Sudan University of Science and Technology

College of Graduate Studies

Physicochemical study and antimicrobial activity of the oil of *Trigonella foenum graecum*

دراسة الخصائص الفيزيوكيميائية لزيت الحلبة وفعاليته كمضاد للميكروبات

A Thesis Submitted Fulfillment of the Requirements of the Master Degree in Chemistry

By

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استـــهلال

بسم الله الرحمن الرحيم

﴿ وَقُل رَّبِّ أَدْخِلْنِى مُدْخَلَ صِدْقٍ وَأَخْرِجْنِى مُخْرَجَ صِدْقٍ وَٱجْعَل لِّى مِن لَّدُنكَ سُلْطَـٰنًا نَّصِيرًا ﴿٨٠﴾ ﴾

الإسراء (الآية: 80)

Dedication

To my

Parents,

Wife, Sons, Daughter,

Brother and Sisters

Acknowledgement

Praise to Allah Almighty for helping me to complete this work. It is a pleasure to record my deep appreciation, and thanks to Dr. Kamal Mohammed Saeed for his wise guidance, which helped me to present this research form.

I am very grateful to the staff of the Chemistry department, Sudan University of science and Technology and to the staff of phytochemistry department, medicinal and aromatic plants research institute for their technical support.

I am also grateful to my friends for their moral support.

Abstract

The aim of this study is to determine the physicochemical properties and antimicrobial activity of the oil from *Trigonella foemnum-graecum*.

The oil yield was (37%). The physicochemical presents that moisture was (1.75%), refractive index at (30°C) (1.4743), density (0.855g/cm^3), saponification value (0.2mg KOH/g), peroxide value (0.5mg O_2/100g) and acid value was (3.4mg KOH/g).

GC-MS analysis shows that the dominant fatty acids are (9.12) Octadecadienoic acid (Z, Z) methyl ester (43.48%), 9, 12, 15- Octadecatrienoid acid, methyl ester, (Z, Z, Z) (20.15%), Hexadecanoic acid, methyl ester (16.35%) and Stearic acid (8.77%).

The oil shows antimicrobial activity against *Candida* moderate.

Significant activity against gram Positive *Staphylococcus aureus*, *Bacillus subtilis* and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*.

المستخلص

هدفت هذه الدراسة لتحديد الخصائص الفيزيوكيميائية والنشاط الميكروبي لزيت الحلبة. تم الحصول على كمية الزيت بنسبة (37%)، نتائج الخصائص الفيزيوكيميائية للزيت الرطوبة (1.75%) ومعامل الانكسار في (30) درجة مئوية (1.4743)، وكانت كثافة الزيت (0.885جم/سم^3)، وقيمة التصبن (0.2 ملجرام هيدروكسيد بوتاسيوم/ جرام زيت)، رقم البيروكسيد (0.5 ملجرم من جزيء الأكسجين/ جرام زيت)، وقيمة الحموضة وجدت (3.4 ملجرام هيدروكسيد بوتاسيوم/ جرام زيت).

التحليل بواسطة جهاز الكروماتوغرافية الغازية– طيف الكتلة أوضح أن الحموض الدهنية بالزيت السائدة هي (43.48%):

9, 12, 15- Octadecatrienoid acid, methyl ester, (Z, Z, Z) (20.15%), Hexadecanoic acid, methyl ester (16.35%) and Stearic acid (8.77%).

أخضع الزيت لاختبارات مضاد الميكروبات حيث أبدى فعالية متوسطة ضد المبيضات Candida، أما النشاطية المهمة ضد gram positive المكورات العنقودية Staphylococcus aureus، البكتريا العصوية Bacillus subtilis وgram negative القولونية Escherichia coli والزائفة Pseudomonas aeruginosa.

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Chapter One

1. Introduction

1.1 Aromatic plants oils

For thousands of years aromatic plants and oils have been used as perfumes, incense and in cosmetics, as well as for culinary and medicinal purposes. Their traditional uses in early cultures are where their religious and therapeutic functions became combined (Lawless, 1992). In recent decades aromatherapy has become more popular, which has renewed interest in essential oils. (Lawless,1992) described "Aromatherapy as a branch of alternative medicine which claimed that specific aromas of essential oils have healing effects". The term aromatherapy was first introduced in 1928 by a French chemist named Gattefosse. He worked for his family's perfume business and became interested with the therapeutic possibilities of the oils. He also found that "many essential oils were more effective in their entirety than their synthetic substituent's or their isolated active ingredients". (Lawless, 1992) New forms of disease are increasingly affecting people that live in today's highly pressurized society and these diseases are less responsive to treatment with conventional medicines. Many people who have been disappointed with mainstream medicine have turned to complementary therapies which do not rely on scientific evidence but rather on historical and observational evidence. (Bowles, 2003) Modern research has largely confirmed the traditional uses regarding therapeutic applications of certain plants. (Lawless, 1992) The therapeutic potential of plant essential oils, still need to be fully discovered. Many medicinal plants have been examined to provide biologically active compounds, on which most of our contemporary drugs are based. However, there is still much more to discover about their exact pharmacology. This is

especially relevant in terms of essential oils which have such a concentrated yet complicated composition. But as reported by Lawless (1992) in the Encyclopedia of essential oils that “only a small proportion of the 2world floral has been examined for pharm logically active compounds, but with the ever-increasing danger of plants becoming extinct, there is a real risk that many important plant sources may be lost”. Therefore, it is important to study and conserve the environment as they may provide important leads for the cure of diseases such as cancer, AIDS and others that society faces these days. A specific plant with many therapeutic uses is *Centella Asiatica*, a perennial herbaceous creeper commonly found in moist places. (Gohil, 2010) It is indigenous to Africa, Asia, and South America. In South Africa, it is found along the moist eastern parts, widely distributed from the Cape Peninsula northwards. (Van Wyk et al, 2002). The plant is commonly known as Pennywort and in Chinese as Gotu Kola. It is an important traditional medicinal herb used by Asian communities and in southern and central Africa since ancient times. *Centella Asiatica* has become very popular due to its effectiveness and versatility. It has, in particular, a reputation of being a brain stimulant and as a wound healing agent. (Zheng, 2007) Extensive experimental and clinical investigations have been conducted by scientists which have been focused on some potential compounds that have low toxicity and a high efficacy which could benefit human health. (Zheng, 2007) There have been many studies on the therapeutic uses of the entire plant. However, the species varies considerably in different parts of the world and is sometimes treated as several distinct species. (Van Wyk et al, 2002) Therefore, the composition of *Foenum graecum* growing in South Africa can vary greatly from that growing in India and other countries due to the different factors such as geo-climatic location, soil type, life

stage of plant, pollution and time and day harvesting is done. (Bowles, 2003) Even though *Centella Asiatica* has been used extensively in traditional medicine in South Africa, many studies have not been carried out in the country. (Oyedeji and Afolayan, 2004) There have been more studies carried out worldwide on the tincture of C*entella Asiatica* compared to the essential oil. Heavy metals found in the environment can easily contaminate many medicinal herbs like *Centella Asiatica* during their manufacturing and growth processes when their ready-made products are produced. Heavy metals can be found in the air, soil and water and examples of such sources are atmospheric dust, rainfall, fertilisers and plant protective agents. Since *Centella* has many therapeutic uses, good quality control is imperative to protect consumers from contamination. (Abu- Darwish, 2009)

1.2 Botanical description of *Trigonella foenum-graecum*:

Trigonella foenum-graecum belongs to the family **Fabacea** and it is popularly known as Fenugreek (Warrior, 1995). It has been in use as a food and flavouring agent since time immemorial. It is native to Eastern Mediterranean Central Asia and Ethiopia, and much cultivated in India and China (Morton, 1990) Plant seeds and leaves are used not only as food but also as an ingredient in traditional medicines (Wallis, 2005). It has been reported in *Ayurveda* and *Siddha* that it is used to treat fever, dysentery and heart diseases, while in Unani system, it is used as an aphrodisiac, diuretic, emmenagogue and tonic (Nadkarni, 1982). Due to its strong flavor and aroma fenugreek leaves and seed are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine (Sharma, 1996). It is a robust, erect, aromatic annual herb reaching up to 60 cm in height. The leaves are compound, up to 5 cm in length, with long pedicel. Leaflets are lanceolate or obovate, about 2.5 cm long, with slight toothed margins. The flowers are axillary, occurring singly or in pairs, sessile, yellow in colour. The fruits are typically

leguminous pods 5-8 cm long, narrow and with a persistent bark, enclosing 10-20 golden yellow seeds, which possess a characteristic savoury aroma (Major, 2002).

1.3 Essential oils

(Elsharkawy ,2014) describes an "essential oil as a concentrated, hydrophobic liquid containing volatile compounds. These oils are manufactured in the green (chlorophyll bearing) parts of the plant and are transported to other plant tissues as the plant matures. (Galadima et al, 2012) It is then stored in small sacs in the roots, shoots, leaves, flowers and seeds of fragrant plants. (Whitton, 1995) Whitton (1995) explains that this oil gives the plant its distinctive smell which "help attract insects for pollination and allow the plant to protect itself from invading bacteria and fungi. We extend this protection to our benefit when we extract and use the oils because they can be antiseptic, bactericidal, viricidal and fungicidal as well as anti-inflammatory, anti-spasmodic, digestive or sedative depending on their individual plant chemistry". Every oil extracted from various plants has a concentrated, distinctive combination of natural chemicals. (Whitton, 1995).

In general, carbon, hydrogen and oxygen make up the composition of essential oils which can be subdived into two groups, oxygenated compounds and hydrocarbons. Examples of oxygenated compounds would be esters, alcohols, aldehydes, phenols, oxides and ketones although sometimes lactones, nitrogen and sulphur compounds can be present as well. Hydrocarbons consist mainly of terpenes (monoterpenes, diterpenes and sesquiterpenes). (Lawless, 1992)

1.4 Chemical compositions:

Essential oils are made up of compounds such as terpenoids, aldehydes, esters, ketones, phenols and alcohols (Radulescu, *et al.*, 2004). The odour and taste of an essential oil is mainly determined by the oxygenated constituents, the fact that they contain oxygen gives them some solubility in water and considerable solubility in alcohol (Tisserand, *et al*., 1995).

1.4.1 Terpenes:

Terpenes are composed of hydrogen and carbon atoms only. All terpenes are based on the isoprene unit, an essential building block in plant biochemistry (Tisserand, *et al*., 1995).

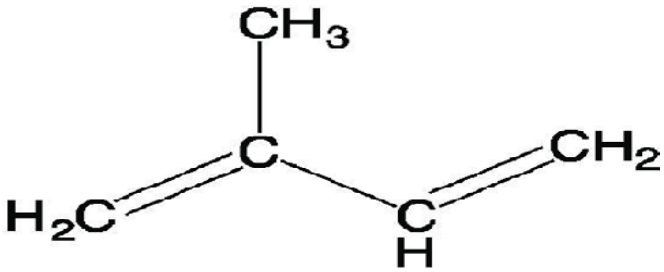

Fig.1 General structure of Isoprene unit

1.4.1.1 Monoterpenes

The hydrocarbons are almost always present in essential oil. Monoterpenes contain ten carbon atoms. They are called monoterpenes, because this is the basic unit as found in nature. These terpenes can also have several functional groups (Leland, *et al.*, 2006). The functional groups are:

I. Aldehyde–any class of compounds characterized by the presence of a carbonyl group (C=O group) in which the carbon atom is bonded to at least one hydrogen atom.

II. Ketones–compounds where the carbon atom of the carbonyl group is bonded to two other carbon atoms.

III. Alcohols–any class of compounds characterized by the presence of a hydroxyl group (-OH group) bonded to saturated carbon atom.

IV. Esters–Esters are any class of compounds structurally related to carboxylic acids but in which the hydrogen atom in the carboxyl group (-COOH group) was replaced by a hydrocarbon group, resulting in a COOR structure where R is the hydrocarbon.

V. Phenol-Phenols constitute a large class of compounds in which a hydroxyl group (-OH group) is bound to an aromatic ring.

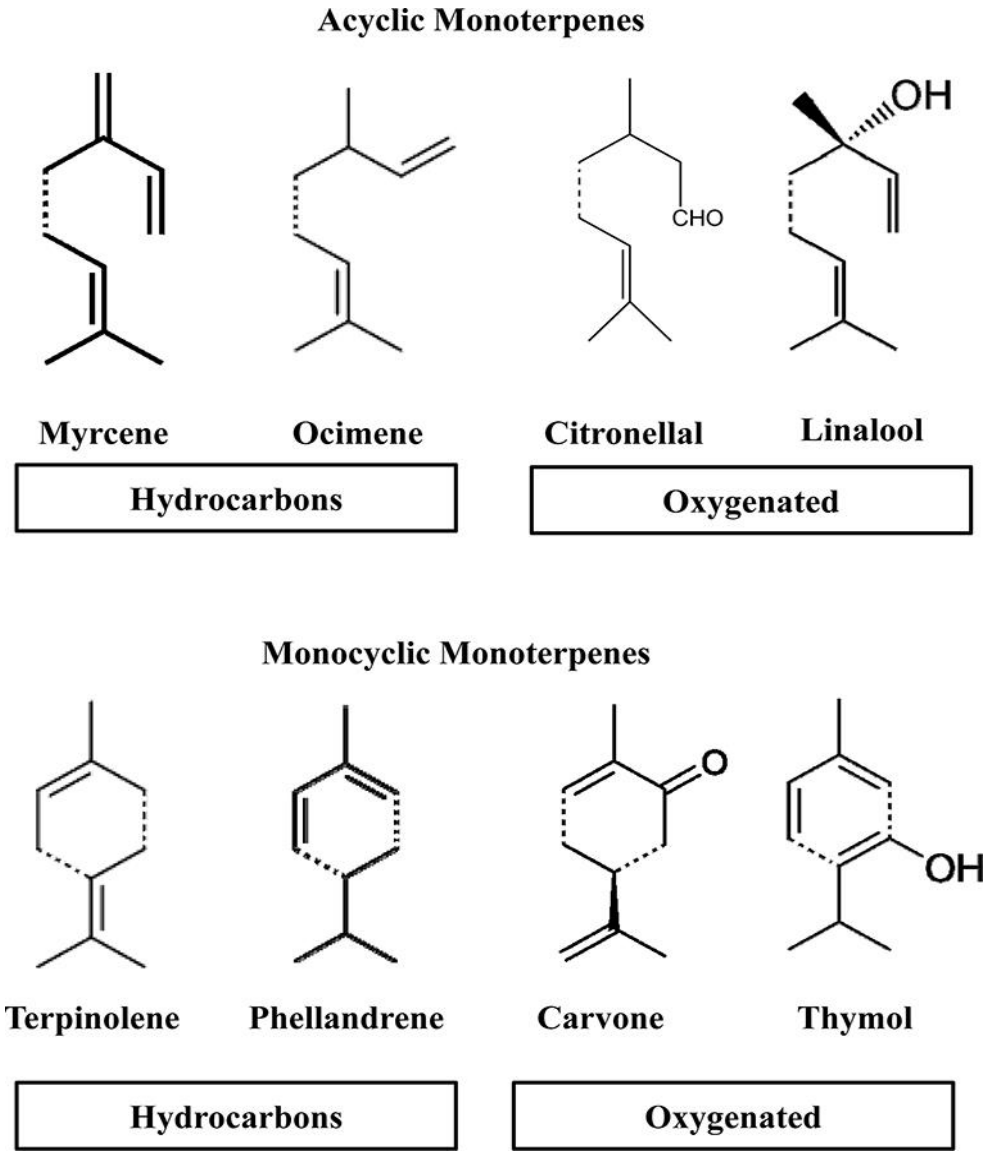

Fig 1.2 structure of A cyclic and Monocyclic Monoterpenes

1.4.1.2 Sesquiterpenes:

Sesquiterpenes are composed of three isoprene units and therefore have 15 carbon atoms. Examples of sesquiterpenes characteristic of essential oils: hydrocarbons (β- bisabolene), alcohols (farnesol), ketones (nootkatone), aldehydes (sinensals) and esters (cedryl acetate).

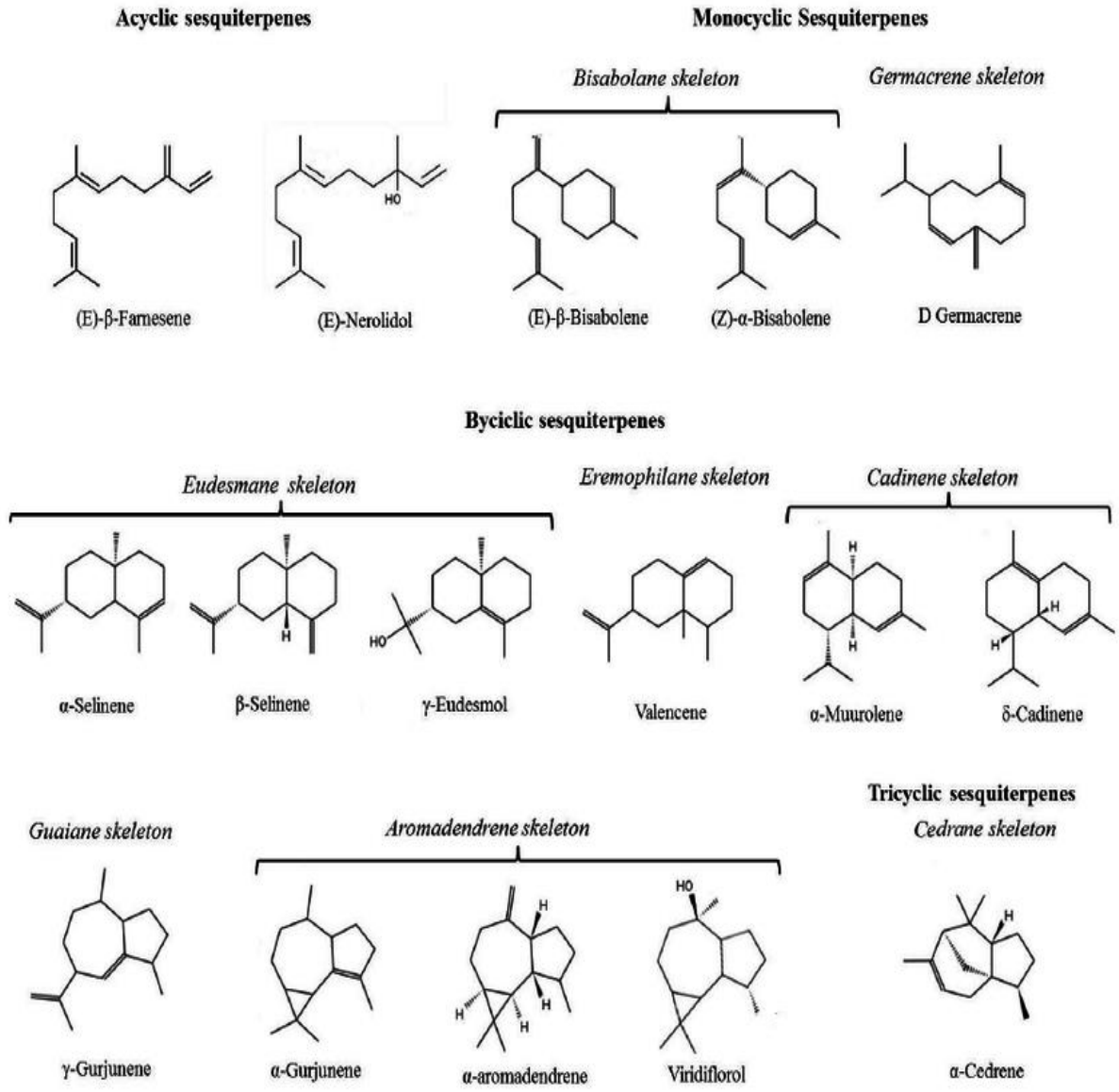

Fig1.3 structure of Monocyclic, Bicyclic and Tricyclic Sesqueterpenes

1.4.2 Phenylpropanoids:

Phenylpropanoids (C6-C3) are far less common than terpenoids. Very often they are allyl (H2C=CH-CH2-R) and phenylphenols and sometimes, they are aldehydes characteristic of certain *Apiaceae* oils (anise, fennel, parsley: anethole, anisaldehyde, apiole, methylchavicol) and also of clove, nutmeg, tarragon, calamus and cinnamons. Also present in essential oils are C6-C1 compounds such as vanillin (rather common) or methyl anthranilate (Norsita, 2003).

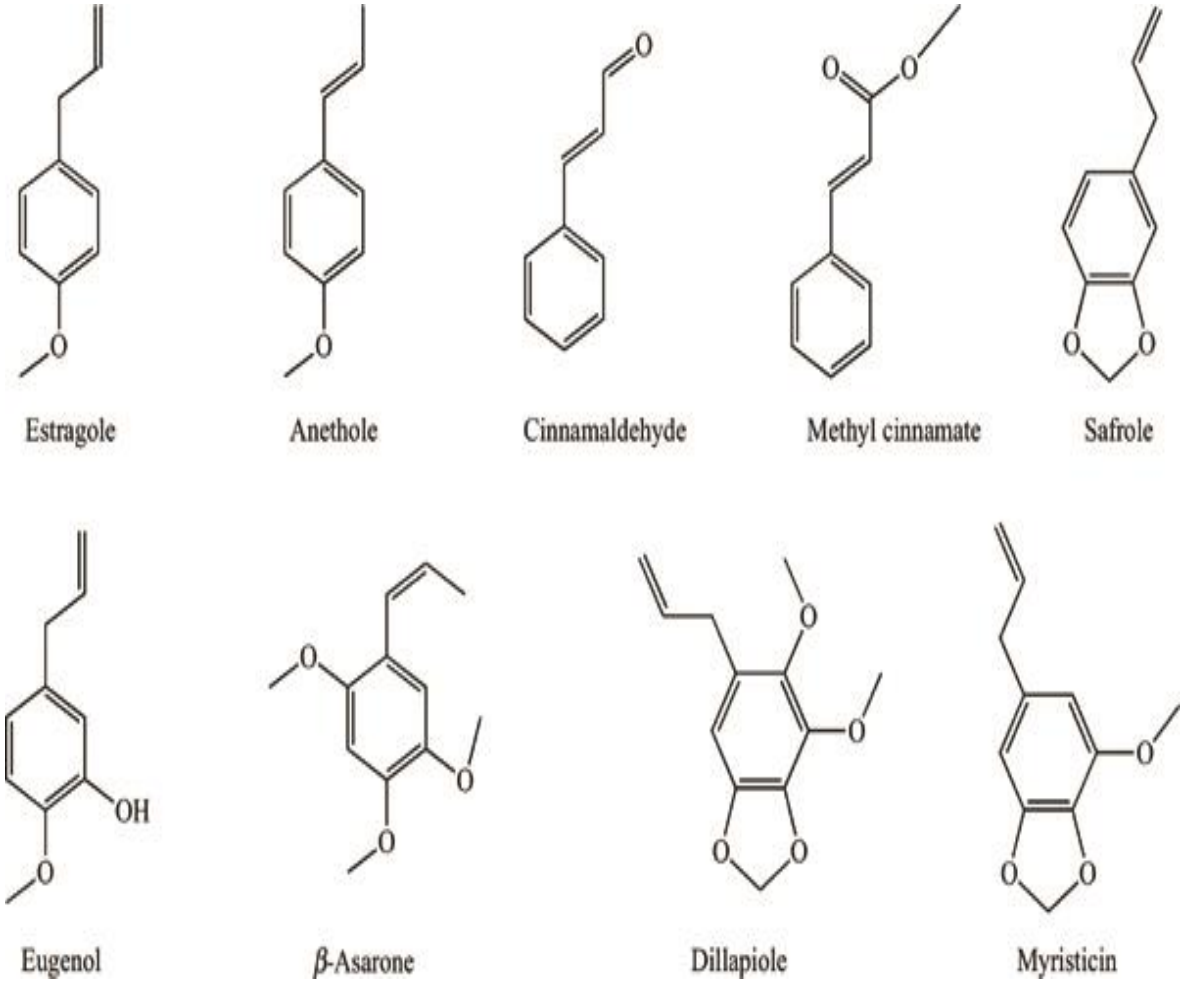

Fig1.4 Structure of some essential oils, Phenylpropanoids

Essential oils may contain various aliphatic compounds, generally of low molecular weight, which are extracted during steam distillation: hydrocarbons (linear or remified, saturated or not, rarely specific), acids (C3 to C10), alcohols, aldehydes, acyclic esters or lactones. Nitrogen or sulfur containing compounds are characteristic of roasted or grilled products and are exceptional among products. Products of higher molecular weight are not uncommon and are not extracted by steam distillation; there are homologs of the phenylpropanoids, diterpenes and coumarins (some of which can actually be steam distilled) among others. Representatives of this group are incidental and often rather specific for a few species or genera. For example, the mustard oils, containing allyl isothiocyanate are found in the family of the Cruciferae; allyl sulfides in the oil of garlic. The oil from *Ferula asafetida* L. belonging to the family of

Umbelliferae, gained reputation from its active component, secondary butyl propenyl disulfide, a competitor of the odoriferous principles of the skunk, primary n-butyl mercaptan and dicrotyl sulfide (Norsita, 2003).

Some plants like garlic, onion, leek, and shallots (Allium spp.) 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-*Rubus nigrum* whereas 1-p-menthene-8-thiol is found in fruit oils (Baser and Demirci 2007; Hunter, 2009). These compounds appear to be important in plant defense and in nitrogen detoxication of plants. Although most sulfur compounds have very unpleasant pungent odors, organosulfur compounds present in essential oils can be aromatically very pleasant. It is also known that sulfur compounds are used in the flavoring of vegetables, fruits as well as processed foods and beverages.

Nitrogen-Containing Compounds are found in a few essential oils. Examples include methyl anthranilate, skatole, indole, pyridine, and pyrazine. Methyl anthranilate is present in several Citrus oils (orange, lemon, and bergamot) and in ylang-ylang– *Cananga odorata* oil. Skatole is a compound in the form of large crystals or powder. It found in orange– Citrus aurantium blossoms and jasmine– Jasminum sp. (Baser and Demirci 2007). This compound has a very interesting aroma with a fecal smell at high concentrations but a floral scent in dilution. It used as a fixative in floral fragrances and a flavor agent in ice-cream and cigarettes. Indole is a white crystalline powder that turns red on exposure to air. It occurs in neroli and some citrus fruit oils. Has a similar odor to skatole and is used in a wide range of fragrances (Hunter, 2009), Pyridines and pyrazines occur in black pepper –*Piper nigrum*, sweet orange– Citrus × sinensis, and vetiver– *Chrysopogon zizanioides* oils (Baser and Demirci 2007).

1.5 Essential oil extraction methods

Essential oils are extracted using different methods such as distillation, solvent extraction, expression, maceration and enfleurage. (Whitton,1995)

1.5.1 Distillation*:*

Distillation is the best, and most commonly used method for obtaining the purest essential oils. The plant material is placed into a flask and heated with water, steam or both. The heat causes the little sacs of oil to burst, releasing the volatile contents into the resulting vapour. The vapour is then cooled through a condenser and becomes a liquid once more. The liquid is then separated into the essential oil and fragrant water. (Whitton, 1995) This recondensed water is referred to as a hydrosol. The disadvantage of steam distillation is that some delicate chemical components may denature easily by extreme heat. (Whitton, 1995).

1.5.2 Solvent extraction*:*

The plant material is placed in a suitable flask with a solvent (e.g. hexane or supercritical carbon dioxide) and allowed to stand for a period of time (hours to days). (Bowles, 2003) Whitton (1995) reports that "The resulting mixture is then filtered and becomes what is known as a 'concrete'. The concrete is then mixed with alcohol, chilled, filtered and the alcohol evaporated off, leaving behind a highly perfumed oil which is called an 'absolute'. The disadvantage of this method is that some of the solvent is present in the collected essential oil product that is consumed. This could be a problem as a reaction may occur if applied on the skin by a consumer who is allergic to the solvent. Therefore, solvent extraction is usually used for oils that are put into perfumes and not used on the skin". (Kububa, 2009).

1.5.3 Expression:

Expression is only used for citrus oils. The rinds are separated from the fruit either by hand or machine and squeezed to release their oils. (Whitton,1995)

1.5.4 Maceration*:*

The plant material is placed in hot vegetable oil. The heat causes the cell membranes to burst and the vegetable oil absorbs the plant oil creating an "infused" oil. (Whitton, 1995).

1.5.5 Enfleurage*:*

This specialized method is not very popular and mainly used to extract oils from flowers. Whitton (1995) fresh flower petals are placed on glass frames which have been layered with fat. The fat absorbs the oils from the flowers and the petals are replaced after 24 hours until the fat is saturated with oil. The final compound, is now called a “pommade”. It is then washed with alcohol. The alcohol is then evaporated off which leaves a scented oil. (Whitton,1995).

1.6 Factors affecting the composition (quality) of essential oils:

The difference in composition of essential oils have different species of the same genus are evident, however there are many other factors that influence the composition and yield of essential oils are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one another." Geoclimatic location is one of the most significant factors that can cause different chemotypes of essential oils. Some factors that can affect the essential oil molecules ratio produced by the plant include genetic variations, seasonal and maturity variation, the presence of fungal diseases and insects (Hussain, 2009). Other factors include soil type, life stage of plant and even the time of day when harvesting is done, since the volatile content of the leaf increases with time and also with the size of the leaf. (Bowles, 2003) The part of the plant used, the post-harvest drying and storage are also important factors that can affect the composition of the oil. (Hussain, 2009) The extraction methods used also has an effect on the essential oil quality. Steam distillation for example can affect the chemical composition of the essential oils, as heat and water vapour can cause molecular rearrangement, hydrolysis of double bonds, and in general will produce substances not originally found in plant. (Bowles, 2003) In solvent extraction, it is impossible to remove every single molecule from the concrete or absolute which will also affect its composition. Another issue is the degradation of essential oils; some of their constituents, such as monoterpenes

and monoterpenoid aldehydes, readily combine with oxygen from the air, especially if there is free energy in the form of heat or light. Some will form resins and others will be oxidized. The position of the double bonds can change, open chains can close to from rings and the nature of functional groups can change. These processes will alter the therapeutic effects of the essential oil constituents so it is important to store the essential oils in such a way as to minimize contact with air and free energy (i.e. store in amber bottles and refrigerate). However, no definitive studies have been done on the length of time for significant degradation of constituents, or the effects of such degradation on the therapeutic qualities of oils. For example, oxidation of some oils may enhance their therapeutic properties. (Bowles, 2003) Pollution is another factor that influences the quality of the essential oil. For example, *Centella Asiatica* in India and South-East Asia, suffers from high levels contamination because it is collected from sewage ditches. Because *Centella Asiatica* is an aquatic plant, it can easily take up pollulants from water into the plant. Devkota (2013) reported that essential oil production in *Centella Asiatica* plants are also affected by the variation of sunlight the plant receives. Accumulation of essential oil in herbs directly or indirectly depends upon light. *Centella Asiatica* produces high amounts of α-carophyllene and carophyllene oxide in open areas compared to shaded areas. Another factor that is explained by Lawless (1992) is synthetic essential oils called “natural identical” oils versus natural oils. Currently many oils and perfumes that use to be extracted from different plants are being produced almost entirely synthetically. These synthetic oils are preferred by the perfumery and flavouring industries as they need consistency in their products and any seasonal changes will cause the natural oils to change. When the naturally occurring, essential oils are compared to the so-called ''natural identical'' products, they have an entirely different character. For that reason, the synthetic oils are much cheaper to produce than the genuine oils. Many aromatic oils contain a relatively small number of major constituents, several minor constituents and also a very large number of trace elements. To

reconstruct such a complex combination of components including all trace elements would be virtually impossible. (Lawless, 1992) Most ‘'natural identical’' oils are only 96% pure or accurate, but it is the other 4% which are usually the trace elements that often truly characterize a particular fragrance. A real essential oil has a specific combination of constituents; however, its' therapeutic value is often a result of the trace elements. Lawless (1992) explains the reason for this might be that “these minute amounts of trace elements have synergistic or controlling effect on the main ones. Natural identical oils cannot be used therapeutically as substitutes for the naturally occurring aromatic materials, not only because the subtle balance of constituents is lost but also because they lack the vital ‘life force’ of oils of natural origin”.

1.7 Analysis of oil

1.7.1 Gas chromatography-Mass spectrometry:

Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios (*m/z* and relative abundances (Tood, 1995) The analyte may be ionized thermally, by an electric Feld or by impacting energetic electrons, ions, or photons. During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis. In addition, the potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of data generated by GC-MS instruments was fully exploited. The

most frequent and simple identification method in GC-MS consists of the comparison of the acquired unknown mass spectra with those in a reference MS library. A mass spectrometer produces an enormous amount of data, especially in combination with chromatographic sample inlets (Vekey, 2001) Over the years, many approaches for analysis of GC-MS data have been proposed using various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantify all of the chromatographic peaks. Library search algorithms are commonly provided with mass spectrometer data systems to assist in the identification of unknown compounds (McLafferty, 1999), However, as is well known, compounds such as isomers, when analyzed by means of GC-MS, can be incorrectly identified; a drawback which is often observed in essential oil analysis. As is, widely, acknowledged, the composition of essential oils is mainly represented by terpenes, which generate very similar mass spectra; hence, a favorable match factor is not sufficient for identification and peak assignment becomes a difficult, if not impracticable, task. In order to increase the reliability of the analytical results and to address the qualitative determination of compositions of complex samples by GC-MS, retention indices can be an effective tool. The use of retention indices in conjunction with the structural information provided by GC-MS is widely accepted, and routinely used to confirm the identity of can elute within 100 retention index units on a methyl silicone-based compound. Besides, retention indices when incorporated to MS libraries can be applied as a filter, thus shortening the search routine for matching results, and enhancing the credibility of MS identification (Costa, 2007). According to D. Joulain and W. A. König (Joulian, 1998), provided data contained in mass spectral libraries have been recorded using authentic samples, it can be observed that the mass spectrum of a given sesquiterpene is usually sufficient to ensure its identification when associated with its retention index obtained on methyl silicone stationary phases. Indeed, for the aforecited class of compounds, there would be no need to use a polyethylene glycol phase, which

could even lead to misinterpretations caused by possible changes in the retention behavior of sesquiterpene hydrocarbons as a result of column aging or deterioration. Moreover, according to the authors, attention should be paid to the retention index and the mass spectrum registration of each individual sesquiterpene, since many compounds with rather similar mass spectra elute in a narrow range; more than 160 compounds column, for example, 1400–1500).

1.7.2 Fast GC for essential oil analysis:

Nowadays in daily routine work, apart from increased analytical sensitivity, demands are also made on the efficiency in terms of speed of the laboratory equipment. Regarding the rapidity of analysis, two aspects need to be considered: (i) the costs in terms of time required, for example, as is the case in quality control analysis, and (ii) the efficiency of the utilized analytical equipment. When compared to conventional GC, the primary objective of fast GC is to maintain sufficient resolving power in a shorter time, by using adequate columns and instrumentation in combination with optimized run conditions to provide 3–10 times faster analysis times (Korytar, 2002, Cramer, 1999). The technique can be accomplished by manipulating a number of analysis parameters, such as column length, column I.D., stationary phase, flm thickness, carrier gas, linear velocity, oven temperature, and ramp rate. Fast GC is typically performed using short, 0.10 or 0.18mm I.D. capillary columns with hydrogen carrier gas and rapid oven temperature ramp rates. In general, capillary gas chromatographic analysis may be divided into three groups, based solely on column internal diameter types; namely, as conventional GC when 0.25mm I.D. columns are applied, fast GC using 0.10–0.18mm I.D. columns, and ultrafast GC for columns with an I.D. of 0.05 mm or less. In addition, GC analyses times between 3 and 12 min can be defend as “fast”, between 1 and 3 min as “very fast,” and below 1 min as “ultrafast”. Fast GC requires instrumentation provided with high split ratio injection systems because of low sample column capacities, increased inlet pressures, rapid oven heating rates, and fast electronics for detection and data

collection (Mondello, 2003). The application of two methods, conventional (30m ¥ 0.25mm I.D., 0.25mm *d*f column) and fast (10m ¥ 0.10mm I.D., 0.10mm *d*f column), on five different citrus essential oils (bergamot, mandarin, lemon, bitter oranges, and sweet oranges) has been reported (Mondello, 2003). The fast method allowed the separation of almost the same compounds as the conventional analysis, while quantitative data showed good reproducibility. The effectiveness of the fast GC method, through the use of narrow bore columns, was demonstrated. An ultrafast GC lime essential oil analysis was also performed on a 5 m ¥ 50 mm capillary column with 0.05mm stationary phase film thickness (Mondello, 2004) The total analysis time of this volatile essential oil was less than 90 s; a chromatogram is presented in Figure 6.2. Another technique, ultrafast module-GC (UFM-GC) with direct resistively heated narrow-bore columns, has been applied to the routine analysis of four essential oils of differing complexities; chamomile, peppermint, rosemary, and sage (Bicchi, 2004). All essential oils were analyzed by conventional GC with columns of different lengths; namely, 5 and 25m, with a 0.25mm I.D., and by fast GC and UFM-GC with narrow-bore columns (5m ¥ 0.1mm I.D.). Column performances were evaluated and compared through the Grob test, separation numbers, and peak capacities. UFM-GC was successful in the qualitative and quantitative analysis of essential oils of different compositions with analysis times between 40 s and 2 min versus 20–60 min required by conventional GC. UFM-GC allows to drastically reduce the analysis time, although the very high column heating rates may lead to changes in selectivity compared to conventional GC, and that are more marked than those of classical fast GC. In a further work the same researchers (Bicchi, 2005) stated that in UFM-GC experiments the appropriate flow choice can compensate, in part, the loss of separation capability due to the heating rate increase.

1.8 Uses of essential oils:

The main uses of essential oils are in the fragrance industry as perfumes and after shaves, as flavorings in the food industry and for their functional properties in the pharmaceutical industry. (Burt, 2004) Essential oils are very important in medicine. Their therapeutic uses are usually based on historical purposes rather than scientific evidence. Their uses range from skin treatments to remedies for cancer (Elsharkawy, 2014) as well as for their antiviral and antibacterial properties. (Burt, 2004).

1.9 Importance of Essential Oil in Pharmaceuticals

Essential Oils have flexible applications in pharmaceutics. A percentage of the applications are recorded. The germicidal properties of Essential Oil make them dynamic against extensive variety of microorganisms as anti-microbial safe strains. Notwithstanding this they are used likewise against parasites and yeasts. The most well-known wellsprings of essential oils utilized as cleaning agents seem to be: Cinnamon, thyme, clover, eucalyptus, culinsavory, and lavender. citral, geraniol, linalool and thymol are considerably stronger than phenol. At the point when utilized remotely, essential oils (L'essence de terebenthine) expand microcirculation and give a slight neighborhood sedative activity. Till now, essential oils are utilized as part of various treatments. They are known not exceptionally to be viable in diminishing sprains and other articular agonies. Oral administration of essential oils like eucalyptus or pin oils, arouse ciliated epithelial cells to emit bodily fluid. On the renal system, these are known to increase vasodilation and in consequence bring about diuretic effect (K. Satish Kumar, 2010). Fundamental oils from the *Umbellifereae* family, *Mentha* species and *verbena* are alleged to diminish or dispense with gastrointestinal fits. These essential oils expand discharge of gastric juices. In different cases, they are known to be powerful against sleep deprivation (K. Satish Kumar, 2010).

1.9.1 Antimicrobial activity:

The antimicrobial activity of essential oils of Zingiberaceae species has been demonstrated by several researchers (Seenivasan, *et al.* (2006); Oonmetta-aree, *et al.* (2006). In this present study, the antimicrobial activity of the essential oils has been tested against two dermatophytic fungi namely *Microsporum canis* and *Trichophyton rubrum*, two *Candida* species, *Candida albican* and *Candida glabrata* and five strains of *Staphylococcus aureus* (*Sa 2α, Sa 3, Sa 7, VISA* and *VRSA*) using minimal inhibitory concentration (MIC) assay. MIC is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganism after overnight incubation. *Microsporum canis* dermatophytosis is an infectious fungal skin disease, which appears in the form of different lesions in the fur of the animal. It is caused by *M. canis*, a pathogenic fungus that grows in the hair, and in the top layer of the skin. The disease is zoonotic, meaning that it can be transmitted from animals to human. While, *Trichophyton rubrum* is an anthropophilic dermatophyte. The downy strain has become the most widely distributed dermatophytes of man. It frequently causes chronic infections of skin, nails and rarely scalp. *Candida albican* is the fungi that live in our gastrointestinal tract and this species belong to the family of Saccharomycetaceae. It can cause vaginal yeast infections. *Candida* can spread throughout the intestinal tract causing bloating, gas, food reactions, allergies, diarrhea and many other diseases. It also can spread to the vaginal area, the prostate, the heart, lungs, liver and cause numerous symptoms and illnesses. The other species of *Candida* used in this study is *Candida glabrata*. *C. glabrata* is often the second or third most common cause of candidiasis after *C. albicans. Candida glabrata* can be found in the environment, particularly on leaves, flowers, water and soil. This species can cause candidiasis in men at any age. *Staphylococcus aureus* is a gram-positive bacteria belonging to the family Micrococcaceae and are frequently found living on the skin in the nose of a healthy person. This microbe is a versatile pathogen of humans and animals that

has evolved resistance to all antibiotic classes' causes a wide variety of diseases in humans, ranging in severity such as boils and furuncles to more serious diseases such as septicaemia, pneumonia and endocarditis (Crossley, *et al*. 1997 and Lowy, 1998). VISA and VRSA are strains of *S. aureus* which can cause a variety of infections to the body. VISA stands for *Staphylococcus aureus* with intermediate resistance to vancomycin. Vancomycin is an antibiotic often used to treat very serious infections. VISA strains have minimum inhibition concentrations (MIC) of vancomycin in the range of 8 to 16 µg/ml due to a thickening of the bacterial cell wall. While, VRSA stands for *S. aureus* with complete resistance to vancomycin and the vancomycin minimum inhibition concentrations (MIC) is more than 32 µg/ml. It is probable that *S. aureus* bacteria with intermediate or complete resistance to vancomycin would be resistant to most antibiotics commonly used for staphylococcal infections.

1.9.2 Antioxidant activity:

Consumption of fruits and vegetables with high content of antioxidative phytochemicals such as phenolic compounds may reduce the risk of cancer, cardiovascular disease and many other diseases (Robbins and Bean, 2004 and Shui and Leong, 2006) and can inhibit the propagation of free radical reactions and protect the human body from diseases (Kinsella. *et al*. 1993). Therefore, the interest in naturally occurring antioxidants has increased considerably in recent years for use in food and pharmaceutical products (Djeridane, *et al.*, 2006). There are various methods to determine the antioxidant activities such as DPPH free radical scavenging assay, reducing power assay, β-carotene bleaching assay, superoxide scavenging assay, tyrosinase inhibitory assay and many others. In this study, only two methods are employed; the DPPH free radical scavenging assay and reducing power assay which are briefly described in the following paragraph. The determination of scavenging stable DPPH is a very fast method to evaluate the antioxidant activity of the extracts. With this method it is possible to determine the antiradical power of an antioxidant activity by measuring the

decrease in the absorbance of DPPH at 515 nm. Colour change from purple to yellow when DPPH radical is scavenged by antioxidant, through the donation of hydrogen to form a stable DPPH molecule reduced the absorbance. In the radical form this molecule had an absorbance at 515 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus, 2002).

1.9.3 Anti-inflammatory activity:

Anti-inflammatory refers to the property of a substance or treatment to reduce inflammation. There are several assays for anti-inflammatory activity such as platelet activating factor, nitric oxide, hyaluronidase, lipoxygenase and many other assays. In this study, two assays were applied; hyaluronidase assay and lipoxygenase assay. Hyaluronidase is a mucopolysaccharide hydrolyzing enzyme that degrades hyaluronic acid (HA), a viscous lubricating agent in synovial fluid in joints and which is also present on the skin. Hyaluronidase enhances the spreading of inflammatory mediators throughout the body tissues, thereby contributing to the pathogenesis of inflammatory diseases such as allergic effects, migration of cancer cells, inflammation and the permeability of the vascular system (Ling *et al*., 2005). Lipoxygenase is a biological target for many diseases such as asthma, cancer and many others diseases. Lipoxygenases are classified with respect to their positional specificity of arachidonic acid oxygenation; in particular, the reticulocyte-type 15-LOX and the human 5-LOX are well characterized with respect to their structural and functional properties (Celotti and Laufer, 2001).

1.9.4 Cancer Chemo protective Activity:

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

turmeric, are good sources of anticancer agents (Pyun, 2006) Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer (Milner, 2001). Diallylsulfide, diallyldisulfide, and diallyltrisulfide are examples. According to (Wu, 2005) these compounds activate, in rats, the enzymes involved in the detoxification process of hepatic phase 1 (disintegration of chemical bonds that link carcinogenic toxins to each other) and phase 2 (bonds to toxins released detoxifying enzymes, such as glutathione *S*-transferase). Metabolism happens mainly in the liver, the body's largest internal organ. The portal vein carries blood from the small intestine directly to the liver. Sixty percent of liver tissue is made up of hepatic cells. More chemical processes happen in these than in any other group of cells in the body. Phase 1 metabolism involves chemical reactions, such as oxidation (most common), reduction, and hydrolysis. There are three possible results of phase 1 metabolism. The drug becomes completely inactive. In other words, the metabolites are pharmacologically inactive. One or more of the metabolites are pharmacologically active, but less so than the original drug. The original substance is not pharmacologically active, but one of its metabolites is. The original substance is called a prodrug. Phase 2 metabolism involves reactions that chemically change the drug or phase 1 metabolites into compounds that are soluble enough to be excreted in urine. In these reactions, the molecule (drug or metabolite) is attached to an ionisable grouping. This is called conjugation and the product is called a conjugate. Metabolites formed in phase 2 are unlikely to be pharmacologically active. Some drugs undergo either phase 1 or phase 2 metabolism, but most undergo phase 1 metabolism followed by phase 2 metabolism.

1.10 Dangers of essential oils

Most essential oils are not used in their concentrated form but are diluted in a vegetable-based carrier oil such as olive oil or sweet almond oil usually at a concentration in the range 0.5-3%. If applied directly to the skin in its

concentrated form it could cause contact dermatitis usually caused by an allergic reaction. Some essential oils can increase the light sensitivity of the skin making it more likely to burn. (Tisserand and Young, 2014) If a pure essential oil is ingested at amounts greater than its therapeutic use, it can be poisonous. Medical practitioners prefer organically produced oils as there is some concern of contamination of essential oils with pesticides. (Tisserand and Young, 2014) When essential oils are burned, they release volatile organic compounds, which can have health effects. They may also release polycyclic aromatic hydrocarbons which are known carcinogens. The LD50 of most essential oils or their main components are 0.5-10 g kg-1 (orally or skin test). (Tisserand and Young, 2014).

1.11 Objectives of this study

This study was aimed to:

- To Extract The oil from *Trigonella Foenum graecum*.
- To Study The physicochemical properties of the extracted oil.
- To Evaluate the antimicrobial potential of the oil.

Chapter Two

2. Materials and Methods

2.1 Materials:

2.1.1 Plant material

Seed of *Trigonella Foenum graecum were* collected from Khartoum bahry local market , Sudan and authenticated by direct comparison with herbarium sample . The seed were shade- dried at room temperature and powdered .

2.2 Methods

2.2.1 Solvent extraction

Powdered shade–dried seed of *Trigonella Foenum graecum* (350g) were extracted with n-hexane at room temperature. The solvent was removed by *vacuum.*

2.2.2 Esterification of the oil

The extracted oil was esterified as follows: (2ml) was placed in a test tube and 7 ml of alcoholic sodium hydroxide was added followed by 7 ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously shaken 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 for

five minutes. The hexane layer was then separated. (5 µl) of hexane extract were mixed with 5 ml of diethyl ether. The solution was filtered and the filtrate (1µl) Was injected in the GC–MS vial.

2.2.3 GC– MS analysis

The GC– MS analysis was performed on a Shemadzo GC- MS– QP 2010 Ultra instrument with RTX – 5MS column (30m, length ; 0.25 mm diameter; 0.25 µm, thickness).

2.2.4 Test organisms

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

2.3 Physicochemical properties of the oil

2.3.1 Specific gravity

Determined by AOAC**.** The dry pycnometer filled with 25 mL prepared sample in such a manner to prevent trap of air bubbles after removing the cap of the side arm. The stop [per was inserted in the pycnometer immersed immediately in water bath at 30.0 $\pm$ 0.2ºC and held for 30 minutes. Any oil came off the capillary opening of the pycnometer stopper was wiped out carefully. The bottle removed from the bath, cleaned and dried thoroughly. The cap of the side arm removed and quickly the bottle weighed ensuring the temperature did not fall below 30^0C.

$$\textbf{Specific gravity at } 30\ ^0\text{C}/\ 30\ ^0\text{C} = \frac{A-B}{C-B}$$

Where:

A: weight in gm of specific gravity bottle with oil at 30 ^{0}C.

B: weight in gm of specific gravity bottle at 30 ^{0}C.

C: weight in gm of specific gravity bottle with water 30 ^{0}C.

2.3.2 Refractive index

The refractive index of the oil was determined by (AOAC, 1990). The refract meter was first adjusted at 1.3330 at 20^0 C using distilled water as a blank. A drop of the oil was placed in the instrument and telescope was adjusted so that the cross hairs were distinct and in focus. The adjustment of the knob was rotated until the lower part of the field was dark and the upper part was light and a clear definite boundary appeared. The coarse adjustment knob was moved first and then the fine adjustment knob until the boundary line coincided with the intersection of the cross hair in the telescope. The instrument was read when temperature is stable.

2.3.3 Determination of color

Colour was determined according to handbook of food analysis (AOAC, 2000). The sample liquid and filtered through a filter paper to remove any impurities and traces of moisture till is sure that the sample was absolutely clear and free from turbidity. The glass cell of desired size cleaned with carbon tetrachloride and allowed to dry. The cell filled with the oil and placed in position in the tintometer. The colour matched with sliding red, yellow and blue colours.

Report the colour of the oil in terms of Lovibond units as follows:

Colour reading = (a Y + 5 b R) or (aY+ 10 b R).

Where a = sum total of the various yellow slides (Y) used

b = sum total of the various red (R) slides used

Y + 5R is the mode of expressing the colour of light coloured oils; and

Y + 10 R are for the dark-coloured oils.

2.3.4 Viscosity

Approximately 25 mL of oil were placed in the Tube DIN 1 outer cylinder, and then the bob MK Din-9 was inserted. The radius of the tube (R_a) is 16.25 mm and the radius of the bob is (R_i) 15.5 mm. The length of the bob is 54 mm. The correct mode was set for the appropriate measuring system (MS 19) and the measurement time was fixed at 60 seconds. The torque of each sample at the different

temperatures was recorded at a range of shear rate (Y) from 64.5 to 4835 s^{-1}. All viscometric measurements of the samples were carried out in triplicate. Every replicate was run twice the mean torque value of the two runs was recorded for each replicate at a given shear rate (AOAC, 2000). The shear stress was obtained from.

2.4 Chemical properties of the oils

2.4.1 Acid value

Acid value was determined according to handbook of food analysis (AOAC, 1990). The oil mixed thoroughly before weighing. About 5 of cooled oil sample accurately weighed in a 250 ml conical flask and 50 ml added to 100 ml of freshly neutralized hot ethyl alcohol and about one ml of phenolphthalein indicator solution. The mixture boiled for about five minutes and titrated while hot against standard sodium hydroxide shaking vigorously during the titration. The weight of the oil taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10 ml.

Calculation

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where:

V = Volume in ml of standard sodium hydroxide used

N = Normality of the Sodium hydroxide solution

W = Weight in g of the sample

The acidity is frequently expressed as free fatty acid for which calculation shall be.

$$\text{Free fatty acids as oleic acid} = \frac{28.2 \times V \times N}{W}$$

Per cent by weight

Acid value = Percent fatty acid (as oleic) × 1.99

2.4.2 Saponification value

Saponification value was determined according to handbook of food analysis (AOAC, 2000). About 1.5 to 2.0g sample were transferred into a 200ml conical flask. A 30 mL of 0.5 N potassium hydroxide ethanol, and fix a cooling pipe to the flask. The flask gently heated and occasionally shaked while adjusting the heat so that back flow ethanol will not reach the top of cooling pipe. After heated for 1 hour, immediately cooled, and titrated with 0.5 N Hcl before the test liquid is solidified. Blank test performed for 3 times to obtain mean value of titration volume of 0.5 N hydrochloric acid.

The saponification was calculated as followed:

Saponification value (mg / g) = $\frac{(\mathrm{BLl} - \mathrm{EPl}) \times \mathrm{TF} \times \mathrm{Cl} \times \mathrm{KI}}{\mathrm{W}}$/ SIZE

Where:

EPl： Titration volume (ml)

BLl： Blank level (25.029ml)

TF： Reagent (HCl) factor (1.006)

Cl: Concentration conversion coefficient (28.05 mg/ml)

(Potassium hydroxide in Eq.:56.11×0.5)

Kl ： Unit conversion coefficient (1)

W ： Sample weight (g).

2.4.3 Unsaponification value

Un saponification matters were determined according to (AOAC,2000). Accurately 5 g of well mixed oil sample were weighed into a 250ml conical flask. 30ml of alcoholic potassium hydroxide solution were added. The content boiled under reflux air condenser for one hour or until the saponification is complete (complete saponification gives a homogeneous and transparent medium). The condenser washed with about 10 ml of ethyl alcohol. The saponified mixture was transferred while still warm to a separating funnel. The saponification flask washed first with some ethyl alcohol and then with cold water, using a total of 50

ml of water to rinse the flask, Cooled to 20 to 25ºC. Fifty ml of petroleum ether were added to the flask, shaked vigorously, and allowed the layers to separate. The lower soap layer transferred into another separating funnel and repeat the ether extraction for another 3 times using 50 ml portions of petroleum ether. The combined ether extract was washed three times with 25 ml portions of aqueous alcohol followed by washing with 25ml portions of distilled water to ensure ether extract is free of alkali (washing is no longer alkaline to phenolpthalen). The solution transferred to 250ml beaker, rinse separator with ether, added rinsing's to main solution. Evaporated to about 5ml and transferred quantitatively using several portions.

2.4.4 Peroxide value

Peroxide value was determined according to Handbook of food analysis. Five grams of the sample were delivered into a stoppered conical flask. About 25 mL of solvent (15 ml acetic acid+10 ml choloroform) were added and gently shake to dissolve the sample completely. The air inside flask gently replace with nitrogen to remove remaining oxygen. One ml of saturated potassium iodide was added and the flask was sealed immediately and gently shaken for one minute. The flask was left at room temperature 15 to 20°C in a dark room. Thirty mL of pure water were added, and the flask sealed and stirred and titrated with 0.01mol/L sodium thiosulphate.

The peroxide value was measured as followed:

Peroxide value (meq / kg) = $= \frac{(\mathrm{BLl} - \mathrm{EPl}) \times \mathrm{TF} \times \mathrm{R}}{\mathrm{W}}$

Where:

EP1 : Titration volume (mL)

BL1 : Blank level (0.00mL)

TF : Factor of reagent (1.006)

R : Constant (10)

W : Sample weight (g)

2.4.5 Iodine value

Iodine value was determined according to Handbook of Food Analysis (AOAC, 2000). To 300 ml stopper conical flask 0.1g sample were added. Twenty ml of carbon tetrachloride were added and Twenty-five mL Hanus solution also added and the flask also sealed. The flask content shaked for one minute. And kept sealed and left in a dark room (about 20°C) for 30 minutes with continuous shaking every 5 minutes. Ten mL of 15% potassium iodide and 100 ml of water were added, and the flask sealed and shake for 30 seconds. The flask content titrated with 0.1mol/L sodium thiosulfate to obtain iodine value. Likewise, performed using blank test.

The Iodine value was calculated as follow:

Iodine value (c g / g) = (BL1－EP1) × TF × C1 ×K1 / SIZE

Where:

EP1：Titration volume (mL)

BL1：Blank level (47.074mL)

TF ：Factor of titrant (1.006)

C1：Concentration conversion coefficient (1.269)

(Atomic mass of iodine: 126.9/100)

K1 ：Unit conversion coefficient (1)

SIZE ：Sample size (g)

2.5 GC-MS Analysis

The extracted oil was investigated by gas chromatography – mass spectrometry using a Shimadzo GC-MS– QP 2010 Ultra instrument using helium as carrier gas.

2.6 Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and fungis respectively. Broth cultures (5.0×10^7 cfu/ml) were streaked on the surface of the solid medium containing in Petri dishes. Filter

paper discs (Oxide, 6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria the plates were incubated at 37ºC for 24h, while for fungi the plates were incubated at 25ºC 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.

Chapter Three

3. Results and Discussion

3.1 Physicochemical properties

Table 3.1 Physical properties

Property	Value
Specific gravity	0.912 g/cm^3
Viscosity	16.149 poise
Refractive index	1.4743
Mousier content	1.75 %

Table 3.2 Chemical properties

Property	Value
Acid value	6.732 mg / goil
Peroxide value	0.5 mg / 100 g
Saponification value	0.2
Free fatty acid	3.4

Discussion for table 3.1 and 3.2

Specific gravity of *Trigonella Foenum graecum* essential oil 0.912 g/cm^3 which near the density of water. The refractive index1.4743 we found it is acceptable according to references (1.4741– 1.4749). The viscosity of extracted oil 16.146 poise and this value is acceptable because it is fixed oil. Peroxide value of oil 0.5mg/100 g which indicated the presents of peroxide in this oil . The acid value obtained is 6.732mg/g oil this result means partial hydrolysis of oil during distillation.

3.2 GC-MS analysis of *Trigonella Foenum graecum* oil

The oil extracted from *Trigonella Foenum graecum* was investigated by GC-MS analysis. The fatty acid content of the oil was determined by retention time and the observed fragmentation pattern. Seventeen components were detected in total

ion chromatogram. The typical 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 (Z, Z) methyl ester (43.48%).

ii – 9,12,15- Octadecatrienoid acid, methyl ester, (Z, Z, Z) (20.15 %).

iii – Hexadecanoic acid, methyl ester (16.35%).

iv – Methyl stearate (8.77 %).

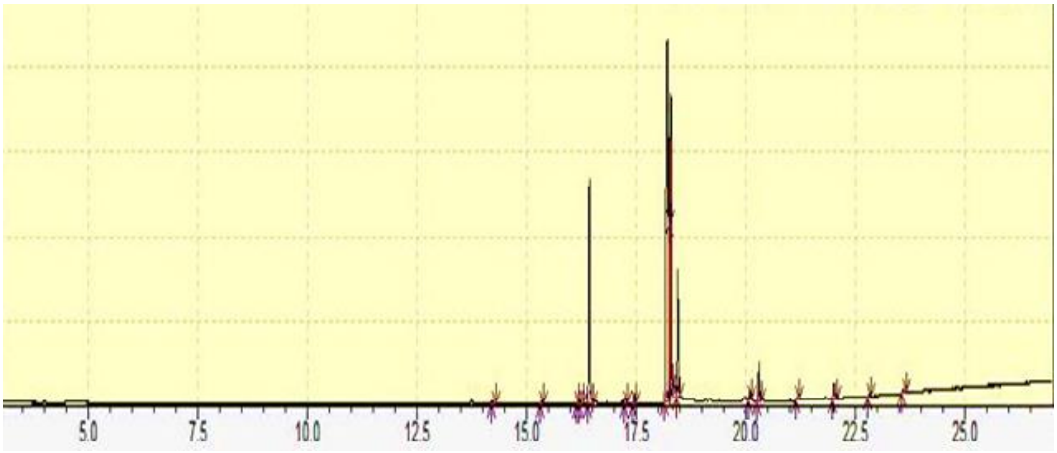

Fig.3.1: GC chromatograms of *Trigonella Foenum graecum* oil

Table 3.3: constituents of *Trigonella Foenum graecum*

ID#	Name	Ret.Time	Area	Area%
1	Methyl tetradecanoate	14.215	335306	0.20
2	Pentadecanoic acid, methyl ester	15.346	403348	0.24
3	7-Hexadecenoic acid, methyl ester, (Z)-	16.183	126364	0.08
4	9-Hexadecenoic acid, methyl ester, (Z)-	16.227	304054	0.18
5	Hexadecanoic acid, methyl ester	16.430	26931074	16.35
6	cis-9-Hexadecenal	17.244	703745	0.43
7	Heptadecanoic acid, methyl ester	17.458	1070240	0.65
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.209	71583034	43.48
9	9-Octadecenoic acid (Z)-, methyl ester	18.260	6638309	4.03
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.287	33188445	20.15
11	Methyl stearate	18.448	14434278	8.77

12	cis-11-Eicosenoic acid, methyl ester	20.093	1097054	0.67
13	Eicosanoic acid, methyl ester	20.296	4473874	2.72
14	Heneicosanoic acid, methyl ester	21.167	344173	0.21
15	Docosanoic acid, methyl ester	22.004	2033019	1.23
16	Tricosanoic acid, methyl ester	22.810	380509	0.23
17	Tetracosanoic acid, methyl ester	23.586	624971	0.38

The characterization of these major components is briefly discussed below:

i– 9,12 Octadecadienoic acid (Z, Z) methyl ester (43.48%)

The mass spectrum of 9,12 Octadecadienoic acid (Z, Z) methyl ester is depicted in Fig. 2. The signal which was observed at m/z 294 (R.T 18.20) is due to M^+ $[C_{19}H_{34}O_2]^+$, while the signal at m/z 264 corresponds to loss of methoxyl.

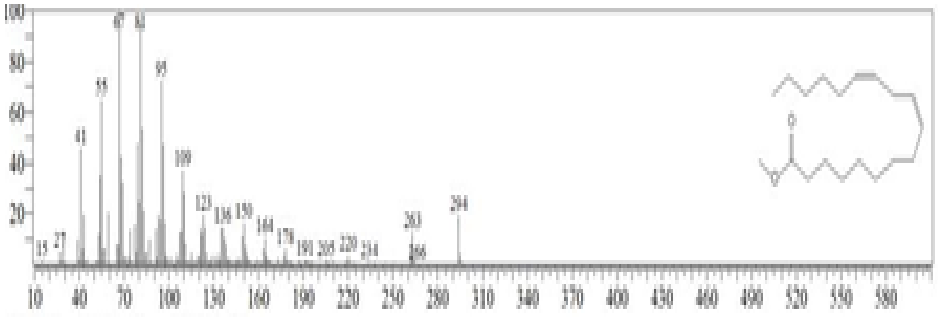

Fig.3.2 mass spectrum of 9,12,15- Octadecatrienoid acid, methyl ester, (Z, Z, Z)

ii– 9,12,15- Octadecatrienoid acid, methyl ester, (Z, Z, Z) (20.15%)

Mass spectrum of ii– 9,12,15- Octadecatrienoid acid, methyl ester is depicted in Fig.3 at m/z 292, which appeared at R.T.18.287 corresponds to M^+ $[C_{19}H_{44}O_2]^+$, while the peak at m/z 277 is attributed to loss of methoxyl.

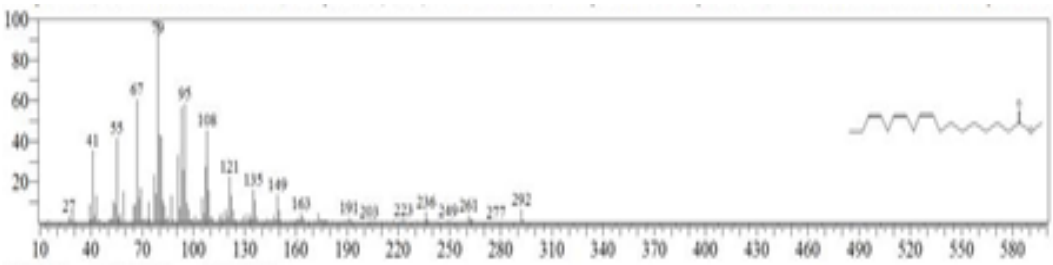

Fig.3.3 mass spectrum of hexadecanoic acid, methyl ester

iii– Hexadecanoic acid, methyl ester (16.35%)

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

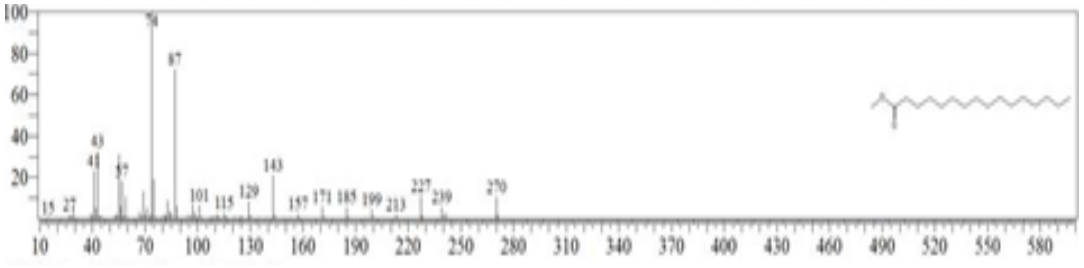

Fig. 3.4 mass spectrum of hexadecanoic acid, methyl ester

iv– Methyl stearate (8.77%)

The mass spectrum of methyl stearate is Shawn in Fig.5. The peak at m/z 298 (R.T. 18.448) is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at 267 m/z corresponds to loss of methoxyl.

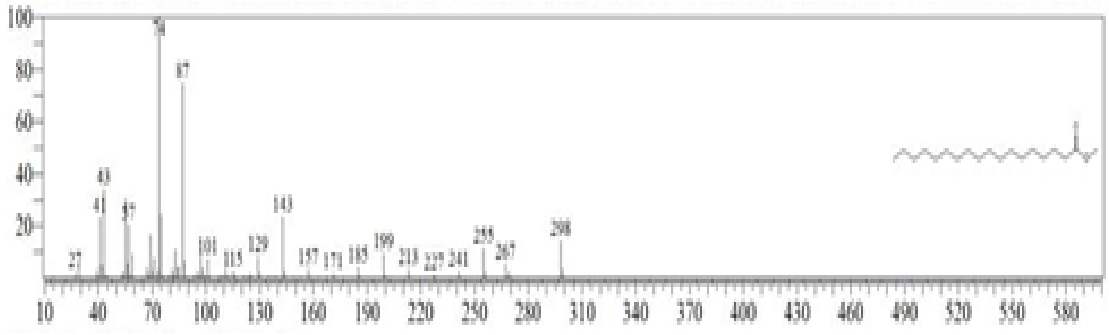

Fig. 3.5 mass spectrum of methyl stearate

3.3 Antimicrobial activity

Trigonella Foenum graecum essential oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard pathogenic bacteria. The average of the diameters of the growth inhibition zones are displayed in Table 3.4. Results were interpreted as follows: 9 considered inactive; 9-12: weak activity; 13-18: active and <18: very active.

Ampicilin, gentamycin and clotrimazole have been used as positive controls.

Table 3.4: Antimicrobial activity of the oil

Type	onc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa*: Staphylococcus aureus*

Ec.*: Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

An.: *Aspergillus niger*

Ca.: *Candida albicans*

Bs.: Bacillus subtilis

At a concentration of 100mg/ml, the oil showed moderate anticandidal activity. It also exhibited significant activity against Gram Positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Escherichia coli.* and Gram-negative *Pseudomonas aeruginosa.*

3.4 Antioxidant assay

Evaluation of the antioxidant activity was carried out by measuring the capacity of the test compound against stable DPPH radical. As depicted in Table 3.5 crude extract exhibited very active anti- oxidant activity.

Table 3.5: Radical scavenging activity of crude extract

Sample	Antioxidant activity
Propyl gallate	91.00%±0.01
Crude extract	71.00%±0.02 %

Chapter Four

Conclusion and Recommendation

4.1 Conclusion

The oil extracted from *Trigonella Foenum graecum* was investigated by GC-MC analysis. The fatty acid content of the oil was determined by retention times and the observed fragmentation pattern. Seventeen components.

- The bulk of the oil is unsaturated fatty acids.
- The oil Shaw acid and saponification values are very law.
- Significant antimicrobial activity against both gram positive and gram-negative pathogens.

4.2 Recommendation

The following is recommended:

- Other phytochemical (steroids, alkaloids, flavonoids... etc). present in the studied plant may be isolated and thoroughly investigated.
- The isolates could be evaluated for other biological activities.

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