Sudan University of Science and Technology

College of Graduate Studies

Characterization of Oils from some Sudanese Medicinal Plants and their Antimicrobial Activity

توصيف الزيوت من بعض النباتات الطبية السودانية وفعاليتها المضادة للميكروبات

A Thesis Submitted in Fulfillment of the Requirements of the Ph.D. Degree in Chemistry

By

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إستهلال

قَالَ تَعَالَىٰ:

﴿ وَقُلِ ٱعۡمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمۡ وَرَسُولُهُۥ وَٱلۡمُؤۡمِنُونَۖ وَسَتُرَدُّونَ إِلَىٰ عَٰلِمِ ٱلۡغَيۡبِ وَٱلشَّهَٰدَةِ فَيُنَبِّئُكُم بِمَا كُنتُمۡ تَعۡمَلُونَ ﴾

صدق الله العظيم

سورة التوبة ١٠٥

DEDICATION

TO ,

MY Parents

MY Wife

MY Children

Brothers and sisters

Acknowledgement

First of all I would like thank to almighty Allah for giving me the will and strength, to finish this work.

I would like to express my deepest gratitude to my supervisor Prof . Mohammed Abdel Karim for his suggestion ,guidance, encouragement and useful criticism throughout the course of the study .

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Abstract

In this study, six Sudanese plant species(*Eucalyptus camaldulensis*, *Kegalia Africana*, *Beta vulgaris*, *Tephrosia apollina*, *Dichrostachys cinera and Annona squamosa*) have been investigated. These plant species are of medicinal attributes and potentials. They have been studies by GC-MS. The target plants have also been assessed for their antimicrobial activity. The GC-MS analysis of *Beta vulgaris* oil showed the presence of 35 components.. Fatty acids constituted major bulk of the oil(99.67%). Terpenes(0.03%) and hydrocarbons(0.03%) appeared as minor constituents. The studied oil showed significant activity against *Bacillus subtilis* and moderate anticandidal activity. *Tephrosia apollina* oil was analyzed by GC-MS . The analysis showed 22 components. Fatty acids constituted 97.96% , the rest is ϐ-sitosterol (2.04%).The studied oil showed significant activity against *Bacillus subtilis* , *Staphylococcus aureus* and *Escherichia coli.* GC-MS analysis of *Eucalyptus camaldulensis* volatile oil was conducted. Thirty seven constituents were identified by GC-MS.Major constituent was : 9,12-octadecadienoic acid methyl ester (54.74%).The oil showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* GC-MS analysis of *Annona squamosa* oil was conducted. Twenty two constituents were identified. The oil showed moderate anticandidal activity. *Dichrostachys cinera* oil was analyzed by GC-MS. Twenty three constituents were identified.. Major components are: 9,12-octadecadienoic acid methyl ester(30.87%) and 9-octadecenoic acid methyl ester(27.02%).The oil showed significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The oil from *Kegalia Africana* was analyzed by GC-MS. Twenty three constituents were identified.Major components are: 9,12,15-octadecatrienoic acid methyl ester(31.32%) and 9,12-octadecadienoic acid methyl ester(29.44%).The oil showed moderate anticandidal activity.

المستخلص

في هذا البحث درست ستة نباتات سودانية هي: المشطور، الثلج ، الكداد، البان، القشطة والعميوقا. أوضحت تحاليل الكروموتوغرافيا الغازية- طيف الكتلة وجود 35 مكونا بزيت الثلج، حيث كونت الزيوت الدهنية الجزء الأكبر من الزيت (99,67 %) بينما كانت نسبة التربين هي (0,03%) وقد وجدت هيدروكربونات بنسبة ضئيلة(0,03%).وفى اختبار مضاد الميكروبات ابدى الزيت فعالية عالية ضد *Bacillus subtilis* وفعالية معتدلة ضد فطر كانديدا. اما العميوقا فقداعطت 22 مكونا وقد كانت نسبة الزيوت الدهنية هي الغالبة (97,96%) بينما ظهر ستوسترول بنسبة متدنية(2,04%). اعطى هذا الزيت في اختبار مضاد الميكروبات فعالية عالية ضد: *Staphylococcus aureus* and *Escherichia col* الزيت الطيار لبذور البان اعطى 37 مكونا أهمها :-9,12 octadecadienoic acid methyl ester (54.74%).وقد ابدى الزيت فعالية معتدلة ضد *Staphylococcus aureus Escherichia coli*. عند تحليل زيت القشطة بكروموتغرافا الغازية – طيف الكتلة اتضح وجود 22 مكونا . وقد اعطى ها الزيت فعالية معتدلة ضد فطر كانديدا. اما زيت الكداد فقد اعطى 23 مكونا أهمها : 9,12-octadecadienoic acid methyl ester(30.87%) and 9-octadecenoic acid methyl ester(27.02%).وفى اختبار مضاد الميكروبات ابدى الزيت فعالية عالية ضد : *Staphylococcus aureus* and *Bacillus subtilis* . اما نبات المشطور فقد اعطى 23 مكونا أهمها : 9,12,15-octadecatrienoic acid methyl ester(31.32%) and 9,12-octadecadienoic acid methyl ester(29.44%)وفى اختبار مضاد الميكروبات ابدى هذا الزبت فعالية معتدلة ضد فطر كانديدا .

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Introduction

1.Introduction

1.1. Natural products

Thousands of years ago natural products have been used for disease prevention and health care. Ancient civilizations of Chinese, North Africans and Indians provide evidence for use of the natural sources in curing various types of diseases. The oldest known document is four thousand years old called Sumerian clay tablet used for various diseases[(1)].

Similarly mandrake was used for relief of pain. Turmeric was used for blood clotting. Gall bladder infections were treated by the roots of endive plants. Raw garlic was used to treat the circulatory disorders [(2)].

These old medicines are still used in many countries as the alternative medicines. Until nineteenth century active components were not isolated from medicinal plants [(3)].

In 1806 Friedrich Sertürner was a scientist who isolated morphine from the *Papaver somniferum.* Then natural products were extensively screened to obtain medicines. Atropine was obtained from the *Atropa belladonna* [(4)].

According to modern search it was revealed by World Health Organization that almost 80% of world's population depends on the traditional medicines. Almost 121 drugs used in USA in these days come from the natural sources. From these 90 drugs come from plant sources indirectly or directly [(5).] .

Almost 47 % of anticancer drugs come from the natural products. Between years from 1981 to 2006 about 100 anticancer agents were developed. From these 25 were the derivatives of natural products, 18 were mimics of natural products and 11 were derived from the natural product called pharmacophore. There were also 9 anticancer agents which were purely natural products. Thus the natural sources are significant source of caring in the health system [(6,7)] .

Plants play a vital role in the management of various diseases and have been heavily utilized in the sustainable development of drugs that provide a major

focus in global health care delivery [8]. Plants have been used for the treatment diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs.[9] Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry[10].

1.2.The target plant species

1.2.1. Annona squamos

The genus name , 'Annona' is from the Latin word 'anon ', meaning 'yearly produce', referring to the production of fruits of the various species in this genus . Annonaceae , the custard apple family trees , shrubs , or rarely lianas are a family of flowering plants consisting of trees , shrubs , or rarely lianas. With about 2300 to 2500 species and more than 130 genera [11]. its type genus is Annona.

Out of varied genus 42 different species were found in *Annona* genus, some of them are:- *Annona glabra* - Pond apple ,*Annona muricata* -Soursop *Annona squamosa* - Sugar apple ,*Annona reticulata* - Custard apple ,*Annona diversitolia* - Lama ,*Annona purpurea* - Soncoya ,*Annona crassiflora* - Marolo ,*Annona manni* - Jungle sop , *Annona montana* - Mountain soursop , *Annona accuminata* - Annona ,*Annona salzmannii* - Beach sugar apple , *Annona cherimola* - Cherimoya custard apple as *Annona squmosa* is widely available[11].

Annona squamosa is a small, well branched tree or shrub [12] from the family Annonaceae that bears edible fruits called sugar-apples or sweetsops. It tolerates a tropical lowland climate better than its relatives *Annona reticulate* and *Annona cherimola*(whose fruits often share the same name helping make it the most widely cultivated of these species[13].*Annona squamosa* is a small,

semi-(or late) deciduous, much branched shrub or small tree 3 meters (9.8 ft) [12] to 8 meters (26 ft) tall very similar to Soursop (*Annona muricata*) [14] .

Annona squamosa

Scientific classification of *Annona squamosa* is presented below:

-Kingdom: Planate

-Order: Magnoliales

-Family: Annonaceae

-Genus: Annona

-Species: *A. squamosa*

Annona squamosa is native to the tropical Americas and West Indies ,but the exact origin is unknown. It is now the most widely cultivated of all the species of *Annona*, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand, and Taiwan; it was introduced to southern Asia before 1590. It is naturalized as far north as southern Florida in the United States and as south as Bahia in Brazil, and is an invasive species in some areas [14] .

In traditional Indian, Thai, and American medicine, the leaves are used in a decoction to treat dysentery and urinary tract infection[15]. In traditional Indian medicine, they are also crushed and applied to wounds [15] . In Mexico, the leaves are rubbed on floors and put in hens' nests to repel lice [13] .leaves

is considered efficacious in prolapsus of children, the crushed leaves are sniffed to overcome hysteria and fainting spells, they are also applied on ulcer and wounds. Roots are employed internally in depression of spirits , spinal diseases and used in dysentery. Bark is known to be a powerful astringent [16] and used in diarrhea. In Ayurveda, fruits are considered as a good tonic; enriches blood, used as expectorant, increases muscular strength; cooling, lessens burning sensation and tendency to biliousness; sedative to heart and relieves vomiting . The seeds are said to be abortifacient and good to destory lice in hair in Yunani medicine. Seed yields oil and resin which acts as detergent and their powder mixed with gram-flour, is a good hair wash. Seeds are powerful irritant of conjunctiva and produce ulcers in the eye [17]. *Annona squamosa* is used as an insecticidal, an antitumor agent, anti-diabetic, antioxidant, anti-lipidimic and anti-inflammatory agent which has been characterized due to the presence of the cyclic peptides[18]. In addition, the crushed leaves are sniffed to overcome the hysteria and fainting spells, and they are also applied on the ulcers and wounds[18]. A leaf decoction is taken in the case of dysentery [18]. Leaves are used as poultice over boils and ulcers and also to kill lice. Leaf infusion is efficacious in prolapsus of children. Bruised leaves with salt make a cataplasm to induce suppuration[18].

Different chemical constituents have been identified from this species including: borneol, camphene, camphor, car-3-ene, carvone, β- caryphyllene, eugenol, farnesol, geraniol, hetriacontanone , hexacontanol , higemamine, isocorydine, limonine from stems, root extracts of *Annona squamosa* Linn. The volatile constituents of *Annona squamosa* Linn bark were identified from the essential oil obtained by the steam distillation and studied by GC/MS [19]. The chloroform extract of the plant contains an active constituent-annotemoyin[20] . Flavonoids isolated from aqueous extract of *Annona squamosa* have shown antimicrobial activity. Bullatacin is one such compounds that possessed antitumoral and pesticidal activity[21] . In another

study two major alkaloids have been isolated. The name of the compounds are liriodenine and oxoanalobine both of the compounds belong to the group of oxoaporphines and were identified by their spectra. The compounds were isolated from the root extract of the plant[22].

1.2.2-Beta vulgare subsp. cila

Vegetables constitute a food source containing carbohydrates, vitamins, minerals and fibers with low fats and protein contents. Leafy vegetables, in particular, are a rich source of beta-carotene, ascorbic acid, minerals and fibers [23] . Fruits and vegetables in the diet have been found in epidemiological studies to be a protective agent against several chronic diseases associated with ageing such as cancer, cardiovascular disease, cataracts, brain and immune dysfunction [24] . These natural protective effects have been attributed to various components, such as carotenoids, vitamin C, E, and phenolic and thiol (SH) compounds [25] . Many studies have focused on the biological activities of phenolics which are potent antioxidants and free radical scavengers [26] .

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [27] .

Swiss chard (*Beta vulgaris* subsp. *cila*, BVc) is a member of the family Chenopodiaceae. This family contains important food crops, such as *Spinacia oleracea* (spinach), which is the most consumed Chenopodiaceae leafy vegetable in Europe. *Beta vulgaris* has been used for food since 1000 B.C. by all populations of the Mediterranean basin. The Romans utilized the leaves for food, while the roots were used for medicinal applications. Seeds, leaves and roots are rich in phenolic compounds, whose concentration is dependent on the stage of plant development [28] . Swiss chard (*Beta vulgaris*) is a herbaceous biennial leafy vegetable cultivated in many parts of the world. It is of low cost and wide use in many traditional dishes [29] . The leaves can be

used in salads or cooked like spinach, and the stems are usually chopped and cooked like celery.The plant is more robust and easier to grow than spinach and celery. The leaves of chard contain nutritionally significant concentrations of vitamins A, C and B, calcium, iron and phosphorus [30] . *Beta vulgaris L.* species are used as a popular folk remedy for liver and kidney diseases, for stimulation of the immune and hematopoietic systems ,and as a special diet in the treatment of cancer [31] . Phytochemical screening of chard have revealed the presence of some fatty acids (palmetic, stearic, oleic, linoleic and linolenic acids), phospholipids ,glycolipids, polysaccharides, ascorbic acid, folic acid, pectin, saponins, flavonoids, phenolic acids, betalains [32] and apigenin [29] . *Beta vulgaris* subsp. *cycla* has been indicated in folk medicine as a hypoglycemic [33] , anti-inflammatory and hemostatic herb [34] . It has been proposed that another cultivar of *Beta vulgaris*, chard contains apigenin flavonoids, namely vitexin, vitexin-2-O-rhamnoside and vitexin-2-O-xyloside, which show antiproliferative activity on cancer cell lines[35] . The root has an anticancer activity. The cancer chemo-preventive potential of the beet root is thought to be due to the betalains[36] which are composed of two main groups: the red betacyanins and the yellow betaxanthins. Both are used as natural additives for food and are powerful radical scavengers ([37]. The betacyanins have been shown to inhibit the proliferation of tumor cells *in vitro*[38,39] .

Beta vulgaris

Scientific classification of *Beta vulgaris* is shown below:

-Kingdom:Plant

- (unranked):Angiosperms

- (unranked):Eudicots

- (unranked): Core eudicots

-Order:Caryophyllales

-Family: Amaranthaceae

-Genus: Beta

- Species: B. vulgaris

Beta vulgaris L. is an herbaceous biennial or, rarely, perennial plant up to 120 cm (rarely 200 cm) height; cultivated forms are mostly biennial. The roots of cultivated forms are dark red, white, or yellow and moderately to strongly swollen and fleshy (subsp. *vulgaris*); or brown, fibrous, sometimes swollen and woody in the wild subspecies. The stems grow erect or, in the wild forms, often procumbent; they are simple or branched in the upper part,[(40)] and their surface is ribbed and striate.[(41)] The basal leaves have a long petiole (which may be thickened and red, white, or yellow in some cultivars). The simple leaf blade is oblanceolate to heart-shaped, dark green to dark red, slightly fleshy, usually with a prominent midrib, with entire or undulate margin, 5–20 cm long on wild plants (often much larger in cultivated plants).

The upper leaves are smaller, their blades are rhombic to narrowly lanceolate.[40]

The flowers are produced in dense spike-like, basally interrupted inflorescences. Very small flowers sit in one- to three- (rarely eight-) flowered glomerules in the axils of short bracts or in the upper half of the inflorescence without bracts.[40] The hermaphrodite flowers are urn-shaped, green or tinged reddish, and consist of five basally connate perianth segments (tepals), 3-5 × 2–3 mm, 5 stamens, and a semi-inferior ovary with 2-3 stigmas.[40] The perianths of neighbouring flowers are often fused.[42] Flowers are wind-pollinated.

In fruit, the glomerules of flowers form connate hard clusters. The fruit (utricle) is enclosed by the leathery and incurved perianth, and is immersed in the swollen, hardened perianth base.[40] The horizontal seed is lenticular, 2–3 mm, with a red-brown, shiny seed coat. The seed contains an annular embryo and copious perisperm (feeding tissue).[41]

The species description of *Beta vulgaris* was made in 1753 by Carl Linnaeus in "Species Plantarum", at the same time creating the genus *Beta*.[43] Linnaeus regarded sea beet, chard and red beet as varieties (at that time, sugar beet and mangel wurzel had not been selected yet). In the second edition of "Species Plantarum" (1762), Linnaeus separated the sea beet as its own species, *Beta maritima*, and left only the cultivated beets in *Beta vulgaris*.[44] Today sea beet and cultivated beets are considered as belonging to the same species, because they may hybridize and form fertile offspring. The taxonomy of the various cultivated races has a long and complicated history, they were treated at the rank of either subspecies, or convarieties or varieties. Now rankless cultivar groups are used, according to the International Code of Nomenclature for Cultivated Plants.

The roots and leaves of the beet have been used in traditional medicine to treat a wide variety of ailments.[45] Ancient Romans used beet root as a

treatment for fevers and constipation, amongst other ailments. Apicius in De re coquinaria gives five recipes for soups to be given as a laxative, three of which feature the root of beet.[46] Platina recommended taking beetroot with garlic to nullify the effects of 'garlic-breath'.[47][

Beet greens and Swiss chard are both considered high oxalate foods which are implicated in the formation of kidney stones.[47]

1.2.3. Dichrostachys cinerea (L.) Wight .Arn.

Dichrostachys cinerea (L.) Wight et Arn (Mimosaceae) known as Sicklebush, Bell mimosa, Chinese lantern tree or Kalahari Christmas tree, is a semi-deciduous to deciduous fast growing tree, typically grows up to 7 meters in height. It is characterized by strong alternate smooth spines (up to 8 cm long), dark grey-brown fractures on old branches and stems and bark on younger branches [48].

D. cinerea is one of the very useful wild medicinal plants used in folk medicine across Africa and Asia[49] . Its bark is used to prepare concoction for treatment of dysentery, headache and elephantiasis. The root infusions are used to treat gonorrhea coughs, syphilis and sore eye and also used as laxative, anthelmintic and strong diuretic. Leaves are good fodder for domesticated animals and seeds are edible[50] . Pharmacological studies on *D. cinerea* have shown antibacterial, antiviral, antilice, antiplasmodial, and antitrypanosomal effects [51,52,53] Phytochemical studies on *D. cinerea* revealed the isolation of various constituents, such as sterols, alkaloids, tannins, triterpenes, polyphenols, phenolic acids, flavonoids [54] and cardiotonic heterosides [55] .

Dichrostachys cinerea. *L*

-Taxonomic name : *Dichrostachys cinerea*. L

-Class : Dicotyledons

-Order : Fabales

-Family : Fabaceae

-Genus : Dichrostachys

-Species : Dichrostachys cinerea.L

Fruit and seeds from D. cinerea are edible. Cattle, camels and game giraffe, buffalo relish the juicy pods that drop to the ground and even eat the young twigs and leaves. Leaves are highly palatable, rich in protein (11-15% crude protein) and mineral content. Young shoots and pods are also browsed by smaller domestic animals. Pods and seeds do not contain hydrocyanic acid, minimizing the chance of poisoning animals. The flowers are a valuable honey source.The wood is dense, burns slowly with few sparks and emits a non-toxic smoke, making it excellent firewood. It often grows many small trunks, ideal in size for carrying in a head load. The bark yields a strong fibre used for various applications such as twine. The debarked roots are used for strong plaiting work such as for racks and baskets. *D. cinerea* yields a medium to heavy, durable hardwood with a density of 600-1190 kg/cubic m at 15% mc. Heartwood red or dark purple with darker streaks, sharply differentiated from the yellowish-brown sapwood; grain straight or slightly interlocked; texture rather fine and even. Due to its generally small

dimensions, its utilization is limited making such items as walking sticks, handles, spears and tool handles. Fencing posts are durable and termite resistant, easily lasting up to 50 years[56,57,58].

The bark is used to treat dysentery, headaches, toothaches, elephantiasis and acts as a vermifuge. Root infusions are taken for leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. Pounded roots and leaves are used to treat epilepsy. The roots are chewed and placed on the sites of snake bites and scorpion stings, and the leaves, which are believed to produce a local anaesthesia, are used for the same purpose and also as a remedy for sore eyes and toothache. Leaves are taken as a diuretic and laxative, and used for gonorrhoea and boils; powder from leaves is used in the massage of fractures. The plant is used as a veterinary medicine in India. [56,57,58]

1.2.4. Eucalyptus camaldulensis

The genus *Eucalyptus* is in the family *Myrtacea*. The species is second to pines in global importance as plantation trees. In the tropics and subtropics it is the most widely planted genus. Globally, *Eucalypts* comprises more than 900 species and unknown hybrids and varieties[59]. The essential oil of *Eucalyptus* is endowed with antiseptic properties established in vitro and unequivocal on many germs [60]

Very few studies have been conducted on the antibacterial and antifungal activities of the essential oil of *E.camaldulensis*. However most of the work done has focused on other species . Traoré et al.[61] tested the antimicrobial activity of essential oils of *Eucalyptus citriodora* and *Eucalyptus houseana* by agar diffusion method. They found that they have some activity against *Staphylococcus aureus* and *Escherichiacoli*; in addition they are active against *Candida albicans. E. camaldulensis* has been historically reported in folklores to be an anaesthetic, antiseptic, astringent folk remedy for cold, colic, cough, diarrhoea, dysentery, haemorrhage, laryngalgiaand laryngitis[62].

Eucalyptus camaldulensis

Scientific classification of *Eucalyptus camaldulensis* is shown below:

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Myrtales

Family: Myrtaceae

Genus: Eucalyptus

Species: *E. camaldulensis*

The oil from *Eucalyptus camaldulensis* was used traditionally for the treatment of cystitis, diabetes, gastritis, kidney disease, laryngitis, leukorrhoea, malaria, pimples, ringworm, wounds, ulcers of the skin, urethritis and vaginitis. It was also used as an expectorant for symptomatic treatment of mild inflammation of the respiratory tract, bronchitis, asthma, and inflammation of the throat[(63)] . In south Europe, oil was used for fever, neuralgic pain, asthma, lung tuberculosis and as an antiseptic agent[(64)] . It is used externally for wounds, acne, poorly healed ulcers, stomatitis, bleeding gums, rheumatism and neuralgia [(65)] . However, There were three broad categories of uses for Eucalyptus oil (medicinal, industrial and perfumery/flavouring) [(66)]

1.2.5. Kigelia africana (Lam.)

Kigelia africana belongs to the family Bignoniaceae. It is a multipurpose tree species with many medicinal attributes and potentials. Its common names include sausage tree (Eng); worsboom (Afr);[67] pandoro (West Nigeria): acase study of Enugu state. Educ. Res. Rev. 1(1):16-22). It is a tree that grows up to 20m tall or more [68] . The tree is evergreen where rainfall occurs throughout the year, but deciduous where there is a long dry season. The leaves are opposite or in whorls of three, 30 – 50 cm long, pinnate, with six to ten oval leaflets up to 20 cm long and 6 cm broad; the terminal leaflet can be either present or absent. The flowers (and later the fruit) hang down from branches on long flexible stems (2-6 m long). Flowers are bisexual and produced in panicles; they are bell shaped (similar to those of the African tulip tree but darker and more waxy), orange to reddish or purplish green and about 10 cm wide. Individual flowers do not hang down but are oriented horizontally [69] and some birds are attracted to these flowers. Their scent is most notable at night indicating their reliance on pollination by bats, which visit them for pollen and nectar [70] .

K. africana is abundant in the tropics and is widely used traditionally in Southern Nigeria as a herbal remedy for various ailments.The plant is traditionally used as anticancer, antiulcer, anti-ageing, antioxidant, and anti malarial. It is also widely applied in the treatment of genital infections, gynecological disorders, renal ailments, fainting, epilepsy, sickle-cell anemia, eczema, central nervous system depression, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation, tumours etc., especially in developing nations where orthodox medicine are expensive or inaccessible [71,72] .

K. africana

Scientific classification of *K. Africana* is shown below:

Kingdom : Plantae – plantes, Planta, Vegetal, plants

Subkingdom : Viridiplantae – green plants

Infrakingdom : Streptophyta – land plants

Superdivision : Embryophyta

Division: Tracheophyta – vascular plants, tracheophytes

Subdivision : Spermatophytina – spermatophytes, seed plants, phanérogames

Class : Magnoliopsida

Superorder: Asteranae

Order: Lamiales

Family: Bignoniaceae – bignonias

Genus : Kigelia DC. – sausage tree

Species: Kigelia africana (Lam.) Benth. – sausage tree

The kigelia plant have medicinal properties not only because of its perceived characteristics such as bitterness, astringent taste or smell,but because it has a long history of use by rural and African countries particularly for medicinal properties. Several part of the plant are employed for medicinal purposes by certain people. In Malwi during famine the seeds are roasted to eat. Baked fruits are used to ferment beer and when boiled yield a red dye.It is also used in cosmetics preparation by Tonga women of Zambezi valley. In the folk

medicine, the fruits are used as dressing for ulcers, purgative and to increase the flow of milk in lactating women. Roots are said to yield a bright yellow dye. The barks is traditionally used for syphilis and gonorrhea. Unripe fruit is used in central Africa as a dressing for wounds, haemorrhoids and rheumatism [73] .

1.2.6.Tephrosia apollina (Delile.) link

The genus *Tephrosia* PERS. (Fabaceae, Papilionoideae) comprises between 300 to 400 species of annual and perennial woody herbs, distributed in tropical and subtropical regions of the world [74,75]. The plant is known for its medicinal properties and has significant anti-bacterial properties; the leaves and the root have been used to treat bronchitis, cough, earache, wounds and bone fractures by herbalists in countries like Oman[76]. The ground leaves of *Tephrosia apollinea* are also inhaled to reduce nasal congestion, or boiled with water to make eardrops.[77]. Powdered bark can be mixed with water and poured into the ears of camels to alleviate ticks, and powdered leaves can be used as a paste in the treating of wounds [78] It can also be rubbed on limbs in conjunction with *Fagonia indica* and *Ocomim basilicum* to treat people affected with polio [79]. Although unpalatable when consumed raw, when boiled the leaves of *Tephrosia apollinea* and numerous other plants are used to make hot drinks by the Bedouin in parts of Sinai and the Negev. [80]. But herbal doctors in Oman warn that *Tephrosia apollinea* can be potentially harmful to humans. Since 1993 it has not been fully analyzed chemically to assess the wider impact it could have on health [81].

Tephrosia apollinea

Scientific classification

Kingdom:Plantae

(unranked):Angiosperms

(unranked):Eudicots

(unranked):Rosids

Order:Fabales

Family:Fabaceae

Genus:Tephrosia

Tephrosia apollinea can be used to make indigo dyes [82] . The species was noted to be commonly cultivated for this purpose in Nubia in the 1800s.

1.3. Gas chromatography-Mass spectrometry (GC-MS)

The hyphenated technique - GC-MS- is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances constituting a sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples, including that of material samples obtained from planet Mars during probe missions as early as the 1970s. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in

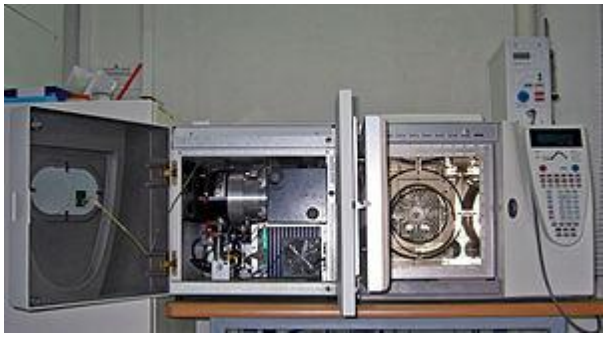
materials that were previously thought to have disintegrated beyond identification. Like liquid chromatography–mass spectrometry, it allows analysis and detection even of tiny amounts of a substance[83].

GC-MS can perform a 100% specific test which positively identifies the presence of a particular substance, hence this technique has been widely used for forensic substance identification. A nonspecific test merely indicates that any of several in a category of substances is present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification[83].

As detector, the use of a mass spectrometer has been developed during the 1950s after being originated by James and Martin in 1952. These comparatively sensitive devices were originally limited to laboratory settings.[1]The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1964, Electronic Associates, Inc. (EAI), a leading U.S. supplier of analog computers, began development of a computer controlled quadrupole mass spectrometer [84-88].

In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. By the 2000s computerized GC/MS instruments using quadruple technology had become both essential to chemical research and one of the foremost instruments used for organic analysis.

Nowadays, computerized GC-MS machines are widely used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine[89].



GC-MS instrument - column of the gas chromatograph in the oven on the right

As far as instrumentation is concerned, the GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio[90].

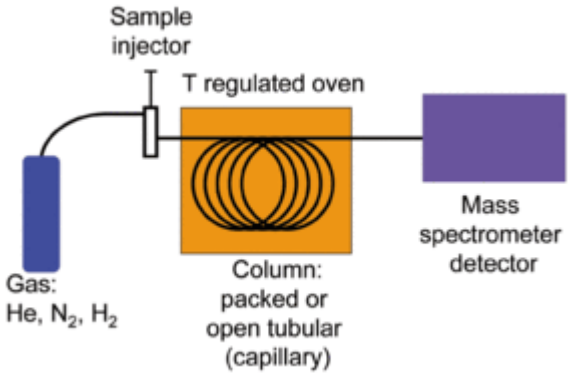


Figure2: Schematic of GC-MS

Gas chromatography and mass spectrometry, used together, allow a much finer degree of substance identification than either unit used separately.

An accurate identification of a particular molecule by gas chromatography or mass spectrometry alone is not possible. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample [90].

1.3.1. Different types of detectors

Common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupled mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD).

Another detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadruples (MS-MS) (see below), or in the case of an ion trap MS^n where n indicates the number mass spectrometry stage[91].

1.3.2. The process of ionization

When molecules leave the column they pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.The ionization technique chosen is independent of using full scan or SIM[92].

A common type of ionization is the so called electron ionization (EI). The molecules enter into the MS (the source is a quadrupled or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectrometrists as the employ of molecular electron bombardment, whereas "soft ionization" is done by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA). Spectral

library searches employ matching algorithms such as Probability Based Matching and dot-product matching that are used with methods of analysis written by many method standardization agencies[92].

1.3.2.1. Cold electron ionization

Electron ionization could be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information[11]. In this method named cold electron ionization (Cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic molecular beam (SMB) Collisions with the make up gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process[93]. Cold-EI mass spectra are characterized by an abundant molecular ion while the usual fragmentation pattern is retained, thus making Cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae[94].

1.3.2.2. Chemical ionization

During the process of chemical ionization a reagent gas - methane or ammonia - is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interes[95].

During soft ionization the test sample is fragmented to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.In positive chemical ionization

(PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts[95].

During a negative chemical ionization , the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply[95].

A mass spectrometer is typically utilized in one of two ways: full scan or selected ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

In the GC-MS analysis , the primary goal of the analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data. Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically, this identification

done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample[96].

While a full spectrum analysis considers all peaks within a spectrum, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced[96].

For a full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be *m/z* 50 to *m/z* 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as *m/z* 28 due to nitrogen), carbon dioxide (*m/z* 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

One advantage of a full scan is the determination unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method. In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass

fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard[96].

1.3.3. Some applications of GC-MS

Some applications of GC-MS are briefly outlined below:

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies[96].

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate[96].

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased[96].

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids[96].

Explosive detecting systems have become a part of all international airports. These systems run on a host of technologies, many of them based on GC-MS.

There are only three manufacturers certified by the FAA to provide these systems one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith 's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems[97].

Traditional GC-MS units with transmission quadruple mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, so man, and VX. These complex and large GC-MS systems have been modified and configured with resistively heated low thermal mass (LTM) gas chromatographs that reduce analysis time to less than ten percent of the time required in traditional laboratory systems. Additionally, the systems are smallerand systems that are hand-carried by two-person teams or individuals, much ado to the smaller mass detectors. Depending on the system, the analyses can be introduced via liquid injection, desorbed from sorbent tubes through a thermal desorption process, or with solid-phase micro extraction (SPME) [98].

GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass [99].

Dozens of congenital metabolic diseases also known as inborn error of metabolism are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better

outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS[100].

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

Several GC-MS have left earth. Two were brought to Mars by the Viking program[100]. Venera 11 and 12 and Pioneer Venusanalysed the atmosphere of Venus with GC-MS[101]. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan[102,103].

1.4. Antimicrobials

Antimicrobials are defined as agents that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi.

Antimicrobials are categorized according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis[104].The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones. The term also used to be restricted to

antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth[105].

Substances with antimicrobial properties have been used by several civilizations for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection[135]. More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine[105].

Alexander Flemingn, in 1928, became the first to discover a natural antimicrobial fungus known as *Penicillium rubens*. The substance extracted from the fungus he named penicillin and in 1942 it was successfully used to treat a *Streptococcus* infection[106]. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients until then[24].Nowadays several antimicrobial agents exist, for use against a wide range of infectious diseases[106].

Several essential oils are claimed to possess antimicrobial potential. Examples include the oils of bay, cinnamon, clove and thyme[107,108]. Active constituents include terpenoids chemicals and other secondary metabolites.

However, despite being of prevalent use in alternative medicine, essential oils have seen limited use in mainstream medicine. While 25 to 50% of pharmaceutical compounds are plant-derived, none are used as antimicrobials, though there has been increased research in this direction. Barriers to increased usage in mainstream medicine include poor regulatory oversight and quality control, mislabeled or misidentified products, and limited modes of delivery[109].

1.5. Essential oils

Essential oil may be defines as a highly concentrated hydrophobic liquid of complex mixtures containing volatile aroma compounds and can be extracted from several parts of plant , for example ,leaves, peels, barks, flowers , buds, seeds, and so on which serve as the major source of essential oil[110].

Essential oils have been used through history by many cultures for their medicinal and therapeutic benefits. In medicinal practice, the Egyptians used essential oils extensively for beauty treatment, food preparation, and in religious ceremony. Frankincense, sandalwood, myrrh and cinnamon were considered very valuable cargo along the ancient caravan trade routes and were sometimes exchanged for gold.

The Greeks also used essential oils in their practices of therapeutic massage and aromatherapy. The Romans utilized essential oils to promote health and personal hygiene.

Being influenced by the Greeks and Romans, the Persians began to refine distillation methods for extracting essential oils from aromatic plants [111]. Essential oil extracts were used throughout the dark ages in Europe for their antibacterial and fragrant properties.

The powerful healing properties of essential oils were rediscovered in 1937 by a French chemist, Rene Maurice Gattefosse, who plunged his badly burnt hand into a vat of lavender oil (mistaking it for water) and was surprised to see no injury or scarring. Therapeutic-grade essential oils have been used successfully to treat injured soldiers during World War II[111].

With the invention of synthetic drugs, the use of plants and herbs for their therapeutic properties temporarily declined. That trend is reversing as health scientists and medical practitioners continue to research and validate the benefits of therapeutic-grade essential oils to protect the body, boost the immune system, and revitalize the mind and influence mood, without the adverse effects commonly associated with synthetic drugs [111].

1.5.1. Essential oils in plants

Essential oils are isolated from various parts of the plant. such as leaves (patchouli. pines. cedar). hits (mandarin); bark(cinnamon), root (ginger). grass (citronella); gum (myrrh and balsam oils). Bemes(pimenta); seed (caraway). tlorvers (rose) ; twiigs (clove stem) ; buds (cloves), wood(amyris) ; heartwood(cedar).and saw dust(cedar oil). These plants are processed to yield their quintessence or essential oils by separation from cellulose, glycerides, starches.sugars, tannins, salts and minerais in the botanicals.

One of the most widespread physical method for isolating essential oils from plants is co-istillation with steam[112].A small group of products are exception to the simple definition of an essential oil.

To extract some oils like garlic oil , mustard oil, enzymatic release of the volatile components is required before they can be freed from their matrix via steam distillation. There are other flower oils or resin oils obtained by extraction which contain only a small portion of volatile oil, but nonetheless are called essential oils.

Some products obtained through dry-distillation contain only a limited amount of volatiles. Such products do not fall within the designation of essential oil[142].Getting the odorous principal from the botanical is called ‘expressing' the essential oil.

Citrus oils were formerly obtained by tedious hand-pressing or sponge-pressing, but now they are produced by modern high speed. Multi-functional units. Many flower oils are extracted with a purified petroleum solvent[113].

A process of extraction known as Enfleurage is an old process in which delicate flower petals were physically stuck onto a purified fat, is no longer in common use.One popular extraction procedure is known as maceration[114-116].

Yield of essential oils from their natural matrix varies largely. Nutmegs yield (10-12wt %) of oil, whereas onions yield less than 0.01 % after enzymatic

treatment. Thuga wood oil yield is within the range : 0.60-1.00% wet material .Essential oils are typically liquid at room temperature.

The function of essential oil in the plant is not fùlly understood. Microscopic examination of plant parts that contain the oil sacs readily shows their presence when the sac on the foliage is pricked and the aroma appears.

The odour oraroma of the flowers are said to act as attractants for insects involved in pollination. Andthus aid in preservation of species and natural selection. Essential oils are almost alwaysbacteriostatic and often bactericidal. Many components of essential oils are chemicallyactive. They are sources of plant metaboiic energy if present in large quantities althoughsome chemists have labelled them as waste products of plant metabolism. Exudates suchas balsam and resins which contain essential oils act as protective seals againstdisease or parasite. They prevent loss of sap and are formed readily when the tree trunks are damaged[113].

1.5.2. Uses of essential oils

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oil uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments. Essential oil benefits come from their antioxidant, antimicrobial and anti-inflammatory properties.

The amount of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

The Essential oils are good source of several bioactive compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly

water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved.

Essential oils are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products and have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most countries[114,115].

Aim of this study

This study was designed to fulfill the following goals:

1- Extraction of oils from six Sudanese medicinal plants(*Annona squamos*, *Beta vulgare* subsp. *cila*, *Dichrostachys cinerea*, *Eucalyptus camaldulensis*, *Tephrosia apollina*,*Kigelia africana*).

2- Investigation of oil constituents by GC-MS.

3- Evaluation the antimicrobial potential of the target oils.

Chapter Two

Materials and Methods

2.Materials and Methods

2.1.Materials

2.1.1.Plant material

Seeds of *Annona squamos*, *Beta vulgare* subsp. *cila*, *Dichrostachys cinerea*, *Eucalyptus camaldulensis*, *Tephrosia apollina*, *Kigelia africana* were purchased from the local market-Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2. Instruments

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

2.1.3.Test organisms

The studied oils were screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 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
6	*Candida albicans*	fungi

2.2- Methods

2.2.1-Extraction of oils

Powdered seeds of studied plants (400g) were exhaustively extracted with n-hexane by maceration.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

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 oils were analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas.Oven temperature program is presented in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

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

Table 3: Chromatographic conditions

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

2.2.3.Antimicrobial assay

i). Preparation of bacterial suspensions

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

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

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

ii). Preparation of fungal suspensions

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

iii). Testing for antibacterial activity

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

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

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

Chapter Three

Results and Discussion

3.Results and Discussion

In this study, six Sudanese plant species(*Eucalyptus camaldulensis*, *Kegalia Africana, Beta vulgaris, Tephrosia apollina, Dichrostachys cinera and Annona squamosa*) have been investigated. These plant species are of medicinal attributes and potentials. They have been studies by GC-MS. The target plants have also been assessed for their antimicrobial activity in an attempt to provide a scientific evidence for their folklore uses.

3.1. *Beta vulgaris*

3.1.1. GC-MS analysis

The GC-MS analysis of *Beta vulgaris* oil showed the presence of 35 components. Total ions chromatograms is depicted in Fig.1, while the different constituents of the oil are presented in Table 1. Fatty acids constituted major bulk of the oil(99.67%). Terpenes(0.03%) and hydrocarbons(0.03%) appeared as minor constituents .The GC-MS analysis revealed the following major components:

-9,12-Octadecdienoic acid methyl ester(34.10%)

-Hexadecanoic acid methyl ester(19.05%)

-9-Octadecenoic acid methyl ester(17.57%)

-Methyl stearate(8.68%).

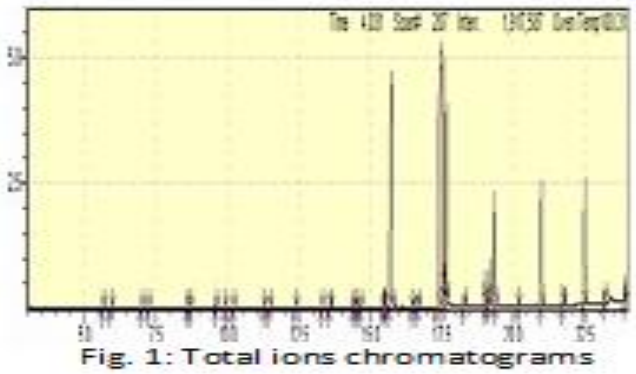


Fig. 1: Total ions chromatograms

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 2.The peak at m/z 294, which appeared at R.T. 17.480 in total ion

chromatogram, corresponds: $M^{+}[C_{19}H_{34}O_{2}]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

The mass spectrum of hexadecanoic acid methyl ester is displayed in Fig. 3.The peak at m/z 270(R.T. 15.749) is due to the molecular ion: $M^{+}[C_{17}H_{34}O_{2}]^{+}$ .The peak at m/z239 accounts for loss of a methoxyl.

Fig. 4 shows the mass spectrum of 9-octadecenoic acid methyl ester .The peak at m/z 296, with R.T. 17.522 , corresponds the molecular ion: $M^{+}[C_{19}H_{36}O_{2}]^{+}$, while the signal at m/z265 is due to loss of a methoxyl.The mass spectrum of methyl stearate is presented in Fig. 5.The signal at m/z 298, which appeared at R.T. 17.653 is attributed to $M^{+}[C_{19}H_{38}O_{2}]^{+}$ .The peak at m/z267 accounts for loss of a methoxyl.

Table 1: Constituents of *Beta vulgaris* oil

ID#	Name	Ret.Time	Area%
1.	Benzoic acid, methyl ester	5.641	0.02
2.	Hexanoic acid, 3-ethyl-, methyl ester	5.941	0.03
3.	L-.alpha.-Terpineol	6.993	0.03
4.	8-Nonenoic acid, methyl ester	7.231	0.01
5.	4-Decenoic acid, methyl ester	8.604	0.01
6.	Decanoic acid methyl ester	8.710	0.00
7.	1-Pentadecene	9.582	0.03
8.	10-Undecenoic acid, methyl ester	9.926	0.00
9.	Nonanoic acid, 9-oxo-, methyl ester	10.204	0.01
10.	Dodecanoic acid, methyl ester	11.265	0.01
11	10-Oxodecanoic acid. methyl ester	11.464	0.01
12.	Methyl myristoleate	12.364	0.01
13.	cis-5-Dodecenoic acid, methyl ester	13.308	0.01
14.	Methyl tetradecanoate	13.583	0.45
15.	6-Octadecenoic acid, methyl ester, (Z)-	14.391	0.04
16	5-Octadecenoic acid. methyl ester	14.496	0.02
17.	Pentadecanoic acid, methyl ester	14.657	0.10
18.	7-Hexadecenoic acid, methyl ester, (Z)-	15.449	0.09
19	9-Hexadecenoic acid. methyl ester. (Z)-	15.492	0.44
20.	Hexadecanoic acid, methyl ester	15.749	19.05
21.	cis-10-Heptadecenoic acid, methyl ester	16.455	0.21
22.	Heptadecanoic acid. methyl ester	16.664	0.44
23.	9.12-Octadecadienoic acid (Z.Z)-. methyl ester	17.480	34.10
24.	9-Octadecenoic acid (Z)-, methyl ester	17.522	17.57
25.	Methyl stearate	17.653	8.68
26.	trans-Geranylgeraniol	18.271	0.27
27.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.014	1.09
28	cis-11-Eicosenoic acid. methyl ester	19.162	1.67
29.	Eicosanoic acid. methyl ester	19.366	4.02
30.	Heneicosanoic acid, methyl ester	20.179	0.16
31.	Docosanoic acid, methyl ester	20.986	4.75
32.	Tricosanoic acid. methyl ester	21.738	0.63
33.	Tetracosanoic acid, methyl ester	22.489	4.90
34	Pentacosanoic acid. methyl ester	23.187	0.31
35.	Hexacosanoic acid, methyl ester	23.875	0.83

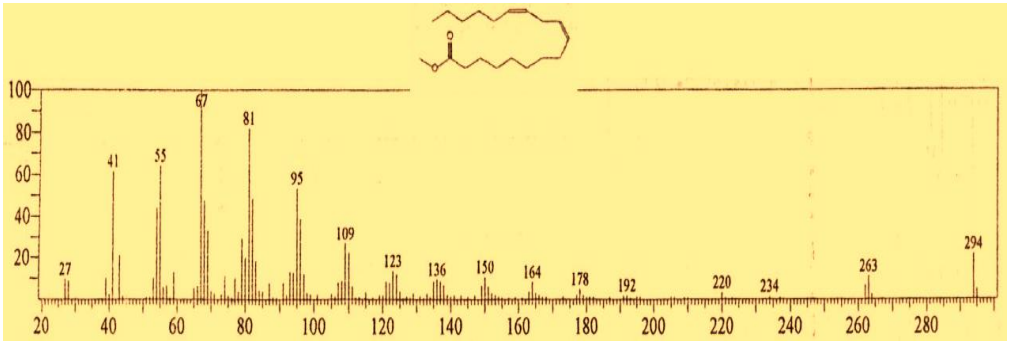


Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

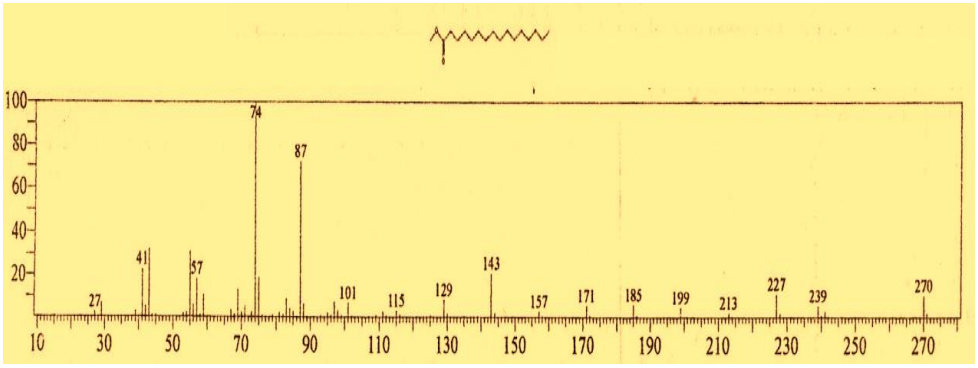


Fig. 3: Mass spectrum of hexadecanoic methyl ester

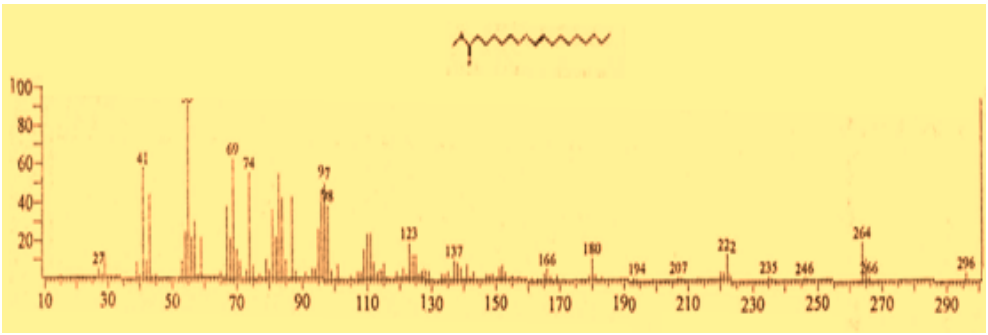


Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester

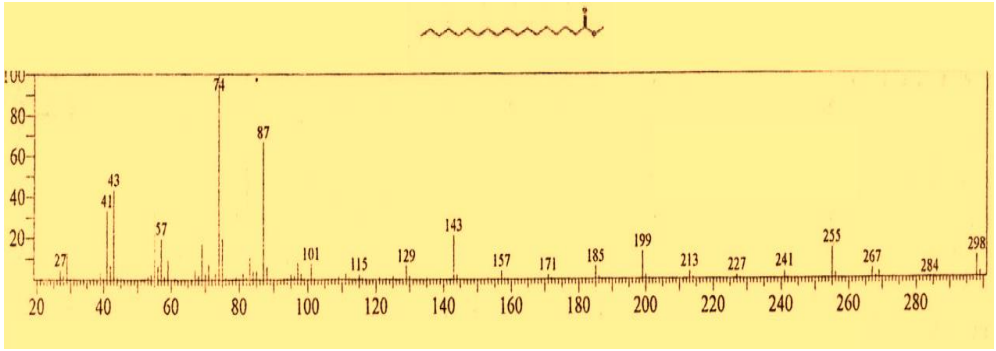


Fig. 5: Mass spectrum of methyl stearate

3.1.2. Antimicrobial assay

Beta vulgaris oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the

growth inhibition zones are presented in Table (2).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Bacillus subtilis* and moderate anticandidal activity.It exhibited partial activity against other test organisms.

Table 2: Inhibition zones of *Beta vulgaris* oil

Sample	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	10	18	10	12	15
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.*: Staphylococcus aureus*

Ec.*: Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Bs.*: Bacillus subtilis*

Ca.: *Candida albicans*

3.2. *Tephrosia apollina*

3.2.1.Constituents of the oil

Tephrosia apollina oil was analyzed by GC-MS . The analysis showed 22 components. Fatty acids constituted 97.96% , the rest is ϐ -sitosterol (2.04%). The oil was dominated by : (i)9-octadecenoic acid methyl ester(31.79%) ; 9,12-octadecadienoic acid methyl ester(21.34%); hexadecanoic acid methyl

ester(17.86%) and methyl stearate(16.07%). The total ions chromatogram is shown in Fig. 6, while the constituents of the oil are displayed in Table 3.

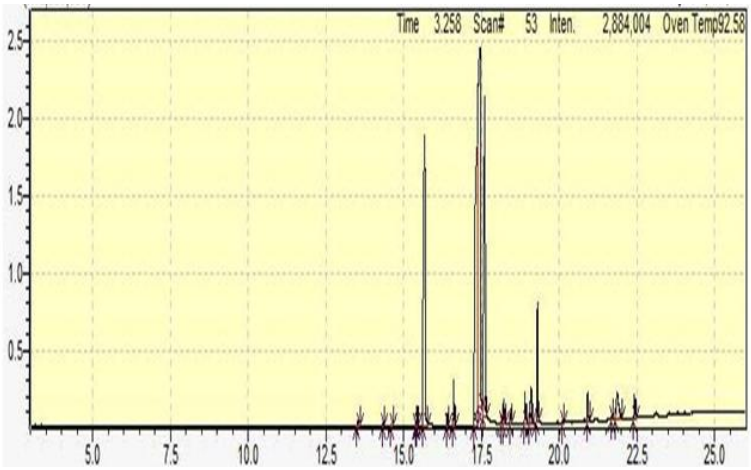


Fig.6 : Total ions chromatograms

Major components of the oil are discussed below:

(i)9-octadecenoic acid methyl ester(31.79%)

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 7.The peak at m/z 296, which appeared at R.T. 17.460 , in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_2]^{+}$ .The peak at m/z265 corresponds to loss of a methoxyl function.

(ii)9,12-octadecadienoic acid methyl ester(21.34%)

Fig. 8 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester .The signal at m/z 294, which appeared at R.T. 17.370 corresponds : $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 is due to loss of a methoxyl .

(iii) hexadecanoic acid methyl ester(17.86%)

The mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 9.The peak at m/z 270 (R.T. 15.681) accounts for the molecular ion: $M^{+}[C_{17}H_{34}O_2]^{+}$ .The signal at m/z239 is attributed to loss of a methoxyl .

(iv) methyl stearate(16.07%)

Fig. 10 illustrates the mass spectrum of methyl stearate .The signal at m/z 298, which appeared at R.T. 15.783 corresponds : $M^{+}[C_{19}H_{38}O_2]^{+}$ .The peak at m/z267 is due to loss of a methoxyl function.

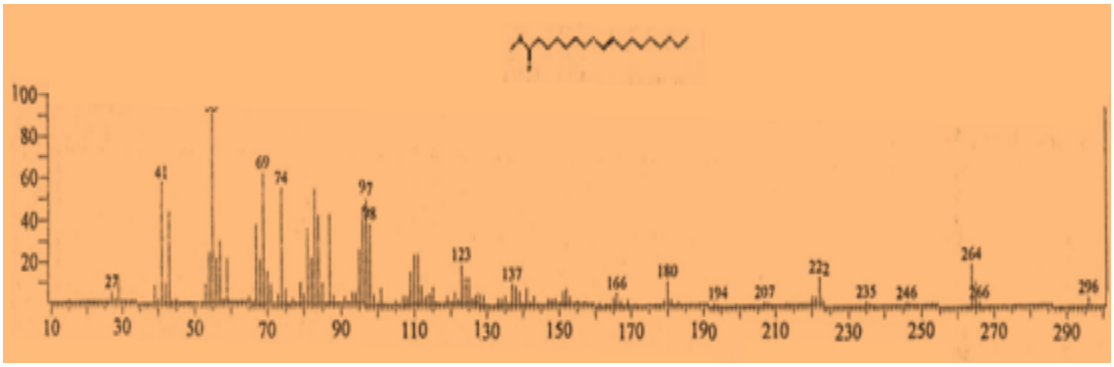


Fig. 7: Mass spectrum of 9-octadecenoic acid methyl ester

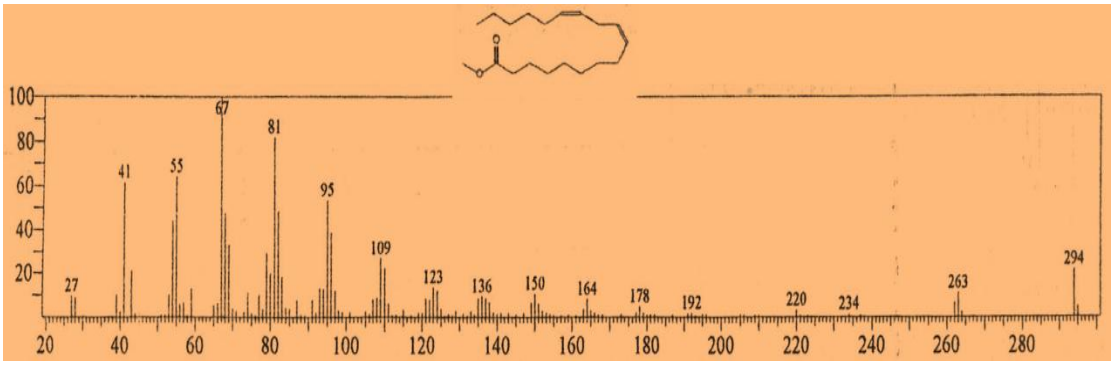


Fig. 8: Mass spectrum of 9,12-octadecadienoic acid methyl ester

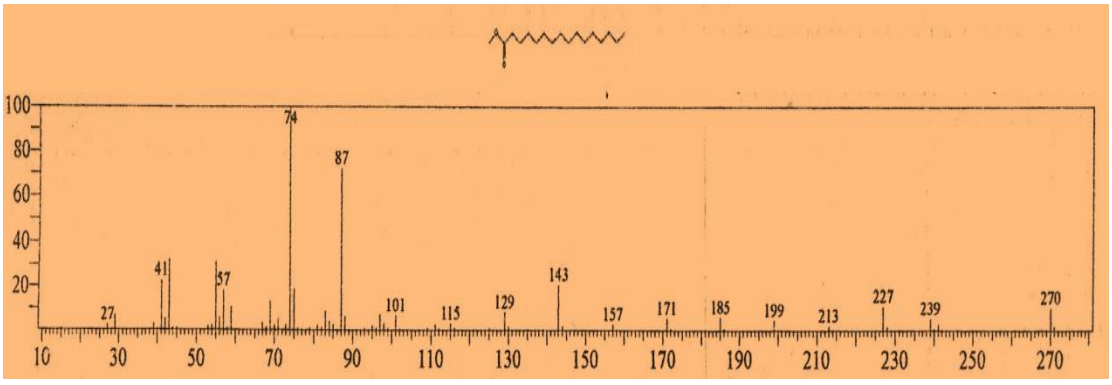


Fig. 9: Mass spectrum of hexadecanoic methyl ester

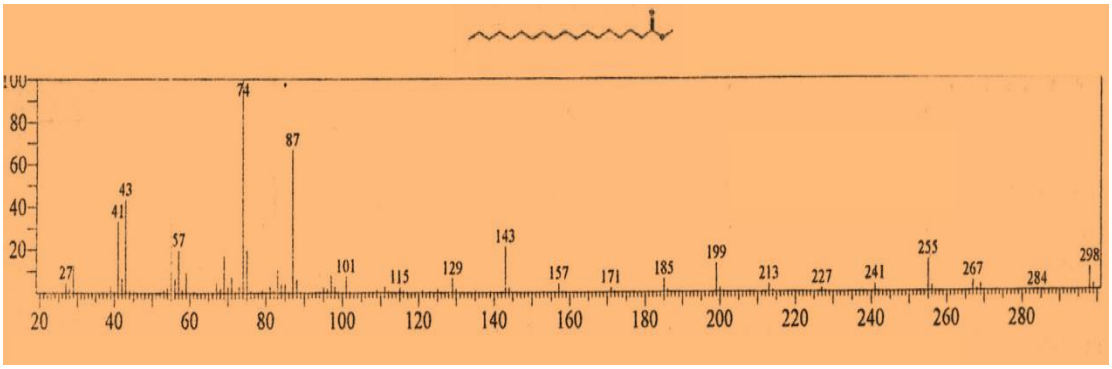


Fig. 10:Mass spectrum of methyl stearate

Table 3: Constituents of the oil

ID#	Name	Ret.Time	Area%
1	Methyl tetradecanoate	13.531	0.13
2.	cis-5-Dodecenoic acid, methyl ester	14.341	0.03
3.	Pentadecanoic acid, methyl ester	14.606	0.09
4	7-Hexadecenoic acid. methyl ester. (Z)-	15.397	0.11
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.440	0.45
6.	Hexadecanoic acid, methyl ester	15.681	17.86
7.	cis-10-Heptadecenoic acid, methyl ester	16.404	0.27
8.	Heptadecanoic acid, methyl ester	16.613	1.21
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.370	21.34
10.	9-Octadecenoic acid (Z)-, methyl ester	17.460	31.79
11.	Methyl stearate	17.615	16.07
12.	cis-11,14-Eicosadienoic acid, methyl ester	18.155	0.14
13.	cis-10-Nonadecenoic acid, methyl ester	18.226	0.60
14.	Nonadecanoic acid, methyl ester	18.445	0.39
15.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.907	0.92
16.	cis-11-Eicosenoic acid, methyl ester	19.104	1.46
17.	Eicosanoic acid, methyl ester	19.307	3.42
18.	Heneicosanoic acid, methyl ester	20.129	0.07
19.	Docosanoic acid, methyl ester	20.921	0.75
20.	Tricosanoic acid, methyl ester	21.687	0.10
21.	.gamma.-Sitosterol	21.872	2.04
22.	Tetracosanoic acid, methyl ester	22.426	0.76

3.2.2.Antimicrobial assay

Tephrosia apollina oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (4). Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed significant

activity against *Bacillus subtilis* , *Staphylococcus aureus* and *Escherichia coli* .It exhibited moderate activity against other test organisms.

Table 4 : Inhibitory effect of *Beta vulgaris* oil

Sample	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	23	22	21	15	15
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.*: Staphylococcus aureus*

Ec.*: Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Bs.*: Bacillus subtilis*

Ca.: *Candida albicans*

3.3. *Eucalyptus camaldulensis*

3.3.1.GC-MS analysis

GC-MS analysis of *Eucalyptus camaldulensis* volatile oil was conducted and the identification of the constituents was based on retention times and computer matching of the MS data with the (NIST) mass spectral library . Excellent matching was observed when comparing the observed mass spectra with the database on the MS library

Thirty seven constituents were identified by GC-MS. The typical total ion chromatogram (TIC) is given in Fig. (11) while the constituents of the oil are outlined in Table 5 .

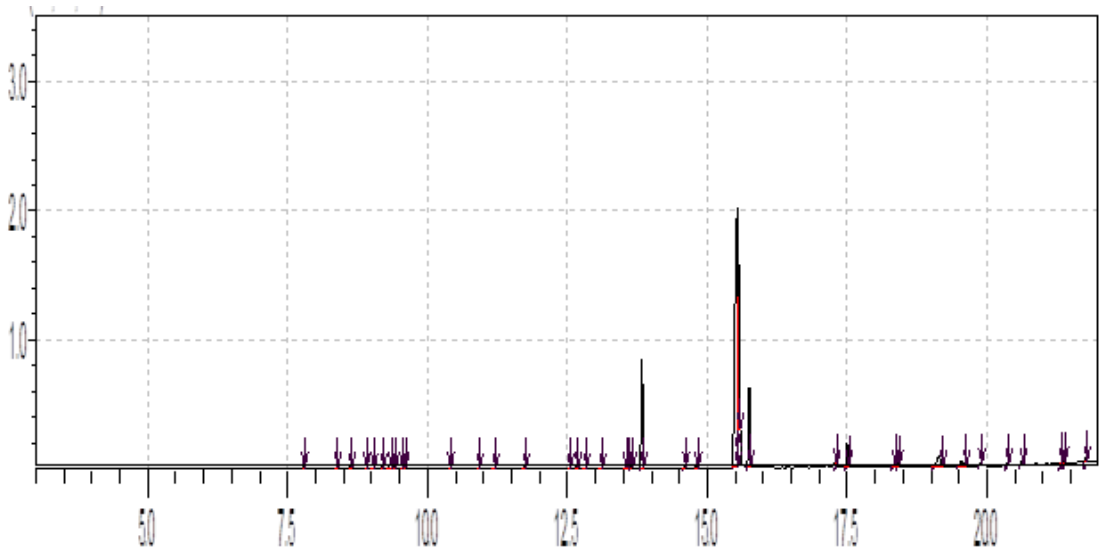


Fig.11 : Total ions chromatograms

Table 5 : Constituents of the oil

ID#	Name	Ret.Time	Area	Area%
1.	Copaene	7.778	192912	0.14
2.	Caryophyllene	8.371	66849	0.05
3.	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	8.613	386325	0.29
4.	Alloaromadendrene	8.890	93961	0.07
5.	.gamma.-HIMACHALENE	9.025	88977	0.07
6.	Bicyclo[3.1.1]heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)-, [1R-(1.alpha.,5.alpha.,6.beta.)]-	9.177	144546	0.11
7.	.alpha.-Guaiene	9.304	309595	0.23
8.	Butylated Hydroxytoluene	9.397	246248	0.18
9.	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	9.541	30901	0.02
10.	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	9.579	62646	0.05
11.	Globulol	10.387	48856	0.04
12.	Agarospirol	10.906	27408	0.02
13.	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.155	96277	0.07
14.	Methyl tetradecanoate	11.725	197014	0.15
15.	cis-5-Dodecenoic acid, methyl ester	12.534	40039	0.03
16.	.alpha.-Phellandrene	12.666	266180	0.20
17.	Pentadecanoic acid, methyl ester	12.801	135484	0.10
18.	.gamma.-Terpinene	13.104	48143	0.04
19.	7,10-Hexadecadienoic acid, methyl ester	13.529	52439	0.04
20.	Methyl hexadec-9-enoate	13.588	222877	0.17
21.	9-Hexadecenoic acid, methyl ester, (Z)-	13.632	197738	0.15
22.	Hexadecanoic acid, methyl ester	13.833	16031387	11.87
23.	cis-10-Heptadecenoic acid, methyl ester	14.592	152024	0.11
24.	Heptadecanoic acid, methyl ester	14.801	312841	0.23
25.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.534	73949570	54.74
26.	9-Octadecenoic acid (Z)-, methyl ester	15.564	17046962	12.62
27.	Methyl stearate	15.747	11213763	8.30
28.	11-Eicosenoic acid, methyl ester	17.297	890185	0.66
29.	Methyl 18-methylnonadecanoate	17.495	3159543	2.34
30.	Heneicosanoic acid, methyl ester	18.321	373819	0.28
31.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	18.407	435193	0.32
32.	Hexatriacontane	19.141	4099263	3.04
33.	.beta.-Sitosterol acetate	19.536	2042107	1.51
34.	Tricosanoic acid, methyl ester	19.880	334991	0.25
35.	Tetracontane	20.362	207394	0.15
36.	Tetracosanoic acid, methyl ester	20.618	705760	0.52
37.	Methyl 23-methyl-tetracosanoate	21.331	177698	0.13

Major components are briefly discussed below:

a) 9,12-Octadecadienoic acid methyl ester (54.74%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.12 .The peak at m/z294 (R.T. 15.534) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl .

9,12-Octadecadienoic exists in lipids and cell membrane. It belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human body and are available through diet

b) 9-Octadecenoic acid methyl ester(12.62%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 13.The peak at m/z 296 (R.T. 15.564) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z266 is attributed to loss of a methoxyl .

9-Octadecenoic acid(oleic acid) is included in animal fats and vegatables , hence it is included in the normal human diet. It is used as emollient. The acid is used in small amount as excipient in pharmaceutical industries. 9-Octadecenoic acid could be responsible for the hypotensive effect of olive oil[15]. It has been claimed that the presence of oleate in olive oil is associated with decreased risk of breast cancer.

c) Hexadecanoic acid methyl ester(11.87%)

Figure 14 shows the mass spectrum of hexadecanoic acid methyl ester .The molecular ion : $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270 at R.T. 13.833 in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

hexadecanoic acid(palmitic acid) is a saturated fatty acid and it is considered as the most common fatty acid in animals and humans.During the synthesis of fatty acids ,Palmitic acid is the precursor of long-chain fatty[(11)] .This acid is a major lipid component of human breast milk[(12,13)] . Palmitic acid , beside being used in soap industry, it is widely used in food industry.

d) Methyl stearate(8.30%)

Figure 15 displays the EI mass spectrum of methyl stearate .The molecular ion : $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T. 15.747).The peak at m/z267 corresponds to loss of a methoxyl function.

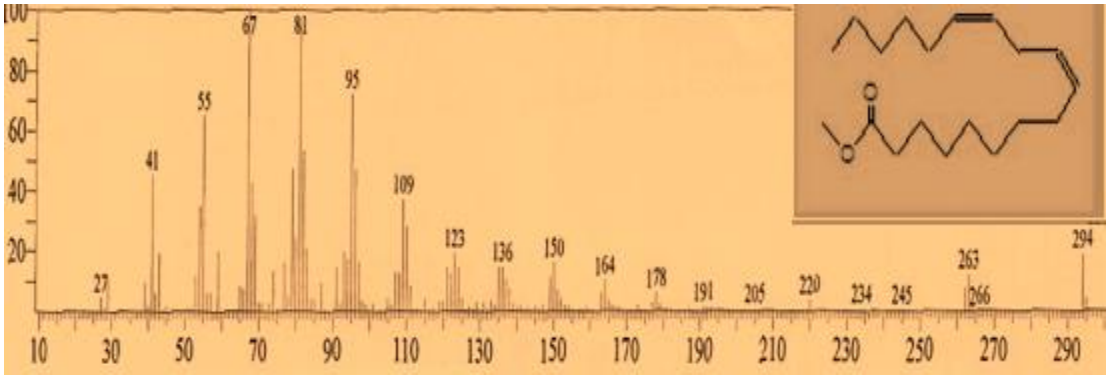


Fig. 12: Mass spectrum of 9,12-octadecadienoic acid methyl ester

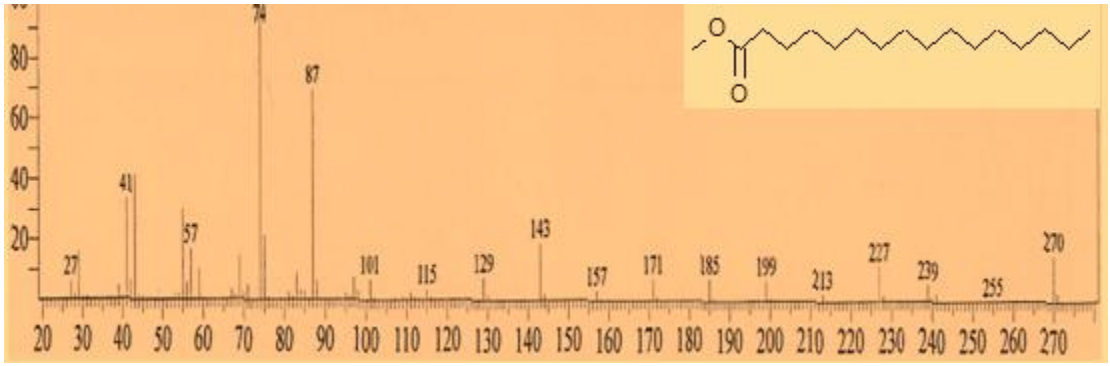


Fig. 13: Mass spectrum of hexadecanoic acid methyl ester

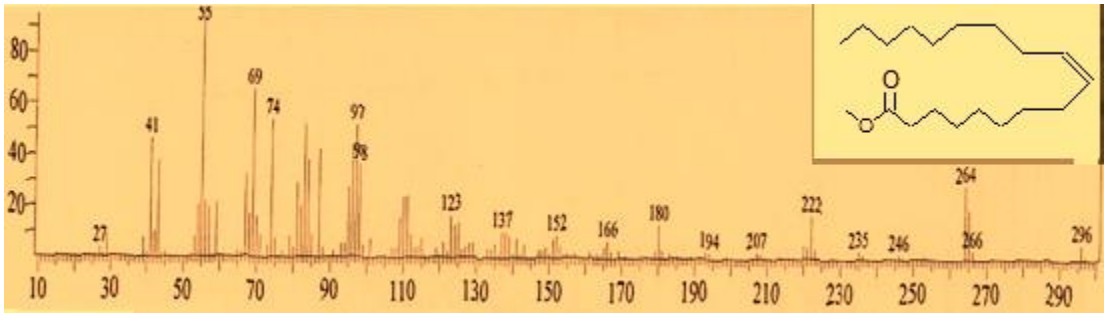


Fig. 14: Mass spectrum of 9-octadecenoic acid methyl ester

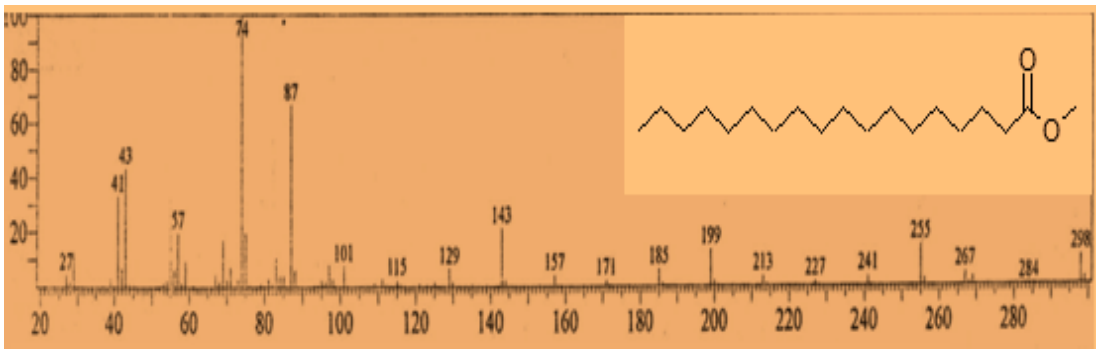


Fig. 15: Mass spectrum of methyl stearate

3.3.2. Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (6) . The oil showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. .It also showed partial activity against other test organisms.

Table 6: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
oil (100mg/ml)	15	12	15	12	10

Table 7 : Antibacterial activity of standard drugs

Drug	Conc. (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5 : Antifungal activity of standard drug

Drug	Conc. (mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

3.4-*Annona squamosa*

3.4.1- GC-MS analysis

GC-MS analysis of *Annona squamosa* oil was conducted. Twenty two constituents were identified by GC-MS. The typical total ion chromatogram (TIC) is given in Fig. (16) while the constituents of the oil are outlined in Table 8

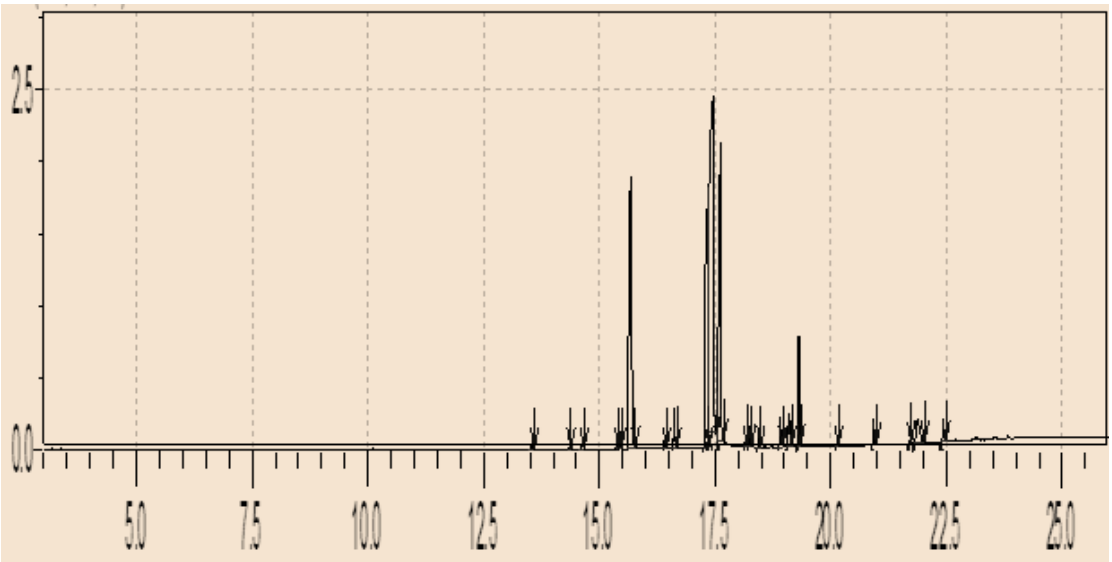


Fig.16: Total ions chromatograms

Major components are:

i)-9-Octadecenoic acid methyl ester(31.79%)

ii)-9,12-Octadecadienoic acid methyl ester(21.34%)

iii)- Hexadecanoic acid methyl ester(17.86%)

iv)-Methyl stearate(16.07%)

Table 8: Constituents of the oil

D#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.531	0.13
2.	cis-5-Dodecenoic acid, methyl ester	14.341	0.03
3.	Pentadecanoic acid, methyl ester	14.606	0.09
4.	7-Hexadecenoic acid, methyl ester, (Z)-	15.397	0.11
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.440	0.45
6.	Hexadecanoic acid, methyl ester	15.681	17.86
7.	cis-10-Heptadecenoic acid, methyl ester	16.404	0.27
8.	Heptadecanoic acid, methyl ester	16.613	1.21
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.370	21.34
10.	9-Octadecenoic acid (Z)-, methyl ester	17.460	31.79
11.	Methyl stearate	17.615	16.07
12.	cis-11,14-Eicosadienoic acid, methyl ester	18.155	0.14
13.	cis-10-Nonadecenoic acid, methyl ester	18.226	0.60
14.	Nonadecanoic acid, methyl ester	18.445	0.39
15.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.907	0.92
16.	cis-11-Eicosenoic acid, methyl ester	19.104	1.46
17.	Eicosanoic acid, methyl ester	19.307	3.42
18.	Heneicosanoic acid, methyl ester	20.129	0.07
19.	Docosanoic acid, methyl ester	20.921	0.75
20.	Tricosanoic acid, methyl ester	21.687	0.10
21.	.gamma.-Sitosterol	21.872	2.04
22.	Tetracosanoic acid, methyl ester	22.426	0.76

i) 9-Octadecenoic acid methyl ester(31.79%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 17.The peak at m/z 296 (R.T. 17.460) corresponds $M^{+}[C_{19}H_{36}O_2]^{+}$, while the signal at m/z266 is attributed to loss of a methoxyl .

ii)9,12-Octadecadienoic acid methyl ester (21.34%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.18 .The peak at m/z294 (R.T. 17.370) coincides with $M^{+}[C_{19}H_{34}O_2]^{+}$, while the peak at m/z263 is due to loss of a methoxyl .

iii) Hexadecanoic acid methyl ester(17.86%)

Figure 19 shows the mass spectrum of hexadecanoic acid methyl ester .The molecular ion : $M^{+}[C_{17}H_{34}O_2]^{+}$ appeared at m/z 270 at R.T. 15.681 in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

iv) Methyl stearate(16.07%)

Figure 20 displays the EI mass spectrum of methyl stearate .The molecular ion : $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T. 17.615).The peak at m/z267 corresponds to loss of a methoxyl function.

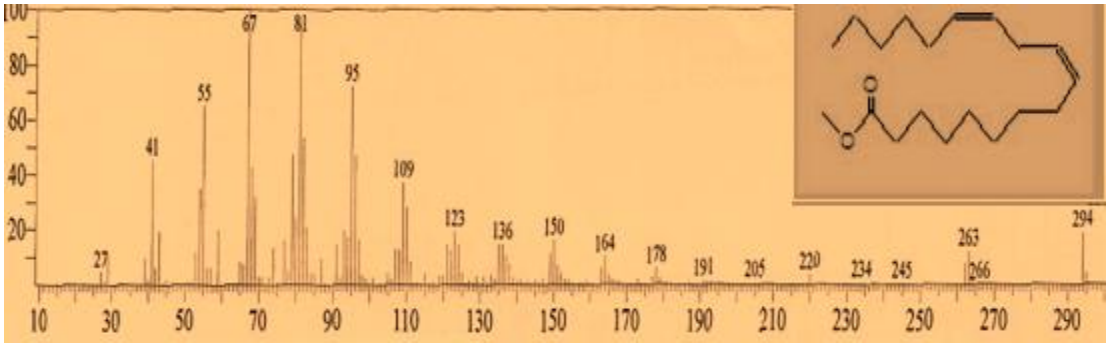


Fig. 17: Mass spectrum of 9,12-octadecadienoic acid methyl ester

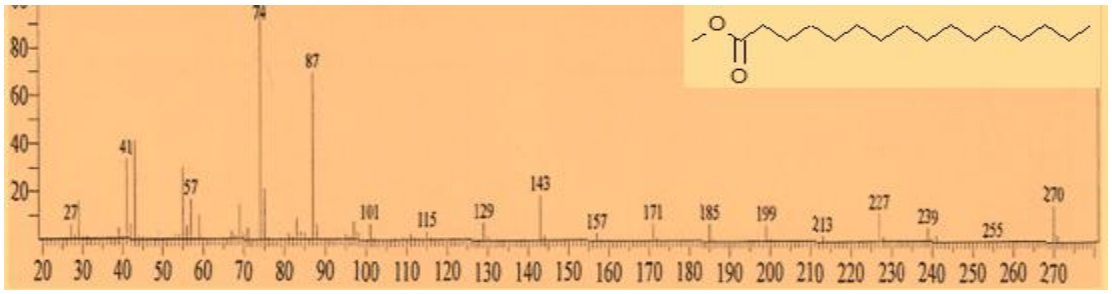


Fig. 18: Mass spectrum of hexadecanoic acid methyl ester

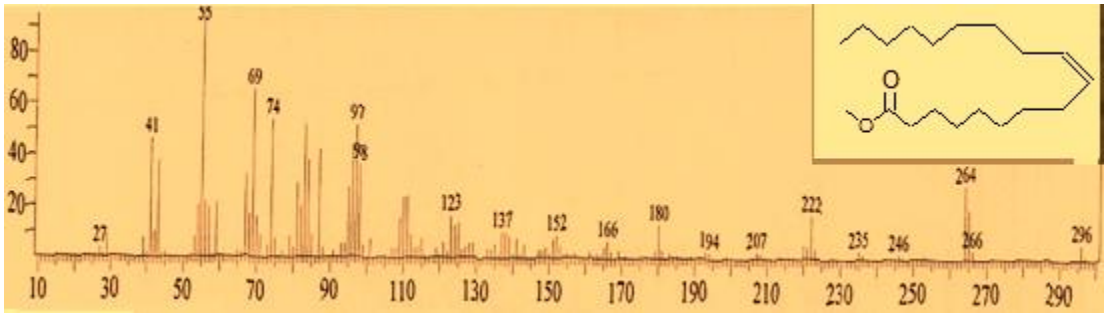


Fig. 19: Mass spectrum of 9-octadecenoic acid methyl ester

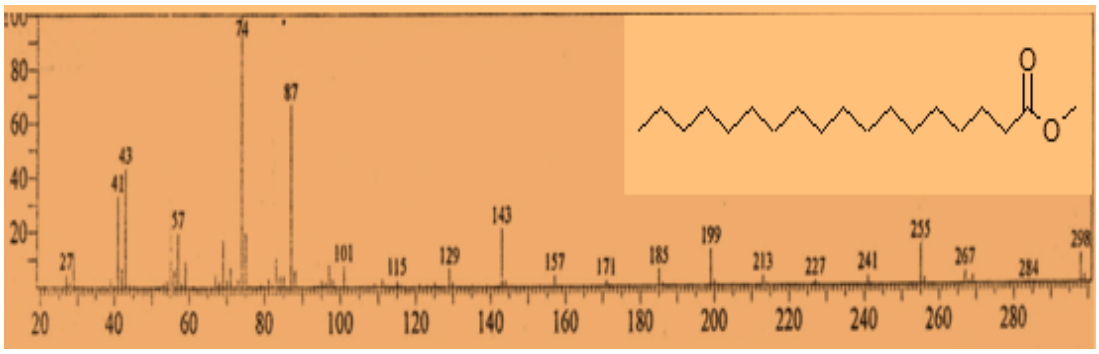


Fig. 20: Mass spectrum of methyl stearate

3.4.2 Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (9) . The oil showed moderate anticandidal activity. However, it exhibited partial activity against *Bacillus subtilis* and *Escherichia coli.*

Table 9: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
oil (100mg/ml)	10	--	--	10	15

3.5. Dichrostachys cinera

3.5.1.GC-MS analysis

Dichrostachys cinera oil was analyzed by GC-MS. Twenty three constituents were identified. The typical total ion chromatogram (TIC) is given in Fig. (21) while the constituents of the oil are outlined in Table 10 .

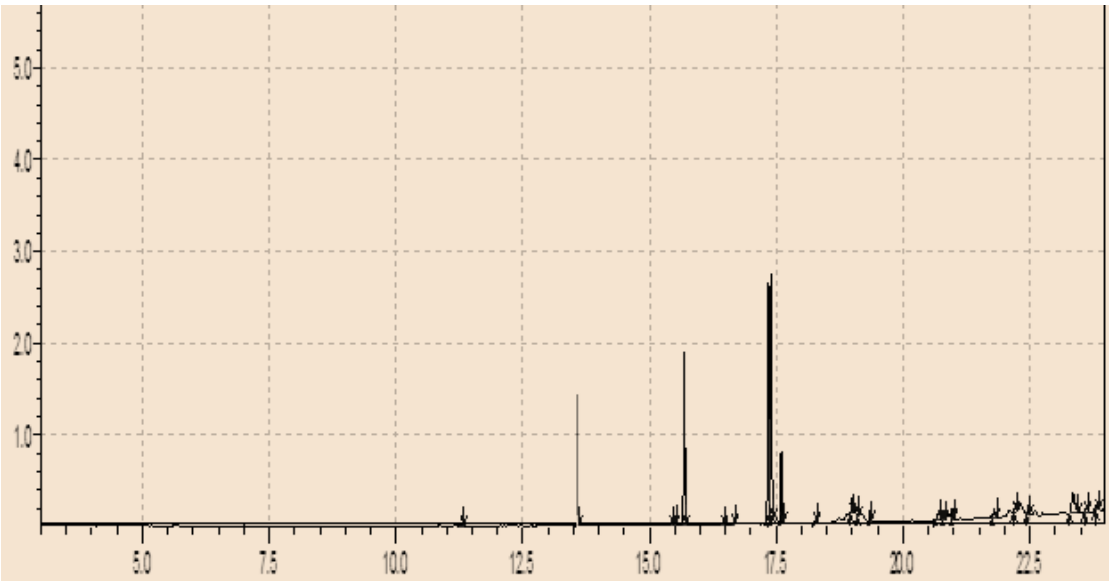


Fig. Total ions chromatograms

Table 10: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	Dodecanoic acid, methyl ester	11.268	0.12
2.	Methyl tetradecanoate	13.582	0.55
3.	7-Hexadecenoic acid, methyl ester, (Z)-	15.446	0.16
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.489	0.44
5.	Hexadecanoic acid, methyl ester	15.688	17.94
6.	cis-10-Heptadecenoic acid, methyl ester	16.451	0.12
7.	Heptadecanoic acid, methyl ester	16.659	0.29
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.350	30.87
9.	9-Octadecenoic acid (Z)-, methyl ester	17.396	27.02
10.	Methyl stearate	17.598	6.83
11.	trans-Geranylgeraniol	18.260	0.27
12.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.993	1.70
13.	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	19.116	0.34
14.	Eicosanoic acid, methyl ester	19.352	1.05
15.	Eicosane	20.701	1.49
16.	13-Docosenoic acid, methyl ester, (Z)-	20.794	0.26
17.	Docosanoic acid, methyl ester	20.971	1.09
18.	Vitamin E	21.785	1.04
19.	2-methylhexacosane	22.215	0.99
20.	Tetracosanoic acid, methyl ester	22.471	0.64
21.	Octadecanal	23.347	5.13
22.	Tetracontane	23.618	0.81
23.	Hexatriacontane	23.825	0.85

Major components are:

i)-9,12-Octadecadienoic acid methyl ester(30.87%)

ii)-9-Octadecenoic acid methyl ester(27.02%)

iii)- Hexadecanoic acid methyl ester(17.94%)

iv)-Methyl stearate(6.83%)

i)9,12-Octadecadienoic **acid methyl ester (30.87%)**

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.22 .The peak at m/z294 (R.T. 17.350) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl .

ii)9-Octadecenoic acid methyl ester(27.02%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 23.The peak at m/z 296 (R.T. 17.396) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z266 is attributed to loss of a methoxyl .

iii) Hexadecanoic acid methyl ester(17.94%%)

Figure 24 shows the mass spectrum of hexadecanoic acid methyl ester .The molecular ion : $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270 at R.T. 15.688 in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

iv) Methyl stearate(6.83%)

Figure 25 displays the EI mass spectrum of methyl stearate .The molecular ion : $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T. 17.598).The peak at m/z267 corresponds to loss of a methoxyl function.

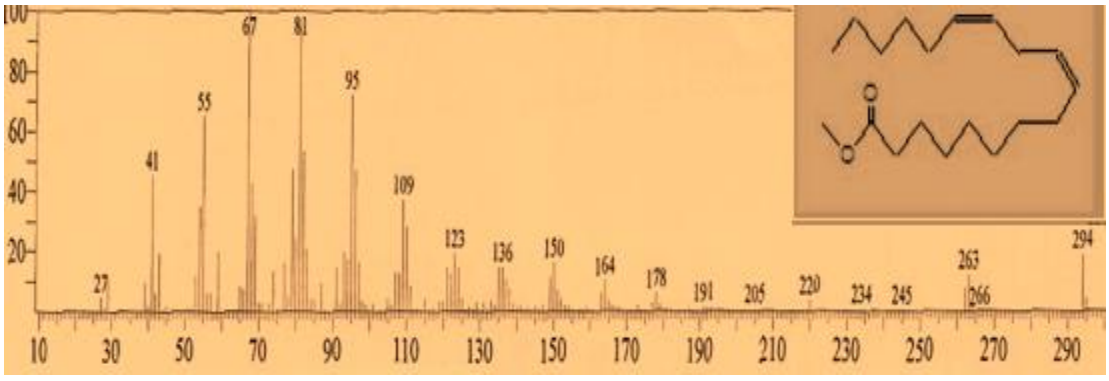


Fig. 22: Mass spectrum of 9,12-octadecadienoic acid methyl ester

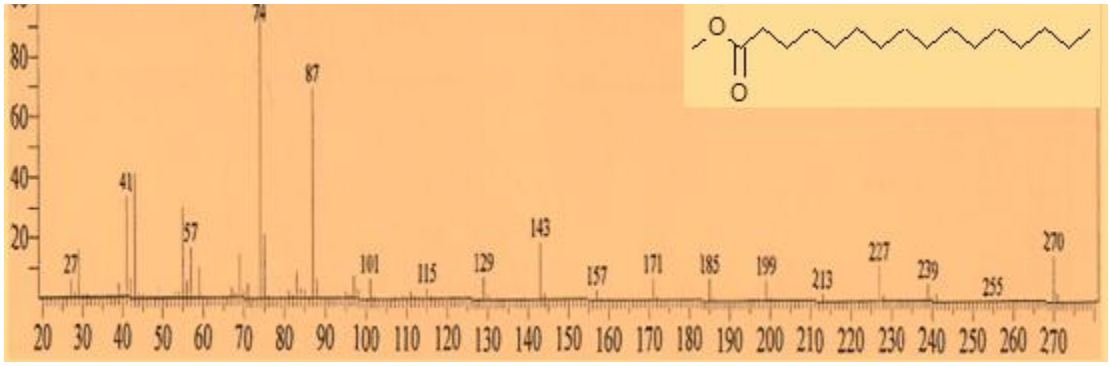


Fig. 23: Mass spectrum of hexadecanoic acid methyl ester

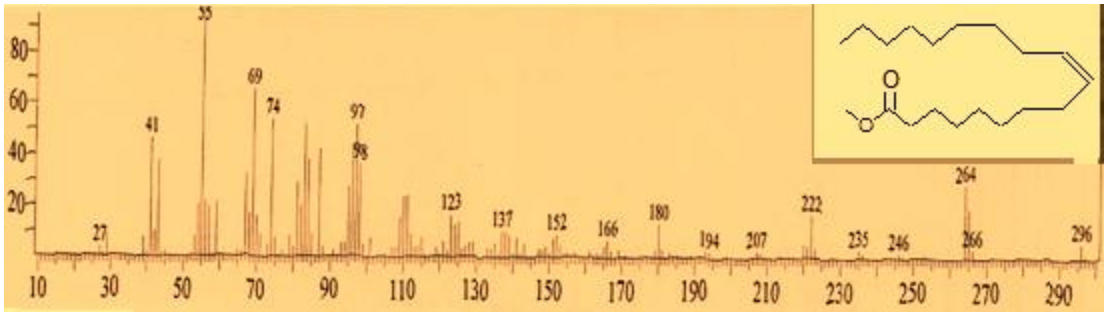


Fig. 24: Mass spectrum of 9-octadecenoic acid methyl ester

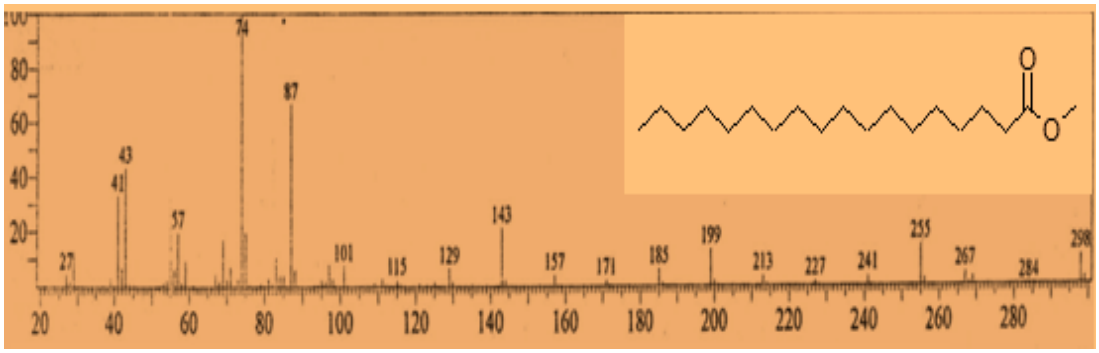


Fig. 25: Mass spectrum of methyl stearate

3.5.2-Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (11).

The oil showed significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* It also showed significant anticandidal activity. However, it exhibited moderate activity against *Escherichia coli.*

Table 11: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
oil (100mg/ml)	16	7	18	19	18

3.6-Kegalia Africana

3.6.1-GC-MS analysis

The oil from *Kegalia Africana* was analyzed by GC-MS. Twenty three constituents were identified. The typical total ion chromatogram (TIC) is given in Fig. (26) while the constituents of the oil are outlined in Table 12 .

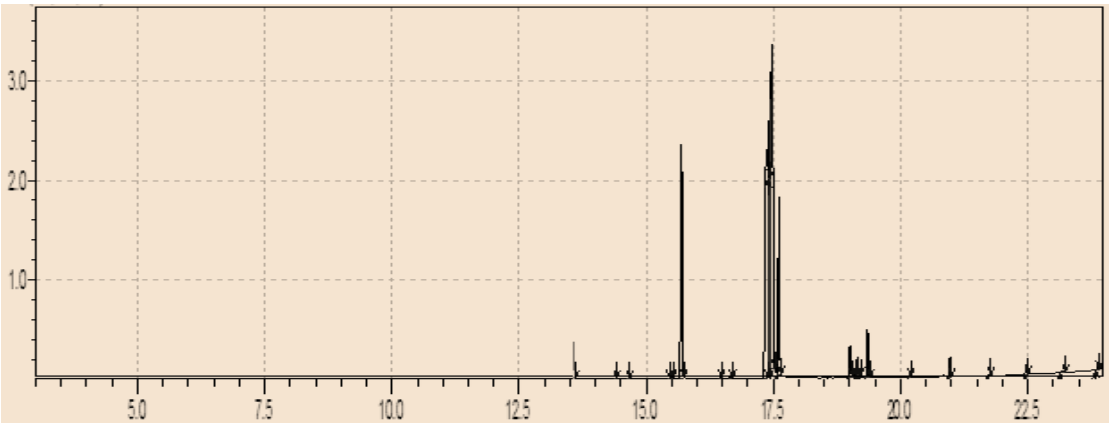


Fig.26 Total ions chromatograms

Table 12: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.581	0.06
2.	5-Octadecenoic acid, methyl ester	14.390	0.01
3.	Pentadecanoic acid, methyl ester	14.654	0.02
4.	7-Hexadecenoic acid, methyl ester, (Z)-	15.448	0.05
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.487	0.24
6.	Hexadecanoic acid, methyl ester	15.695	15.57
7.	cis-10-Heptadecenoic acid, methyl ester	16.449	0.09
8.	Heptadecanoic acid, methyl ester	16.658	0.23
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.370	29.44
10.	9-Octadecenoic acid (Z)-, methyl ester	17.440	4.38
11.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.481	31.32
12.	Methyl stearate	17.609	10.52
13.	.gamma.-Linolenic acid, methyl ester	19.004	1.95
14.	cis-11,14-Eicosadienoic acid, methyl ester	19.124	0.34
15.	cis-11-Eicosenoic acid, methyl ester	19.150	0.48
16.	11,14,17-Eicosatrienoic acid, methyl ester	19.192	0.28
17.	Eicosanoic acid, methyl ester	19.349	2.70
18.	Heneicosanoic acid, methyl ester	20.175	0.07
19.	Docosanoic acid, methyl ester	20.968	1.13
20.	Tricosanoic acid, methyl ester	21.733	0.10
21.	Tetracosanoic acid, methyl ester	22.471	0.63
22.	Pentacosanoic acid, methyl ester	23.183	0.16
23.	Hexacosanoic acid, methyl ester	23.870	0.23

Major components are:

i) 9,12,15-Octadecatrienoic acid methyl ester(31.32%)

ii)-9,12-Octadecadienoic acid methyl ester(29.44%)

iii)- Hexadecanoic acid methyl ester(15.57%)

iv)-Methyl stearate(10.52%).

i)9,12,15-octadecatrienoic acid methyl ester(31.32%)

The EI mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester is shown in Fig.27.The peak at m/z 292(R.T. 17.481) corresponds to $M^+[C_{19}H_{32}O_2]^+$ .The signal which appeared at m/z277 is due to loss of a methyl group while the signal at m/z261 accounts for loss of a methoxyl function.

ii)9,12-Octadecadienoic acid methyl ester (29.32%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.28 .The peak at m/z294 (R.T. 17.481) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl .

iii)Hexadecanoic acid methyl ester(15.57%)

Figure 29 shows the mass spectrum of hexadecanoic acid methyl ester .The molecular ion : $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270 at R.T. 15.695 in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

vi)Methyl stearate(10.52%)

Figure 30 displays the EI mass spectrum of methyl stearate .The molecular ion : $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T.17.609).The peak at m/z267 corresponds to loss of a methoxyl function.

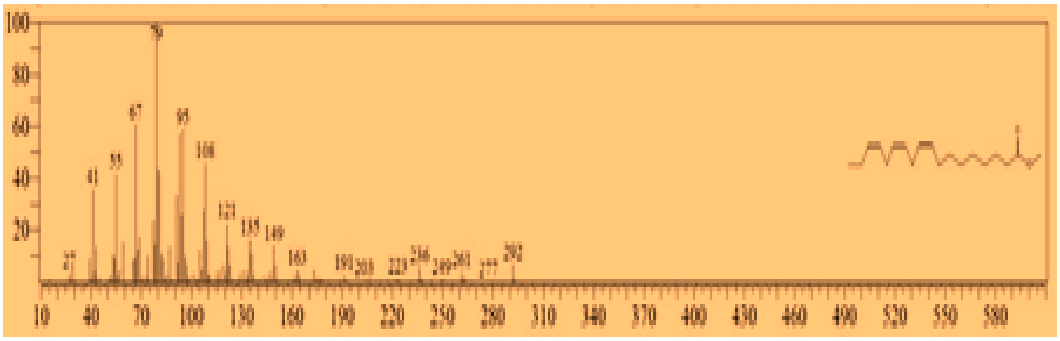


Fig.27: Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester

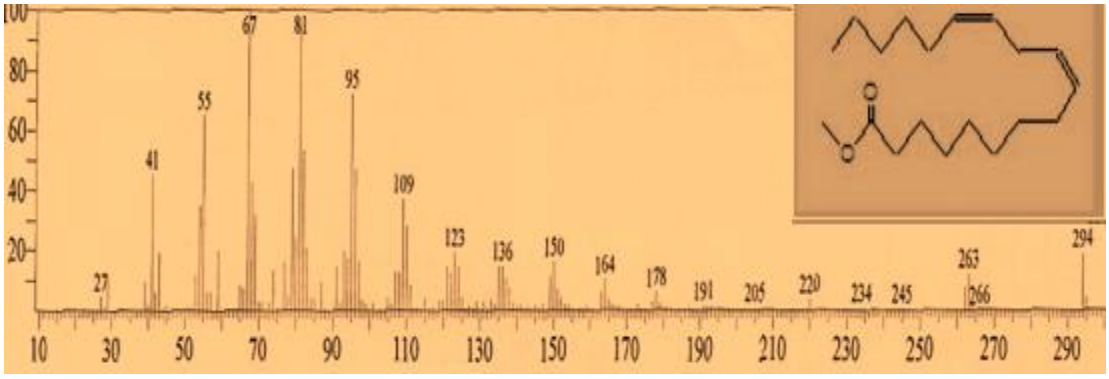


Fig. 28: Mass spectrum of 9,12-octadecadienoic acid methyl ester

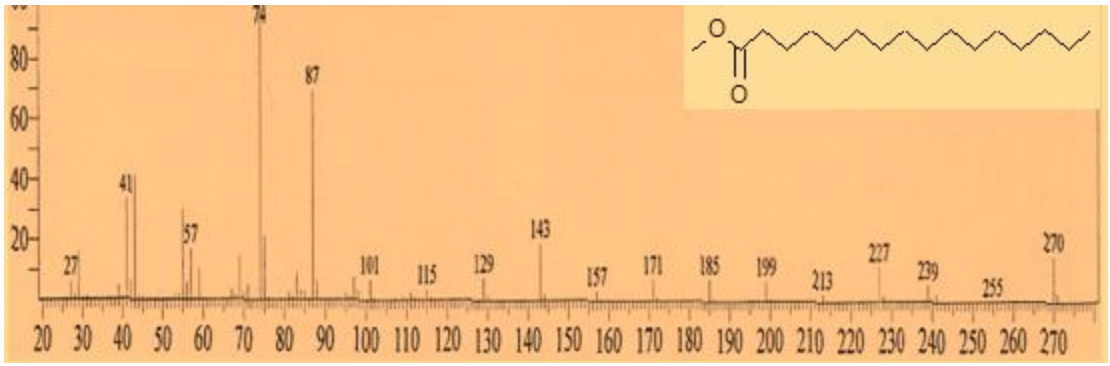


Fig. 29: Mass spectrum of hexadecanoic acid methyl ester

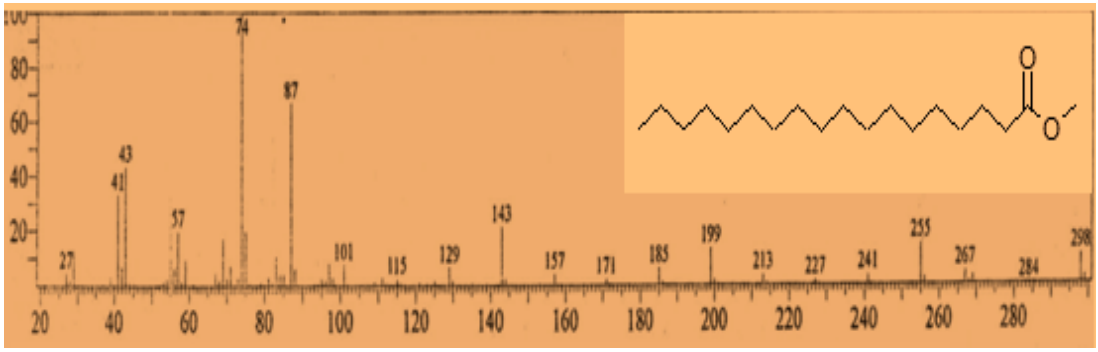


Fig. 30: Mass spectrum of methyl stearate

3.6.2.Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (13) .Ampicilin , gentamicin and clotrimazole were used as positive controls.

The oil showed moderate anticandidal activity.It also showed significant anticandidal activity. However, it exhibited partial activity against *Bacillus subtilis* and *Escherichia coli.*

Table 13: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
Oil (100mg/ml)	10	-	-	10	15

Conclusion

In this study, six Sudanese plant species(*Eucalyptus camaldulensis ,Kegalia Africana, Beta vulgaris, Tephrosia apollina, Dichrostachys cinera and Annona squamosa*) have been investigated. These plant species are of medicinal attributes and potentials. They have been studies by GC-MS. The target plants have also been assessed for their antimicrobial activity and different antimicrobial responses has been observed.

Recommendations

The following is highly recommended:

1-Other phytochemicals of the studied species may be isolated and their structures may be elucidated and the biological activity could be screened..

2-The isolated oils may be screened for other biological effects such as antiinflammatory, antidiabetic, antiviral, antilashmenial ..etc.

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