Sudan University of Science and Technology

College of Postgraduate Studies

Investigation of Chemical Constituents of *Ocimum basilicum* Oil and its Antimicrobial Activity

دراسة المكونات الكيميائية لزيت الريحان وفعاليته المضادة للمكروبات

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

By

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July, 2019

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ
إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴿١٠٥﴾

صدق الله العظيم

(التوبة-105)

Dedication

Dedicated to :

my parents,

my sisters and brothers.

Acknowledgment

First of all I would like to thank **Allah Almighty** for helping me to complete this work.

It is a pleasure to record my deep appreciation, and thanks to Prof. Mohammed Abdel Karim Mohamed for his wise guidance, which helped me to present this research in this shape.

I am very grateful to the Staff of the Department of Phytochemistry , Medicinal and Aromatic Plants Research Institute for all Facilities.

Thanks for the staff , Chemistry Dept. , Sudan University of Science and Technology for their kind help and support.

I am very grateful to my friends for their support to complete this work .

Abstract

The oil extracted from *Ocimum basilicum* was investigated by GC-MS analysis. The fatty acid content of the oil was determined by retention times and the observed fragmentation pattern. Twenty four components were detected in total ion chromatograms being dominated by :

(i)9,12-Octadecadienoic acid methyl ester(40.77%)

(ii)-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

(iii)-Hexdecanoic acid methyl ester(14.74%)

(iv)-Methyl stearate (9.83%).

The antimicrobial activity of the oil against five standard human pathogens has been attempted . However , no responses were recorded.

المستخلص

استخلص الزيت الثابت لبذور نبات الريحان ودرس بواسطة تقنية الكروماتوغرافية الغازية –طيف الكتلة حيث تم تحديد الحموض الدهنية بالزيت بزمن الاستبقاء ونمط التكسر. اتضح ان الزيت يحتوى على اربعة وعشرون مكونا ,المكونات الرئيسة هي :

(i)9,12-Octadecadienoic acid methyl ester(40.77%)

(ii)-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

(iii)-Hexdecanoic acid methyl ester(14.74%)

(iv)-Methyl stearate (9.83%).

ثم اخضع زيت الريحان لاختبارات مضاد الميكروبات حيث لم يبدى اى فعالية ضد الميكروبات القياسية المستخدمة في الاختبار.

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Chapter one

Introduction

1- Introduction

1-1 General approach

Medicinal and aromatic plants play an important role in primary health care and they have been used since antiquity to fight diseases. For instance, aromatic plants have been used for thousands of years for their preservative and medicinal properties or to impart flavor and aroma to food.

It is known that Hippocrates prescribed perfume fumigations. The presence of essential oils is one of the main causes of the pharmaceutical properties of plants.

The term 'essential oil' was introduced in the 16th century by, Paracelsus von Hohenheim- the founder of the discipline of toxicology. He termed the active component of a drug, 'Quinta essential'[1].

Essential oils are phytochemicals that consist largely of small molecule, such as terpenes, usually formed from only carbon and hydrogen, but sometimes oxygen is found.

Essential oils are used in perfumes and make-up products, as food preservers and additives, in sanitary products, in agriculture, and as natural remedies. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths, but most frequently in aromatherapy.

Several techniques are available for the extraction of essential oils including: water distillation, steam distillation, solvent extraction, expression under pressure, supercritical fluid extractions and subcritical water extractions.

The type of extraction is chosen according to the purpose of the use. For medicinal uses it is preferred to extract the oil by steam distillation or by expression.

When essential oils are to be used in perfumes, extraction with lipophilic solvents and sometimes with supercritical carbon dioxide is favored. Thus, the chemical profile of the essential oil products differs not only in the number of molecules, but also in the stereochemical types of molecules extracted, according to the type of extraction.

Many factors play a role in the quality, quantity and in composition of the essential oil including:climate, soil composition, plant organ, age and vegetative cycle stage[2,3]. Thus to get essential oils characterized by constant composition, they have to be extracted under the same conditions from the same organ of the plant, which has been growing on the same soil, under the same climate, and has been picked in the same season.

Many reports have been published[4-7] addressing good quality of essential oils. Due to their antimicrobial and insecticidal properties, essential oils have been largely employed according to their properties already observed in natural matrix.

Now more than 3000 essential oils are known but about three hundreds essential oils are considered commercially important, especially for industries. Some essential oils have particular medicinal properties that have been praised to cure certain organ dysfunction or systemic disorder[8-10].

Scientific knowledge on the physiological activity of essential oils will undoubtedly allow to developing new applications in the pharmaceutical and food industries. Some essential oils are known to constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the same secondary effects[11].

1-2-Definition of essential oils

Essential oils are comprising a complex mixtureof lipophilic organic matter characterized by a low molecular weight. Due to their highly volatility, essential oil differfrom fixed oils (usually obtained from seed) .

Essential oils are strong smelling ,relatively fluid and generally immiscible with water. They are colorless or slightly colored and are stable in the presence of light, heat, and air, in addition to having a characteristic pleasant odor[11].

Essential oils are extracted from aromatic and medicinal plants. They are abundant in certain plant families including:Lauraceae, Myrtaceaeand Rutaceae.

In their natural matrix, essential oils are arranged in the form of droplets between cells, and can act like hormones, regulators, and catalysts.

Some literature reports suggest that the role of essential oils in plant is to help the plant to adapt to the environment and they may have stimulated production in stress situations. Some plants that live in very hot climates, such as in the desert, use essential oils to protect themselves from the sun. Another example is the tenuous cloud of essential oils formed around bushes of myrrh and frankincense in order to filter out the suns rays and freshen theairaroundtheplant[12,13]. Essential oils arephytochemicals formed by several volatile compounds[14,15]

An essential oil is defined as the product obtained from plant raw material by hydrodistillation, steam distillation or dry distillation or by a suitable mechanical process (for Citrus fruits). Cold pressing without heat is usually used for Citrus fruit oils because their constituents are heat-sensitive and unstable, converting into artifacts under heat and pressure[16-18]. Sometimes, essential oils are frequently associated with gums and resins that are separated by the distillation process[19] .The above definition of essential oils does not include other aromatic/volatile products obtained by different extractive techniques like extraction with solvents (concretes, absolutes), supercritical fluid extraction, and microwave-assisted extraction. Needless to mention that essential oils differ from fixed oils or fatty oils in both chemical and physical properties. Fatty oils contain glycerides of fatty acids and leave a permanent stain on filter paper, whereas essential oils contain volatile compounds and vanish rapidly without leaving any stain.Essential oils are complex mixtures of volatile (around 100 u) to semi-volatile compounds (around 300 u), usually with a strong odor, rarely colored, soluble in organic solvents, and insoluble in water. Essential oils consist of volatile constituents of terpenoid and non-terpenoid origin, synthetized through different biosynthetic routes and with distinct primary metabolic precursors. Terpenoids biosynthesis involves both the mevalonate and non-mevalonate (deoxyxylulose phosphate) pathways, whereas phenylpropanoids are formed via the shikimate pathway [20-23].

Essential oils are dominated by monoterpenes and sesquiterpenes. Phenylpropanoidsare frequent present in volatile oils.

However, some essential oils may also contain fatty acids and their esters and, more rarely, nitrogen and sulfur derivatives[24,25]. .

1.3-Constituents of essential oils

A large number of essential oils contain hydrocarbons; these categorycontains molecules composed merely of hydrogen and carbon only and are classified into terpenes. Such hydrocarbons could be acyclic, alicyclic or aromatic in nature. Limonene, myrcene, p-menthane, pinene, pinene, sabinene, pcymene, myrcene, phellandrene, thujane, fenchane, farnesene, azulene, cadinene and sabinene are some examples of this family of products. These compounds are known to be endowed witha wide range of therapeutic activities .

1.3.1-Terpenes

Terpenes are known to comprise the largest single class of phytochemicals found in essential oils[26]. Terpenes consist of isoprene units. Each isoprene unit consists of a C_5H_8 unit. The simplest terpenes are monoterpenes that contain two isoprene units. Sesquiterpenes have three isoprene molecules and diterpenes have four .

Since each isoprene unit possesses five carbon atoms, it is easy to calculate the number of carbon atoms per molecule .Terpenes can be subdivided into many categories including: acyclic or cyclic which indicate their structure. Acyclic terpenesare linear(β-myrcene is an example), while cyclic terpenes form a ring (p-cymene is an example). Monocyclic, bicyclic, and tricyclic monoterpenes frequently occur in essential oils[27].

Terpenoids are derived from aliphatic precursors such as geraniol for the formation of monoterpenes, farnesol for sesquiterpenes, and geranylgeraniol for diterpenes[28].

Terpenoids may be categorized into different structural and functional classes. According to the number of isoprene units in their structure, terpenes can be classified into: hemiterpenes (one isoprene unit), monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), diterpenes (four isoprene units)…etc[28].

Theoccurrence of monoterpenes($C_{10}H_{16}$) sesquiterpenes($C_{15}H_{24}$). in essential oils is very common.These constituents have many isomeric cyclic or linear structures, various degrees of unsaturations, substitutions, and oxygenated derivatives, being generally called terpenoids[28].

1.3.2-Monoterpenes

Nearly all essential oils contain monoterpenes. Monoterpenes are the most representative constituents, attaining around 90% of a large number of essentialoils[29] . They are formed by a head – head joining of two isoprene units. These compounds oxidize easily because of their rapid reaction to air and heat sources.

However,monocyclicmonoterpenic hydrocarbons are the most common in essential oils but acyclic and bicyclic compounds are also found. The main linear monoterpenic hydrocarbons found in essential oils have a typical 2,6-dimethyloctane structure with three double bonds while bicyclic compounds have a second ring with three, four, or five carbons besides the hexane ring.

Biochemical modification of essential oils which includesoxidations or rearrangements processes give rise to several other phytochemicals (known as monoterpenoids). These compounds include highly functionalized chemical entities[30].

1.3.3- Sesquiterpenes

Sesquiterpenes are characterized by less volatilitity in contrast to terpenes and they have a greater potential for stereochemical diversity[31] and have stronger odors. Sesquiterpenes possessantiinflammatoryactivity[32]. They also possess some bactericidal effect[33] .

Sesquiterpenes oxidized over time into alcohols known as sesquiterpenols. In case of some oils, this oxidation is thought to improve the odor. One of the most antiinflammatorysesquiterpenes, chamazulene, only has 14 carbon atoms but is usually included with sesquiterpenes. Chamazulene and caryophyllene have strong antioxidant[34,] and antitumor activity[35,36] . Sesquiterpenescould be monocyclic, bicyclic or tricyclic and are a very diverse group[37]. Examples include α-bisabolene in black pepper (Piper nigrum) and β-caryophyllene in ylangylang (*Canangaodorata*)[38]. Some sesquiterpenes such as α-farnesene can be effective against the bacteria that cause tooth decay[39] .

1.3.4- Diterpenes

Only a limited number ofditerpenesoccur in essential oils. This is due to the fact that they are big, heavy molecules with correspondingly high boiling points.

Diterpenes can occurduring solvent extraction.correspondingly high boiling points.

Diterpenes are formed by ahead-to-tail arrangements of four isoprene units . These compounds are generally found in resins but some can occur is essential oils. Since they are heavier than monoterpenes and sesquiterpenes, longer distillations are required. Examples of diterpenes include phytol and geranylcitronellol (acyclic); camphorene (monocyclic); scareol and its derivatives, manool and labdane (bicyclic like) and kaur-15-ene, phyllocladene, pimaradiene, and sandaracopimaradiene (tricyclic)[40] .

Two major types of irregular monoterpenes have been identified. The first are substituted cycloheptanemonoterpenes (tropones), such as eucarvone and nezukone. The second group includes those compounds that do not fit the regular head-to-tail coupling[41]. Examples of these compounds are artemisia ketone, santolinatriene, and chrysanthemol[42]. Other irregular monoterpenes namely necrodane derivatives also occur primarily in Lavandulaluisieri oils[43].

1.4-Polypropanoids

Polypranoidsconsist of one or more C_6–C_3 units, the C6 unit being a benzene ring. There is no widely accepted classification for these compounds[44].

Phenylpropanoidsare found in some plant species mainly from the Apiaceae, Lamiaceae, Myrtaceae, and Rutaceae families[45] . Examples of phenylpropanoids occurring in essential oils include :anetholewhich occurs in high amounts in Pimpinellaanisum and Foeniculumvulgare[46], and star anise found inIlliciumverum[47] . Anethole is characterized by sweet anise odor and is used in liquors, soap and perfumes[48,49].

Some sulfur-containing phytochemicals are found in some plant species including : garlic, onion, leek, and shallots . These constituents contain volatile sulfur compounds, namely allyl sulfide, dimethyl sulfide, diallyl disulfide, and dimethylthiophene. Other sulfur-containing compounds like 4-mercapto-4-methyl-pentanone occurs in blackcurrant—

Rubusnigrum whereas 1-p-menthene-8-thiol is found in fruit oils[50].

The biological function of these compounds appear to be associated with plant defense and in nitrogen detoxication of plants. Although most sulfur compounds have very unpleasant pungent odors, organo-sulfur compounds present in essential oils can be aromatically very pleasant. It is also known that sulfur compounds are relevant in the flavoring of vegetables, fruits as well as processed foods and beverages[50].

Only few essential oils contain nitrogen-containing constituents such as methyl anthranilate, skatole, indole, pyridine, and pyrazine. Methyl anthranilate is present in several Citrus oils and in Canangaodorata oil. Skatole is a compound in the form of large crystals or powder. It occurs in orange – Citrusaurantiumblossoms and Jasminum species[51] . This compound has a very interesting aroma with a fecal smell at high concentrations but a floral scent in dilution. Used as a fixative in floral fragrances and a flavor agent in ice-cream and cigarettes.

Indolewhich contains nitrogen is a white crystalline powder that turns red on exposure to air. It occurs in neroli and some citrus fruit oils. It has an odor to skatole and is used in a wide range of fragrances[52]. Pyridines and pyrazines are found inPipernigrum, sweet orange – Citrussinensis, and Chrysopogonzizanioides oils[53] .

1.5- Esters

Some essential oils contain esters .Esters are sweet smelling compounds and give a pleasant smell to the oils and are very commonly found in a large number of essential oils. They include for example,geraniol acetate, eugenol acetate,linalyl acetate, and bornyl acetate. Esters are endowed with anti-inflammatory, spasmolytic, sedative, and antifungal properties[53].

1.6- Oxides

Oxides or cyclic ethers are characterized by their strong odor and by far the most known oxide is 1,8cineole, as it is the most omnipresent one in essential oils. Bisabolone oxide, linalool oxide, sclareol oxide and ascaridole are other examples. Their therapeutic benefits are expectorant and stimulant of nervous system[53].

1.7- Lactones

Lactones are und usually in pressed oils. They are relatively of high molecular weight. Examples are: nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone, epinepetalactone, aesculatine, citroptene, andpsoralen . Such lactones are used therapeutically as : antipyretic, sedative and hypotensive , but they may be associated with allergic reactions[53].

1.8- Alcohols

Alcohols are the most therapeutically beneficial of essential oil components with no reported contraindications. Alcohols are used as : antimicrobial, antiseptic, tonifying, balancing and spasmolytic . Examples include :linalol, menthol, borneol, santalol, nerol, citronellol and geraniol[54] .

1.9- Phenols

Phenols are among the most reactive, potentially toxic and irritant constituents of essential oils. They are especially harmful for the skin and the mucous membranes. Their properties are similar to alcohols but more pronounced.

Phenols are used as : antimicrobial, rubefacient properties, stimulate the immune and nervous systems and may reduce cholesterol . Phenols are often found as crystals at room temperature, and the most common ones are thymol, eugenol, carvacrol and chavicol[54] .

1.10- Aldehydes

Aldehydes are unstable and easily oxidized common essential oil components. Many cause mucous membrane irritation . They possess sweet, pleasant fruity odors and are found in some of our most well known culinary herbs such as cumin and cinnamon. Therapeutically some aldehydes possess: antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic and spasmolytic properties. Examples include:citral(geranial and neral), myrtenal, cuminaldehyde, citronellal, cinnamaldehyde and benzaldehyde[54] .

1.11- Ketones

Ketones have al limited occurrence in essential oils; they are relatively stable and are not particularly important as fragrances or flavor substances. Some ketones-camphor and thujone- are neurotoxic and abortifacients[55] but have some therapeutic effects. They are described as mucolytic, cell regenerating; sedative, antiviral, analgesic and digestive .

Ketones, which are relatively stable, are not easily metabolized by the liver. Examples of essential oilsketones include :carvone, menthone, pulegone, fenchone, camphor, thujone and verbenon

Table 1: Different classes of essential oils and their bioactivity

Calss of compounds	Examples	Bioactivities
Hydrocarbons	Limonene, myrcene, pinene, pinene, sabinene, cymene, myrcene, phellandrene.	Stimulant, antiviral, antitumour, decongestant, antibacterial, hepatoprotective
Esters	linalyl acetate, geraniol acetate, eugenol acetate, bornyl acetate	spasmolytic, sedative, antifungal, anaesthetic, anti-inflammatory

Oxides	Bisaboloneoxide, linalool oxide, sclareol oxide, ascaridole	antiinflammatory, Expectorant, stimulant
Lactones	nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone	Antimicrobial; antiviral; Antipyretic, sedative, hypotensive; analgesic
Alcohols	nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone.	Antimicrobial; antiviral; Antipyretic, sedative, hypotensive; analgesic
Phenols	thymol,eugenol, carvacrol, chavicol	antimicrobial,spasmo lytic, anaesthetic, irritant, immune stimulating
Aldehydes	citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde	Antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic, sedative, spasmolytic
Ketones	carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone	mucolytic,cell regenerating, sedative, antiviral, neurotoxic, analgesic, digestive, spasmolytic

1.12- The therapeutical potential of essential oil

For centuries, essential oils have been used therapeutically for various health purposes .This mainly due to their antidepressant, stimulating, detoxifying, antibacterial, antiviral and calming properties[55].

Recently, essential oils are gainingtremendous popularity as a natural, safe and cost-effective therapy for a number of health concerns. The benefits of essential oils are diverse and their uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments[55].

1.12.1 –Hormone balance property of essential oil

Some essential oils are known to balance body estrogen, progesterone, cortisol, thyroid and testosterone and other hormones levels. Some volatile oils, like clary sage, geranium and thyme, help to balance out estrogen and progesterone levels in the body, which can improve conditions like infertility and PCOS, as well as PMS and menopause symptoms. Some reports indicated that some essential oils, notably geranium and rose, have the ability to influence the salivary concentration of estrogen in women. This may be helpful for women who are experiencing menopausal symptoms that are caused by declining levels of estrogen secretion. Essential oils can also lower cortisol levels, which can help to improve mood and reduce symptoms of depression, and increase testosterone levels[55].

1.12.2-Effect of essential oils on immunity

Many essential oils are endowed with anti-inflammatory, antiviral, antibacterial, antiseptic and anti-fungal properties that help to boost your immune system and fight infections. The chemical substances found in essential oils, such as terpenes, esters, phenolics, ethers and ketones, have the potential to fight foreign pathogens that can threaten your health. Some of the best essential oils for improving immunity include : oregano, myrrh, ginger, lemon, eucalyptus, frankincense, peppermint (or Menthapiperita) and cinnamon.ManyStudies demonstrated that essential oils effectively destroy several fungal, viral and bacterial pathogens, including methicillin-resistant *Staphylococcus aureus, Helicobacter pylori* and *Candida albicans*infections[55].

Now that antibiotic resistance is becoming such a major threat in modern health care, using essential oils as a form of independent or combination therapy can help to fight bacterial infections in a safer and more natural wayOregano oil, for instance, has powerful immune-boosting properties and has displayed both antiviral and antibacterial properties in some studies. Oregano oil contains carvacrol and thymol, two compounds that have antimicrobial effects and can inhibit the synthesis and growth of several types of bacteria[55].

1.12.3 – Effect of essential oils on digestion

Another well-researched essential oils benefit is their role in aiding and improving digestion. Some oils help to relieve upset stomach, indigestion, diarrhea, stomach spasms and even conditions of the gastrointestinal system, like IBS. Essential oils can also aid your digestion by helping to stimulate digestive enzymes that make it easier to break down and absorb the nutrients, fats and protein that are needed by the human body[55].

It has been reported thatginger essential oil can promote the digestive health by easing indigestion, constipation and ulcers. Ginger oil stimulated gastric emptying in people with indigestion. Ginger oil is also used to relieve gas, reduce nausea and ease abdominal pain[21].

Some research showed that peppermint oil can provide rapid relief of IBS symptoms. In a 4-week, randomized, double-blind, placebo-controlled clinical trial, 72 patients with IBS received either peppermint oil or placebo. The peppermint group experienced a 40 percent reduction in total IBS symptoms after 4 weeks, which was superior to the 24 percent decrease of symptoms reported by the patients in the placebo group. After just 24 hours of using peppermint oil, the treatment group experienced a decrease in symptoms of 19.6 percent[22].

1.12.4-Effect of essential oils on body energy Levels

Essential oils couldboost body energy levels . Some oils have stimulating effects and can actually increase brain oxygen. This property will eventually leave the body refreshed, focused and energized.

It has been shown that pepper mint oil increased brain oxygen concentration, improved exercise performance and reduced exhaustion in healthy male athletes who consumed peppermint oil with water for 10 days[23].

1.12.5- Neuroprotective effects of essential oils

Essential oils are known to possess a neuroprotective property and could improveimprove cognitive performance. This is one of the most impressive essential oil benefits and it has helped many people who are suffering from neurodegenerative diseases like Alzheimer’s and dementia. In a scientific review published in Frontiers in Aging Neuroscience, researchers found that because essential oils possess powerful antioxidants that work to inhibit free radical scavenging, they help to naturally improve brain function and reduce inflammation[24].

It has been shown that essential oils may improve memory and ability to focus. Oils like peppermint can improve sustained attention over a longer period of time, while oils like lavender can be useful for people going through tough exercises or situations. Furthermore, essential oils can be useful in relieving agitation in individuals with dementia. This is due to their calming and sedative effects[24.]

1.13- *Osmium bailicum* L.

Basil (*Osmium bailicum* L.) is the most common species in Lamiaceaefamily.This family includes 350 species distributed between the genders as herbaceous plants , sometimes shrubs, but rarely as trees , with a significant content of essential oils.[56,57]

The essential oil is a liquid with lightly yellowish color and a characteristic smell.The yield of essential oil from different plant parts varies between 0.15-1.59%, and it depends also on the seasonal factor and locality . Basil essential oil contains among others :linalool, methychariol, estragol ,thymol and $_p$ – cymen . The oil also contains saponins ,tannins, flavonoids , glycosides , enzymes and some organic acids.[56]

The useful parts of the plants are leaves and seeds. Hot tea of basil plant leaves has been used traditionally in treating nausea , dysentery and flatulence[56].

Externally , basil formulations can be used for different skin infections such as treatment of acne ,snake bites and insect stings . In addition to these, basil has been used as a remedy for a number of ailments, including cancer, convulsion, deafness, diarrhea, epilepsy, sore throat, toothaches, and whooping eough.[58-62]

-Taxonomy

Kingdom : Plantae.

Sub-kingdom: Tracheobionta.

Division :Magnoliophyta.

Super division: Spermatophyte.

Class: Magnoliopsid.

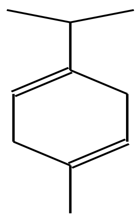
Sub-class: Asteridae.

Family: Lamiaceae.

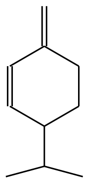
Genus: Ocimum.

Species: basilicum.[63]

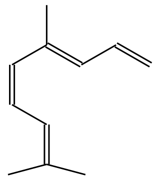
Chemical structure of some foremost compounds isolated from essential oils of Ocimum :



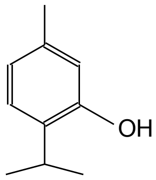
Gamma-Terpine



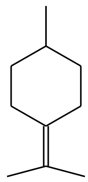
Phellandrene



Geraniol



Thymol



Lemonin

Basil is useful in diseases of heart and blood includingleucoderma .The juice relieves joints pain , gives luster to eyes, it is also good for toothache, earache and cures some diseases when used with camphor.[61] The juice of plant is dropped into ears to cure dullness of hearing.[64,65] The infusion of the plant is given to treat gouty joints and is used as gargle for foul breath .Basil cures headache , aids digestion and acts as a mild laxative.[61] The plant is useful in treatment of stomach complaints , fever , cough and gout.[65] The warm leaves juice of this plant along with honey is used to treat croup.[66] It also forms an excellent nostrum for the cure of ringworm. It is used as a lotion for sore eyes too.[61] The seeds washed and pounded are used in poultices for unhealthy sores and sinuses. An infusion of seeds is given in fever. The seeds are chewed in case of snake - bite, one portion is swallowed and the other portion is applied to the bitten part.[61]A cold infusion of seeds is said to relieve the pain of parturition. Seeds are also given internally to treat cystitis, nephritisand internal piles.[65] Due to the mucilaginous and cooling effect , an infusion of basil seed is given to treat gonorrhea, diarrhea and chronic dysentery.[66,67]

Methanolic extract of *Ocmiumbasilicum* was evaluated for its analgesic activity by tail immersion method in Swiss mice. The extract was able to show analgesic activity at 200 mg/ kg concentration which was well comparable with the standard drug, aspirin.[68]

Both the hexane and methanol extracts .but not the ethanol extracts, inhibited three isolates out of 23 strains of *Candida albicans*.The hexane extract showed strong and broader spectrum of antibacterial activity followed by methanol and ethanol extracts. The minimal inhibition zones of the hexane, methanol and ethanol extracts ranged from 125 to 250 µl/ml, respectively[68].

In vitro antioxidant activities of 50% hydroalcoholic extract of Ocimum species namely *Ocimumbasilicum* and *Ocimum sanctum* were achieved at varying concentrations (10-15µg / ml) using DPPH radical scavenging activity.The results showed that *Ocimumbasilicum* had more antioxidant activity than *Ocimum sanctum* . In another study the ethanolic extract of *Ocimumbasilicum* exhibited potent antioxidant effects. Eugenol ,thymol, carvacrol, and 4-allylphenol showed stronger antioxidant activities than did the other components tested in the assay . They all inhibited the oxidation of hexanal by almost 100% for aperiod of 30 days at a concentration of 5µg /ml.Their antioxidant activites were comparable to those of the known antioxidants,α-tocopherol and butylatedhydroxy toluene.[58]

Aim of this study

This study was aimed to:

-Extracting oil from the medicinally important species-

-Conducting a GC-MS analysis for the extracted oil.

-Evaluating the antimicrobial potential of the target oil.

2-Materials and Methods

2.1-Materials

2.1.1--Plant material

Seedsof *Ocimumbasilicum*were collected from Khartoum, Sudan and authenticated by direct comparison with a herbarium sample. The seeds were shade – dried at room temperature and powdered

2.1.2-GC-MS analysis

The GC-MS analysis was performed on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)**.**

2.1.3-Test organisms

The oil from *Ocimumbasilicum*seeds was screened for antimicrobial activity using the standard microorganisms :*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and the fungal species *Candida albicans*.

2.2-Methods

2.2.1-Solvent extraction

Powdered shade-dried seeds of *Ocimumbasilicum*(350g) were extracted with n-hexane at room temperature.The solvent was removed *in vacuo* leaving the oil.

2.2.2-Esterification of the oil

The extracted oil was esterified as follows :the oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5μl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1μl) was injected in the GC-MS vial.

2.2.3-Analysis by GC-MS

The extracted oil was investigated by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument using helium as carrier gas under the following Chromatographic conditions :

- *Oven temperature program*

Rate : --- ; Tempt. , 150.0^{0}C ; Hold time(min.$^{-1}$) ,1.00

Rate : 4.00 ; Tempt. , 300.0^{0}C ; Hold time(min.$^{-1}$) ,0.00

Column oven temperature	150.0°C
Injection temperature	300.0°C
Rate	4/min
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

2.2.4-Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to manufacture instructions.

Broth cultures(5.0x10^{7}cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs(Oxid,6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria the plates were incubated at 37^{o}Cfor 24h., while for fingi the plates were incubated at 25^{o}C for 3days.The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin,gentamycin and clotrimazole were used as positive control and DMSO as negative control.

3-Results and Discussion

3.1-Constiruents of *Ocimumbasilicum*

Theoilextracted from *Ocimumbasilicum*was investigated by GC-MS analysis. The fatty acid content of the oil was determined by retention times and the observed fragmentation pattern. Twenty four components were detected in total ion chromatogram. The typical total ion chromatogram (TIC) is presented in Fig. (1) . The constituents of the oil are outlined in Table 1 .The GC-MS analysis revealed the following major constituents:

i-9,12-Octadecadienoic acid methyl ester(40.77%)

ii-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

iii-Hexdecanoic acid methyl ester(14.74%)

iv-Methyl stearate (9.83

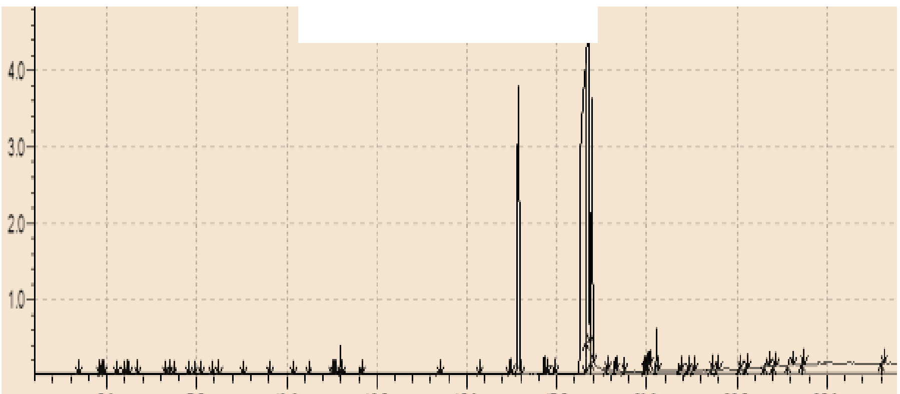


Fig. 1: Total ion chromatograms

Table2 : Constituents of *Ocimumbasilicum* oil

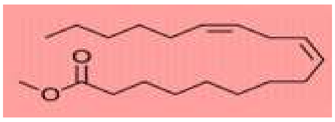
No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.189	0.09
2.	Pentadecanoic acid, methyl ester	15.320	0.04
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.156	0.06
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.201	0.35
5.	Hexadecanoic acid, methyl ester	16.435	14.74
6.	Hexadecanoic acid, 14-methyl-, methyl ester	17.147	0.54
7.	cis-10-Heptadecenoic acid, methyl ester	17.217	0.09
8.	Heptadecanoic acid, methyl ester	17.432	0.19
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.305	40.77
10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.387	26.56
11.	Methyl stearate	18.473	9.83
12.	cis-10-Nonadecenoic acid, methyl ester	18.889	0.11
13.	Octadecanoic acid, 17-methyl-, methyl ester	19.112	0.36
14.	Nonadecanoic acid, methyl ester	19.362	0.03
15.	.gamma.-Linolenic acid, methyl ester	19.933	0.41
16.	cis-11-Eicosenoic acid, methyl ester	20.062	0.43
17.	Eicosanoic acid, methyl ester	20.265	1.18
18.	Methyl 18-methylicosanoate	20.903	0.17
19.	Heneicosanoic acid, methyl ester	21.132	0.04
20.	13-Docosenoic acid, methyl ester, (Z)-	21.790	0.03
21.	Docosanoic acid, methyl ester	21.966	0.25
22.	Methyl 20-methyl-docosanoate	22.563	0.08
23.	Tricosanoic acid, methyl ester	22.771	0.12
24.	Tetracosanoic acid, methyl ester	23.543	0.16

The characterization of these major components is briefly discussed below:

i-9,12-Octadecadienoic acid methyl ester(40.77%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.2. The signal which was observed at m/z294(

R.T. 18.305) is due to M+[$C_{19}H_{34}O_2$]+, while the signal at m/z263 corresponds to loss of a methoxyl.



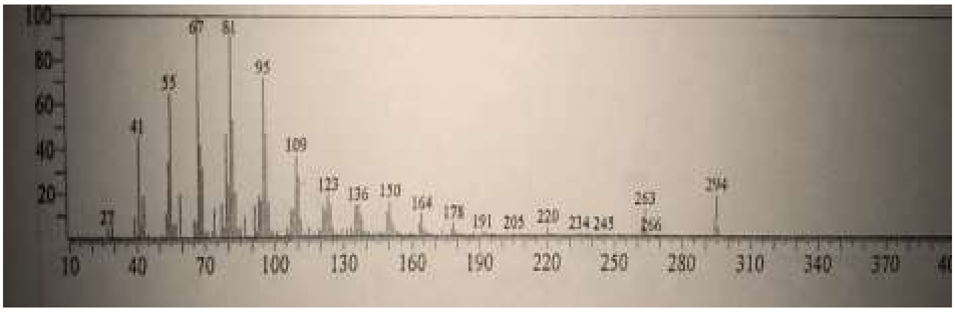


Fig.2: mass spectrum of 9,12-octadecadienoic acid (Z,Z)-methyl ester

ii-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

Mass spectrum of 9,12,15-octadecatrienoic acid,methyl ester is depicted in Fig. 3.The peak at m/z292,which appeared at R.T.18.387 corresponds to M+ [$C_{19}H_{44}O_2$]$^+$, while the peak at m\z261 is attributed to loss of methoxyl.

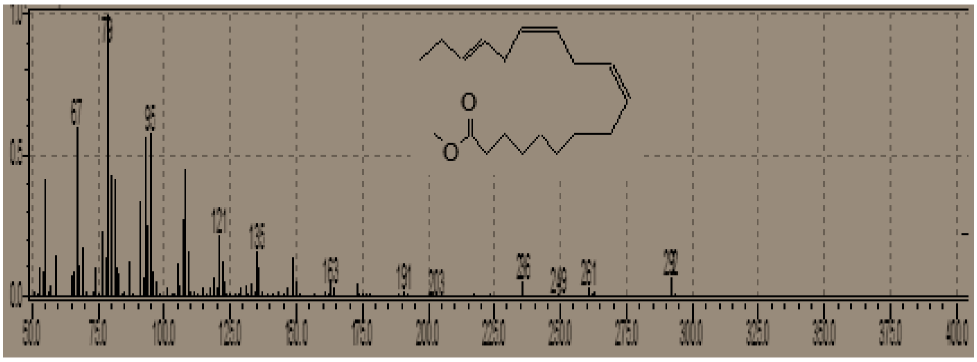


Fig3. Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

iii-Hexdecanoic acid methyl ester(14.74%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.4. The peak at m/z 270, which appeared at R.T. 16.435 accounts for $M^+ [C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of methoxyl .

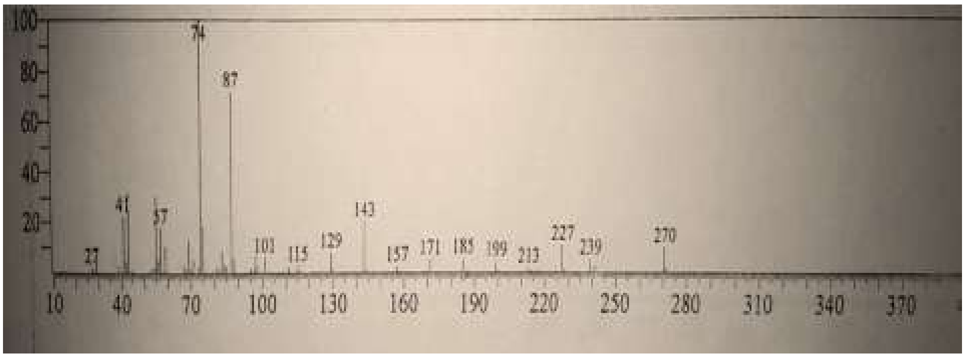
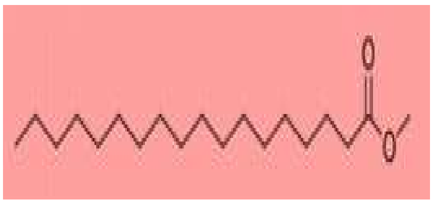


Fig.4: mass spectrum of hexadecanoic acid, methyl ester

iv-Methyl stearate (9.83%)

The mass spectrum of methyl stearate is shown in Fig.5. The peak at m/z 298 (R.T.18.473) is due to $M^+ [C_{19}H_{38}O_2]^+$, while the signal at m/z 267 correspond to loss of a methoxyl .



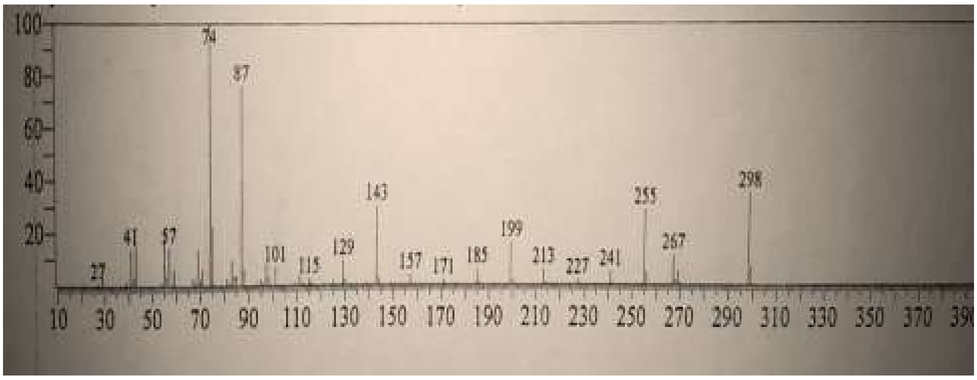


Fig.5: mass spectrum of methyl stearate

3.2-Antimicrobial assay

*Ocimumbasilicum*oil was investigated for antimicrobial activity against five standard microbial isolates. However, the oil failed to give antimicrobial potential against the following microbial isolates :*Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

Conclusion

Theoilextracted from *Ocimumbasilicum*was investigated by GC-MS analysis. The fatty acid content of the oil was determined by retention times and the observed fragmentation pattern. Twenty four components.

In the cup plate agar diffusion bioassay, the oil failed to show any responses.

Recommendations

The following is recommended:

-Other phytochemicals (steroids, alkaloids, flavonoids ..etc) present in the studied plant may be isolated and thoroughly investigated.

-The isolated oil could be evaluated for other biological activities including antimalarial, antiinflammatory effects.

References

1. Guenther, E ., In The Essential Oil. D.VanNostrand, New York, USA, Vol IV, (1950).

2. Masotti ,V., Juteau ,F., Bessière ,J.M., Viano , J., Seasonal and phonological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. J Agric Food Chem 51, 7115-7121(2003).

3. Angioni , A., Barra ,A., Coroneo , V ., Dessi , S., Cabras , P., Chemical composition, seasonal variability, and antifungal activity of Lavandulastoechas L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem 54,4364-4370 (2006).

4. WHO Food safety and foodborne illness. World Health Organization Fact sheet 237, Geneva, Switzerland (2002).

5. WHO World health report Reducing risks, promoting healthy life. World Health Organization, Geneva, Switzerland 248(2002).

6. WHO Guideline: Sodium intake for adults and children, World Health Organization (WHO), Geneva, Switzerland (2012).

7. Smith, R.L ., Cohen, S.M., Doull, J ., Feron, V.J ., Goodman, J.I., et al. A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: Essential oils. Food ChemToxicol 43, 345-363(2005).

8.Simic, A., Sokovic, M.D., Ristic, M., Grujic-Jovanovic, S., Vukojevic, J.J., et al. The chemical composition of some Lauraceae essential oils and their antifungal activities.Phytother Res 18, 713-717(2004).

9.Hajhashemi, V., Ghannadi, A., Sharif, B., Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandulaangustifolia Mill.J Ethnopharmacol 89, 67-71(2003).

10.Perry,N.S., Bollen,C., Perry, E.K., Ballard, C., Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol.BiochemBehav 75, 651-659(2003).

11. Carson, C.F., Riley, T.V., Non-antibiotic therapies for infectious diseases(2003).

12. Morais, Lilia, A. S., Essential oils in phytosanitary control.In :Bettiol, W., Morandi, M. A. B., (Ed.) Biocontrol of plant diseases: useand prospects. Jaguariúna-SP: EmbrapaEnviroment, Cap. 1, p.341(2009).

13. Lavabre, Marcel. , “Aromatherapy: healing by essential oils; translationRaffaelladeFilippis.” 7ª ed. Rio de Janeiro: Nova Era (2009).

14.Sangwan, N.S., Farooqi, A.H.A., Shabih, F., et al Regulation of essential oil production in plants. Plant Growth Regul 34,3–21(2001).

15. Baser, K.H.C., Demirci, F., Chemistry of essential oils. In: Berger, R.G., (ed) Flavours and fragrances—chemistry, bioprocessing and sustain nability. Springer, Berlin, pp 43–86(2007).

16.Council of Europe European pharmacopoeia, 5th edn. Council of Europe, Strasbourg(2004).

17.Litchenthaler, H.K., The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant MolBiol 50,47–65(1999).

18. Dewick, P.M., Chap_4 Theshikimate pathway: aromatic amino acids and phenylpropanoids. In: Medicinal natural products: a biosynthetic approach, 2nd edn. Wiley, United Kingdom, pp 121–164(2002a).

19. Sell, C., Chap.5 _The chemistry of essential oils. In: Baser, C.K.H., Buchbauer, G. , (eds) Handbook of essential oils: science,technology, and applications. CRC Press, Taylor & Francis Group, Boca Raton ,pp 121–150(2010).

20. Bakkali, F., Averbeck, S., Averbeck, D., et al Biological effects of essential oils—a review. Food ChemToxicol 46,446–475(2008).

21. Hunter, M. , Essential oils: art, agriculture, science, industry and entrepreneurship (a focus on the Asia-Pacific region).

Agriculture issues and policies series. Nova Science Publishers, Inc, New York(2009).

22. Waterman,A.S.,Tow conceptions of happiness:Contrasts of personal expressiveness (eudaimonia) and hedonic enjoyment.J0urnal of personality and social psychology,64(4),678(1993).

23. Jeena, K., Liju, V.B., Kuttan, R., Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J PhysiolPharmacol 57(1),51–62(2013) .

24. Ishnava,K.B., Chauhan,J.B., &Barad, M.B., Anticariogenic and phytochemical evaluation of Eucalyptus globules Labill. Saudi journal of biological sciences, 20 (1), 69-74(2013).

25.Orano,L.,Venditti,A.,Ballero,M.,Sanna,C.,Quassiniti,L.,Bramucci,M.,&Bianco,A.,Chemopreventive and Antioxidant Activity of the Chamazulene-Rich Essential Oil Obtained from Artemisia arborescens L. Growing on the IsIe of La Maddalena, Sardinia,Italy .Chemistry &biodiversity, 10(8),1464-1474(2013).

26. FERAZ, L., Portella,M.J.,Vallez,M., Gutierrez,F.,Martin-Blanco,A., Martin-Santos,R.,&Subira,S.,Hostility and childhood Sexual abuse as predictors of suicidal behavior in borderline personality Disorder.Psychiatry research,210(3), 980-985(2013).

27. Dee,D.P., Uppala,S.M., Simmons,A.J.,Berrisford, P., Poli,P., Kobayashi,S., &Bechtold ,P., The ERA-Interim reanalysis: Configuration and performance of the data assimilation system. Quarterly Journal of the royal meteorological society, 137 (656) , 553-597(2011).

28. Chakona, A., E. R., Swartz, P. H. ,Skelton, D., Tweddle, R. ,Bills, E., Swartz, W., Coetzer, et al. "The status and distribution of freshwater fishes." Zootaxa 3686 , 121-137(2013).

29. Dewick, P.M., Chap. 5 Themevalonate and deoxyxylulose phosphate pathways: terpenoids and Steroids. In: Medicinal natural products: a biosynthetic approach, 2nd edn. Wiley, United Kingdom, pp 167–285(2002b).

30. Zuzarte, M., Gonçalves, M.J., Cruz, M.T., Lavandulaluisieri essential oil as a source of antifungal drugs. Food Chem 135,1505–1510(2012a).

31. Sharif, R., Kiani, H., Farzaneh, M., et al Chemical composition of essential oils of Iranian Pimpinellaanisum L. and Foeniculumvulgare Miller and their antifungal activity against postharvest pathogens. Jeobp 11(5),514–522(2008).

32. Huang, Y., Zhao, J., Zhou, L., et al Antifungal activity of the essential oil of Illiciumverum fruit and its main component trans-anethole. Molecules 15(11),7558–7569(2010).

33. Bauer, K.,Garbe,D.,Common fragrance and flavour materials. Preparation ,properties and uses .VCH Verlagsgesellschaft, Weinheim(2001).

34. Gali-Muhtasib, H., Hilan, C.,&Khater, C., Traditional uses of Salvia libanotica (East Mediterranean sage) and the effects of its essential oils. Journal ofEthnopharmacology, Vol. 71 pp. 513–520(2000).

35. Ozbek, H., Ugras, S. ,Dulger, H., Bayram, I., Tuncer, I., Ozturk, G., & ztürk,A.,Hepatoprotective effect of F. vulgare essential oil. Fitoterapia, Vol.74, pp. 317-319(2003).

36. Pengelly,A.,The Constituents of Medicinal Plants; Allen &Unwin (2004).

37. Bowles, E.J., The Chemistry of Aromatherapeutic Oils; 3rd Edition Griffin Press,(2003).

38. Svoboda, K., Hampson, J.,& Hunter, T., Secretory tissues: Storage and chemical variation of essential oils in secretory tissues of higher plants and their bioactivity. International Journal of Aromatherapy, Vol.9, pp. 124-131(1999).

39. Deans,S.G., Svoboda,K.P.,Gundidza,M., &Brechany, E.Y., Essential oil profiles of several temperate and tropical aromatic plants: their antimicrobial and antioxidant activities. ActaHortic., Vol.306, pp. 229-232(1992).

40. Griffin, S.G., Wyllie, S.G., Markham, J.L., & Leach, D.N., The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour and Fragrance Journal, Vol.14, pp. 322-332(1999).

41. Edris, A.E., Pharmaceutical and Therapeutic Potentials of Essential Oils and Their Individual Volatile Constituents. Phytotheray Research, Vol. 21, pp.308-323(2007).

42. Baser, K.H.C., &Buchbauer,G., Handbook of essential oils: Science, Technology, and Applications, CRC Press NW(2010).

nior,G.A.S., Andrade, L.N. , & Batista, J.S., 43.DeSousa,D.P.,J Spasmolytic Activity of Chiral Monoterpene Esters. Rec. Nat. Prod., Vol.5, pp. 117-122(2011).

44. Sugawara,Y., Hara,C., Tamura, K.,Fujii,T., Nakamura, K., Masujima, T., & Aoki, T. , Sedative effect on humans of inhalation of essential oil of linalool: Sensory evaluation and physiological measurements using optically active linalools. AnalyticaChimicaActa Vol.365, pp. 293-299(1998).

45. Peana, A.T.,D’Aquila,P.S., Panin, F., Serra, G., Pippia, P., &Moretti, M.D.L., Antiinflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine, Vol.9, pp. 721-726(2002).

46. Ghelardini,C., Galeotti,N., &Mazzanti,G., Local anaesthetic activity of the essential oil of Lavandulaangustifolia. PlantaMedica, Vol.65, pp. 700–703(1999).

47. Ghelardini,C.,Galeotti,N., &Mazzanti, G., Local anaesthetic activity of monoterpenes and phenylpropanes of essential oils. PlantaMedica, Vol. 67, pp. 564– 566(2001).

48. Miceli, N., Taviano, M.F., Giuffrida D., Trovato, A., Tzakou, O., & Galati E.M., Antiinflammatory activity of extract and fractions from Nepetasibthorpii Bentham. Journal of Ethnopharmacology, Vol.97, pp. 261-266(2005).

49. Gomes, N.G.M., Campos, M.G., Orfao,J.M.C.,&Ribeiro,C.A.F., Plants with neurobiological activity as potential targets for drug discovery. Progress in Neuro- Psychopharmacology & Biological Psychiatry, Vol. 33, pp. 1372-1389(2009).

50. Dorman, H.J.D. ,& Deans, S.G., Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, Vol. 88, pp. 308-316(2000).51. De Sousa, D.P., Junior, G.A. , Andrade, L.N., Calasans, F.R., Nunes, X.P., Barbosa-Filho, J.M., & Batista, J.S., Structure and spasmolytic activity relationships of analogues found in many aromatic plants. Z. Naturforsch. C. Vol.63, pp. 808-812(2008).

52. Hawk,C., Ndetan, H., Evans,MW., Jr.Potential role of complementary and alternative health care providers in chronic disease prevention and health promotion: an analysis of National Health Interview Survey data. Preventive Medicine. 54(1),18–22. [PubMed]v(2012).

53. Leach, M.J, A critical review of natural therapies in wound management. Ostomy Wound Manage; 50,36–40. 42,44–6 passim. [PubMed] (2004).

54. Buckle, J., Clinical Aromatherapy in Nursing. London: Edward Arnold Ltd, (1997).

55. Swamy, M. K., Sinniah, U. R. Patchouli (PogostemoncablinBenth.): botany, agrotechnology and biotechnological aspects. Industrial Crops and Products.87,161–176.doi: 10.1016/j.indcrop.2016.04.032. [Cross Ref](2016).

56-Ozcan.N.M;,Chalchat.C.C (2002):Essential oil Composition of Ocimumbasilicum L and Ocimum minimum L.in Turkey , Czech , Food sci ,20,:223-22

57-Sebic.S.A; Dinic Z; llicic.R ; pivic.R ; Josic.D (2017): Effect of Indigenous pseudomonas chlororaphis strains on Morphological and Maim chemical Growth parameters of Basil (OcimumL)ratarpovrt 54(2):42-47

58-Bilal.A; Jahan.N; Ahmed.A; Bilal.N.S; Habib.S; Hajra.S(2012): phytochemical and pharmacological studias on OcimumBasilicumL. Areview.IJCRR; 04:23

59-Pande.K.A; Singh.P; Tripathi.N.N (2014): Chemistry and bioactivities of essential oils of somOcimum species: on overview . Asian pae J Trop Biomed 4(9):682-694

60-Koul.O; Suresh.W ,Dhaliwal.G.S.(2008): Essential oils as a green pesticides : potential and constraints . biopesticInt ;4:63-84

61-Kirtikar .K.R ;Basu.B.D Indian Medicinal Plants with illustrations . 2nd ed. volVIII.Uttaranchal : Oriental Enterpeises;2003. 2701-2705 .

62-Khatri .M; Nasir.M.K,A; Saleem.R; andNoor.F (1995) Evaluation of Pakistani sweet basil oil for commercial exploitation Pakistan journal of scientific and industrial research ,38,281-282.

63-Verna.S; Chemical Constituent and pharmacological action of Ocimum sanctum (Indian holy basil-tulsi) , JPHYTO 2016,5(5): 205-207.

64-Khare.C.P . Indian medicinal plants , an illustrated dictionary new Delhi :springer India (p) Ltd ;2007. 442-4.

65-Nod kami. K.M ; The Indian plants and Drugs . New Delhi :Shrishti Book Distributors;2005.263.

66-Chopra.R.N; Nayar.S.L; Chopra. I. C. Glossary of Indian Medicinal Plants , 6th ed . New Delhi :NISCIR; 2002-178-79.

67-Anonymous . The Wealth of India . Vol IV .New Delhi : National Institute of Sciences Communication and Information Resources , 2003.216-218.

68-Ahmet.A; Medine.G; Meryem.S; Hatice.O; Fikrettin.S;Isa.K.Antimicrobial Effects of Ocimumbasilicm (labiatae) extract. TurK J Biol 2005;29:155-60.