1. INTRODUCTION

The plant kingdom is important to human beings, the increasing use of plant materials as a way to improve health and to heal specific elements is known as herbs. In general, plants are used as medicinal plants or herbs. Recently, researchers in many countries and numerous publications have a growing interest in developing the cultivation of medicinal plants.

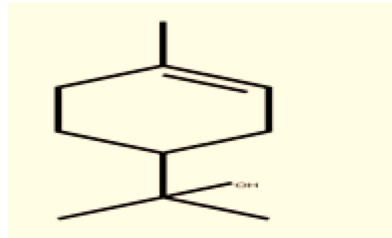
In traditional medicines, in all the available literature, medicinal plants are defined as plants that have the potential to treat many diseases, and act as sources of various types of chemical compounds with complex structures while the botanical definition of a herb is a plant whose stem is not woody and tenacious. The meaning of the word herb is open to the controversy by botanists and herbalists (AOAC, 1990)

1.1. Natural products classification

It is according to the type of chemical skeleton they are aliphatic or non-aliphatic fatty compound of the open-chain. Acyclic and cyclo-aliphatic compounds are mainly terpenoids, steroids, and some alkaloids. .Some natural products are heterocyclic compounds such as alkaloids, flavonoids (Kamel 2000).

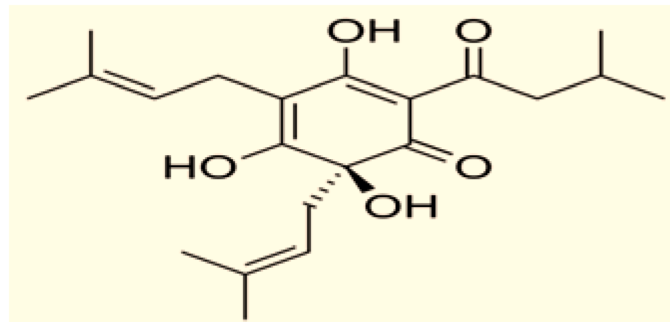
1.1.1 Terpenoids

Terpenoids are more or less based upon the specific 'Isoprene molecule' .

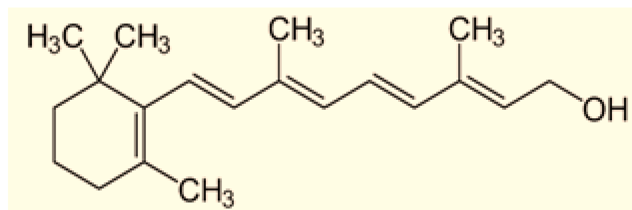


Isoprene unit

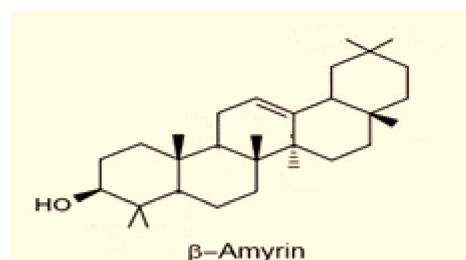
i)-Mono terpenoids: two isoprene units [$C_{10}H_{16}$]



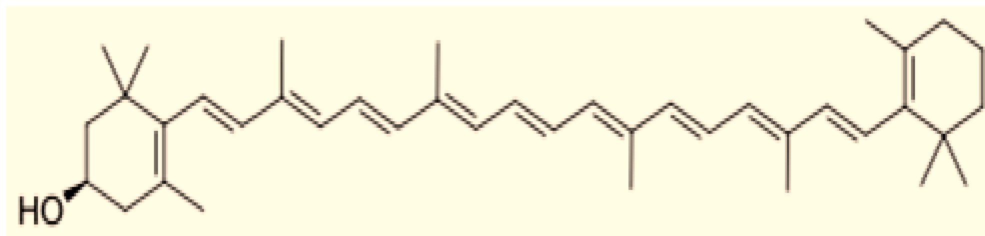
ii)-Sesquiterpenoids: three isoprene units [$C_{15}H_{24}$]



iii)-Diterpenoid: four isoprene units [$C_{20}H_{32}$]



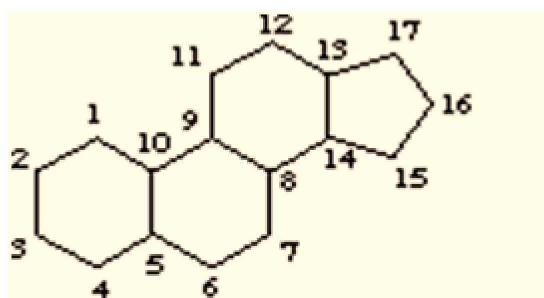
iii)-Triterpenes are a class of chemical compounds consisting of three terpene units with the molecular formula $C_{30}H_{48}$; it can also be ruminated as it consists of six isoprene units. Animals, plants, and fungi all produce triterpenes, including squalene, which is a precursor to all stimulant.



iv)-Tetra terpenoids: eight isoprene units [$C_{40}H_{64}$]

1.1.2 Steroids

Steroids are the most important natural products. They are well distributed in animals and plants and have been extensively studied since the isolation of cholesterol. The steroids are found in two sources. (Khalid, 1974); animals (cholesterol) and plants (stigma sterol).

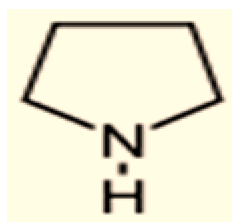


Steroids:basic skeleton

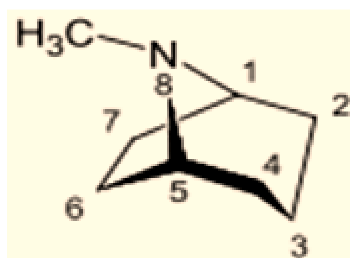
1.1.3 Alkaloids

First, the term (alkaloid) was critically assigned to all those natural materials that react almost like a base or alkali**.** An attempt was made to classify alkaloids into: pyrimidine alkaloids**,** pyridine alkaloids (or piperdine alkaloids)**,** pyrrolidine alkaloids, tropane alkaloid**,** quinoline alkaloids**,** isoquinolone alkaloid ,indole alkaloids, and imidazole alkaloids**.**

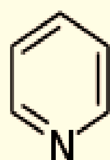
These previously mentioned eight typical N-heterocyclic basic ring categories of ‘alkaloids (Khalid, *et al* 1974).



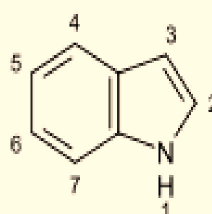
Pyrrolidine



Tropane



Pyridine

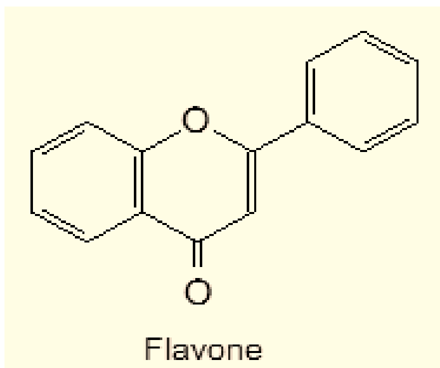


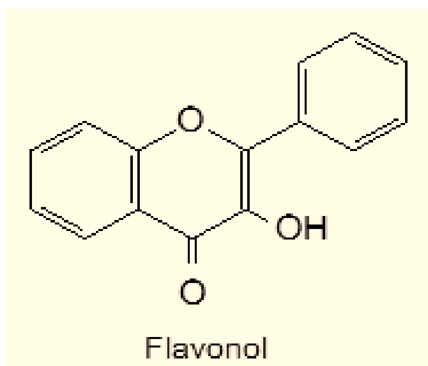
Indole

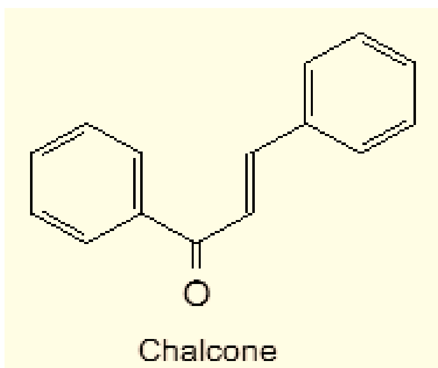
1.1.4 Flavonoids

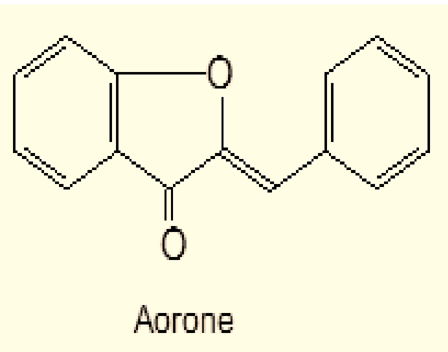
The flavonoids from the Latin word "flavous" which means yellow, is generally used to describe a wide range of natural products that process 15 carbon atoms with a C_6-C_3-C_6 carbon frame, comprising two benzene rings linked through a pyrane(or pyrene) heterocycle.

Classification of flavonoid aglycones includes: flavones, flavonols ,chalcones, aurones flavanones ,isoflavones**,** dihydroflavonols, dihydrochalcones ,anthocyanidins and flavans**.**





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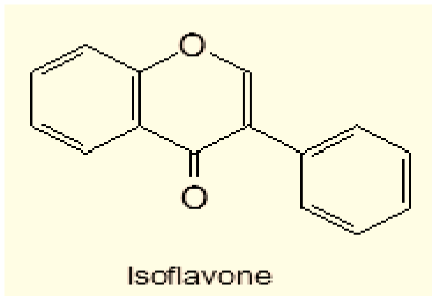
Aorone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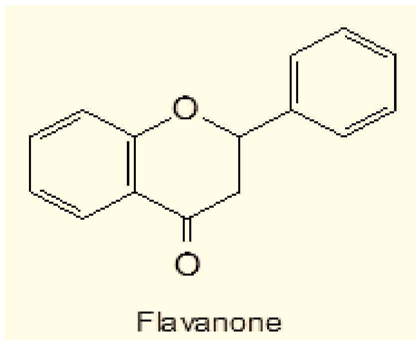
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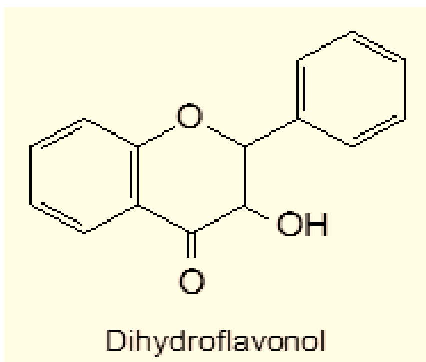
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OH
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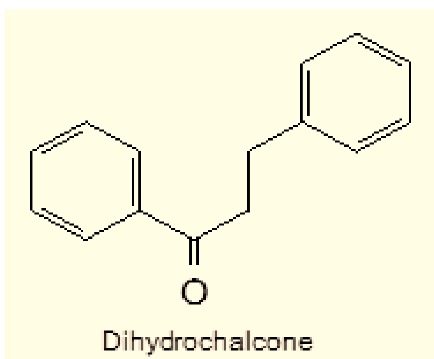
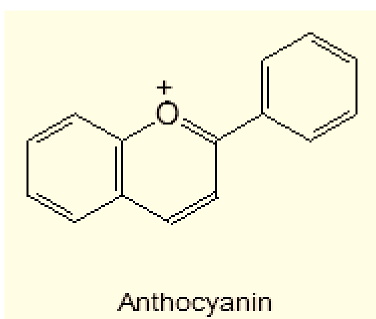
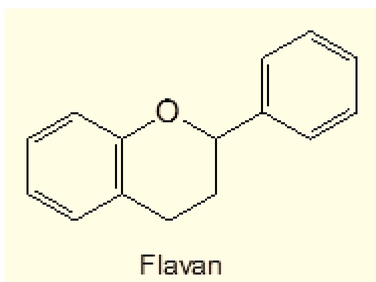
Dihydroflavonol







1.2. Essential oils

Essential oils (EOs) are obtained from aromatic and medicinal plants as a volatile mixture of chemical compounds with strong odor. EOs are extracted from the aromatic and medicinal plants using steam or hydrodistillation or Soxhlet extraction (solvent extraction or continuous extraction) methods developed in the middle ages by Arabs (Bakkali et al. 2008). EOs are considered as one of the most predominant plant products in agriculture, as they exhibit antifungal, antibacterial, antioxidant, anticancer, antidiabetic, antiviral, insect repellent, and anti-inflammatory

properties (Buchbauer 2010; Teixeira et al. 2013; Said et al. 2016; Swamy et al. 2016).

Research on artificial pharmaceutical substances reveals the significance of EOs extracted from medicinal and aromatic plants, as their therapeutic properties have numerous applications. Consequently, researchers and farmers have been motivated to expand the cultivation and market of these substances (Swamy and Sinniah 2015, 2016). Presently, about 100 herbs are known for their EOs, while more than 2000 herbs are scattered across 60 families, such as Umbelliferae, Lamiaceae, Lauraceae, Brassica Myrtaceae, etc., could produce medicinally valued EOs. In global markets, only 300 among 3000 known types of EOs are deemed to be of commercial importance. EOs have found application in agricultural sectors and can be potentially used in other industries, such as pharmaceuticals, drugs, food, perfumes, makeup products, sanitary products, dentistry, food preservatives, additives, cosmetics, and natural remedies (Swamy et al. 2016; Mahmoudi 2017). EOs, such as limonene, patchoulol, geranyl acetate, etc., have been widely used. Moreover, essential oil blends are used in bath products and in aromatherapy. Further, many EOs are particularly valued for their medicinal properties (Swamy and Sinniah 2015, 2016; Arumugam et al. 2016). For example, menthol EOs are used as natural bug repellent, as well as for treating joint pain, respiratory allergies, muscle pain, headache, hair growth, and fever relief, as well as in cancer treatment (menthol protects against cell death and DNA damage).

EOs or natural products are widely used as fragrances. However, their application in human health, agricultural industry, and environmental protection requires a better understanding of their biological properties. Some of the EOs and their chemical constituents are viable as alternatives to synthetic compounds, presently widely used in the chemical industry. This is because EOs are not associated with harmful side effects (Carson and Riley 2003). In nature, EOs play an important role in providing plant protection against pathogenic bacteria, viruses, and fungi and preventing the attack by insect pests. In addition, EOs can attract or repel insects when present in pollen and seeds. To protect chemical compounds' ecological equilibrium, the use of EOs in pharmaceutical, food, bactericidal, and fungicidal is becoming more prevalent in recent times. EOs yielding medicinal and aromatic plants are normally native to warm countries, where they represent an important traditional pharmacopeia (Arumugam et al. 2016). EOs are less dense than water. They are volatile and mostly colorless, as well as soluble in organic solvents. All plant parts, such as buds, leaves, fruits, bark, root, stems, twigs, and flowers, can contain EOs.

1.2.1 Biological effects of essential oils

At present, around 60 plant families are known to produce EOs, which are valued in medicinal, pharmaceutical, flavor and fragrance, and agricultural industries. Several plant species belonging to the Apiaceae, Alliaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae, and Rutaceae family produce EOs with medicinal and industrial values (Vigan 2010; Hammer and Carson

2011). EOs are rich in terpenes, while phenylpropanoids more frequently occur in Apiaceae, Alliaceae, Lamiaceae, Myrtaceae, and Rutaceae plant families (Chami et al. 2004). These family plants are used for the commercial level manufacture of EOs. For example, patchoulol, coriander, anise, dill, and fennel EOs are extracted from *P. cablin, C. sativum, P. anisum, A. graveolens and F. vulgare*, respectively. These EOs are well known for their antimicrobial and anticancer activities. The plants belonging to the Lamiaceae and Apiaceae family are popular for antimicrobial, anticancer, antibacterial, antimutagenic, anti-inflammatory, and antioxidant activities (Swamy and Sinniah 2015; Swamy et al. 2016). Some of the plants from Lamiaceae family produce EOs (Burt 2004; Hammer et al. 2006; Hussain et al. 2008), such as *M. piperita, R. officinalis, O. basilicum, S. officinalis, M. officinalis, S. hortensis, T. vulgaris, L. angustifolia, and O. vulgore* (Swamy and Sinniah 2015; Swamy et al. 2016). Likewise, EOs from Lauraceae and Myrtaceae families also exhibit antimicrobial, antitumor, anticancer, antibacterial, and antiviral activities (Burt 2004; Hammer et al. 2006). *Cinnamomum verum* (Lauraceae) and *Syzygium aromaticum* (Myrtaceae) EOs are particularly rich in eugenol. Many EOs have been screened for their pharmacological potential.

1.2.1.1 Essential oils as antibacterial agents

Many essential oils have been investigated for their antibacterial and antifungal activities, as well as their potential against Gram-positive and Gram-negative bacteria (Swamy et al. 2016). EOs show good antibacterial properties against Salmonella, Staphylococcus, and

other bacterial pathogens. Thus, it is essential to study their effects as very good alternatives to antibiotics (Fujita et al. 2015; Karbach et al. 2015; Sienkiewicz et al. 2015). *O. basilicum* essential oil exhibits good antibacterial properties against Gram-positive bacteria (Al Abbasy et al. 2015; Avetisyan et al. 2017). In the investigations of antibacterial effects, manuka oil has been shown to exhibit good antibacterial activity. Similarly, eucalyptus, rosmarinus, Lavandula oil, and tree oil were found effective against *Streptococcus mutans, S. sobrinus, Fusobacterium nucleatum,* and *Porphyromonas gingivalis* (Takarada et al. 2004). Tea tree (*Melaleuca alternifolia*) oil is demonstrated to be sensitive to 15 genera of oral bacteria, indicating its potential applications in oral hygiene (Hammer et al. 2003). *Pittosporum undulatum* and *Hedychium gardnerianum* EOs show the highest antibacterial activities against *Staphylococcus epidermis* and *S. aureus.*

Despite the discovery of new antibiotics, bacterial infectious/diseases still pose a serious threat to human health, predominantly due to the appearance of antibiotic- resistant strains. In addition, as the global people continues to expand, this will result in a greater prevalence of bacterial diseases, low immunity, and increased drug resistance. Therefore, bacterial infections will be more likely to be fatal (Ahmad and Beg 2001; Hall-Stoodley et al. 2004; Swamy et al. 2016; Rudramurthy et al. 2016). To decrease the risk of infectious diseases, high concentrations of antibacterial drugs are usually employed, resulting in toxicity and adverse side effects. Hence, there is a need to explore alternative approaches and

develop new molecules against human pathogenic bacteria (Rudramurthy et al. 2016). In this context, plant EOs exhibit a good potential due to their proven activity against both Gram-positive and Gram-negative bacteria as shown in Table 9.3 (Edris 2007; Lang and Buchbauer 2012; Hassanshahian et al. 2014; Teixeira et al. 2013). Some EOs show potential antibacterial activity against Gram- positive bacteria only, such as Santalum album, Leptospermum scoparium, and Chrysopogon zizanioides (Hammer and Carson 2011).

According to the available evidence, cinnamon, lemongrass, thyme, clove, rosewood, orange, rosemary, peppermint, bay, basil, and eucalyptus EOs exhibit the most effective antimicrobial activity. EOs are very active at <1% minimum inhibition concentrations (MICs). *Escherichia coli* exhibits zone of inhibition at 0.02, 0.04, and 0.06% concentrations against clove, grass, oregano, bay, and thyme EOs, respectively (Hammer and Carson 2011). Some EOs show less activity, but their major constituent molecules are observed to possess higher activity. For example, eugenol, carvacrol, and 4-terpinenol display greater antibacterial activity than their corresponding EOs. In extant literature, phenols and aldehydes are reported as potential antimicrobial activity (Lambert et al. 2001; Ultee et al. 2002; Carson et al. 2006). A large number of the EOs have been shown to be successful against drug-resistant strains, antibiotics, and biofilms (May et al. 2000; Bozin et al. 2006). EOs of *A. fragrantissima, A. ligustica, A. absinthium, A. biennis, A. cana, A. dracunculus, A. longifolia, A. frigida, C. officinalis, C.*

sativum, C. cyminum, C. longus, D. littoralis, E. erythropapps, E. rostkoviana, F. margarita, L. nobilis, L. angustifolia, L. longifolia, J. excelsa, M. suaveolens, N. sativa, O. vulgare, T. vulgaris, O. basilicum, P. cablin, T. kotschyanus, S. cumini, T. ammi, and *S. sparganophora* show potential antibacterial activity against *S. aureus, S. epidermidis, E. coli, S. mutans, B. thermosphacta, L. innocua, L. monocytogenes, P. putida, B. cereus, B. subtilis, N. gonorrhoeae, K. pneumoniae, C. botulinum, C. perfringens, S. sonnei, S. lutea, P. putida, M. flavus, L. innocua, E. faecalis,* and *S. putrefaciens* (Lopes-Lutz and Alviano 2008; Maggi et al. 2009; Matasyoh et al. 2009; Begnami et al. 2010; Runyoro et al. 2010; Ait-Ouazzou et al. 2012; Bejaoui et al. 2013; Teixeira et al. 2013; Yang et al. 2013; Amatiste et al. 2014; Andrade et al. 2014; Bilcu et al. 2014; Bisht et al. 2014; Flores *et al*. 2014; Kasim et al. 2014; Khoury et al. 2014; Petretto *et al*. 2014; Pullagummi et al. 2014; Santurio et al. 2014; Singh et al. 2014;; yousefbeyk et al. 2014; Zeedan et al. 2014; Ahmadi et al. 2015; Beatovia *et al.* 2015; Santos et al. 2015; Ibrahim *et al*. 2015a, b; Novy et al. 2015).

1.2.1.2 Essential oils as antioxidant agents

Modern era has brought about different health problems, such as noncommunicable diseases (e.g., cancer, diabetes, and Alzheimer's, Parkinson's, and heart diseases) which are attributed to oxidative stresses. EOs exhibit a significant antioxidant activity due to their phytocompounds, such as flavonoids, terpenoids, and phenolic compounds (Edris 2007; Miguel 2010; Sanchez-Vioque et al. 2013; Bouzabata et al. 2015). Among many EOs, *O. majorana, T. filifolia,*

B. monnieri, C. longa, S. cryptantha, A. millefolium, S. multicaulis, M. officinalis, M. alternifolia, Ocimum, and *Mentha* sp. have been reported to possess significant antioxidant activity (Tepe et al. 2004; Maheshwari et al. 2006;Hussain et al. 2008). Thymol and carvacrol containing EOs in particular show strong antioxidant properties (Tepe et al. 2004). Likewise, EOs of *Cuminum cyminum, Petroselinum sativum, S. cumini, and Coriandrum sativum* also exhibit efficient antioxidant (Romeilah et al. 2010; Eshwarappa et al. 2014). In addition, clove oil shows a much stronger antioxidant and radical scavenging activity compared to cinnamon, basil, oregano, nutmeg, and thyme EOs (Tomaino et al. 2005).

1.2.2 Extraction of essential oils

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process (Bruneton, 1995). The oil droplets being stored in the oil glands or sacs can be removed by either accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are:

1.2.2.1 Hydrodistillation

The technique involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger

set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapour and oil are removed through a water cool condenser.

1.2.2.2 Hydrodiffusion

Hydrodiffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma of the plants (Buchbauer, 2000).

1.2.2.3 Maceration

An extraction process that consists of maintaining contact between the plant and a liquid (solvent) for a period. Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.) inside an airtight container, for a variable time based on the plant material and liquid used.

Before being processed, the plant must be properly washed and separated from foreign material such as topsoil, pebbles or rocks, weeds, and materials non-suitable for extraction. The plant material can be used fresh or dry based on the desired product.In order to increase contact between the plant material being extracted and the liquid (solvent), the plant needs to be cut into small pieces.The pieces should not be too big; otherwise, the solvent will not be able to penetrate the innermost cells. They also should not be reduced to powder; that would result in losing the volatile active ingredients

(essential oils) contained inside the plant, and losing the difficult separation by filtration of the plant material from the liquid used once maceration is completed.

The solvent must be chosen based upon the chemical nature of the compounds contained within the plant. Solubility and the desired use of the extraction should be considered when choosing the solvent. That is, recognizing their solubility and the desired use of the extraction. Generally, alcohol is the most used substance because it is able to extract a greater part of the molecules (active ingredients) contained within the plant, including molecules which are hydrophilic, soluble in water, or lipophilic and therefore, soluble in oil or other organic solvents.

1.2.2.4 Enfleurage

This process is applicable to flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odourless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called Enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind only the essential oil, hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

1.2.2.5 Cold pressing

Another method of extracting essential oil that has not found high application in scientific research is cold pressing. It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

1.2.2.6 Steam distillation

This is the most common method of extracting oils and is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel. Next steam is added and passed through the plant that contains the plants aromatic molecules or oils. Once upon, the plant releases these aromatic molecules and in the state, the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapours. As they cool, they condense and transform into a liquid state.

1.2.2.7 Solvent extraction

This method involves the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used for extraction. For instance, leaves, roots, fruits are extracted with benzene with or without mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and colour. After the extraction, the solvent is removed by

distillation under reduced pressure leaving behind the semisolid concentrate, this concentrate are extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil.

1.2.2.8 Microwave assisted process (MAP)

The MAP process uses microwave to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants (Belanger et al., 1995). This technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction . This technique has also been applied for the extraction of saponins from some medicinal plants (Safir et al., 1998).

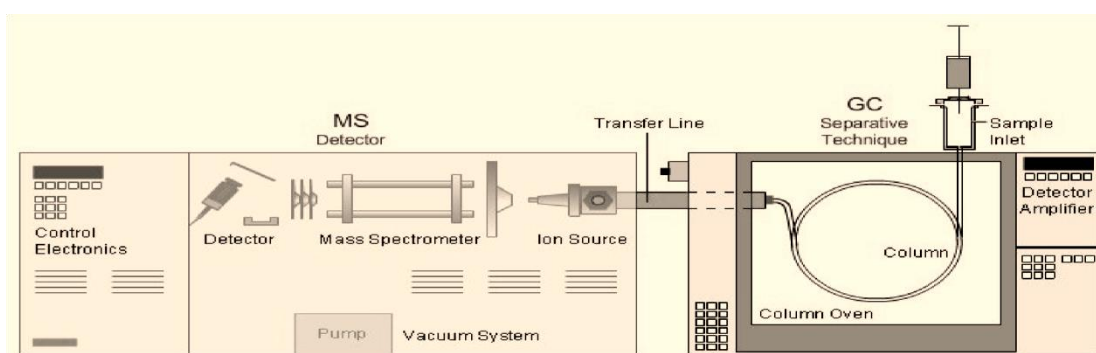
1.2.2.9 Carbondioxide extraction

In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition

1.3 Gas chromatography

Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary

phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. The detector for the GC is the mass spectrometer (MS).



GC-MS instrumentation

GC/MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS).It is used to analyze complex matrices (Skoog et al., 2007). Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the organic and biochemical mixtures (Skoog et al., 2007). The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure.

In the injector the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column

1.3.1 Instrumentation and working of GC-MS

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter (GC columns from Theory and Instrumentation of GC).

Mass analyzer: There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis**.**

Vacuum system: Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way.

Detector: The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons,

which are further amplified, or by inducing a current (generated by moving charges).

Control Electronics: The MS parameters can be selected and controlled from this panel. Modern instruments will also allow to control MS parameters from a computer by using specially designed software. The mobile-phase called as carrier gas, must be chemically inert. The helium gas is most commonly used, however, argon, nitrogen, and hydrogen are also used. These gases are held in pressurized tanks and use pressure regulators, gauges, and flow meters to control the flow rate of the gas. Flow rates usually range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular

capillary columns, and are assumed to be constant if inlet pressure is constant. This is often accompanied by a molecular sieve to purify the gas before it is used.

Samples are introduced as a plug of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port, which should be about 50°C above the boiling point of the least volatile constituent of the sample. After the sample is introduced, it is carried to the column by the mobile phase. The temperature of the column is an important variable, so the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As the mobile phase carrying the

sample is passed through the stationary phase in the column, the different components of the sample are separated.

After being separated, the sample is run through a detector (Skoog et al., 2007), which ionizes the sample and then separates the ions based on their massto-charge ratio. This data is then sent to a computer to be displayed and analyzed. The computer linked to the GCMS has a library of samples to help in analyzing this data . Data for the GC-MS is displayed in several ways. One is a total-ion chromatogram, which sums the total ion abundances in each spectrum and plots them as a function of time. Another is the mass spectrum at a particular time in the chromatogram to identify the particular component that was eluted at that time. A mass spectra of selected ions with a specific mass to charge ratio, called a mass chromatogram, can also be used.

GC-MS is widely used for research purposes in many fields and is emerging as an important technique in food science as well.

1.4 *Brassica hirta*

Binomial name is *Sinapis alba* L. and some Synonyms as *Brassica alba* (L.) Rabenh. *Brassica hirta* Moench.

Sinapis alba L. (white or yellow mustard, also known as *Brassica hirta*) is an annual plant of the family Brassicaceae that originates from the Mediterranean. It is found worldwide as a cultivated plant species as well as a weed. It is a winter–spring plant that can be grown in short cycles, commonly in rotation with other cereal crops, with the possibility of second-crop cultures . In Europe, white

mustard is the most used mustard species and in North America, it is the only species in commercial production for the food processing and condiment industries .

White mustard has many cropping applications, including edible oil seeds , fast-growing salads , condiments, fodder, and green manure . The plant can extract toxic heavy metals from soil . Young seedling leaves, which are rich in vitamin A, C, and E, are edible as fresh and tasty salad leaves and have a medicinal value to purify blood . White mustard seed has significant agronomic value due to its high protein and oil contents and low starch content . Its well-balanced amino acid profile makes the seed an attractive source of food-grade proteins. It is widely used as a binding agent and protein extender in meat processing and for hot dog mustard, mayonnaise, and salad dressings. The seeds have strong disinfectant properties and can be used as a food preservative . Its essential oil can be used to preserve foods due to its potent antimicrobial activity . Industrially, white mustard seed oil is used as a lubricant and for lighting . Moreover, the seed is used in traditional medicine for its anti-tumor, antiviral, and analgesic activities it also has expectorant, stimulant, and antimicrobial activities that are useful for digestive and respiratory ailments.

Recently, white mustard seed oil has garnered interest for its use as a feedstock for biodiesel production (Ahmad et al., 2008). The oil itself can be used as an alternative fuel .

Aim of this research

This study was carried out to:

-Extract the oil from the medicinally important plant species *Brassica hirta* .

-Identify the constituents of the oil by GC-MS analysis.

-Evaluate the oil for its antimicrobial potency.

2-Materials and Methods

2.1-Materials

2.1.1--Plant material

Seeds of *Brassica hirta* were purchased from the local market-Khartoum-Sudan. The plant material was authenticated by direct comparison with a reference herbarium sample.

2.1.2-GC-MS analysis

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

2.1.3-Test organisms

The studied oils were screened for antimicrobial activity using the standard microorganisms shown in Table(2.1).

Table 2.1: Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

2.2-Methods

2.2.1-Extraction of oil

Powdered seeds (300g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil.

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

2.2.2-GC/MS analysis

The studied oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas. Chromatographic conditions are presented below:

- *Oven temperature program*

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

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

Column oven temperature	150.0°C
Injection temperature	300.0°C
Rate	4/min
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

2.2.3-Antimicrobial assay

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

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

3-Results and discussion

3.1. *Brassica hirta*

In this study *Brassica hirta* seed oil was investigated by GC-MS analysis.The oil was also evaluated for its antimicrobial potency.

3.1.1.GC-MS analysis of *Brassica hirta* oil

Brassica hirta oil was analyzed by GC-MS. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the GC-MS library.

Twenty nine components have been detected by GC-MS analysis (Table 3.1).The typical total ion chromatograms (TIC) is presented in (Fig:3. 1).

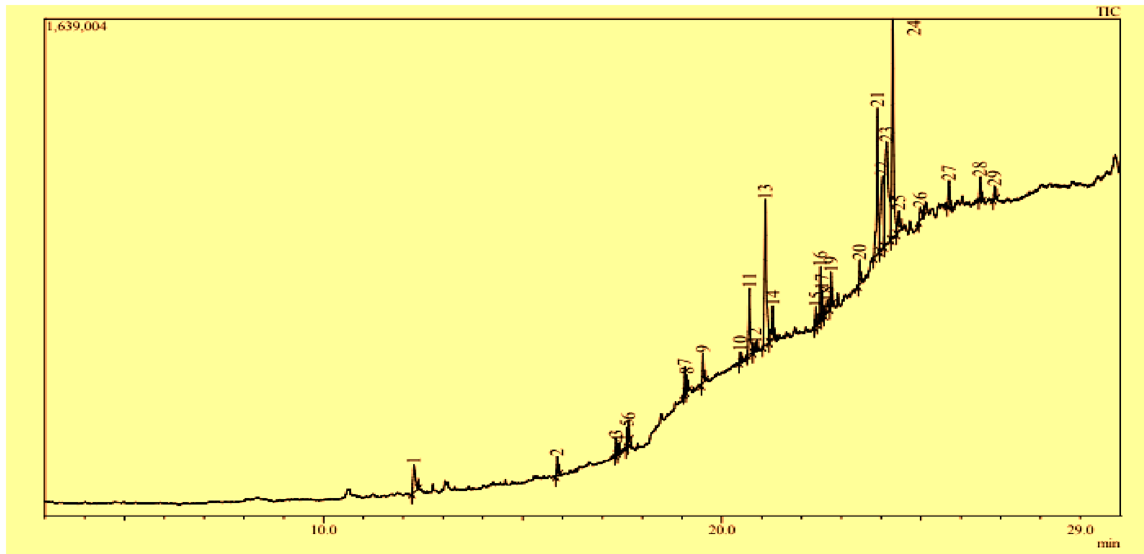


Fig.3.1: Total ions chromatograms

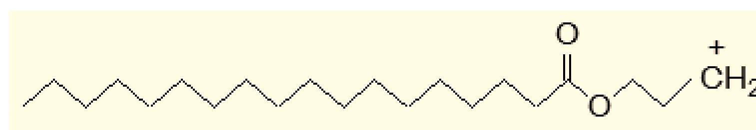
Table 3.1: Constituents of *Brassica hirta* oil

Peak#	Name	R.Time	Area	Area%
1	Diethyl Phthalate	12.271	385650	2.91
2	Hexadecanoic acid, methyl ester	15.869	126223	0.95
3	1-Nonadecene	17.339	99131	0.75
4	Octadecanoic acid, 2-propenyl ester	17.401	57613	0.43
5	Methyl 10-trans,12-cis-octadecadienoate	17.618	117811	0.89
6	9-Octadecenoic acid (Z)-, methyl ester	17.662	111307	0.84
7	Pentadec-7-ene, 7-bromomethyl-	19.073	172349	1.30
8	Palmitoyl chloride	19.117	111441	0.84
9	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-et	19.526	215818	1.63
10	13-Tetradecenal	20.464	77602	0.58
11	Oleoyl chloride	20.693	615349	4.64
12	Pentadec-7-ene, 7-bromomethyl-	20.830	110719	0.83
13	9-Octadecenoic acid, 1,2,3-propanetriyl ester	21.093	1556567	11.73
14	Octadecanoic acid, 2-hydroxy-1,3-propanediy	21.278	369578	2.79
15	cis-9-Hexadecenal	22.357	117670	0.89
16	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpro	22.477	350448	2.64
17	Heptanoic acid, docosyl ester	22.520	204897	1.54
18	Oleic acid, trimethylsilyl ester	22.635	123454	0.93
19	9-Octadecenoic acid, 1,2,3-propanetriyl ester	22.743	215893	1.63
20	18.alpha.-Olean-3.beta.-ol, acetate	23.458	174701	1.32
21	cis-13-Docosenoyl chloride	23.904	1398294	10.54
22	9-Octadecenoic acid (Z)-, 3-[(1-oxooctadecyl	24.035	1336512	10.07
23	9-Octadecenoic acid (Z)-, 3-[(1-oxooctadecyl	24.141	2230147	16.81
24	9-Octadecenoic acid, 1,2,3-propanetriyl ester	24.289	2049681	15.45
25	Octadecanoic acid, 2-hydroxy-1,3-propanediy	24.442	254671	1.92
26	9-Octadecenoic acid (Z)-, 3-[(1-oxooctadecyl	24.975	135733	1.02
27	6-Ethyl-3-trimethylsilyloxydecane	25.707	239623	1.81
28	Stigmastan-3,5-diene	26.488	198952	1.50
29	Olean-12-ene-3,28-diol, (3.beta.)-	26.852	108679	0.82
			13266513	100.00

The mass spectra of major constituents of the oil are briefly discussed below:

i) 9-Octadecenoic acid (Z)-,3-[(1-oxooctadacyl) oxy]propyl ester-(16.81)%

The mass spectrum of 9-octadecenoic acid (Z)-,3-[(1-oxooctadacy)oxy]propyl ester is shown in Fig.3.2. This constituent appeared at retention time 24.141. The signal at m/z325 is due to loss of the following group:



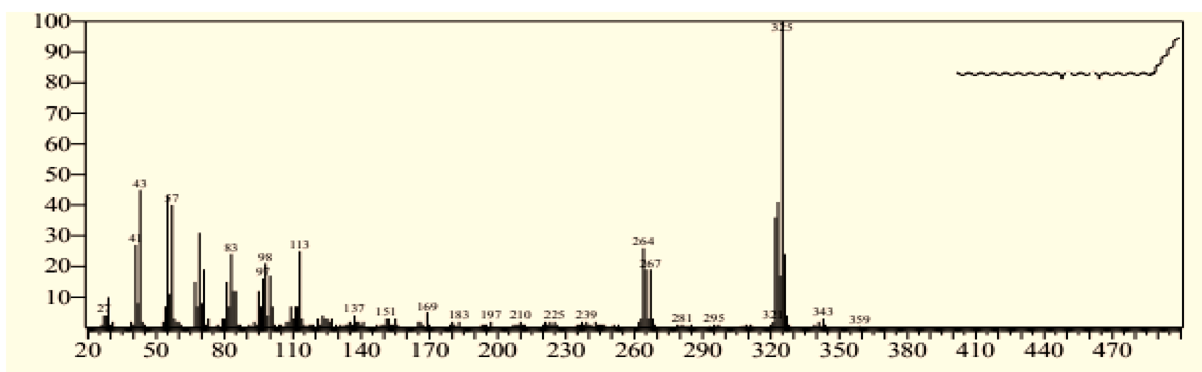
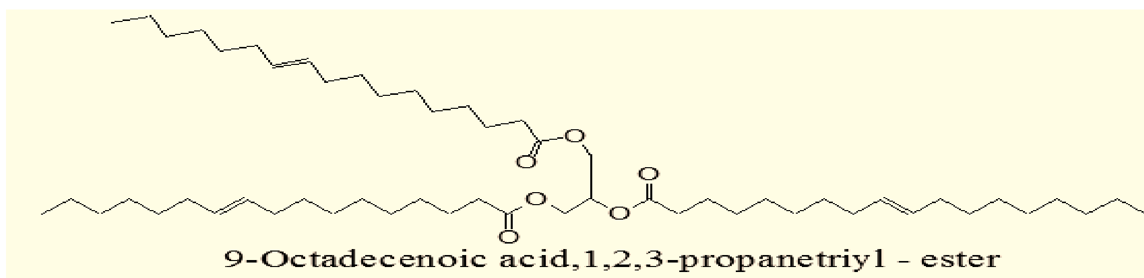


Fig. 3.2: Mass spectrum of 9-octadecenoic acid (Z)-,3-[(1-oxooctadacyl) oxy]propyl ester

ii)- 9-Octadecenoic acid,1,2,3-propanetriyl -ester(15.45)%

Fig.3.3 presents the mass spectrum of 9-Octadecenoic acid,1,2,3-propanetriyl **-** ester. The peak at m/z884 with retention time 24.289 is due to the molecular ion M^+ $[C_{57}H_{104}O_6]^+$.



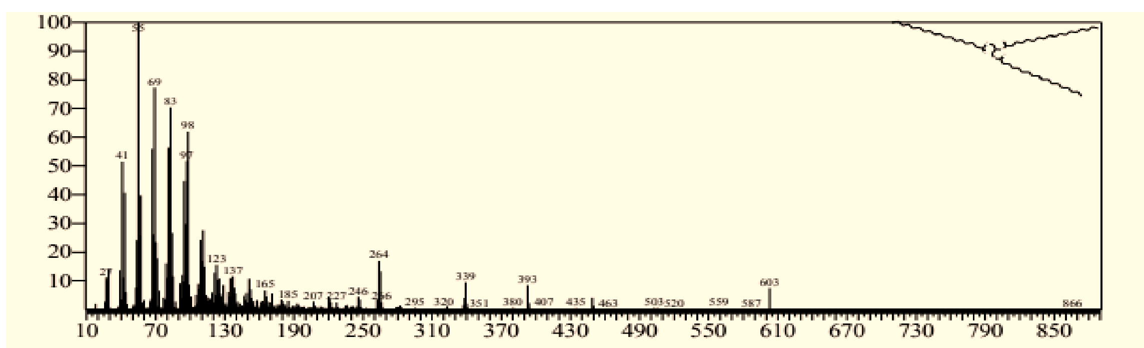
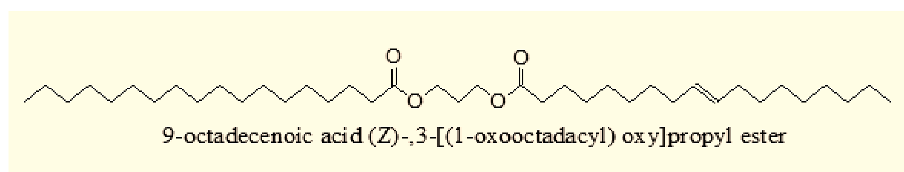


Fig. 3.3: Mass spectrum of 9-octadecenoic acid,1,2,3-propanetriyl - ester

iii- 9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-(11.73)%



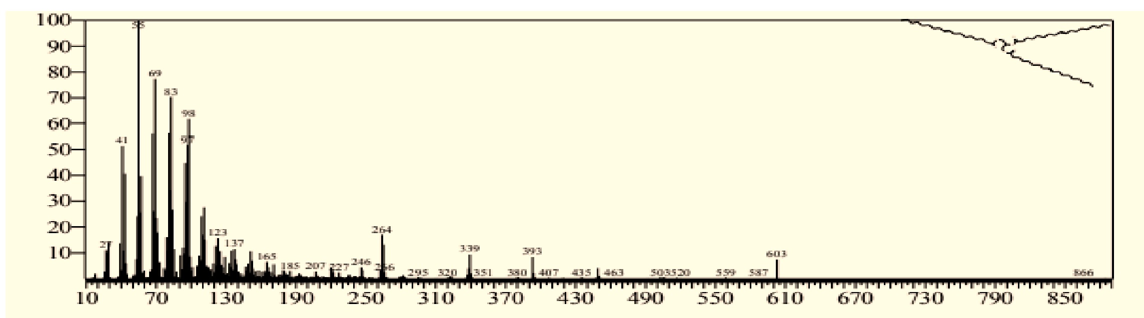
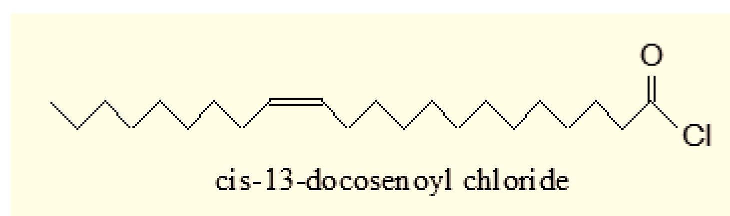


Fig. 3.4: Mass spectrum of 9-octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-

Fig. 3.4 shows the mass spectrum of 9-octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)- . The signal at m/z 884 (retention time:21.093) is due to the molecular ion M^+ $[C_{57}H_{104}O_6]^+$.

iv- cis-13-Docosenoyl chloride (10.54 %)

The mass spectrum of cis-13-docosenoyl chloride is shown in Fig.3.5. The peak at m/z 356 with retention time 23.904 accounts for the molecular ion M^+ $[C_{22}H_{41}ClO]^+$.



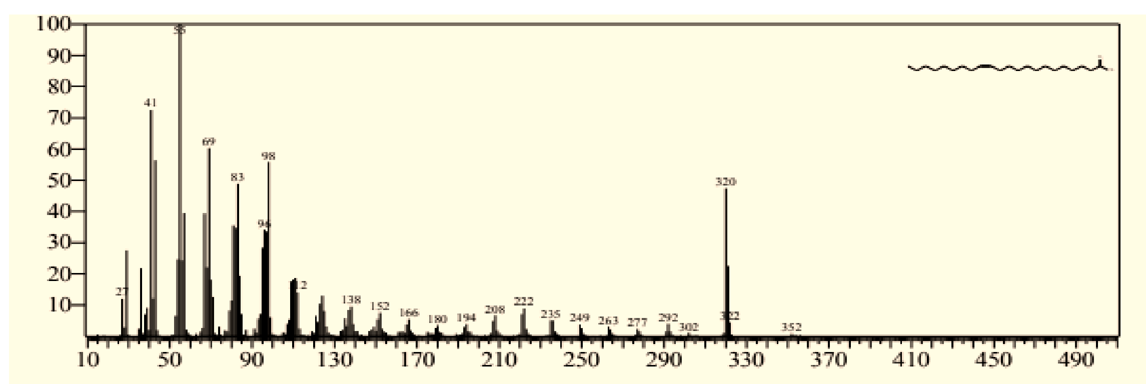


Fig. 3.5:Mass spectrm of cis-13-docosenoyl chloride

3.1.2-Antimicrobial activity

Brassica hirta seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table(3.2).The results were interpreted in the following manner: (<9mm: inactive;9-12mm:partially active; 13-18mm: active; > 18mm:very active) .The oil showed weak anticandidal activity.

Table 3.2: Antimicrobial Activity of the *Brassica hirta* oil

Oil	Antibacterial activity				
	Gram positive		Gram negative		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	--	7	8	7.6	11.3

Conclusion

Brassica hirta oil was analyzed by GC-MS. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the GC-MS library. Twenty nine components have been detected by GC-MS analysis. The oil was assessed for its antimicrobial potential via the cup plate agar diffusion bioassay. *Brassica hirta* oil exhibited weak anticandidal activity.

Recommendations

The following is highly recommended:

-The biologically active constituents of the oil may be isolated by HPLC and evaluated for their biological potential.

-The secondary metabolites of *Brassica hirta* may be isolated and characterized by spectral tools and evaluated for their biological activity.

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