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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(B.Sc.( Hons) Chemical Laboratories)

Supervisor:

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بسم الله الرحمن الرحيم

وقل أعملوا في سيرى الله عملك ورسله والمؤمنون وسُبِرَتْ
إلى عدا الْغَيْبَ والْشَّهِيدَةَ فِيْنَ يُشْكِرُونَ ما كَنَّا نَعْمَالُونَ

(النور -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 الحمض الدهني بالزيت بزمن الاستبقاء وننم التكسر. اتضح أن الزيت يحتوي على أربعة وعشرون مكونة المكونات الرئيسية 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 أي فعالية ضد الميكروبات القبلية 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{11}.

1-2-Definition of essential oils

Essential oils are comprising a complex mixture of lipophilic organic matter characterized by a low molecular weight. Due to their highly volatility, essential oil differ from 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\textsuperscript{11}.

Essential oils are extracted from aromatic and medicinal plants. They are abundant in certain plant families including: Lauraceae, Myrtaceae and 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 sun's rays and freshen the air around the plant\textsuperscript{12,13}. Essential oils are phytochemicals formed by several volatile compounds\textsuperscript{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\textsuperscript{16-18}. Sometimes, essential oils are frequently associated with gums.
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\textsuperscript{20-23}.

Essential oils are dominated by monoterpenes and sesquiterpenes. Phenylpropanoids are frequent present in volatile oils.
However, some essential oils may also contain fatty acids and their esters and, more rarely, nitrogen and sulfur derivatives\textsuperscript{24,25}.

1.3-**Constituents of essential oils**

A large number of essential oils contain hydrocarbons; these category contains 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, \( \text{p-menthane} \), \( \text{pinene} \), \( \text{pinene} \), \( \text{sabinene} \), \( \text{pcymene} \), \( \text{myrcene} \), \( \text{phellandrene} \), \( \text{thujane} \), \( \text{fenchane} \), \( \text{farnesene} \), \( \text{azulene} \), \( \text{cadinene} \) and \( \text{sabinene} \) are some examples of this family of products. These compounds are known to be endowed with a wide range of therapeutic activities.

**1.3.1-Terpenes**

Terpenes are known to comprise the largest single class of phytochemicals found in essential oils\textsuperscript{26}. Terpenes consist of isoprene units. Each isoprene unit consists of a \( \text{C}_5\text{H}_8 \) 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 terpenes are
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\textsuperscript{27}.

Terpenoids are derived from aliphatic precursors such as geraniol for the formation of monoterpenes, farnesol for sesquiterpenes, and geranylgeraniol for diterpenes\textsuperscript{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\textsuperscript{28}.

The occurrence 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\textsuperscript{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 essential oils\textsuperscript{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, monocyclic monoterpenic 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 includes oxidations or rearrangements processes give rise to several other phytochemicals (known as monoterpenoids). These compounds include highly functionalized chemical entities.

1.3.3- Sesquiterpenes

Sesquiterpenes are characterized by less volatility in contrast to terpenes and they have a greater potential for stereochemical diversity and have stronger odors. Sesquiterpenes possess antiinflammatory activity. They also possess some bactericidal effect.

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 antiinflammatory sesquiterpenes, chamazulene, only has 14 carbon atoms but is usually included with sesquiterpenes. Chamazulene and caryophyllene have strong antioxidant, and
antitumor activity\textsuperscript{35,36}. Sesquiterpenes could be monocyclic, bicyclic or tricyclic and are a very diverse group\textsuperscript{37}. Examples include $\alpha$-bisabolene in black pepper (Piper nigrum) and $\beta$-caryophyllene in ylangylang (\textit{Cananga odorata})\textsuperscript{38}. Some sesquiterpenes such as $\alpha$-farnesene can be effective against the bacteria that cause tooth decay\textsuperscript{39}.

\subsection*{1.3.4- Diterpenes}

Only a limited number of diterpenes occur in essential oils. This is due to the fact that they are big, heavy molecules with correspondingly high boiling points. Diterpenes can occur during 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 in essential oils. Since they are heavier than monoterpenes and sesquiterpenes, longer distillations are required. Examples of diterpenes include phytol and geranylgeranylcitronellol (acyclic); camphorene (monocyclic); scareol and its derivatives, manool and labdane (bicyclic like) and kaur-15-ene, phyllocladene, pimaradiene, and sandaracopimaradiene (tricyclic)\textsuperscript{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\textsuperscript{41}. Examples of these compounds are artemisia ketone, santolinatriene, and chrysanthemol\textsuperscript{42}. Other irregular monoterpenes namely necrodane derivatives also occur primarily in Lavandulaluisieri oils\textsuperscript{43}.

1.4-Polypropanoids

Polypranoids consist of one or more C\textsubscript{6}–C\textsubscript{3} units, the C6 unit being a benzene ring. There is no widely accepted classification for these compounds\textsuperscript{44}.

Phenylpropanoids are found in some plant species mainly from the Apiaceae, Lamiaceae, Myrtaceae, and Rutaceae families\textsuperscript{45}. Examples of phenylpropanoids occurring in essential oils include : anethole which occurs in high amounts in Pimpinella anisum and Foeniculum vulgare\textsuperscript{46}, and star anise found in Illicium verum\textsuperscript{47}. Anethole is characterized by sweet anise odor and is used in liquors, soap and perfumes\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.

Indole which 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\textsuperscript{52}. Pyridines and pyrazines are found
in *Pipernigrum*, *sweet orange* – *Citrussinensis*, and *Chrysopogonzizanioides* oils.

### 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.

### 1.6- Oxides

Oxides or cyclic ethers are characterized by their strong odor and by far the most known oxide is 1,8 cineole, 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.

### 1.7- Lactones

Lactones are usually in pressed oils. They are relatively of high molecular weight. Examples are: nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone, epinepetalactone, aesculatine, citroptene, and porsoralen. Such lactones are used therapeutically as: antipyretic, sedative and
hypotensive, but they may be associated with allergic reactions\textsuperscript{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\textsuperscript{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\textsuperscript{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.\textsuperscript{54}

1.11- Ketones
Ketones have 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\textsuperscript{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 oil ketones include: carvone, menthone, pulegone, fenchone, camphor, thujone and verbenone.

Table 1: Different classes of essential oils and their bioactivity

<table>
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<tr>
<th>Class of compounds</th>
<th>Examples</th>
<th>Bioactivities</th>
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<tr>
<td>Hydrocarbons</td>
<td>Limonene, myrcene, pinene, pinene, sabinene, cymene, myrcene, phellandrene.</td>
<td>Stimulant, antiviral, antitumour, decongestant, antibacterial, hepatoprotective</td>
</tr>
<tr>
<td>Esters</td>
<td>linalyl acetate, geraniol acetate, eugenol acetate, bornyl acetate</td>
<td>spasmolytic, sedative, antifungal, anaesthetic, anti-inflammatory</td>
</tr>
<tr>
<td>Oxides</td>
<td>Bisaboloneoxide, linalool oxide, sclareol oxide, ascaridole</td>
<td>antiinflammatory, Expectorant, stimulant</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Lactones</td>
<td>nepetalactone, bergaptene, costuslactone, dihydronepentalactone, alantrolactone</td>
<td>Antimicrobial; antiviral; Antipyretic, sedative, hypotensive; analgesic</td>
</tr>
<tr>
<td>Alcohols</td>
<td>nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone.</td>
<td>Antimicrobial; antiviral; Antipyretic, sedative, hypotensive; analgesic</td>
</tr>
<tr>
<td>Phenols</td>
<td>thymol, eugenol, carvacrol, chavicol.</td>
<td>antimicrobial, spasmodytic, anaesthetic, irritant, immune stimulating</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde.</td>
<td>Antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic, sedative, spasmolytic</td>
</tr>
<tr>
<td>Ketones</td>
<td>carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone</td>
<td>mucolytic, cell regenerating, sedative, antiviral, neurotoxic, analgesic, digestive, spasmolytic</td>
</tr>
</tbody>
</table>

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\textsuperscript{55}.

Recently, essential oils are gaining tremendous 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\textsuperscript{55}.

\textbf{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\textsuperscript{55}.

\textbf{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 Mentha piperita) and cinnamon. Many studies demonstrated that essential oils effectively destroy several fungal, viral and bacterial pathogens, including methicillin-resistant *Staphylococcus aureus, Helicobacter pylori* and *Candida albicans* infections. \(^5\)

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 way. Oregano 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. \(^5\)

**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\(^5\). It has been reported that ginger 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\(^2\). 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\(^2\).

**1.12.4-Effect of essential oils on body energy Levels**
Essential oils could boost 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\textsuperscript{23}.

1.12.5- Neuroprotective effects of essential oils

Essential oils are known to possess a neuroprotective property and could improve 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\textsuperscript{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\textsuperscript{24}.

1.13- \textit{Osmium balsicum} L.

Basil (\textit{Osmium balsicum} L.) is the most common species in Lamiaceae family. 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.\textsuperscript{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, methylchavicol, estragol, thymol and \textit{p}–cymen. The oil also contains saponins, tannins, flavonoids, glycosides, enzymes and some organic acids.\textsuperscript{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\textsuperscript{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 cough.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:
Basil is useful in diseases of heart and blood including leucoderma. The juice relieves joints pain, gives luster to eyes, it is also good for toothache, earache and cures some diseases when used with camphor. The juice of plant is dropped into ears to cure dullness of hearing. 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. The plant is useful in treatment of stomach complaints, fever, cough and gout. The warm leaves juice of this plant along with honey is used to treat croup. It also forms an excellent nostrum for the cure of ringworm. It is used as a
lotion for sore eyes too. 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. A cold infusion of seeds is said to relieve the pain of parturition. Seeds are also given internally to treat cystitis, nephritis and internal piles. Due to the mucilaginous and cooling effect, an infusion of basil seed is given to treat gonorrhea, diarrhea and chronic dysentery.

Methanolic extract of *Ocimum basilicum* 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.

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.

In vitro antioxidant activities of 50% hydroalcoholic extract of Ocimum species namely *Ocimum basilicum* and *Ocimum sanctum* were achieved at varying concentrations (10-15 μg/ml)
using DPPH radical scavenging activity. The results showed that *Ocimum basilicum* had more antioxidant activity than *Ocimum sanctum*. In another study the ethanolic extract of *Ocimum basilicum* 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 a period of 30 days at a concentration of 5μg/ml. Their antioxidant activities 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 *Ocimum basilicum* (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 : --- ; Temp. , 150.0°C ; Hold time(min.⁻¹) , 1.00

Rate : 4.00 ; Temp. , 300.0°C ; Hold time(min.⁻¹) , 0.00

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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 manufacturer instructions.

Broth cultures (5.0x10⁷ 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 100 mg/ml of test sample. For bacteria the plates were incubated at 37°C for 24h., while for fungi the plates were incubated at 25°C for 3 days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicillin, gentamycin and
clotrimazole were used as positive control and DMSO as negative control.
3-Results and Discussion

3.1-Constiruents of *Ocimumbasilicum*

The oil extracted 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](image-url)
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/z 294(
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-Hexadecanoic 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](image)

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.

![image](image)
3.2-Antimicrobial assay

*Ocimum basilicum* 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

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.

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


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


