl,(Z) is shown in Fig. 3.3. The peak at m/z 152, which appeared at R.T. 11.230 in total ion chromatogram, corresponds to $M^{+}[C_{10}H_{16}O]^{+}$ .The peak at m/z134 corresponds to loss of a methyl function.

5, hepten-2-one, 6-methyl (3.87%)

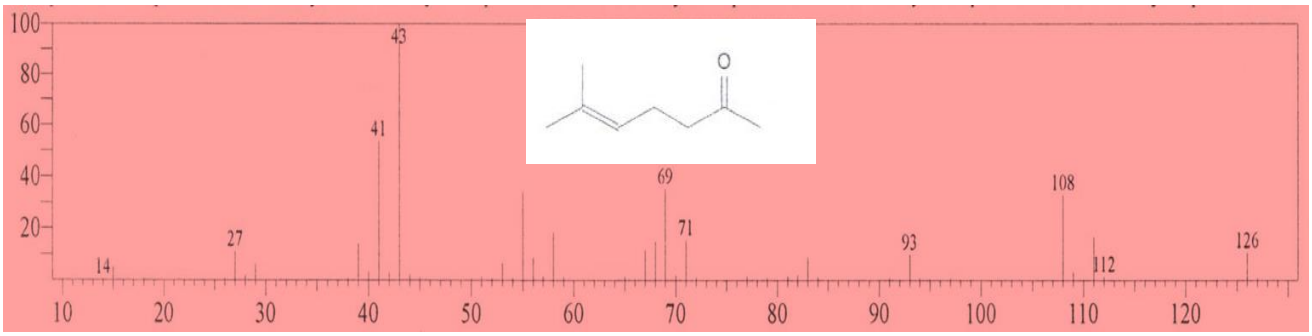


Fig.3.4: Mass spectrum of 5, hepten-2-one, 6-methyl

The EI mass spectrum of 5, hepten-2-one, 6-methyl is shown in Fig.3.4. The peak at m/z 126, which appeared at R.T. 5.843 in total ion chromatogram, corresponds to $M^{+}[C_8H_{14}O]^{+}$ .The peak at m/z112 corresponds to loss of a methyl function.

3.2.GC-MS analysis of *Brassica nigra* essential oil

*Brassica nigra*essential oil was analyzed by GC-MS. Twenty components appeared in total ion chromatogram (Fig.3.5). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in table (3.2).

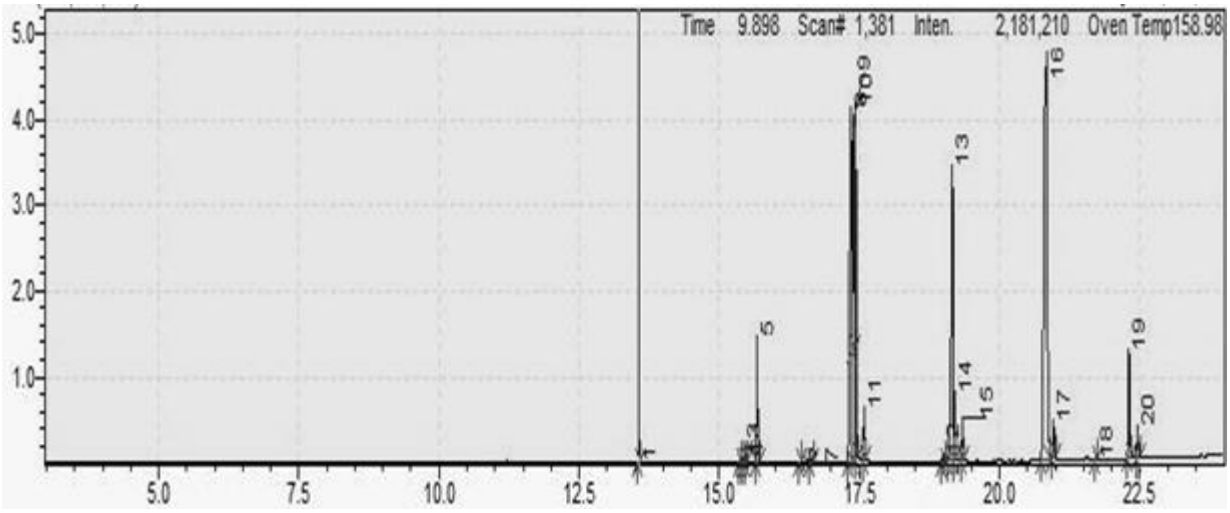


Fig.3.5: Cromatograms of *Brassica nigra* essential oil

Table 3.2: Constituents of *Brassica nigra* essential oil

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	13.573	247957	0.04	Methyl tetradecanoate
2	15.374	238013	0.04	7,10-Hexadecadienoic acid, methyl ester
3	15.440	624927	0.11	7,10,13-Hexadecatrienoic acid, methyl ester
4	15.477	612771	0.10	9-Hexadecenoic acid, methyl ester, (Z)-
5	15.675	22579525	3.83	Hexadecanoic acid, methyl ester
6	16.441	203337	0.03	7-Hexadecenoic acid, methyl ester, (Z)-
7	16.649	230222	0.04	Heptadecanoic acid, methyl ester
8	17.351	107225826	18.20	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
9	17.410	52413554	8.90	9-Octadecenoic acid (Z)-, hexadecyl ester
10	17.429	50680492	8.60	9,12,15-Octadecatrienoic acid, methyl ester, (Z
11	17.588	10009685	1.70	Methyl stearate
12	18.994	1580284	0.27	.gamma.-Linolenic acid, methyl ester
13	19.158	75533877	12.82	11-Eicosenoic acid, methyl ester
14	19.201	11763879	2.00	cis-11-Eicosenoic acid, methyl ester
15	19.341	8077585	1.37	Eicosanoic acid, methyl ester
16	20.843	209992913	35.65	13-Docosenoic acid, methyl ester, (Z)-
17	20.966	7483914	1.27	Docosanoic acid, methyl ester
18	21.725	340884	0.06	Tricosanoic acid, methyl ester
19	22.312	22224509	3.77	15-Tetracosenoic acid, methyl ester, (Z)-
20	22.463	6942210	1.18	Tetracosanoic acid, methyl ester
		589006364	100.00	

The following major constituents were detected by GC-MS:

13-Docosenoic acid, methyl ester (35.65%)

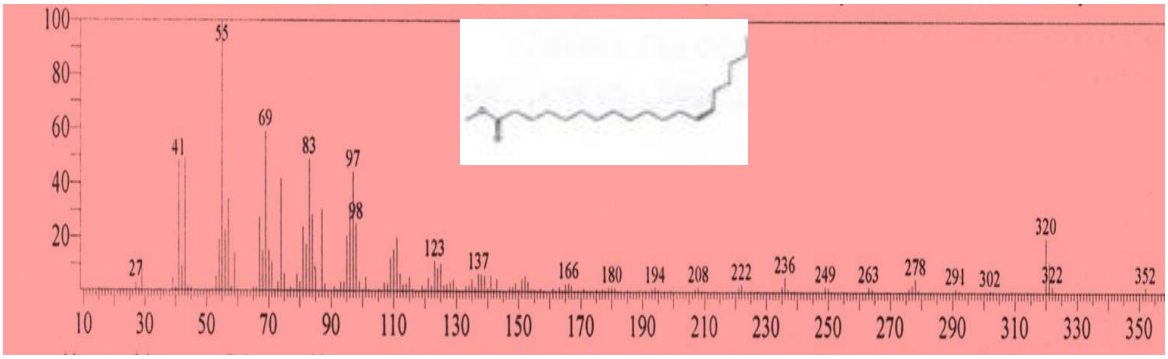


Fig. 3.6: Mass spectrum of 13-docosenoic acid methyl ester

The EI mass spectrum of 13-docosenoic acid, methyl ester is shown in Fig.3.6.The peak at m/z 352, which appeared at R.T. 20.843 in total ion chromatogram, corresponds to $M^{+}[C_{23}H_{44}O_2]^{+}$.The peak at m/z320 corresponds to loss of a methoxyl function.

9,12- Octadecadienoic acid (Z,Z) , methyl ester(18.20%)

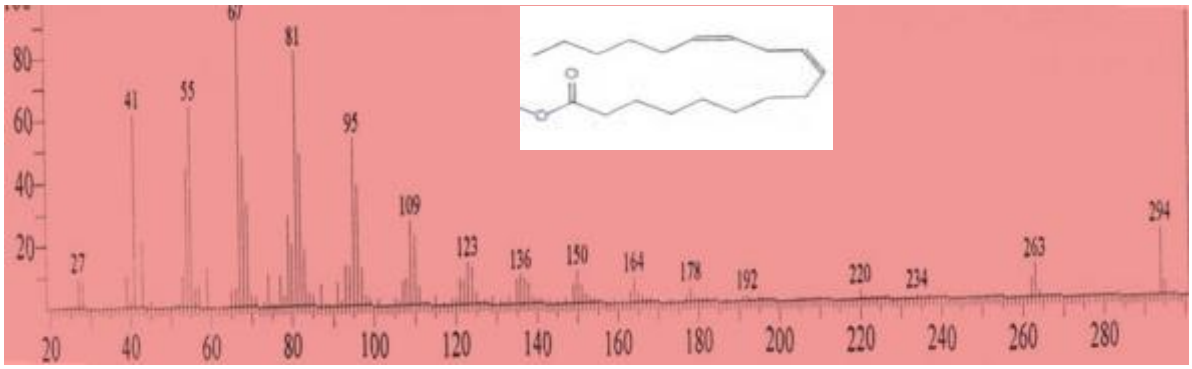


Fig. 3.7: Mass spectrum of 9,12-octadecenoic acid (z,z), methyl ester

The EI mass spectrum of 9,12 -octadecadienoic acid (Z,Z), methyl ester shown in Fig.3.7.The peak at m/z 294, which appeared at R.T.

17.351 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function

11-Eicosenoic acid, methyl ester (12.82%)

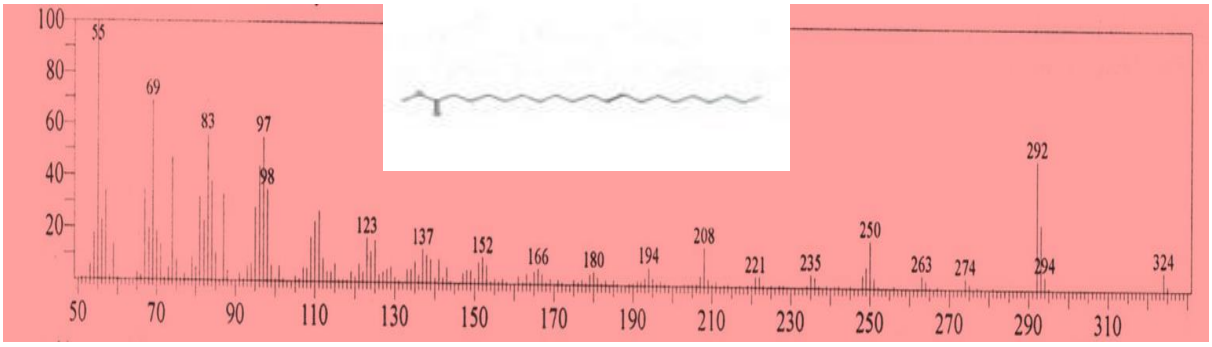


Fig. 3.8: Mass spectrum of 11-eicosenoic acid methyl ester

The EI mass spectrum of 11-eicosenoic acid, methyl ester is shown in Fig.3.8.The peak at m/z 324, which appeared at R.T. 19.158 in total ion chromatogram, corresponds to $M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z292 corresponds to loss of a methoxyl function.

9-Z-Octadecenoic acid, methyl ester (8.90%)

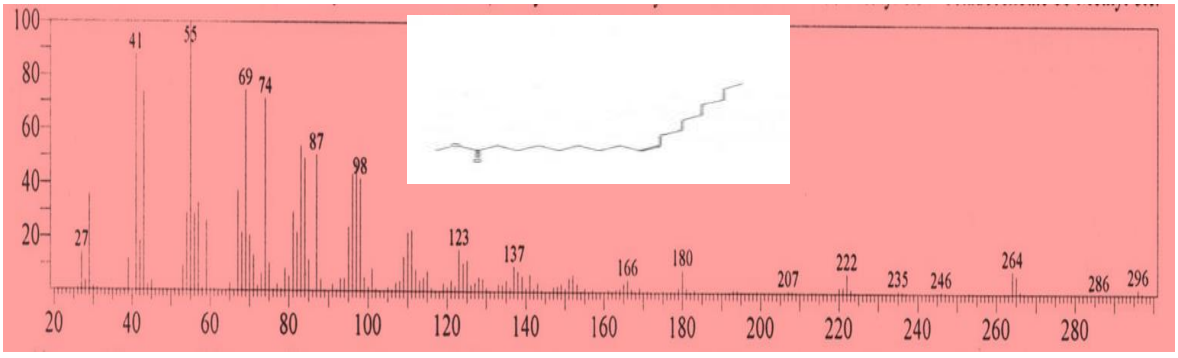


Fig. 3.9: Mass spectrum of 9-Z-octadecenoic acid methyl ester

The EI mass spectrum of 9-Z-octadecenoic acid methyl ester is shown in Fig.3.9.The peak at m/z 296, which appeared at R.T. 17.410 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak

at m/z264 corresponds to loss of a methyl group while the signal at m/z264 is due to loss of a methoxyl function.

9,12,15-Octadecatrienoic acid, methyl ester (8.60%)

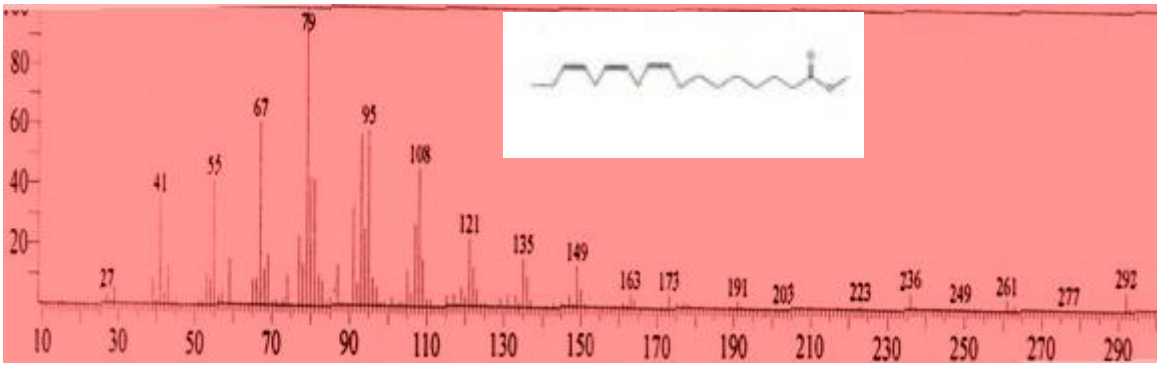


Fig.3.10: Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester

The EI mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester is shown in Fig.3.10.The peak at m/z 292, which appeared at R.T. 17.429 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{32}O_2]^{+}$ .The peak at m/z261 corresponds to loss of a methyl group while the signal at m/z261 is due to loss of a methoxyl function.

Hexadecanoic acid methyl ester (3.83%)

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig.3.11.The peak at m/z 270, which appeared at R.T. 15.657 in total ion chromatogram, corresponds to $M^{+}[C_{17}H_{34}O_2]^{+}$ .The peak at m/z239 corresponds to loss of a methoxyl function.

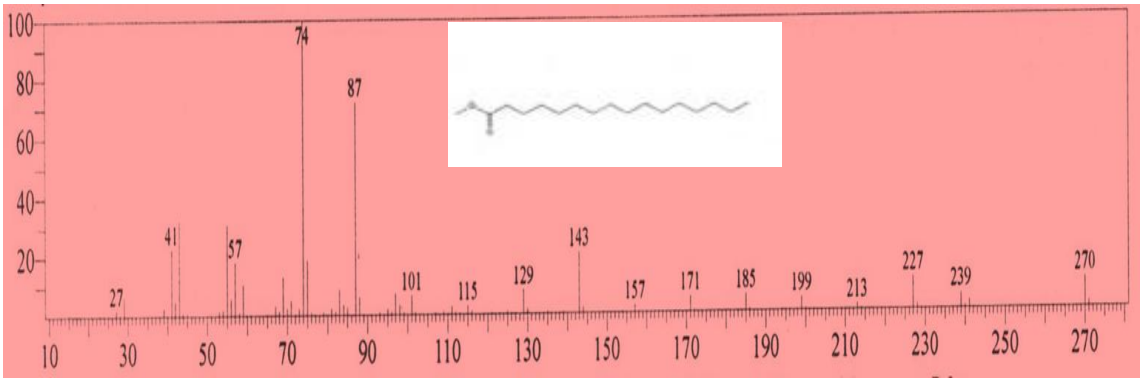


Fig. 3.11: Mass spectrum of hexadecanoic acid methyl ester

15-tetracosenoic acid, methyl ester (3.77)

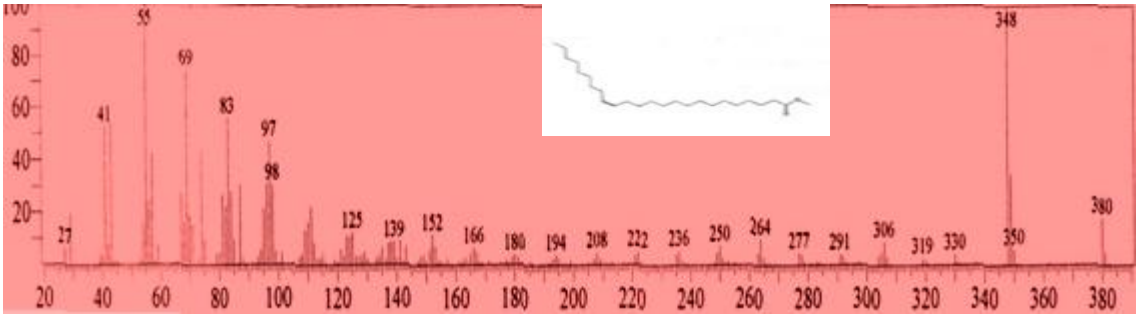


Fig. 3.12: Mass spectrum of 15-tetracosenoic acid, methyl ester

The EI mass spectrum of 15-tetracosenoic acid, methyl ester is shown in Fig.3.12.The peak at m/z 380, which appeared at R.T. 22.312 in total ion chromatogram, corresponds to $M^+[C_{25}H_{48}O_2]^+$ .The peak at m/z348 corresponds to loss of a methoxyl function.

Cis-11-eicosenoic acid, methyl ester (2%)

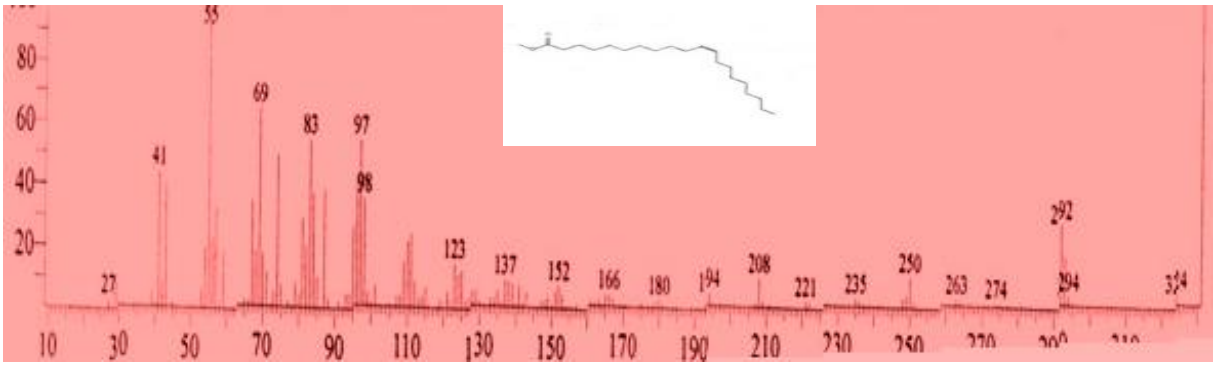


Fig. 3.13: Mass spectrum of cis-11-eicosenoic acid methyl ester

The EI mass spectrum of cis-11-eicosenoic acid, methyl ester is shown in Fig.3.13.The peak at m/z 324, which appeared at R.T. 19.201 in total ion chromatogram, corresponds to $M^{+}[C_{21}H_{40}O_2]^{+}$ .The peak at m/z292 corresponds to loss of a methoxyl function.

3.3. The GC-MS analysis of *Cymbopogon nervatus* essential oil

The essential oil constituents of *Cymbopogon nervatus* was identified by their retention time and computer matching of their mass spectra with those found in NIST and Wiley libraries database. The percentage of composition of the identified compounds was computed from the GC peak area. The GC-MS spectrum of the *Cymbopogon nervatus* revealed the presence of 36 components (Table 3.3). .The typical total ion chromatograms (TIC) are shown in Fig.3.14.

Table 3.3: Constituents of *Cymbopogon nervatus* essential oil

Peak#	R.Time	Area	Area%	Name
1	5.965	99268	0.07	2,3-Dehydro-1,8-cineole
2	6.229	367958	0.25	Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methyl
3	6.600	415034	0.29	5-Hepten-2-one, 5,6-dimethyl-
4	6.632	1023769	0.70	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-
5	6.713	3508273	2.41	D-Limonene
6	7.995	1066716	0.73	o-Isopropenyltoluene
7	8.167	564670	0.39	(1R)-(-)-Myrtenal
8	8.682	19286520	13.28	trans-p-Mentha-2,8-dienol
9	8.990	13697611	9.43	cis-p-Mentha-2,8-dien-1-ol
10	9.038	749927	0.52	Limonene oxide, trans-
11	9.331	277554	0.19	Cyclohexene, 1-methyl-4-(1-methylethyl)-
12	9.444	538554	0.37	4-Isopropenylcyclohexanone
13	9.744	147134	0.10	Cyclohexene, 4-methyl-1-(1-methylethenyl
14	9.854	169736	0.12	3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-tri
15	10.071	2159040	1.49	Ethanone, 1-(4-methylphenyl)-
16	10.149	35264001	24.27	p-Mentha-1(7),8-dien-2-ol
17	10.408	8206551	5.65	[1,1'-Bicyclopentyl]-2-one
18	10.496	945313	0.65	Cyclohexanone, 2-methyl-5-(1-methylethe
19	10.702	238336	0.16	2-Propenal, 2-methyl-3-phenyl-
20	10.789	15146424	10.43	Carveol
21	10.996	30616374	21.07	trans-p-mentha-1(7),8-dien-2-ol
22	11.325	7661485	5.27	(-)-Carvone
23	11.433	497750	0.34	Dehydroelsholtzia ketone
24	11.588	400155	0.28	Naphthalene, 1,2,3,5,8,8a-hexahydro-
25	11.746	132006	0.09	3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cycl
26	11.922	170621	0.12	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl
27	11.970	200583	0.14	1-Cyclohexene-1-carboxaldehyde, 4-(1-me
28	12.029	380352	0.26	1-methyl-4-(prop-1-en-2-yl)-7-oxabicyclo[4
29	12.115	196629	0.14	Dispiro[2.1.2.4]undecane, 8-methylene-
30	13.550	149908	0.10	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylet
31	14.141	104657	0.07	3-Octadecene, (E)-
32	18.855	70652	0.05	Hexanoic acid, 2-phenylethyl ester
33	21.219	37055	0.03	.alpha.-Phellandrene
34	21.714	178856	0.12	2-Pentadecanone, 6,10,14-trimethyl-
35	24.747	474836	0.33	Bicyclo[2.2.1]heptane-2,3-diol, 1,7,7-trime
36	25.753	135076	0.09	Triazido-(1,2,3,4,5-pentamethylcyclopenta
		145279384	100.00	

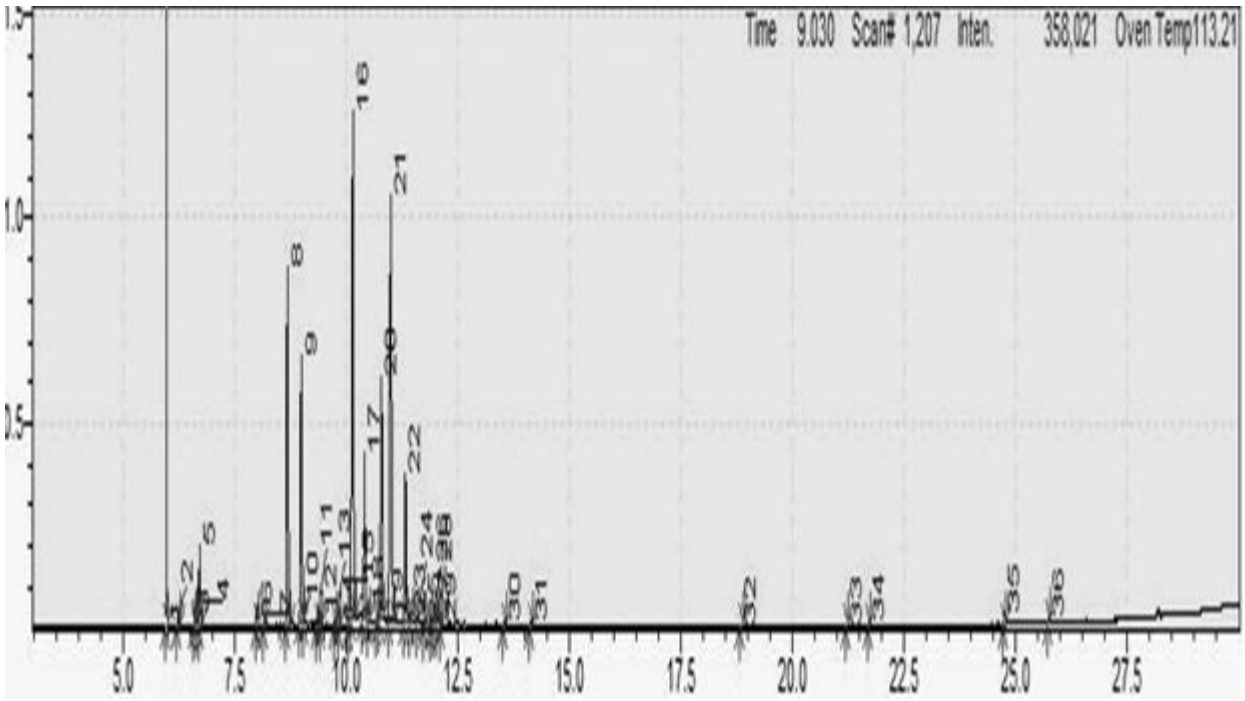


Fig.3.14: Cromatograms of *Cymbopogon nervatus* essential oil

The following major constituents were detected by GC-MS

P-Mentha-1(7),8-dien-2-ol (24.27%)

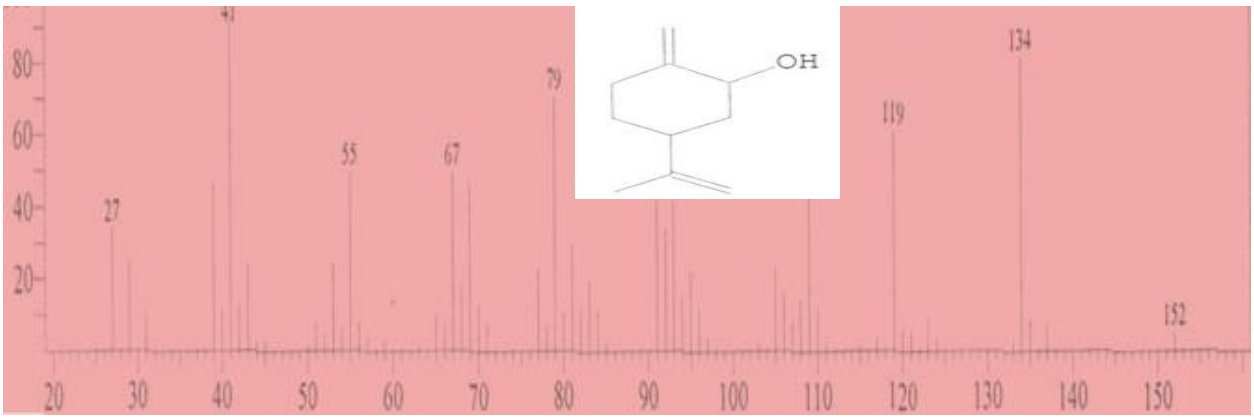


Fig. 3.15: Mass spectrum of P-Mentha-1(7),8-dien-2-ol

The mass spectrum of p-Mentha-1(7),8-dien-2-ol is shown in Fig. 3.15. The signal at m/z 152 (R.T.10.149) corresponds $M^{+}[C_{10}H_{16}O]^{+}$. the peak at m/z 134 corresponds to loss of methyl group.

Trans-p-mentha-1(7),8-dien-2-ol (21.07%)

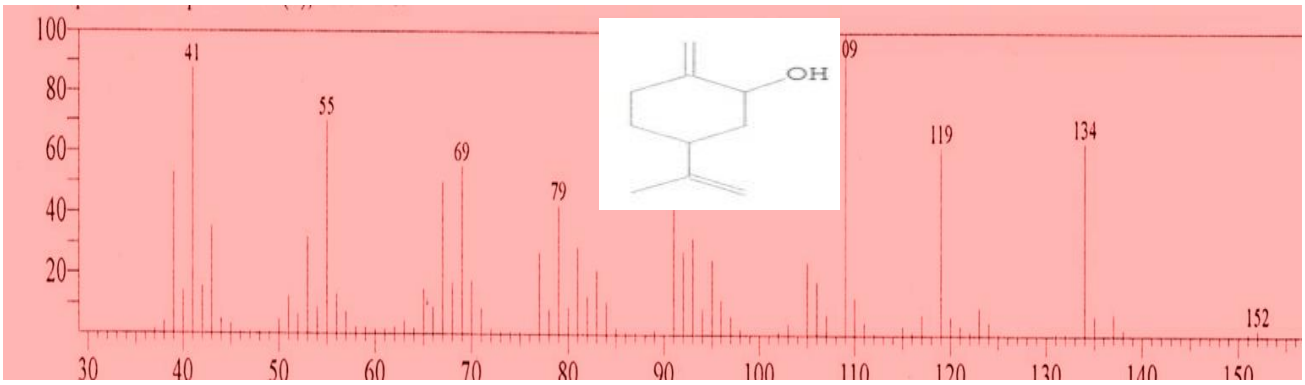


Fig. 3.16: Mass spectrum of trans P-Mentha-1(7),8-dien-2-ol

The mass spectrum of trans p-mentha-1(7),8-dien-2-ol is shown in Fig. 3.16. The signal at m/z 152 (R.T.10.996) corresponds $M^{+}[C_{10}H_{16}O]^{+}$. the peak at m/z 134 corresponds to loss of methyl group.

Trans-p-mentha-2,8-dienol (13.28%)

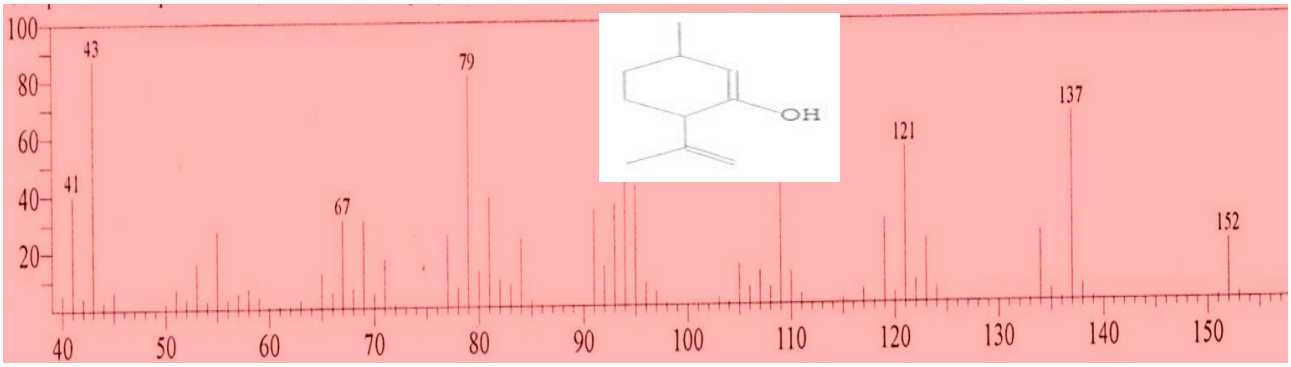


Fig. 3.17: Mass spectrum of trans-P-Mentha-2,8-dienol

The mass spectrum of trans-p-mentha-2,8-dienol is shown in Fig. 3.17. The signal at m/z 152 (R.T.8.682) corresponds $M^{+}[C_{10}H_{16}O]^{+}$. the peak at m/z 137 corresponds to loss of methyl group.

Carveol (10.43%)

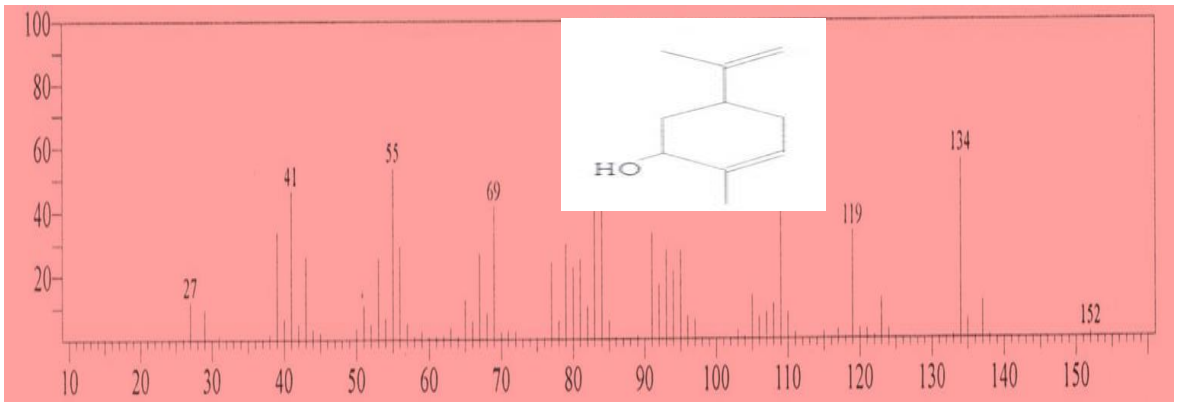


Fig. 3.18: Mass spectrum of carveol

The mass spectrum of carveol is shown in Fig. 3.18. The signal at m/z 152 (R.T.10.789) corresponds $M^+[C_{10}H_{16}O]^+$. the peak at m/z 134 corresponds to loss of methyl group.

Cis-p-mentha-2,8-dien-1-ol (9.43%)

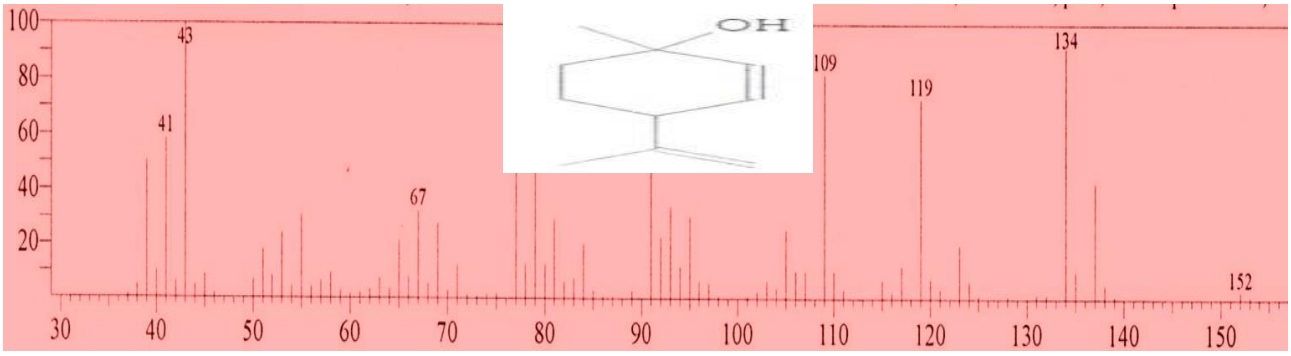


Fig. 3.19: Mass spectrum of cis-P-Mentha-2,8-dienol

The mass spectrum of cis-p-mentha-2,8-dien-1-ol is shown in Fig. 3.19. The signal at m/z 152 (R.T.8.990) corresponds $M^+[C_{10}H_{16}O]^+$. the peak at m/z 134 corresponds to loss of methyl group.

[1,1-Bicyclopentyl]-2-one (5.65%)

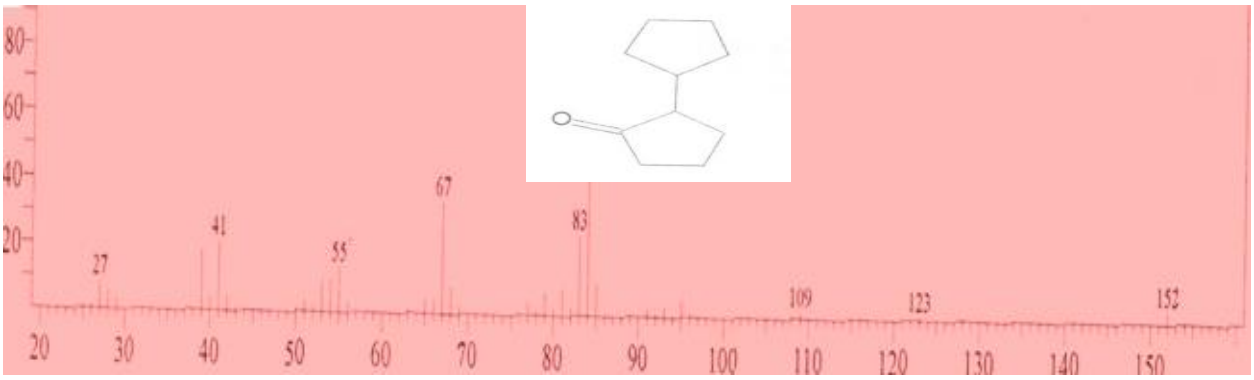


Fig. 3.20: Mass spectrum of [1,1-Bicyclopentyl]-2-one

The mass spectrum of [1,1-Bicyclopentyl]-2-one is shown in Fig. 3.20. The signal at m/z 152 (R.T.10.408) corresponds $M^{+}[C_{10}H_{16}O]^{+}$.

(-)-carvone (5.27%)

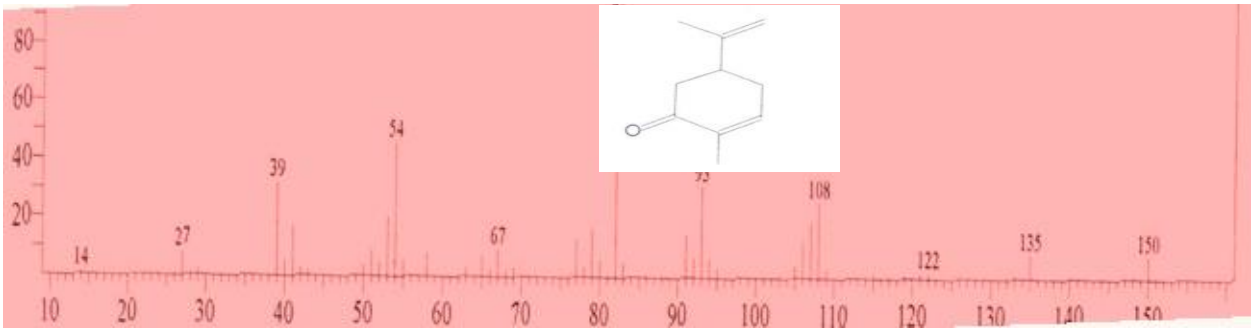


Fig. 3.21: Mass spectrum of (-)-carvone

The mass spectrum of (-)-carvone is shown in Fig. 3.21. The signal at m/z 150 (R.T.10.408) corresponds $M^{+}[C_{10}H_{14}O]^{+}$. the peak at m/z 135 corresponds to loss of methyl group.

D-Limonene (2.41%)

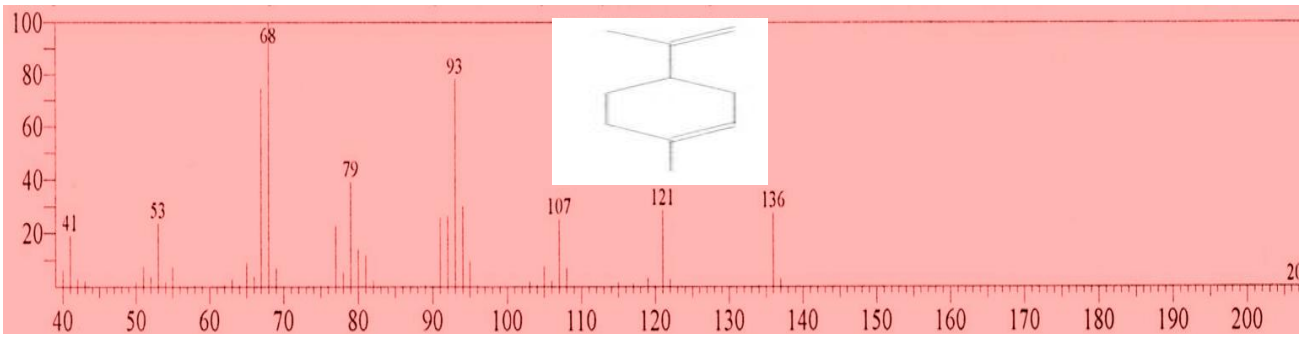


Fig. 3.22: Mass spectrum of D-Limonene

The mass spectrum of D-Limonene is shown in Fig. 3.22. The signal at m/z 136 (R.T.6.713) corresponds $M^{+}[C_{10}H_{16}]^{+}$. the peak at m/z 121 corresponds to loss of methyl group.

3.4. Antimicrobial activity

The target essential oils were evaluated for antimicrobial activity via the cup plate agar diffusion assay. The average of the diameters of the growth inhibition zones are shown in Tables (3.6-3.8) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (3.4) and (3.5) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

In the disc diffustion bioassay, *Cymbopogon citrates* oil showed excellent antibacterial activity against all test bacteria at 100 mg/ml but it not exhibit any anticandidal activity. It also showed excellent activity against *S. aureuo* and *P. aeruginosa* at 50 and 25 mg/ml.

Significant antimicrobial activity was observed for *Cymbopogon nervatus* oil at 100 mg /ml. *Brassica nigra* oil showed excellent antimicrobial activity at 100 mg/ml against all test organisms except for *S. aureuo*. It also exhibited significant activity against *B. subtilis* and *E. coli* at 50 and 25 mg/ml.

Table 3.4 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Pa
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 3.5 : Antifungal activity of standard fungi

Drug	Conc. mg/ml	Ca.
Clotrimazole	30	38
	15	31
	7.5	29

- S.a*: Staphylococcus aureus*
- E.c*: Escherichia coli*
- P.a: *Pseudomonas aeruginosa*
- C.a: *Candida albicans*
- *B.a: Bacillus subtilis*

Table (3-6): The antimicrobial activity of *Cymbopogon citratus* oil

Micro-rganism	Species	Type	Concentration (mg/ml)			
			100%	50%	25%	12.5%
Bacteria	*Bacillus subtilis*	+ ve	30	11	10	-
	Staphylococcus aureus	+ ve	22	17	16	15
	Escherichia coli	- ve	20	-	-	-
	Pseudomonas aeruginosa	- ve	20	18	17	16
Fungi	*Candida albicans*		8	-	-	-

Table (3-7): The antimicrobial activity of *Cymbopogon nervatus* oil

Micro-rganism	Species	Type	Concentration (mg/ml)			
			100%	50%	25%	12.5%
Bacteria	*Bacillus subtilis*	+ ve	27	8	7	-
	Staphylococcus aureus	+ ve	27	16	15	14
	Escherichia coli	- ve	25	-	-	-
	Pseudomonas aeruginosa	- ve	21	-	-	-
Fungi	*Candida albicans*		26	18	14	12

Table (3-8): The antimicrobial activity of *Brassica nigra* oil

Microo-rganism	Species	Type	Concentration (mg/ml)			
			100%	50%	25%	12.5%
Bacteria	*Bacillus subtilis*	+ ve	18	18	18	14
	Staphylococcus aureus	+ ve	10	10	-	-
	Escherichia coli	- ve	18	18	16	15
	Pseudomonas aeruginosa	- ve	18	-	-	-
Fungi	*Candida albicans*		18	-	-	-

Conclusion

The essential oil from *cymbopogon citratus*, *brassica nigra* and *cymbopogon nervatus* were studied by GC-MS. The analysis revealed the presence of 2,6-octadienal, 3,7-dimethyl (E), 2,6-octadienal,3,7-dimethyl (Z) and 5, hepten-2-one, 6-methyl, 13-docosenoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z), methyl ester, 11-eicosenoic acid, methyl ester, 9-Z-octadecenoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, hexadecanoic acid methyl ester, 15-tetracosenoic acid, methyl ester and Cis-11-eicosenoic acid, methyl ester, P-mentha-1(7), 8-dien-2-ol, trans-p-mentha-1(7),8-dien-2-ol, trans-p-mentha-2,8-dienol, carveol, cis-p-mentha-2,8-dien-1-ol, [1,1-bicyclopentyl]-2-one , (-)-carvone and D-limonene components of *cymbopogon citratus*, *brassica nigra* and *cymbopogon nervatus* oils respectively. The oils were evaluated for antimicrobial activity and significant activity was recorded for some test organisms.

Recommendations

1- The target oils may be screened for other biological activities e.g. anti-inflammatory, antilesmenial ….. etc.
2- Other constituents of the target plants steroids, alkaloids ….. etc. may be investigated.

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