Sudan University of Science and Technology

College of Postgraduate Studies

Chemical Constituents and Biological Activity of *Eruca sativa* Oil

المكونات الكيميائية والفعالية البيولوجية لزيت الجرجير

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

By

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الإستهلال

قال تعالي (إقرأ بإسم ربك الذي خلق (1) خلق الانسان من علق (2) إقرأ وربك الاكرم (3) الذي علم بالقلم (4) علم الانسان ما لم يعلم (5)).

سورة العلق (الأيات1-5)

صدق الله العظيم

Dedication

To

my mother

To my father

To my sisters and brothers

Acknowledgement

Firstly, I would like to thank **Allah Almighty** who gave me health, strength and patience to complete this work.

I would like to express my deep gratitude to my supervisor Prof. Mohammed Abdel Karim, for his guidance, support, valuable comments and advice. I would also like to express my sincere thanks to my family for financial support.

Finally, I would like to thank my friends for their moral support and continuous encouragement.

Abstract

The oil from *Eruca sativa* was analyzed by GC-MS.The analysis showed 16 components. The major components are : 13-docosenoic acid, methyl ester (33.72%) , cis- 13-eicosenoic acid, methyl ester (14.67%) **,** 9- octadecenoic acid (z)-, methyl ester (13.21%) **,** 9,12- octadecenoic acid (z,z)-, methyl ester (11.08%) **,** 9,12,15- octadecatrienoic acid, methyl ester (8.57%) **,** hexadecanoic acid, methyl ester (6.48%) . Antimicrobial activity of Eruca sativa oil was conducted. The oil showed partial activity against ***Acinetobacter baumanni and Candida albicans*.**

المستخلص

استخلصت بذور نبات الجرجير بالهكسان حيث تم استخلاص الزيت الثابت. ثم حلل الزيت بتقنية كروماتوغرافيا الغاز- مطياف الكتلة والتي أوضحت وجود 16 مكونا أهمها:

13-docosenoic acid, methyl ester (33.72%)

cis- 13- eicosenoic acid, methyl ester (14.67%)

9- octadecenoic acid (Z)-, methyl ester (13.21%)

9,12- octadecenoic acid (Z,Z)-, methyl ester (11.08%)

9,12,15- octadecatrienoic acid, methyl ester (8.57%)
hexadecanoic acid, methyl ester (6.48%).

وفي اختبار مضاد الميكروبات أظهر الزيت فعالية جزئية ضد :

(Acinetobacter baumanni) and (*Candida albicans).*

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Introduction

1. Introduction

1.1-General Approach

Nature has served as a rich source of medicinal plants for thousands of years which are major source of organic compounds. Natural organic compounds are extracted from all non woody parts of plants including leaves, flowers, roots, seeds, barks and fruits by hydrodistillation, steam distillation, soxhlet extraction and chromatographic methods [1].

Natural products offer huge diversity of chemical structures and can be classified into several families: terpenoids, alkaloids, carotenoids, flavonoids, phenolics and tannins on the basis of their chemical structures. Terpenoids are the most abundant and structurally diverse class of natural products. Among terpenoids, monoterpenes are remarkably diverse in terms of their structure, properties and functions. Monoterpenes are abundant natural compounds that meet the non-toxicity and low cost criteria requirement for new pesticides [2]. Monoterpenes can be classified into two major groups: monoterpene hydrocarbons such as myrcene, pinene, terpinene, limonene, p-cymene, α and β-phellandrene and oxygenated monoterpenes which include acyclic monoterpene alcohols (geraniol, linalool etc.), monocyclic

alcohols (menthol, terpineol, carveol, borneol etc.), aliphatic aldehydes (citral, citronellal etc.), aromatic phenols (carvacrol, thymol, safrol, eugenol etc.). Semi-synthetic derivatives of natural products are generally assumed to be more bioactive than natural products and produced by chemical manipulation of these natural compounds [3]. Essential oils are aromatic, concentrated hydrophobic oily liquids which are by-products of plant metabolism and are commonly referred to as plant secondary metabolites. Essential oils are volatile in nature because they easily diffuse into the air and give distinctive odor, flavor or scent to a plant. An essential oil may contain a mixture of 20-60 aromatic and aliphatic organic compounds, all of which are responsible for its characteristic fragrance and flavor [4]. Essential oils have long been known and used throughout the world in the preparation of perfumes, cosmetics, beverages, medicinal foods, disinfectants, insecticides, fungicides and condiments [5].

Essential oils are volatile oil, generally odorous, which occur in certain plants or specified parts of plants, and are recovered by accepted procedures, such that the nature and composition of the product is, as nearly as practicable, unchanged by such procedures.

It specifies clearly that the nature and composition of the oil must be unchanged by process of extraction. Oil derived by another

technique might be of slightly different chemical composition and therefore might not be accepted by the market as normal oil.
Essential oils are used widely by the pharmaceutical and cosmetic/ perfumery industries as well as in aromatherapy and alternative medicine [6].

Essential oils have some distinctive characteristics, which make them a very valuable commodity with many industrial uses and applications. Their aromatic value enable them to be used as flavourings in both the food and beverage industries. These oils are also widely used in both the cosmetic pharmaceutical industries [7].

The chemical composition of the essential oils is important in determining their quality and consequently price in the market. It is therefore important to note and understand some of the parameters such as temperature, pressure, and time of extraction may affect the quality and yield of essential oil. Essential oils can be extracted using a variety of methods although some are not commonly used today. These include methods such as solvent extraction, supercritical fluid extraction, cold pressing and microwave extraction. The suitability of extraction method varies from plant and there is significant differences in the capital and operational costs associated. [8].

Analysis of essential oil is done by using Gas Chromatography with Mass Spectrometer. The qualitative and quantitative analysis is done to know the constituents in the oil and the percentage of components present in the oil respectively, by doing so we can know the purity of that particular oil [9].

1.2 Literature review of essential oils

It is estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, D. E. 1996). Moerman (1996) reported that while 625 species of plants have been used by various Native American groups as food, 2,564 have found use as drugs. According to his calculations, this leaves approximately 18,000 species of plants which were used for neither food nor drugs [10].

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years . These purposes vary from the use of rosewood and cedar wood in perfumery, to flavoring drinks with lime, fennel or juniper berry oil, and the application of lemongrass oil for the preservation of stored food crops. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and

processed food preservation, pharmaceuticals, alternative medicine and natural therapies.

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials [11].

The term essential oil dates back to the sixteenth century and derives from the drug Quinta essential named by Paracelsus von Hohenheim of Switzerland [12].

Essential oils or “essences” owe their name to their flammability. Numerous authors have attempted to provide a definition of essential oils. The French Agency for Normalization: Agence Française de Normalisation (AFNOR) gives the following definition (NF T 75-006): “The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or “dry”” distillation. The essential oil is then separated from the aqueous phase by physical means [13].

This definition encompasses products obtained always from vegetable raw material [14]. 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 are 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. The latter is intended as a therapeutic technique including massage, inhalations, or baths using these volatile oils. Essential oils can serve as chemical signals allowing the plant to control or regulate its environment (ecological role): attraction of pollinating insects, repellent to predators, inhibition of seed germination, or communication between plants (emission signals chemically signaling the presence of herbivores, for example. Moreover, essential oils also possesses antifungal or insecticidal and deterrent activities. All parts of aromatic plants may contain essential oils as follows:

-Flowers, of course, including: orange, pink, lavender, and the (clove) flower bud or (ylang-ylang) bracts,

-Leaves, most often, including: eucalyptus, mint, thyme, bay leaf, savory, sage, pine needles, and tree underground organs, e.g., roots (vetiver),

-Rhizomes (ginger, sweet flag),

-Seeds (carvi, coriander),

-Fruits, including: fennel, anise, citrus epicarps,

-Wood and bark, including: cinnamon, sandalwood, rosewood [15].

1.3- Brassicaceae family

Brassicaceae is a family classified in the plant order Brassicales according to the Angiosperm Phylogeny Group system (APC system) which contains an assortment of cruciferous plants and crops that are grown worldwide for oil, food and feed [16]. Brassicaceae family comprises vegetables ranging from cabbage, broccoli, Brussels sprouts, cauliflower, collard greens, kale, mustard, turnip, radish, bok choy to herbaceous vegetables such as watercress and *Eruca sativa* (Arugula). Brassicaceae ranks second behind the plant family Solanaceae in vegetable production and consumption worldwide [17]. *Arabidopsis thaliana*, a model plant system with a complete sequenced genome, is a member of the Brassicaceae family.

1.4- Health benefits of Brassicaceae

Brassicaceae plants have medicinal properties and have been used as an aphrodisiac, for eye infections, for digestion issues, for kidney problems, as a deodorant, as an anti-inflammatory, for blood circulation, and an acne treatment [18] because of the phytochemicals produced by plant. Brassicaceae phytochemicals include, among others, carotenoids, flavonoids, vitamin C, and glucosinolates [19].

Sulfur-containing compounds, such as glucosinolates, indole derivatives, thiocyanates and isothiocyanates, are linked to

anticarcinogenic mechanisms [17]. Epidemiological evidence has provided evidence that the consumption of vegetables in the Brassicaceae family is associated with the reduced incidence of cancer. It has been proposed that anticancer phytochemicals, such as glucosinolates, isothiocyanoates and indole derivatives are responsible for this effect [17, 20].

1.5 *Eruca sativa* (Arugula)

Arugula, Eruca sativa (or sometimes incorrectly listed as E.vesiceria subsp. sativa), is a tangy green in the mustard family (Brassicaceae) also known as rocquette, rocket, garden rocket,rocketsalad or Italian cress. It is native to the Mediterranean,Turkey and northern India, where it grows on dry, disturbedground. It has been cultivated at least since Roman times (and is still common in Italian cuisine), and the seeds were once used in aphrodisiac concoctions. It is a cool-season crop, grown like lettuce or spinach, to use raw in salads or cooked as an accent in a variety of dishes. Although arugula has been around for a long time, it only became popular in the United States in the 1990's as a trendy leafy green. This is an annual, growing quickly from seed, just like radishes. Like other leafy crops, it does tolerate frost so can be planted several weeks before the last frost. It is easily grown from seed, but transplant scan is also used. It is often a component of mesclun or "field greens"mixes. Seeds

germinate within a few days even in cool soil. Seedlings have two rounded cotyledons each with an indentation on the end, while the first young leaves are entire and elongated. Older leaves have lobes of varying degrees, so these leaves look like dark green oak leaf lettuce – often deeply pinnate with 4-10 lobes.

The creamy white flowers have four petals, each with purple to deep brown-red veins, and bright yellow stamens. The sepals are shed soon after the flower opens. The ½-3/4 (2-4cm) inch wide flowers are edible and be used as a garnish on salads or soups, with a milder flavor than the leaves. Whole stems can also be cut as a filler in bouquets. When pollinated by insects, the flowers are followed by erect, elongate pods (siliqua) up to 40 mm long and containing 6-8 brown seeds. When young and tender, these pods are edible. Arugula is somewhat weedy, reseeding readily if the plants are allowed to mature in the garden. To collect the seeds it is best to cut the stems when the plants begin to dry. Hang the cut stems upside down in a paper bag until the pods shatter and the seeds are released. Plant arugula grows in full , well-drained soil. Light shade may help slow bolting during the hottest part of the growing season. Because it is such a fast-growing crop it can be used as an interplanting between other crops in small gardens short on space. Arugula can also be grown in containers, even in the winter on a bright windowsill. Arugula requires little care and

has few pests, although flea beetles can damage leaves or completely devour small plants when numerous. Leaves with the shot-holes typical of flea beetle feeding may not be as appetizing as undamaged leaves, but can still be consumed. There is a fair amount of variability in resistance to bolting, leaf patterns, stem color, branching habit, and seed production, and some selections have been made. However, there are few cultivars generally available. Leaves can be harvested as soon as they are a couple of inches long. The tender young leaves have a distinctive spicy, pungent flavor variously described as nutty-peppery, mustard-like or having a horseradish-like taste[21].

Arugula, a member of the Brassicaceae family is primarily eaten as a salad or used as a spice because of its aromatic and spicy flavor. It is a perennial herb native to the Mediterranean coast [22]. The plant's brown seeds measure between 1.5 – 2.0 mm in length, basal leaves are 10 – 25 cm long, and the plant can be up to 80 centimeters in height [23]. Because of Arugula's uniquely pungent

flavor, cultivation has spread around the globe [20]. For example, in Italy, where it is known as "rucola," is used with garlic and oil to season spaghetti because of its sesame seed scent [23]. In Asia, Arugula is an important source of oil seeds in addition to being a vegetable. Two of the most produced compounds in Arugula are 1, 2-bis (4-isothiocyanatobutyl) disulfane and 4-mercaptobutyl

isothiocyanate , both responsible as flavor determinants in Arugula [25, 26].

1.5.1 Classification of *E.sative*

- Kingdom : plantae
- Unranked : Angiosperms
- Unranked : Eudicots
- Unranked : Rosids
- Class : Magnoliopsida
- Subclass : Rosidae
- Order : Brassilcales
- Family : Brassicaceae
- Genus : Eruca
- Species : E.sative [10].

1.6 History of essential oils

In the east the history of essential oils began; for the process of distillation the technical basis of the essential oil industry was conceived and first employed in the orient, especially in Egypt, Persia, and India. Data on the methods, objectives and results of distillation in ancient times are scarce and extremely vague. Indeed, it appears that the only essential oil of which the preparation (by a somewhat crude distillation) has been definitely

hed is the oil of turpentine and, if we care to mention it in connection with essential oils, camphor [27].

Early in history, man exhibited a great deal of interest in the preservation of the fragrances emitted by plants, and those who ware later to be called chemists occupied themselves with separating the essence from plants[27].

It was probably observed that heating of the plant caused the odoriferous components to evaporate, and that upon cooling and subsequent condensation, droplets united, forming a liquid consisting of two layers- water and oil. While, in such primitive experiments, the water from the plant is used to carry over the oils, additional water or steam was later introduced in extraction chamber to obtain better yield and quality [27].

1.7- History of Arugula

Arugula is a Mediterranean annual herb of the mustard family. It is a species of eruca native to the Mediterranean region, from morocco, Portugal east to Lebanon and turkey.

It has a rich and peppery taste and has an exceptionally strong flavor. It is often used in salads, and sometimes cooked with other vegetables for pasta sauces or meats in Italy and other parts of world [28].

1.7.1- Names

- English- rocket
- French- roquette
- Greek- rokka
- Spanish- arugula, rucula oruga
- Romanian- voinicica
- Serbian, Slovenian and polish- rukola
- Turkish- roka
- Portuguese- rucula

- Itlian- rucola [29]

1.7.2- Plant Description

Arugula is an edible plant and found in different types. It grows to about 70m tall (2.5feet). It has four petaled leaves with white-purple veined flowers [29].

Arugula herb is sometimes mistaken as lettuce leaves. But it is different as it has strong peppery taste being a member of mustard family. Sunny season is required to grow arugula. The best time period for this herb is spring to early summer [29].

1.7.3-**Cultivation**

Arugula seeds can be sow best in any good fertile and well drained soil from April to September. Seeds must be sown deep in the soil maximum in drills (18-24inch) spaced apart. This plant requires well drained fertile soil and sunny season. The seed should be protecting from direct sunlight and from freezing temperature [29].

1.7.4- Part Used

Arugula is a four petaled leaf, and its leaves are used in salads. Its seeds are also used to extract oil and in other food items. Apart from leaves and seeds, flowers are also used [29]].

1.7.5-*Eruca sativa* Characteristics

Arugula is an annual plant which has lance-shaped leaves and four-pelated cream colored flowers . Plant leaves are found all over the year, while flowers just from May to August. It grows up to 80 cm length with erect stem. Lower leaves are lyrate-pinnatisect. Seeds color varies from yellow green to brown and also vary in length from 1.7-3 mm. It is well known for its property of bearing drought. As a result, it is produced in barren regions around the world [30].

1. 7.6-on-food benefits of *Eruca sativa*

Non-food benefits include using it in cosmetics. Grinding arugula seeds and putting them as cream on the face can cure teen's pimples. They can be used as deodorant as well. When eating 6 arugula leaves on an empty stomach, it removes sweat's bad odors. It has been used in early ages to produce an extract that protects from dogs bites. As being type of spices, arugula seeds are grinded and used in meat flavoring [31]. Arugula seeds has antibacterial and antifungal effects, but to a different extent. Those factors are considered as non-food benefits. It is best used as antibacterial

agents and less efficient for fungal growth. In early studies, this plant has shown its antimicrobial effect on Escherichia coli, *Salmonella typhi*, and *Bacillus subtilis* only. Later studies showed that using crude water extract from the plant, methanolic extract and aqueous extract from seeds are the ways to inhibit the growth of those microorganisms. Microorganisms that are inhibited in the presence of mentioned extracts are: *Spadicoides stoveri, Paecilomyces variotii, Penicillium funiculosum, Penicillium lilacinum, Enterobacter agglomerans,* and *Hafnia alvei* [32].

1.7.7- Health Benefits of arugula

Arugula is packed with certain types of vitamins, minerals, antioxidants that offer a lot of benefits to human health. Most people prefer to consume arugula because of its unique taste without knowing that the health benefits of arugula are beyond its unique taste and shape [33].

i-There are a lot of antioxidants beside vitamins C and A found in arugula offering powerful antioxidants that are essential for body metabolism and provide protection that required by the body.

ii- There are two potent anticarcinogenic ingredients found in arugula which are effective to fight cancer, these are : indole- 3- carbinol and sulforaphane. Besides that, arugula is also rich of

antioxidants that are effective to fight free radical which stimulate the development of cancerous cells in the first place.

iii-Sometimes the biggest problem of tumor is not only the tumor itself but when the tumor has spread over the body. Arugula contains properties that could prevent new tumors to appear.

iv-Argla is so rich in omega 3 fatty acid and indole- 3 -carbinol. Such combination could control inflammation.

v-Vitamin C and those antioxidants found in arugula are effective against viral infection.

vi-Vegetables that are rich of minerals are always good for heart and arugula is no exception. The minerals found in this green vegetable are enough to maintain the health of the cardiovascular system.

vii-Arugula which is characterized by high level of proteins is a solution for those who suffering .The same thing is true for cholesterol problem .
Arugula is not only rich in calcium but also contains vitamin K in relatively high amount. This combination will promote healthy bones, beside strong teeth.

viii-The combination of vitamin C and antioxidants found in arugula are the best natural solution to promote healthy skin and prevent some skin conditions like eczema, acnes and dull skin.

ix-There are a lot of factors that could cause premature ageing , but whatever the factors are, consuming arugula could help in preventing premature ageing.

x-Arugula contains powerful antioxidants that could help in preventing degenerative diseases .

xi-Blood clotting is not always bad but this 'bad' effect is caused by the appearance of clots in the wrong place, like in the artery. Arugula contain vitamin K that will promote natural blood coagulant and at the same time protect could artery.

xii-Arugula is one of the vegetables which is so rich in vitamin A. Consuming it regularly is not only giving a better eyesight but also may prevent some vision problems associated with ageing.

xiii- Arugula is rich of vitamin A as well as omega 3 fatty acid.

Consuming arugula in regular basis since young age could prevent all many eye conditions. when rich senior age because ixx- Argula is rich in folate and during pregnancy, consumption foods rich in folate is highly recommended because it promotes the optimal development of fetus inside the womb especially in the first three semesters.

ix-Prevent Baby Born with Birth Defects: One of the factor that causes baby born with birth defect is when during pregnancy the fetus is not getting enough nutrients some the developments are not

optimal. Folate contains in arugula could make sure optimal development of the fetus.

x-mega 3 fatty acids and folate found in arugula could assist in reducing the symptoms of depression.

xi-When it comes to Alzheimer, the best solution to is making sure that your brain is getting enough nutrients like omega 3 fatty acids and folate found in argula.

xii-Cognitive decline will be suffered by everyone in old ages but folate contained in arugula could help slowing down the process.

xiii-To boost energy it is essential that the body is getting enough oxygen distributed by hemoglobin in red blood cell, so consuming foods rich of minerals and folate like argula may boost energy .

ixx-Arugula contains strong detoxifications compounds that are essential for a healthy body.

xx-Bowel movement is essential for metabolism, the slower the movement the slower is body metabolism as well. Arugula is rich of fiber that could promote healthy bowel movement.

xxi-Arugula is rich in fiber and it could help in digesting meals in easier way.

xxii-Vitamin B complex found in arugula is essential to the cells metabolism that could act as supporters in all kinds of different cell activities including energy production and the production of red blood cells.

xxiii-Aphrodisiacs contained in arugula are quite enough to boost the testosterone level in men as well as the quality and quantity of the sperm.

ixxx-The problem with weight loss program is not how to do it but much more about how to control the hunger. Chlorophyll found in arugula is effective to suppress your appetite and control your hunger.

iixxx-Arugula , this green vegetable, is not only tasty but also contains vitamin K. This vitamin is very healthy for liver, kidney as well as bladder because it could prevent the clog caused by excessive calcium in the blood stream and as a result could prevent the possibility of liver damage.

iiixxx-Many studies have proven that arugula is really great in preventing osteoporosis. Consuming arugula regularly is highly recommended even for those who have been diagnosed with this condition because calcium found in arugula will assist in slowing down the bone loss process.

ivxxx-There are some factors that make arugula a great immunity system booster. It contains antioxidants, including as strong vitamin A and vitamin C that maintain proper metabolism and improve of immune system.

iivxxx-One of the properties that are responsible to the production of white blood cells in the body is the trace element - copper.

White blood cells are essential in proper blood clotting and arugula is rich of minerals including copper.

iiivxxx-Minerals found in arugula are essential to promote the production of red blood cells.

ixxxx-Consuming arugula could optimize the mineral absorption and make sure that the body will get enough minerals.

xxxx-Argula is a natural deodorant helping in beating bad breath and eliminating body odor.

xxxxi-Argula is recommended for diabetes subjects . Consuming arugula regularly could help in preventing the sugar spike [33] .

xxxxii-Some researchers showed that arugula can be used as an alternative cure to ulcers[33].

Some scientists concluded that this herb possesses ant-ulcer effects as it reduces stomach acid secretion and meditate the activity of hormones. Arugula oil is also for various medicinal purposes [29].

1.7.8- Cautions of Arugula

When it comes the bad effect of arugula, nothing you should worry about because as long as you consume it in proper amount, there is no direct effect you will suffer but some points below are important for you to know as well [29].

 Though the case of allergic after consuming arugula is rare but nothing that is wrong to be careful, especially those who show some allergic reaction after consuming vegetables that closely related to arugula like kale and cauliflower.

 Just like any other green vegetable, arugula also contains oxalic acid which is mostly associated with kidney stone. However, this kind of case

only happens when you consume it in impossible large amount or consumed by those who already have kidney problem.

 The biggest treat from arugula is actually not from the properties contained in this healthy vegetable but how this green vegetable is cultivated. The use of herbicides are the reasons why you should consume organic arugula**.**

The unique thing about arugula is that the cleansing properties found in arugula could eliminate all the bad effects of herbicides and pesticides found in your body. There are many health benefits of arugula. However, it is still strongly recommended for you to purchase organic arugula because for all the benefits of arugula is best for you to consume it raw, especially for the chlorophyll properties. Unfortunately, in some countries, finding organic arugula is so hard and the available arugula is only the inorganic one. The tips is, before you consume inorganic arugula, you should make sure to rinse them throughly and then soak them in hot water for a while to minimize whatever residues contained [29].

1.8- Important physical and chemical properties of essential oils

The chemical properties of essential oils depend on the natural factors such as type of species. The geographical origin and location of the plant, time of harvesting, plant parts from which the oils are extracted, etc [27].Essential oils components and percentage are different from oil to even for the same botanic plant due to:

a)-**Weather and planting time**: Most of herbs are planted but small amount could also be wild grown or collected plant. By means of an example with spearmint, the percentage from summer crop is double that from a winter crop. The oil percentage from a given summer could be different from a previous summer even from the same field. The component analysis of the oil could also be different from one season to another.

b)-**Soil elements:** the β-phellanderene percentage increases in marjoram oil with higher levels of molybdenum, manganese, copper, calcium, zinc or iron in the soil.

c)-**Irrigation**: the highest yield of plant material results from increasing the leaf area. For example, this will happen if a basil field is irrigated every 4 days. The essential oil is highest at medium levels of soil moisture.

d)-**Time of harvest**: the peppermint oil yield increases as the herb approaches maturity in the full bloom stage.

e)-**Length of distillation operation**:you must consider whether the herbs (species) are fresh, faded, or dry. It would take additional time for distillation if the herb is faded than if it is fresh. Leaves take less time than seeds because leaves are thinner than seeds and cells are more concentrated in leaves than seeds [27].

1.8.1- Physical Properties

1.8.1.1 -Specific gravity

Specific gravity is an important criterion of the purity of an essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at 150 C, in general, the specific gravity is less than 1.188. Hence essential oil can be collected over water.

1.8.1.2- Optical rotation

Most essential oils when placed in a beam of polarized light possess the property of rotating the plane of polarization to right (dextrorotatory), or to the left (levorotatory). The degree of rotation and the direction are important indicators of purity.

1.8.1.3- Refractive index

When a ray of light passes from a less dense to a more dense medium, it is bent or "refracted" toward the normal. If (e) :represents the angle of refraction and (i): the angle of incidence,then according to the low of refraction,

Sin i\sin e = N\n

Where:

n: the index of refraction of the less dense medium.

N : the index of refraction of the denser medium.

Refractometers offer a rapid and convenient method for the determination of this physical constant.

1.8.1.4- Molecular refraction

The index of refraction of a liquid varies with temperature and the wavelength of the light. In order to compare the refractivity of different liquids, the use of molecular refractivity (molecular refraction) is necessary.

1.8.1.5 -Solubility

- **Solubility in water**

Most essential oils of commercial interest are steam volatile, reasonably stable to action of heat and practically insoluble in water and hence suitable for processing by steam distillation.

- **Solubility in Alcohol**

Most essential oils are only slightly soluble in water and are miscible with absolute alcohol. The solubility of oil may change with age.

1.8.1.6 -Boiling range

In the case of isolates and synthetics, the boiling range is an important criterion of purity.

1.8.1.7 -Evaporation residue

An important criterion of purity is the evaporation residue; i.e., the percentage of the oil which is not volatile at 1000 C. It is important to study the odour of oil as it volatilizes during the heating.

1.8.1.8 Flash point

The flash point may prove useful in the valuation of an essential oil. The flash point has value as an indication of adulteration: addition of adulterants such as alcohol and low boiling mineral spirits will greatly lower the flash point [27].

1.8.2 Chemical properties

An essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen [34] . They can be essentially classified into two groups:

-Volatile fraction: these are essential oil containing 90–95% of the oil in weight, and containing monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters [35].

-Nonvolatile residue: This comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

However the properties of these components can change. For example, the components from the oils extracted from plants can change according to how, when and where these plants are grown and harvested. The constituents can be again subdivided into 2 groups, such as the hydrocarbons which are made up mainly of terpenes and their oxygenated

conjugates which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides. Some of the common components are listed below along with their properties [35].

i)-**Alcohols**

Alcohols are generally considered safe and have a very low or totally absent toxic reaction in the body or on the skin and so can be used for children. They are extremely useful due to their antiviral, antibacterial and antiseptic properties. Alcohols are present either as a free compound or combined with a terpene or ester and are found in ylang -ylang and lavender as linalool, geraniol in geranium and palmarosa and citronellol in rose, lemon and eucalyptus. Other alcohols include menthol, nerol and benzyl alcohol [35].

ii)-**Alderhydes**

Aldehydes are found in lemon-scented oils such as melissa, lemon verbena, citronella etc. and include citral, citronellal and neral. They generally have sedative qualities with specific antiseptic properties. Other known aldehydes include benzaldehyde, cinnamaldehyde and perillaldehyde. Essential oils containing aldehydes are helpful in treating inflammation
, *Candida* and some viral infections [35].

iii)-**Hydrocarbon**

Building blocks of essential oil are hydrogen and carbon. Basic hydrocarbon found in plants is isoprene having the following structure [36].

(Isoprene)

iv)- **Terpenes**

These components generally have names ending with "**ene**".Some of them are limonene, pinene, piperene, camphene etc. These components act as an antibacterial, antiviral, anti-inflammatory, antiseptic, antiviral and bactericidal. These are further categorized into : monoterpene, sesquiterpene and diterpenes. When two of the isoprene units are joined head to tail, the result is a monoterpene, when three are joined, it is a sesquiterpene and similarly four linked isoprene units are diterpenes [36].

a-**Monoterpene [C10H16]**

Monoterpene are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10).But some of their oxygenated derivatives Such as alcohols, ketones, and carboxylic acids are known as monoterpenoids.

(Limonene) (Menthol)

Two isoprene units are present in these branched-chain C10 hydrocarbons and are widely distributed in nature with more than 400 naturally occurring monoterpenes. Moreover, besides being linear derivatives (geraniol, citronellol), the monoterpenes can be cyclic molecules (menthol – monocyclic; camphor – bicyclic; pinenes (**α** and **β**) – pine genera as well. Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium* (worm wood) from which the liqueur absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil is used as a disinfectant and deodorant. Camphor is used as a counter-irritant, anesthetic, expectorant, and antipyretic, among many other uses [37].

b- **Sesquiterpene**

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic

They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury. These are having properties like anti-inflammatory, antiseptic, analgesic and anti-allergic [37].

c-**Sesquiterpene lactones**

These are available as farnesene in chamomile and lavender. They not only have proved to be of interest from chemical and chemotaxonomic point of view, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities.

Chemically these compounds can be classified according to their carboxylic skeletons; thus, guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc. can be derived from the germacranolides.

Structural features of all these compounds are associated with much of the biological activity. For example beta-caryophyllene in basil and black pepper [37].

d-Diterpenes

Isoprene has been an integral part in most of the components as there are four isoprene units in diterpenes. By stem distillation method diterpenes can not be detected as these molecule are too heavy to allow for evaporation, so it is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Derivatives of diterpenes are plant hormones gibberellins and phytols occurring as a side chain on chlorophyll. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate (GGPP). Therapeutically diterpenes have limited importance and are used in certain sedatives (coughs) as well as in antispasmodics and anxiolytics [37].

e-Alcohols

Naturally alcohols exist either as a free compound or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the

terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols are not fenerally toxic. Therefore, they are considered safe to use. Some of these properties are anti-septic, anti-viral, bactericidal and germicidal. Some of the examples are linalool found in ylang-ylang and lavender, geraniol in geranium and rose and nerol [37].

f-Aldehydes

Aldehyde- containing essential oils are effective in treating *Candida* and other fungal infections. Some of their properties are :anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, and sedative. Aldehydes are present as citral in lemon, Citronellal in lemongrass, lemon balm and citrus eucalyptus [37].

g-**Acids**

Generally organic acids are found in very small quantities in their free state within essential oils. Plant acids act as components or buffer systems to control acidity. These also act anti-inflammatory. Examples are cinnamic and benzoic acids in benzoin, citric and lactic acids [37].

h-**Esters**

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing and balancing effects.They are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative,

with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system. Examples are linlyl acetate in bergamot and lavender and geranyl formate in geranium [37].

j-**Ketones**

Ketones found in plants are used for upper respiratory complaints. 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 (not always) very toxic**.** The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in penny royal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil [37].

k-**Lactones**

Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action than ketones [37].

1.9- Advantages of essential oils

a-Aromatheraphy

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 [39].

Since ancient times 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 are also 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 [40].

b-Importance of essential oils in pharmaceutics

Essential oils have versatile applications in pharmaceutics. Some of the applications are listed below.

-Antiseptics

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 act against fungi and yeasts. The most common sources of essential oils used as antiseptics are: cinnamon, thyme, clover, eucalyptus, culinsavory, and lavender. Citral, geraniol, linalool and thymol are much more potent than phenol [40].

-Expectorants and diuretics

When 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 vasodilation and in consequence bring about a diuretic effect [40].

-Antispasmolytic 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 [40].

Aim of this study

This study was undertaken to:

-Extract oil from the medicinally important

-Conduct GC-MS analysis for the target oil.

-Evaluate the oil for antimicrobial activity**.**

Chapter Two

Materials and Methods

2-Materials and Methods

2.1-Materials

2.1.1- Instruments

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

2.1.2-Test organisms

corochorus olitorius oil oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli* and *Candida albicans*.

2.1.3-Plant material

Seeds of *Eruca sativa* were purchased from the local market and identified by direct comparison with a herbarium sample.

2.2- Methods

2.2.1-Extraction of oil

Powdered seeds of *Eruca sativa*(300g) were exhaustively macerated with n-hexane.The solvent was removed under reduced pressure to afford the oil.

2.2.2- GC-MS analysis

The oil of *Eruca sativa* was analyzed by GC-MS. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)was used.. Oven temperature program and other chromatographic conditions are shown below:

Table 2: Oven temperature program

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 3: 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 assay

(i)Bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

ii)-Fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

iii)-Antibacterial test

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume micrometer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1- Eruca sativa oil

Eruca sativa oil was extracted by maceration of seeds.a GC-MS analysis was conducted to reveal the constituents of the oil. Furthermore, the oil was assessed for its antimicrobial activity against six standard human pathogens.

3.1.1- GC-MS analysis of the oil

The GC-MS analysis of *Eruca sativa* oil showed 16 components dominated by 13-docosenoic acid, methyl ester (33.72%), cis- 13-eicosenoic acid, methyl ester (14.67%)**,** 9- octadecenoic acid (Z)-, methyl ester (13.21%), 9,12- octadecenoic acid (Z,Z)-, methyl ester (11.08%)**,** 9,12,15- octadecatrienoic acid, methyl ester (8.57%)**,** hexadecanoic acid, methyl ester (6.48%) .The total ions chromatograms of *Eruca sativa* oil is shown in figure 1 and the constituent of the oil are displayed in Table 1.

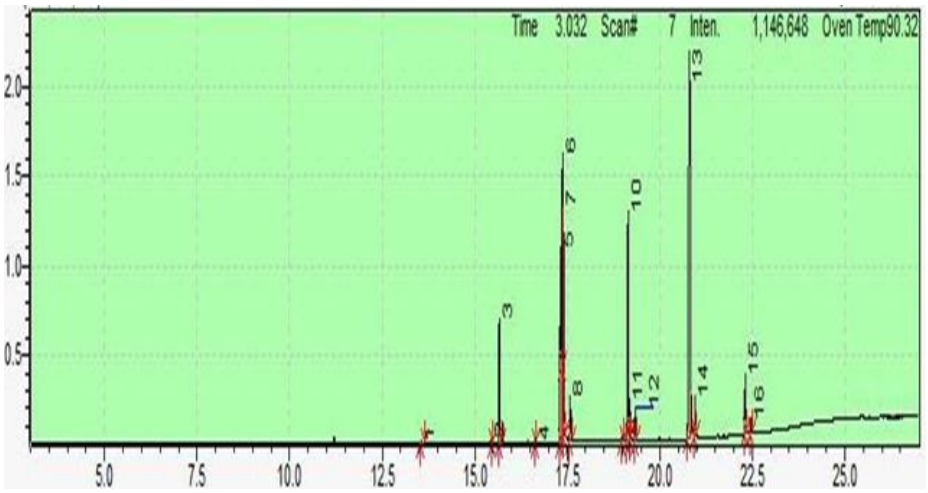

Figure1: Total ions chromatograms of *Eruca sativa* oil

Table 1 Constituents of *Eruca sativa* oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	13.569	150412	0.08	Methyl tetradecanoate
2	15.471	434850	0.24	9-Hexadecenoic acid, methyl ester, (Z)-
3	15.664	11899523	6.48	Hexadecanoic acid, methyl ester
4	16.642	46679	0.03	Heptadecanoic acid, methyl ester
5	17.321	20347539	11.08	9,12-Octadecadienoic acid (Z,Z)-, methyl e
6	17.374	24248131	13.21	9-Octadecenoic acid (Z)-, methyl ester
7	17.395	15728679	8.57	9,12,15-Octadecatrienoic acid, methyl este
8	17.580	4080332	2.22	Methyl stearate
9	18.994	622158	0.34	.gamma.-Linolenic acid, methyl ester
10	19.143	26932687	14.67	cis-13-Eicosenoic acid, methyl ester
11	19.192	3594527	1.96	cis-11-Eicosenoic acid, methyl ester
12	19.337	3072317	1.67	Eicosanoic acid, methyl ester
13	20.806	61902166	33.72	13-Docosenoic acid, methyl ester, (Z)-
14	20.961	3523364	1.92	Docosanoic acid, methyl ester
15	22.311	5806666	3.16	15-Tetracosenoic acid, methyl ester, (Z)-
16	22.466	1208209	0.66	Tetracosanoic acid, methyl ester
		183598239	100.00	

The major components of Eruca sativa oil constituents are shown below:

a) 13-Docosenoic acid, methyl ester (33.72%)

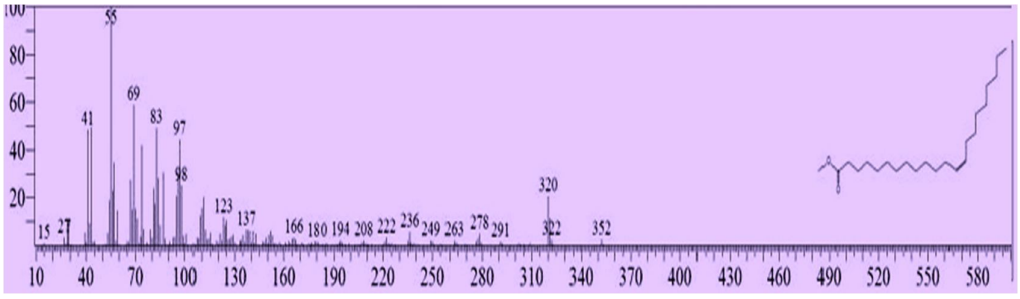

Figure 2 : The mass spectrum of 13- docosenoic acid, methyl ester

The peak at m/z 352 corresponds to the molecular ion :M^+ [C_{23} H_{44} O_2]$^+$. The signal at m/z 322 accounts for loss of a methoxyl .

b) Cis- 13- Eicosenoic acid, methyl ester (14.67%)

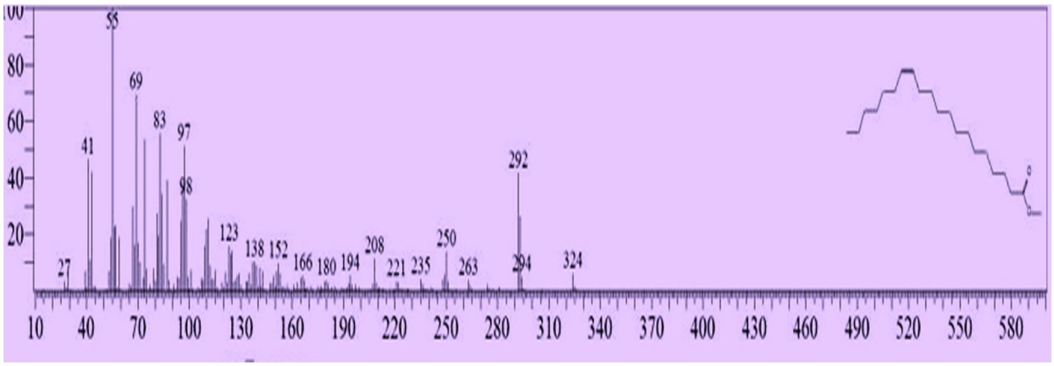

Figure 3 : The mass spectrum of cis- 13- eicosenoic acid, methyl ester

In Fig. 3 ,the peak at m/z 324 is due to $M^+ [C_{21} H_{40} O_2]^+$. The signal at m/z 294 corresponds loss of a methoxyl function.

c) 9- octadecenoic acid (Z)-, methyl ester (13.21%)

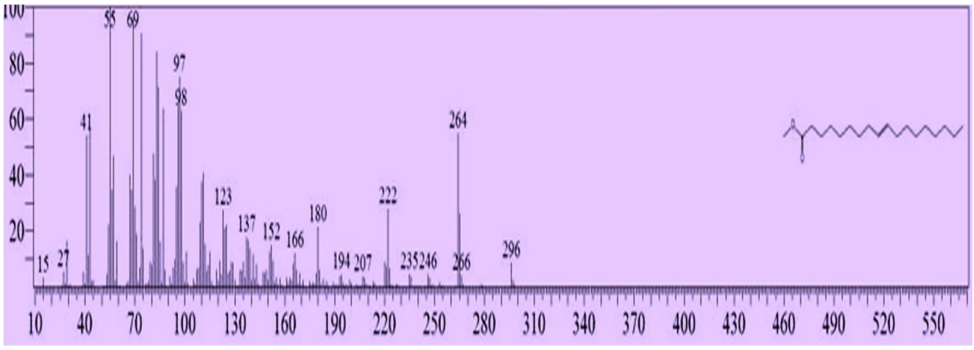

Figure 4 : Mass spectrum of 9- octacenoic acid (Z)-, methyl ester

The peak at m/z 296 accounts for the molecular ion : $M^+ [C_{19} H_{36} O_2]^+$, while the signal at m/z 266 is attributed to loss of a methoxyl .

d) 9, 12- octadecenoic acid (Z, Z)-, methyl ester (11.08%)

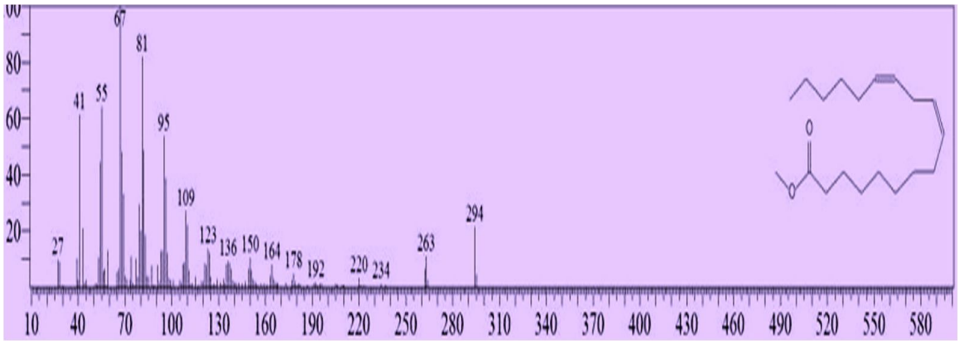

Figure 5 : Mass spectrum of 9, 12- octadecenoic acid (Z, Z)-, methyl ester

In figure 5, the peak which appeared at m/z 294 corresponds M^+ $[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 corresponds loss of a methoxyl group.

e) 9, 12, 15- Octadecatrienoic acid, methyl ester (8.57%)

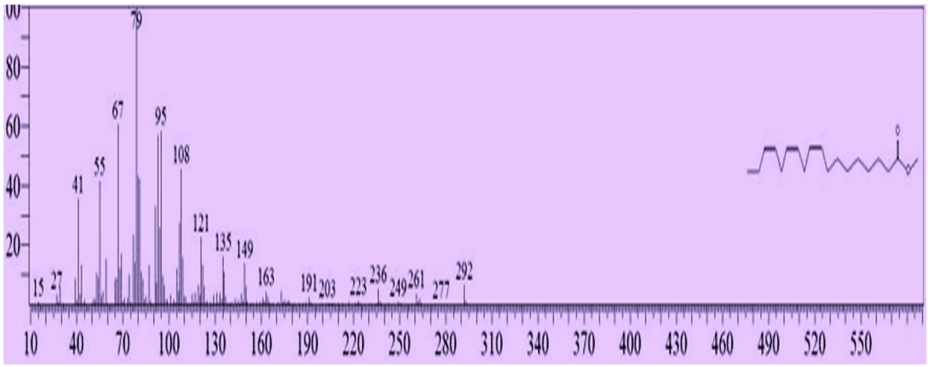

Figure 6 : Mass spectrum of 9, 12, 15- octadecatrienoic acid, methyl ester

The molecular ion : M^+ $[C_{19}H_{32}O_2]^+$ appeared at 292.The signal at m/z 261 is due to loss of a methoxyl function.

f) Hexadecanoic acid, methyl ester (6.48%)

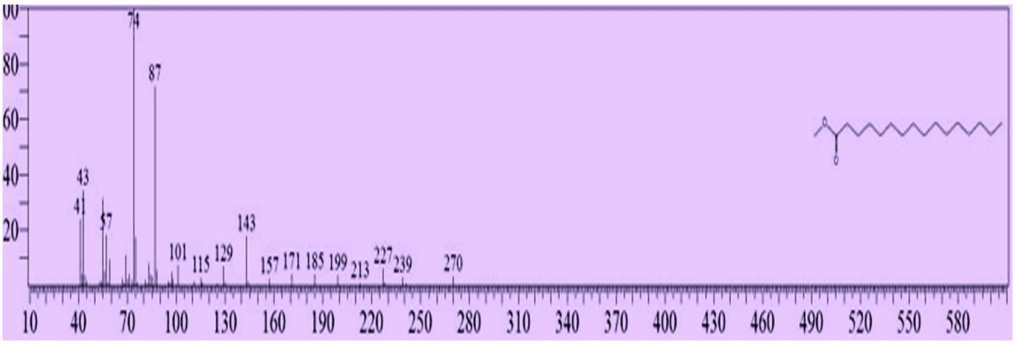

Figure 7 : Mass spectrum of hexadecanoic acid, methyl ester

In Fig. 7, the peak at m/z 270 corresponds to the molecular ion $M^+ [C_{19} H_{38} O_2]^+$, whilst the signal at m/z 239 accounts for a methoxyl.

3.1.2 Antimicrobial activity

Eruca sativa oil was assessed for antimicrobial activity via the cup plate agar diffusion bioassay using six standard human pathogens.. The average of the diameters of the growth inhibition zones are displayed in Table (2) .The results were interpreted in terms of the commonly used terms (>9mm: inative;9-12mm:partially active;13-18mm: active;<18mm:very active).

At a concentration of 100mg/ml , the oil showed partial activity against Acinetobacter baumanni and Candida albicans.

Table 2 : Antimicrobial activity of *Eruca sativa* oil

		Concentration % ug/ml						
		1			Amp	Kan	Nys	
Gram	Strain	0%	50%	100%	0%	10	10	10
-ve	Escherichia coli	-	6±0.1	6±0.1	-	25±0.4	21±0.3	N
-ve	Klebsiella pneumoniae	-	6±0.4	8±0.4	-	22±0.4	24±0.0	N
-ve	Acinetobacter baumannii	-	7±0.5	11±0.4	-	22±0.3	16±0.3	N
-ve	Pseudomonas aureginosa	-	6±0.1	6±0.1	-	29±0.3	11±0.3	N
+ve	Staphylococcus aureus	-	6±0.1	8±0.2	-	32±0.5	19±0.5	N
+ve	Bacillus subtilis	-	6±0.1	6±0.1	-	19±0.4	21±0.4	N
C	Candida albicans	-	6±0.5	9±0.5	-	N	N	12±0.5
F	Aspergillus flavus	-	6±0.2	8±0.4	-	N	N	15±0.5

-ve: gram negative, +ve: gram positive, C: colony forming, F: filamentous, -: no activity, N: Not Valid

Conclusion

The oil from Eruca sativa was analyzed by GC-MS. The analysis showed 16 components. The major components are : 13- docosenoic acid, methyl ester (33.72%) , cis- 13- eicosenoic acid, methyl ester (14.67%), 9-0ctadecenoic acid Z-, methyl ester (13,21%), 9,12- octadecenoic acid (Z,Z) , methyl ester(11.08%) , 9,12,15- octadecatrienoic acid, methyl ester (8.57%) , hexadecanoic acid, methyl ester 6.48 . In the antimicrobial assay partial activity was observed against some of the test organisms.

Recommendations

The following is recommended:

1- Other phytochemicals of Eruca sativa (flavonoids, steroids, saponins .etc) may be isolated and thoroughly studied.
2- The oil of Eruca sativa may be evaluated for its antimalarial, antiflammatory properties.

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