Sudan University of Science and Technology

College of Graduate Studies

Study of Oils and Flavonoids From Some Medicinal Plant Grown in Sudan and Their Biological Activity

دراسة الزيوت والفلافونيدات في بعض النباتات الطبية التي تنمو في السودان وفعاليتها البيولوجية

Submitted in fulfillment for the Requirements of the Ph.D degree in Chemistry

By

Shaza Eltigani Osman Ali

(B.Sc. Chemical Laboratory ; M.Sc. Chemistry)

Supervisor

Prof. Mohammed Abdel Karim Mohamed

March, 2021

الاستـهلال

بسم الله الرحمن الرحيم

قال تعالى :

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَاعَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة – الآية (32).

Dedication

To

My parents

To

My dear husband

To

My brother and sisters

Acknowledgment

Thanks at first and last for light of our light **Allah** who gave us the strength while doing this project and guided us through the way in this life. Then I would like to express my special and deep gratitude to Prof. Mohamed Abedel Karim for his guidance and efforts of supervising and leading me through this study this research. Also I thank the staff members of the chemistry laboratory in both University of Gezira and University of Holey Quran for their facilities and not to forget the technicians in the Agricultural Research Corporation and in Cairo University Finally to every person gave something to light my pathway, I thank them for their support.

Abstract

The research is a study of five Sudanese Medicinal plants . The oils from the five plants were studied by gas chromatography- mass spectra and then the biological activity was evaluated. In addition the major flavonoid of Pomegranate (compound I) was isolated by paper chromatography and identified by NMR and UV - Visible data.

GC-MS analysis of Keaaf showed presence of 26 components the major constituents were : 9, 12-octadecadienoic acid methyl ester (29.48 %), hexdecanoic acid methyl ester(21.72 %), 9-octadecenoic acid methyl ester(18.54%) and methyl stearate (9.33 %).

Devil's Claw showed 15components. Major constituents of the oil were: 9,12-octadecadienoicacid methyl ester(43.99%) ;9-octadecenoic acid methyl ester (25.40%) ; hexadecanoic acid methyl ester (12.01%) and methyl stereate(8.89%). El Defra oil gave 23 components the major were: 13,16-docasadienoic acid: (21.00 %), n- hexdecanoic acid(15.00 %) ; oleic acid (9.61 %) . El hantout showed presence of 20 components the major were: 9, 12-octadecadienoic acid methyl ester (32.29 %), hexdecanoic acid methyl ester(25.27 %), methyl stearate(14.64 %) and 9-octadecnoic acid methyl ester(7.32%) . 17 Components were detected in Um mamleha with major constituent: 9, 12-octadecadienoic acid methyl ester (32.37 %) ; hexdecanoic acid (30.93 %); 9-octadecenoic acid methyl ester(19.13%) and methyl stearate (7.53 %).

The studied oils showed varying antimicrobial responses towards standard bacteria and fungi .Um mamleha gave weak antioxidant activity.

المستلخص

البحث عبارة عن دراسة لستة نباتات سودانية لها أثر طبي هي (الكناف، مخا لب الشيطان، الدفرة، الحنتوت، أم ما مليحة، الرمان) تمت دراسة الزيوت للخمس نباتات الأولي بواسطة كروماتوغرافيا الغاز - الطيف الكتلي وتم التعرف نشاطها الحيوي كما تم استخلاص الفلافينويد الرئيس في نبات بواسطة كروماتوغرافيا الورقة وتم التعرف علي تركيبه بواسطة طيف الرنين المغناطيسي و طيف الاشعة فوق البنفسجية.

احتوي زيت الكناف علي 26 مكونا أهمها: 9، 12- استر ميتيل حمض الأوكتاديكانويك بنسبة (29.48%) و استر ميتيل حمض الهكساديكانويك بنسبة (21.72%) و 9- استر ميتيل حمض الأوكتاديكانويك بنسبة (18.54%) واستيارات الميثيل بنسبة (9.33%).
كما احتوي زيت لسان الدبيب علي 15 مكونا اهمها : 9، 12- استر ميتيل حمض الأوكتاديكانويك بنسبة (43.99%) واستر ميتيل حمض الهكساديكانويك بنسبة (12.01%) و 9- استر ميتيل حمض الأوكتاديكانويك بنسبة (25.40%) واستيارات الميثيل بنسبة (8.89 %).
وتضمنت الدفرة 23 مكونا أهمها احماض : 13,16- دوكسادانيك و هكساديكانويك و الاوليك بنسب (21.00%) و (15.00%) و (9.61%) علي التوالي. بينما تضمن الحنتوت 20 مكون أهمها: 9، 12- استر ميتيل حمض الأوكتاديكانويك بنسبة (32.29%) و استر ميتيل حمض الهكساديكانويك بنسبة (25.27%) واستيارات الميثيل بنسبة (14.64%) و 9- استر ميتيل حمض الأوكتاديكانويك بنسبة (7.32%) بالاضافة الي 27 مكون لزيت نبات أم مامليحة والذي احنوي علي المركبات الرئيسه التاليه: 9، 12- استر ميتيل حمض الأوكتاديكانويك بنسبة (32.37%) و حمض الهكساديكانويك بنسبة (30.93%) و 9- استر ميتيل حمض الأوكتاديكانويك بنسبة (19.13%) واستيارات الميثيل بنسبة (7.53%).
أعطت الزيوت نشاط حيوي متفاوت تجاه بعض أنواع البكتريا والفطريات القياسيه كما أعطي زيت نبات أم ما مليحة نشاط مضاد للأكسدة بصورة ضعيفة.

Table of Contents

No.	Subject	Page No.
	الإستهلال	I
	Dedication	Ii
	Acknowledgement	Iii
	Abstract	Iv
	المستخلص	V
	Table of contents	Vi
	List of figures	ix
	List of tables	Xi
	Chapter One: Introduction	
1	Introduction	1
1.1	General overview	1
1.2	*Hibiscus cannabinus L.*	3
1.3	*Proboscidea louisianica*	7
1.4	*Echinochloa colona*	9
1.5	*Ipomoea sinensis*	11
1.6	*Dembra retraflexa*	12
1.7	*Punica granatum,L*	14
1.8	Essential oil	15
1.8.1	Extraction of essential oils	16
1.8.2	Constituents of essential oils	23
1.9	Gas Chromatography couple to Tandem mass spectrometry	29
1.10	The flavonoids	31

	Chapter two: Materials and Methods	
2	Materials and Methods	35
2.1	Materials	35
2.1.1	Plant material	35
2.1.2	Material of Gas Chromatography	35
2.1.3	Test organism	35
2.1.4	Equipments	36
2.1.5	Solvents	36
2.2	Methods	37
2.2.1	Extraction of oil	37
2.2.2	GC-MS analysis	37
2.2.3	Extraction of flavonoids	37
2.2.4	Isolatiom of flavonoids	37
2.2.5	Structure elucidation of flavonoids	38
2.2.5.1	UV-Visible Spectroscopy	38
2.2.5.1.1	UV – Shift reagent	39
2.2.5.1.2	Nuclear Magnetic Resonance Spectroscopy (NMR)	40
2.2.6	Antimicrobial assay	40
	Chapter Three: Results and Discussion	
3	Results and Discussion	41
3.1	*Echinochloa colona*	41
3.1.1	GC/MS analysis	41
3.2	*Ipomoea sinensis*	45
3.2.1	GC/MS analysis	45
3.2.2	Antimicrobial activity	49
3.3	*Dembra retraflexa*	50

3.3.1	GC - MS analysis	50
3.3.2	Antioxidant activity	53
3.4	*Hibiscus cannabinus*	54
3.4.1	GC/MS analysis	54
3.4.2	Antioxidant activity	57
3.5	*Proboscidea louisianica*	58
3.5.1	GC/MS analysis	58
3.5.2	Antimicrobial activity	62
3.6	*Punica granatum*.L	63
3.6.1	Major flavonoid of *Punica granatum*	63
	Conclusion and recommendations	69
	Refrences	

List of figures

No.	Subject	Page No.
1.2	*Hibiscus cannabinus L.*	4
1.3	*Proboscidea louisianica*	7
1.4	*Echinochloa colona*	9
1.5	*Ipomoea sinensis*	12
1.6	*Dembra retraflexa*	13
1.7	*Punica granatum,L*	14
1.8.1	Hydrodistillation apparatus	16
3.1	Total ion chromatogram of *Echinochloa colona*	41
3.2	Mass spectrum of cis-13,16-docasadienoic acid	43
3.3	Mass spectrum of hexadecanoic acid	43
3.4	Mass spectrum of (9-ocatadecenoic acid) oleic acid	44
3.5	Total ions chromatograms of Ipomoea sinensis	46
3.6	Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester	48
3.7	Mass spectrum of hexadecanoic acid, methyl ester	48
3.8	Mass spectrum of methyl stearate	49
3.9	Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester	49
3.10	Total ions chromatograms of Dembra retraflexa	50
3.11	Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester	52
3.12	Mass spectrum of hexadecanoic acid, methyl ester	52
3.13	Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester	53
3.14	Mass spectrum of methyl stearate	53
3.15	Total ions chromatograms of Hibiscus cannabinus L.	54
3.16	Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-,	56

	methyl ester	
3.17	Mass spectrum of hexadecanoic acid, methyl ester	56
3.18	Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester	57
3.19	Mass spectrum of methyl stearate	57
3.20	Total ion chromatograms of *Proboscidea louisianica*	58
3.21	Mass spectrum of 9 12-octadecdienoic acid methyl ester	51
3.22	Mass spectrum of 9-octadecanoic acid methyl ester	61
3.23	Mass spectrum of hexadecanoic acid methyl ester	61
3.24	Mass spectrum of methyl stearate	62
3.25	UV spectrum of compound I	65
3.26	Sodium methoxide spectrum of compound I	65
3.27	Sodium acetate spectrum of compound I	66
3.28	Aluminium chloride spectrum of compound I	66
3.29	Aluminium chloride / HCl spectrum of compound I	67
3.30	HNMR apectrum of compound I	68

List of Tables

No.	Subject	Page No.
2.2.2	Condition of GC-MS instrument	37
3.1	Constituent of *Echinochloa colona* oil	42
3.2	Inhibtion zones(mm) of *Echinochloa colona* oil	44
3.3	Inhibition zones of standard antibacterial agents	45
3.4	Inhibition zone (mm)s of standard antifungal agent	45
3.5	Constituents of *Ipomoea sinensis* oil	46
3.6	Inhibtion zones(mm) of *Ipomoea sinensis* oil	**50**
3.7	Constituents of *Dembra retraflex* oil	50
3.8	Antioxidant activity of *Dmebra retrafexa* oil	53
3.9	Constituents *of the oil Hibiscus cannabinus L.*	54
3.10	Antioxidant activity of *Hibscus cannabinus* oil Proboscid	57
3.11	Constituents of *Proboscidea louisianica* oil	58
3.12	Inhibtion zones(mm) of *Proboscidea louisianica* oil	62

1-Introduction

1.1- General overview

Vegetable oils are mainly fluid hydrophobic compounds at ambient temperature obtained from crushed seed from different plants such as sunflower, canola, soybean, Jatropha, rapeseed, peanut, cottonseed[1]. Vegetable oils have been used for centuries, oil bearing nuts and animal fats were consumed as sources of energy long before nutrition concepts were envisioned[2].

Largest source of vegetable oils is the seeds of annual plants grown in relatively temperate climates, most of these annual plants not only are cultivated as a source of oil, but are also utilized as protein-rich foods; a second source of vegetable oil is oil-bearing trees.

Some oils like olive, coconut, and palm oils are extracted from the fruit pulp rather than the seed of the fruit. Recently, the production of vegetable oils like sunflower and linseed oils has significantly increased; oils are being considered as a major economic resource[3].

The economic signicance of these oils and fats will increase considerably in the future because they represent a vast potential of naturally renewable raw materials in which the chemical and pharmaceutical industries have a special interest[4].

Oils extracted from natural sources are used in various industrial applications such as emulsifiers, lubricants, plasticizers, surfactants,

plastics, solvents and resins .Most plants contain fats or oils, chiefly in their seeds; the amount varies from very little to as much as 40 - 70%[5] .

The physical and chemical characteristics of fats and oils are largely determined by the nature of their molecule[6]. The fatty acid composition of vegetable oils is the main factor influencing their nutritional value and properties[7].

For a number of practical applications, analyses of fats and oils are required, starting with commodity trading. In every fat and oil processing plant, there are analytical requirements for process quality control. In refining, for example, evaluating the free fatty acids (FFA) content of the oil it is necessary to determine the caustic treat, and to serve as a quality indicator in other areas. Evaluation of melting points, fat solids content, and other physical parameters indicate that the product will function as developed.

For final edible-oil products, organoleptic evaluations, peroxide value, free fatty acids, and other analyses are utilized for assurance that the product has the required bland flavor, with predictive analysis, such as active oxygen method (AOM) stability being utilized to ensure proper shelf life[6].

Currently, there are only about 12 of around 500000 known plant species are exploited for the commercial production of vegetable oils[8]. Oils obtained from plants are an important part of the human diets world-wide. The supply of vegetable oil is in excess of 100 million metric tons in the world.

Plants, With the increasing demands of fats and oils, have become the target of researchers in exploring their uses and functional properties . However, there is an urgent need for exploring the production of alternative sources of vegetable oils[8].

1.2- *Hibiscus cannabinus* L. (**Kenaf**)

Hibiscus cannabinus L. is a valuable fiber and medicinal plant of the Malvaceae family, and is an alternative crop that may be a feasible source of cellulose, which is economically viable and ecologically friendly. The leaves and seeds have also been used in traditional medicine in India and Africa for the treatment of various disease conditions. *Hibiscus cannabinus* fibers are commonly used for paper pulp and cordage, but it is also a promising lingo cellulosic feedstock for bio- energy production. The seed oil can be used for cooking and in different industrial applications[9].

In Sudan *Hibiscus cannabinus* is grown extensively as a cordage fiber in Abu-Namaa area (Central Sudan), characterized by high savanna conditions with heavy rains[10].

i)Botanical description

Fig.1.2 : *Hibiscus cannabinus*

Hibiscus cannabinus stems are generally round, and depending on variety, thorns on the stems range from quite tiny to large bush. Stem color varies from pure green to deep burgundy. This plant plant tends to grow as a single unbranded stem when planted at high production densities of 170,000 to 220,000 plants/ha with a height of 2.5 to 6m. The minor constituents in stem are : 0.4 - 0.8% fats and waxes; 0.6 to 1.2% inorganic matter; 0.8-1.5% nitrogenous matter and traces of pigments The fiber content of the bark is about 50-55%, increasing according to the plant population density, while the less valuable short fibers make up about 45-60% of the inner core[11] .

Leaf shape varies and strongly depends on the variety. Further to that, *Hibiscus cannabinus* varieties are divided into two categories; the varieties (Everglades 71) with deeply lobed leaves (usually called split or divided) and varieties (Everglades 41) with shallowly lobed leaves (usually called entire). The entire leaf type has leaves that resemble those of its relatives like okra and cotton[12]. It should be pointed out that the first few juvenile leaves of all *Hibiscus cannabinus* seedlings have more or less an entire shape[11].

Hibiscus cannabinus flowers are large (7.5–10 cm), bell-shaped, and wide open with five petals. The flower color ranges from light cream to dark purple, with a number of shades between them, but apparently never in bright yellow, pink, or red tones. Many cultivars have flowers with a deep red or maroon center[11]**.**

Hibiscus cannabinus has a long effective tap root system and relatively deep, wide-ranging lateral root system making the plant drought tolerant. Further to that, this plant with its tap root system is considered to be an

excellent user of residual nutrients from previous crops. Root has deep root exploration that reaches to more than 1 m depth.

Following pollination, seed capsules are formed that are 1.9–2.5 cm long and 1.3–1.9 cm wide. The seed develops in five-lobular capsules; each capsule contains five segments with a total of 20–26 seeds/capsules. The seed capsules are covered with small hairy structures that are irritating to the human skin. The capsules of the cultivated varieties are generally not splitting and remain intact for several weeks after reaching maturity. From pollination, the seeds require 60–90 days in frost-free conditions to mature. Seeds take roughly 45 days to ripen[12]. The seed is small (1.5–3.3 g/100 seeds), and retains viability for about 8 months under ordinary storage conditions. Seeds are grayish brown, approximately 6 mm long and 4 mm wide [13].

ii) Environmental Requirements

A-Climate

Hibiscus cannabinus is said to have a wider range of adaptation to climates and soils than any other fiber plant in commercial production. Yields have been highest in regions with high temperatures, a long growing season and abundant soil moisture. It is quite sensitive to cool temperatures and grows slowly when temperatures are below 50°F. Variety development for tolerance to cool air and soil temperatures could greatly expand area of productive adaptation [14].

B-Soils

Hibiscus cannabinus is adapted to a wide range of soil types, but performs best on the heavier, well drained, fertile soils. The plant does not perform

well on soils with severe drainage problems. Prolonged periods of standing water, particularly during the seedling stage, can severely inhibit growth [15].

1.3- *Proboscidea louisianica*

Proboscidea louisianica (**Mill**.), a member of the family Martyniaceae.The plant is also known as devil's claw or as unicorn-plant or ram's horn. It is a spreading annual with stems up to 80 cm long and large, entire, opposite leaves up to 30 cm wide. The entire plant is covered with glandular hairs or trichomes, each tipped by a droplet of oil which makes the plant odoriferous and oily to sight and touch .

Fig.1.2 : *Proboscidea louisianica*

The fruit is a drupaceous dehiscent capsule with a stout fruit body up to 100 mm long. The fruit body is terminated by an incurved beak that is longer than wide; at maturity the outer exocarp dries and falls away and the endocarp beak splits to form a 2-horned claw. The fruits with their vicious claw-like appendages give the plant its common name, Devil's Claw[15].

A white-seeded *Proboscidea louisianica* is sometimes cultivated in the Western U. S.; the young fruit may be pickled for food or the mature fruit may be used as ornaments or as basketry fiber.

The leaves are cordate with crisped edges, and are opposite near the base, but become alternate toward the apex of the plant. The strongly scented flowers are borne in racemes of 8-20 flowers at the summit of the stems and branches. The lavender, pink, or almost white flowers have yellowish and purplish mottling inside the throat. The corolla is 3-6 cm in length and bell-shaped with five lobes forming two lips [16-18].

Proboscidea louisianica is native to the southwestern U. S. and northern Mexico, and is the most widely distributed member of its family, ranging from Florida to California, north to Minnesota and south to Mexico .Proboscidea louisianica (Mill.) *Proboscidea louisianica* can be found growing in disturbed soils and waste places from West Virginia to Illinois and Minnesota and southward to Georgia and Mexico.

Proboscidea louisianica is an erect or prostrate freely branched summer annual which grows 3-8 dm tall. The entire plant is covered with viscid, glandular hairs whose secretions give the plant a fetid odor.

1.4- *Echinochloa colona* (El Defra)

Echinochloa colona (L.) Link. is an annual plant in the family Poaceae. It has become one of the world's most serious grass weeds [19,20]. It is a major weed in many crops, including rice, corn, sorghum, sugar cane,cotton, peanut, and cassava (Holm *et al.*, 1991). Losses in crop yield production due to this weed have been reported in several studies[21].This weed is also an alternate host of diseases, insects, and nematodes[19].

Fig. 1.4 : *Echinochloa colona*

Echinochloa colona is widespread throughout different habitat types and is adominant species of weed communities of summer crops and orchards in Egypt [22,23].

Echinochloa colona is characterized by a high relative growth rate (RGR) together with a high dry matter investment into leaves, during seedling and juvenile stages. This promotes the competitive ability of the species and may ensure a resource turnover from vegetative to reproductive structures later in the plant life cycle[23].

Echinochloa colona begins to produce flower buds early in its lifespan, a behavior that ensures some seed production even in years with a short growing season[23]. Though *Echinochloa colona* is one of the most serious grass weeds, its phytotoxic potential has received little attention.[24] evaluated the allelopathic potential of *Echinochloa colona* leachates on rice. They pointed out that rice root growth was completely inhibited with 10% w/v leachates of 60 days old plant, and that the decomposing and

decomposed leachates reduced rice shoot growth by 57% and 84%, respectively.

Echinochloa colona is: annual grass; tufted, erect and jointed, often flat on the ground, 30-75 cm high. Reddish-purple or green. Leaves linear to10-15 cm long. Seed head is a panicle with 3-10 branches 5-15 cm long. Propagates by seeds. Rooting at nodes. Root is fibrous, white or brown. Stem**;** usually grows outwards at the base before turning upwards and are often purple near the base. Leaves: glabrous, up to 25cm long and 3-8 mm wide, sometimes banded with purple and ligule absent.

Flower**s:** the spikes making up the inflorescence are up 3 cm long and 3-4 mm wide, usually about half their length a part on the main stem, which they join at acute angle. They are made up of numerous almost stalkless spikelets arranged in four distinct rows. Individual spikelets are 2-3 cm long, oval in shape with a pointed tip (but not extended into an awn as in the related E. crus-galli) and contain a single fertile floret. Seed head is a panicle with 3-10 branches 5-15 cm long[24].

Echinochloa colona propagates mostly by seeds but also vegetatively; one jungle rice plant can produce 3000 to 6000 seeds. It germinates during the rainy season or when water levels are on the rise and dies out during the dry season. The flowering starts 3 or 4 weeks after germination, quickly followed by fructification and the first seeds come to maturity 45 days later.

Echinochloa colona is often used in times of food shortage as a famine food. In Chad (central) and Sudan (Kordofan, Darfur) the seeds of this plant are ground into flour from which porridge or bread can be prepared. In Rajasthan in India the seeds are used as rice - hence its English common name of 'jungle rice'.

1.5 - ***Ipomoea*** *sinensis*

Ipomoea *sinensis (*Desr.) Choisy.) is an annual herb with numerous stems growing from a taproot, prostrate or twining, up to 2.5 m long, more or less densely covered with white spreading hairs, particularly when young.
Leaves**:** ovate to ovate-oblong, 2-10 cm long, broadly cordate or rarely sub-hastate at the base, pubescent or hairless; petiole slender, 1-10 cm long.

Fig.1.5 : ***Ipomoea*** *sinensis*

Flowers : axillary, 1-3 on a slender peduncle, up to 6.5 cm long, hairy; pedicels up to 1.9 cm long, hairy, erect in flower, reflexed in fruit. Bracts : minute, lanceolate. Sepals : (in this subspecies) 4-11 mm long, enlarging in fruit up 23 mm long, unequal, outer ones ovate with a broad cordate to subhastate base and a long narrow lanceolate apex; inner ones lanceolate with a somewhat broadened base. Corola : in this subspecies normally white, up to 2.2 cm long. Capsule : globose with an apiculate apex formed by the persistent style-base, hairless.
Stem : pubescent with pilose hairs intermingled in youngest parts, trailing and climbing[24].

1.6- *Dmebra retroflexa*

*Dmebra retroflexa (*Vahl.) Panz**.** is a pilose hairy, tufted annual grass up to 1.2 m high, usually around 0.5 m or smaller growing around Nile banks[24].

Fig.1.6 : *Dmebra retroflexa*

i)Seedling

First leaves ovate-lanceolate, a cute at the apex, slightly narrowed at the base, sparsely pilose hairy on upper surface, not keeled, around 2.0 mm long. Leaf sheath loose, bearing single pilose hairs[24].

ii)Adult plant

Culm striate, glabrous, nodes glabrous, often purplish. Leaves Linear-lanceolate finely acuminate at the apex; more or less pilose hairy on upper surface, rarely glabrous; slightly keeled at the base. Leaf sheath loose, more or less pilose hairy, rarely glabrous; mouth, often with purple ring[24].

iii)Distribution

Dmebra retroflexa is widely spread in River Nile and Gezira but abundant in EL Rahad[24]

1.7- *Punica granatum*

Fig.1.6 : *Punica granatum*

Punica granatum L. (pomegranate) comprises about 500 cultivars with worldwide distribution. It differs in having pink flowers and smaller, less sweet fruit. Pomegranate is used in baking, beverages, and cooking. Moreover, *P. granatum* is largely used for the treatment of several diseases across different cultures and civilizations. Many studies reported the functional properties of pomegranate extracts and juice. This plant has antioxidant, antiinflammatory, antitumor, neuroprotective, cardiovascular, and antidiabetic effects.

P. granatum is a shrub or small tree growing 5 to 10 m (16 to 33 ft) high, the pomegranate has multiple spiny branches and is extremely long-lived, with some specimens surviving for 200 years[24].

1.8- Essential oils

The French Agency for Normalization gives the following definition for essential oils :The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, ordry distillation. The essential oil is then separated from the aqueous phase by physical means [25].

Essential oils may occur in all parts of the plant.Essential oil is a liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as oil of clove. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majorities of essential oils are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits [26].

Essential oil may occur in various parts of aromatic plants:

• Flowers, including: orange, pink, lavender, and the (clove) flowerbud or(ylang-ylang) bracts,

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

• Rhizomes (ginger, sweet flag),

• Seeds (carvi, coriander),

• Fruits, including: fennel, anise, Citrus epicarps,

• Wood and bark, including: cinnamon, sandalwood, rosewood.

1.8.1-Extraction of essential oils

There are several methods for the extraction of essential oils from their natural matrix [27,28]

a)Hydrodistillation

hydrodistillation (HD) is a conventional method used for the extraction of essential oils, in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser.The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively[29,30].

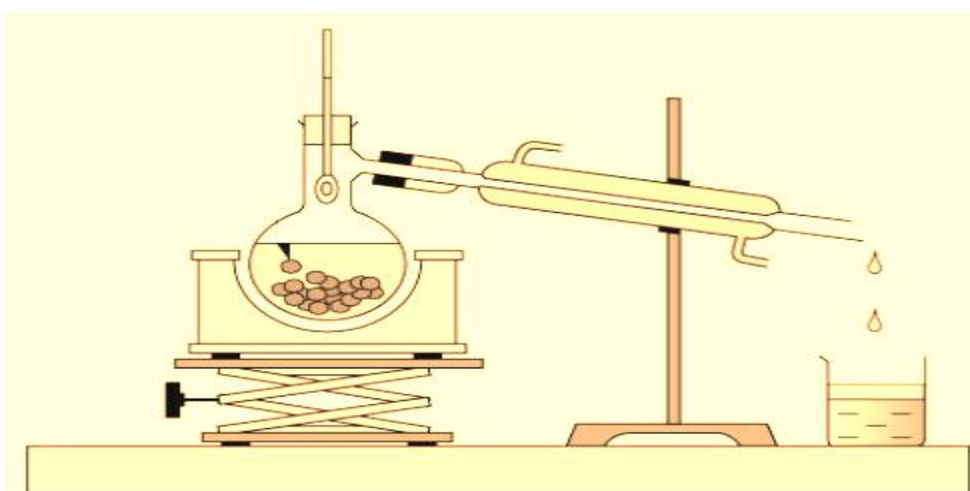


Fig.1.8.1 : Hydrodistillation apparatus

b)Solvent extraction

Solvent exraction, is a separation method to separate a compound based on the solubility of its parts. This is done by using two liquids that don't mix, for example, water and an organic solvent . In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with

perforated trays of essential oil plant material and repeatedly washed with the solvent[31].

c)Soxhlet extraction

The Soxhlet extraction is a technique involving solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid [31].

iv)Cold pressing extraction

In the process of cold pressing the oil is extracted from its matrix at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils [32]. In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

v)Steam Distillation

The technique of extraction known as steam distillation is specially designed for the extraction of heat-sensitive plant constituents such as natural aromatic compounds.

In this technique the plant materials which are charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material . This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected [33]. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.

Some efficient modern extraction techniques have some advantages over the conventional methods mentioned above including: reduction of extraction times, reduction of energy consumption, reduction of volumes of solvents used . Some of these innovative techniques are discussed briefly below:

a)Supercritical fluid extraction

During this innovative technique of extraction a supercritical fluid is employed as the extracting solvent. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. In practice, more than 90% of all analytical

supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32ºC), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [34]. The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes [35]. These essential oils can include limonene and other straight solvents.Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods[36]. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of superior quality, with better functional and biological activities [37]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

b)Extraction by microwave hydrodiffusion

The technique known as microwave hydrodiffusion and gravity (MHG) is a green technique used for the extraction of essential oils. It is originally a microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants [37].Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and

as it does require less energy[38].The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental impact andpower saving [39,40].

b)Solvent-free microwave extraction

Another innovative extraction technique used for the extraction of essential oils is the so called solvent-free microwave extraction (SFME) . The extraction is performed by the water which exists within the matrix without using any solvent [41]. Based on the integration of dry distillation and microwave heating energy, . It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent [42]. In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature, and extraction time were controlled by the panel in the instrument.

d)Ultrasonic-assisted extraction

A technique that can achieve high valuable compounds is the so called ultrasonic-assisted extraction. This process is reputed as an excellent technique and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material[43]. The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive

combinations. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydro distillation . In these applications the power ultrasonic increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer[44]. In ultrasonic-assisted extraction the plant material is immersed in water or another solvent (methanol or ethanol or any other solvent) and at the same time, it is subjected to the work of ultrasound[45]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds [46].

e)Microwave-Assisted Hydrodistillation

A microwave oven is used in the extraction process known as microwave-assisted hydrodistillation. The efficiacy of this extraction process is strongly dependent on the dielectric constant of water and the sample [47]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydro distillation technique(Scheme8). Application of icrowave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [48,49]and consuming only a fraction of the energy used in conventional extraction methods[50].The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of

conditions.Microwave-assisted hydro distillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, olumetric heating and controllable heating process. The principle of heating using Microwave-assisted hydro distillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously[51].

1.8.2-Constituents of essential oils

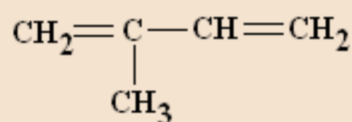
Essential oils are primarily mixtures of a large number of components. They contain terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups[52,53]:

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

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

i)Hydrocarbons

Basic hydrocarbon found in plants are built of isoprene units.The structure of isoprene is illustrated below[53].



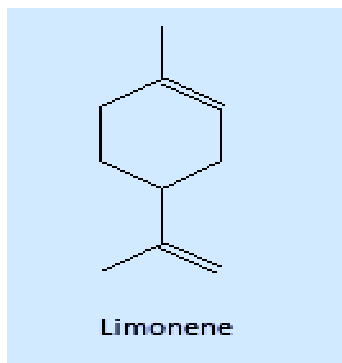
(Isoprene)

ii)Terpenes

Terpenes present in essential oils are known to possess diverse biological activites including: anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes, diterpenes , triterpenes and polyterpenes. Referring back to isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes[53].

i)Monoterpenes

Monoterpenes are naturally occurring compounds. A Mono-terpene has the molecular formula : **[$C_{10}H_{16}$].**The biological activity of monoterpense include: analgesic, bactericidal, expectorant, and stimulant effects. Some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids.An Example of monoterpenes is limonene [53].



Monoterpenes are branched-chain C_{10} hydrocarbons with two isoprene units and they are of wide distribution in nature with more than 400 naturally occurring monoterpenes identified.Some of these being linear derivatives (geraniol, citronellol). The monoterpenes can be monocyclic like camphor – bicyclic like pinenes (**α** and **β**) or tricyclic . Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium* (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses[53].

ii)Sesquiterpenes

Sesquiterpenes constitute a very large group of secondary metabolites .They are endowed with some biological activies including: anti-inflammatory, anti-septic, analgesic, anti-allergic.

The secondary metabolites – sesquiterpenes- are biogenetically derived from farensyl pyrophosphate and their structure may be linear, monocyclic or bicyclic., some have been shown to be stress compounds formed as a result of disease or injury.

More than five hundred sesquiterpene lactones are now known. They are particularly characteristics of the Compositae but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents[53].

Such lactones are classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides , pseudoguaianolides , eudesmanolides , eremophilanolides , xanthanolides, etc[53].

iii)Diterpene

Diterpenes are built from four isoprene units. They are known to possess some biological properties including: hormonal balancers, hypotensive, anti-fungal, expectorant,.

Diterpenes are rarely found in distilled essential oils since they are too heavy to allow for evaporation with steam in the distillation process.

Diterpenes occur many plant families and consist of compounds having a C20 skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate [53].

iv)Alcohols

The biological potential of alcohols include: antiseptic, antiviral, bactericidal and germicidal.

Alcohols are known to exist naturally in a free form, or combined with a terpenes or ester. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use[53].

v)Aldehydes

Natural aldehydes are anantifungal, anti-inflammatory, anti-eptic, antiviral, bactericidal, disinfectant, sedative. Medicinally, essential oils containing aldehydes are effective in treating *Candida* and other fungal infections[53].

vi)Esters

Those essential oils which contain esters are antimicrobial agents and are utilized for their soothing, and balancing effects. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system[53].

viii)Ketones

Ketones extracted from plants are anti-catarrhal, cell proliferant and expectorant.Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually

(not always) very toxic. The most toxic ketone is thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil[53].

ix)Lactone

Plant lactone can reduce prostaglandin synthesis and may act as expectorant. They possess antiinflammatory, antiphlogistic, expectorant and febrifuge activity. Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the actions. Lactones have an even stronger expectorant action than ketones[53].

1.9-Gas Chromatography coupled to Tandem mass spectrometry

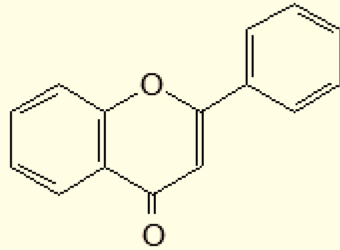
Essential oils(EOs) analysis is based mostly on separation techniques giving the best performance, achieved by the most effective tool. The most popular tool used by scientists for separation techniques is the chromatography and coupled to that is often the mass spectrometry for the identification of components. Analysis of EOs have recently known major developments with varying methods adapted from the conventional gas chromatography coupled to mass spectrometry technique. The driving force of this surge has been the characterization and identification of the structure of known and novel molecules. The advantage of using a gas chromatograph is that it provides the conditions required for achieving the separation of analyte components without lowering the performance of the column when it comes to more complex analysis. However gas Chromatography can be insufficient or difficult to interpret. Presently, we have seen in the literature the use of the gas chromatography coupled to Tandem mass spectrometry. It

is a powerful analytical technique which offers the possibility of detecting specific, targeted compounds whether present in large amount or in trace[54]. Following the separation by gas chromatography, the Tandem mass spectrometry operates by selecting the target ions having specific and known mass. These ions are then dislocated by collision with helium molecules. The product ion resulting from this collision gives a spectrum which confirms the target analyte as even if there is another ion with the same mass, the spectrum will be different. This factor increases the selectivity of the tandem mass spectrometry. The target gas, which can be argon, xenon, helium or other (according to choice of energy desired for the collision ion dissociation process), can play an important role in the results as the pressure and temperature of the target gas affect the internal energy distribution and thus affecting also the mass spectrum. Hence, low energy target gas is less reproducible. Whereas the high energy target gas for the collision ion dissociation process was found to be more reproducible and to give less rearrangement in the mass spectrum making it less complex to analyze[54]. According to the literature, gas chromatography coupled to Tandem mass spectrometry is not only commonly used for the regular analysis of EOs but it remains however an accurate tool for the separation and detection of trace elements found in a complex mixture.

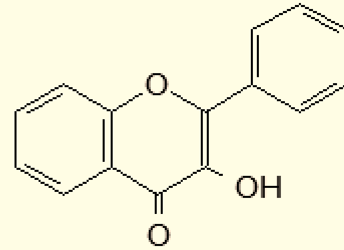
1.10-The flavonoids

Flavonoids are phenolic compounds widely present in plants and foods of plant origin[55,58]. Flavonoids contain fifteen carbon atoms in their basic nucleus- flavan, arranged in a C6-C3- C6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third heterocyclic ring (C). There are six major subgroups:

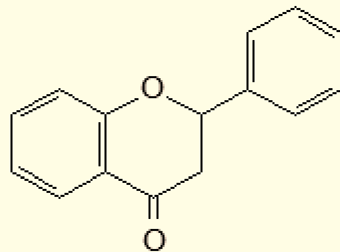
chalcones, flavones, flavonols, flavanones, flavans, dihydrochalcones, dihydroflavonols anthocyanins and isoflavonoids.



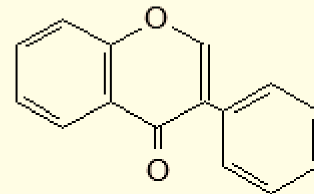
Flavone



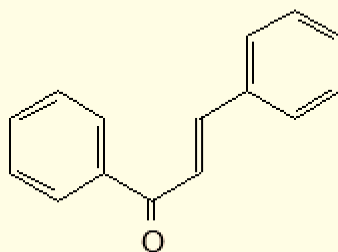
Flavonol



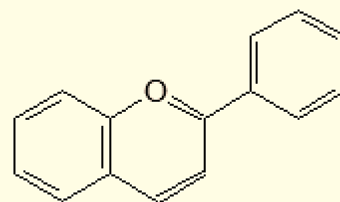
Flavanone



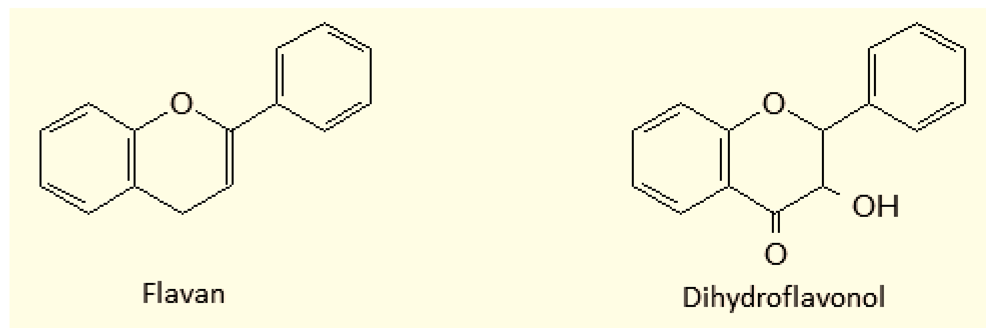
Isoflavone

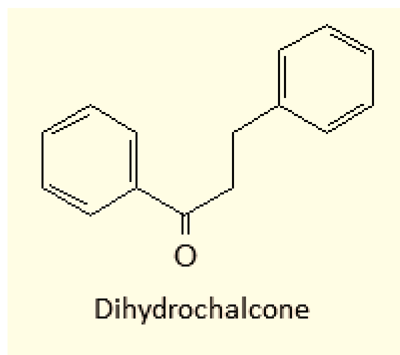


Chalcone



Anthocyanin





Flavonoids encompass a large group of polyphenolic substances with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antivirl and vasodilator effects[59,61].

Aim of this study

This study was designed to:

-Investigate the oils from five Sudanese plants of potential medicinal attributes.

-Analyze the targeted oils by GC/MS to identify and quantify the constituents of the oils.

-Evaluate the targeted oils for antimicrobial and antioxidant activity.

-Extract the major flavonoid of pomegranate.

2-Materials and Methods

2.1-Materials

2.1.1-Plant materials

The seeds of *Echinochloa colona*, *Ipomoea sinensis Dmebra retrafexa*, *Hibscus cannabinus*, *Proboscidea louisianica* and the fruits of *Punica granatum* were purchased from the local market-Khartoum - Sudan. The plants were identified and authenticated by direct comparison with a reference herbarium sample.

2.1.2-Materials for paper chromatography

i. Whatman paper No (3mm) for preparative paper chromatography (Whatman Ltd., England).

ii- Glass jars (rectangular glass tanks 100 x80 x 40cm) for developing PC chromatograms.

2.1.3-Test organisms

- Gram +ve

Bacillus subtilis and Staphylococcus aureus.

- Gram -ve

Escherichia coli and *Pseudomonas aeruginosa* .

- Fungal strains

Candida albicans.

- Positive controls

i. Pencilin: for G+ve bacteria.

ii. Gentamycin: for G-ve bacteria.

iii. Clotrimazole: antifungal standard.

-Media for bacteria

Muller –Hinton agar

- Media for fungi

Sabouraud Agar (oxoid, England) is used as media for fungal growth:

2.1.4- Equipments

1- Ultra - Violet - Visible spectrophotometer (Shimadzu model UV240 and 240PC) .

2- Joel- Nuclear Magnetic Resonance (NMR) pectrophotometer, (Brucker AC-250) operating at 500 MHz.

3- Shimadzo GC-MS-QP2010 Ultra instrument with a RTX- MS column (30m,length ; 0.25mm diameter ; 0.25 μm, hickness) was used **.**

2.1.5. Solvents

Analytical grade solvents were used. Ethanol (Merck, Germany) was used for spectrophotometric analysis . DMSO-d_6 was used as NMR solvent and TMS as internal standard.

2.2- Methods

2.2.1-Extraction of oil

Powdered plant material (250g) was macerated with n-hexane for 48hr.The solvent was removed under reduced pressure giving the oil.

2.2.2-Gas Chromatography –Mass Spectra analysis

The studied oils were analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument - chromatographic conditions are shown below .

Table 2.2.2 : Condition GC-MS instrument

Column oven temperature	150.0ºC
Injection temperature	300.0ºC
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/mi
Spilt ratio	- 1.0

2.2.3- Extraction of flavonoids

The target plant material(1Kg) was macerated with 95% ethanol for 48h. at room temperature.The extract was filtered and the solvent was removed *in vacuo*.

2.2.4-Isolation of flavonoids

Concentrated plant extract was applied on Whatman 3mm paper (46×57 cm) and run in BAW(4:1:5;v:v:v). The dried paper were viewed and examined under visible and ultraviolet light. The chromatograms were then located under UV light , cut off and similar bands were joined and cut into small pieces and slurred with methanol. After several hours of contact the solvent was removed. Compound I was thus isolated in a chromatographically pure form.

2.2.5.Structural elucidation of flavonoids

The structure of the isolated compound was elucidated via a combination of spectral techniques as illustrated below.

2.2.5.1-`UltraViolet-Visible Spectroscopy

UV-Visible spectra of the isolated flavonoids were recorded on a Shimadzu spectrophotometer. Spectra were recorded in a quartz cuvette (1cmx1cmx4.5cm) which did not absorb over the spectral region of interest. One (mg) of the isolated flavonoid was dissolved in HPLC grade methanol (10mL) to create a stock solution. The UV-visible spectrum was taken for this solution at a rapid scan rate (600nm/min) to check the optical density. The concentration of the flavonoid solution was then adjusted so that the optical density of the major absorption peak (between 200-400nm) was within 0.6 to 0.8AU. The spectrum of the flavonoid stock solution was measured at a normal scan speed 50nm/min) over the 200-500nm region. Additional spectra were measured over the regions of peak maxima at a reduced scan rate (10nm/min) to determine the wavelength of maxima accurately. Sodium methoxide solution (3 drops) was added to the cuvette of flavonoid in methanol. The spectrum was then recorded immediately at a normal scan rate. The solution in the cuvette was then discarded. Aluminium chloride solution (6 drops) was then added to fresh flavonoid stock solution (2-3mL) in the cuvette. The spectrum was then recorded at a normal scan rate. Hydrochloric acid (3 drops) was then added to the cuvette containing aluminium chloride and flavonoid in methanol. The spectrum was recorded again and the solution discarded. An excess of powdered anhydrous sodium acetate was added to fresh flavonoid stock solution (2-3mL) in the cuvette and shaken. The powdered sodium acetate formed a layer (1-2mm) at the bottom of the cuvette. The spectrum was recorded immediately.

2.2.5.1.1. UltraViolet - Shift Reagents

-Sodium methoxide solution

Freshly cut 2.5g metallic sodium was dissolved, cautiously, in 100 ml spectroscopic methanol (dry methanol).

-Aluminum chloride solution

(5g) anhydrous aluminium chloride was cautiously dissolved in 100 mL spectroscopic methanol and filtration was carried out after about 24 hours.

- Hydrochloric acid

(50 mL) concentrated hydrochloric acid was mixed with 100 mL distilled water, then keep in glass bottle.

- Sodium acetate

Anhydrous reagent grade sodium acetate was melted and allowed to stand for about 10 minutes. The material was then powdered and stored in a dry bottle.

2.2.5.1.2-Nuclear Magnetic Resonance Spectroscopy of (NMR)

NMR spectrum was obtained on a Bruker Advance DRX instrument. Sample was dried by freeze drying over several days and dissolved in DMSO-*d6* and then analyzed.

2.2.6- Antimicrobial assay

An inoculum suspension (20 mL Mueller-Hinton Agar (MHA) for bacteria was swabbed uniformly to solidify. Then it was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using glass Pasteur pipettes. Aliquot of 20 µl from each test sample (100 mg/mL) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37°C for 24 h.

The resulting inhibition zones were measured in millimeters (mm)The assays were repeated in triplicate and the concurrent values were taken. The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate 13-16 mm and high greater than 17 mm.

3-Results and Discussion

Inthisstudytheoilsfromfivepotentialmedicinalplants(*Echinochloacolona*,*Ipomoeasinensis**Dmebraretrafexa*,*Hibscuscannabinus*and*Proboscidealouisianica*,)hasbeeninvestigatedbyGC.MSandtheantimicrobialactivityhasbeenscreened.APhytochemicalscreeningof*Punicagranatum*indicatedthatthisspeciesisrichinphenolics,henceitwasdecidedtoinvestigatetheflavonoidsofthisspecies.

3.1-*Echinochloacolona*

3.1.1-GasChromatography/MassSpectraanalysis

Gaschromatography-massspectrometryhasbeenusedfortheidentificationandquantificationofthe*Echinochloacolona*oil.Theanalysisrevealedthepresenceof23components-Table(3.1).ThetotalionchromatogramispresentedinFig.3.1.

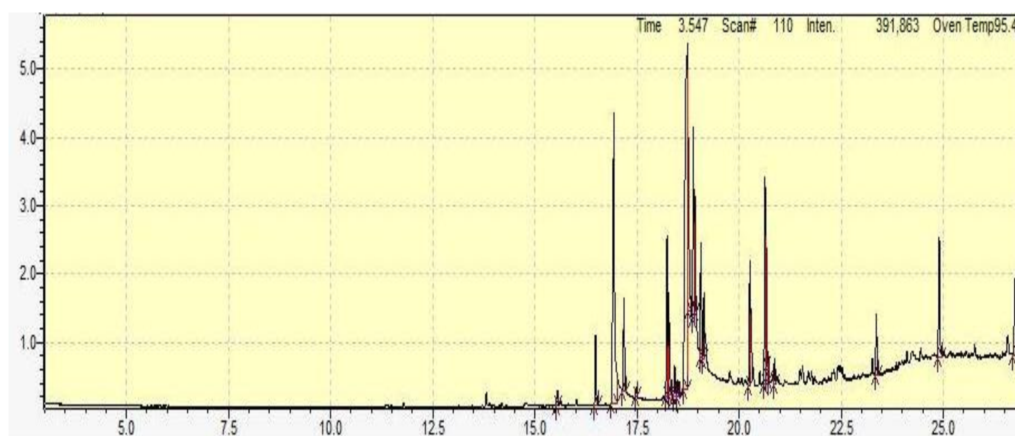


Fig.3.1:Totalionchromatograms

Table3.1: Constituentof*Echinochloacolona*oil

No.	Name	Ret.Time	Area%
1.	3,7,11,15-Tetramethyl-2-hexadecen-1-olS	15.547	0.43
2.	Hexadecanoicacid,methylester	16.488	2.08
3.	n-Hexadecanoicacid	16.933	15.00
4.	Hexadecanoicacid,ethylester	17.183	2.67
5.	Isopropylpalmitate	17.481	0.24
6.	9,12-Octadecadienoicacid(Z,Z)-,methylester	18.239	5.21
7.	9-Octadecenoicacid(Z)-,methylester	18.281	3.28
8.	Phytol	18.424	0.96
9.	Methylstearate	18.505	0.43
10	cis-13,16-Docasadienoicacid	18.714	21.00
11	OleicAcid	18.743	9.61
12	Linoleicacidethylester	18.880	5.98
13	EthylOleate	18.917	3.20
14	Hexadecanoicacid,butylester	19.067	3.52
15	9,12-Octadecadienoicacid,ethylester	19.142	2.07
16	Butyl9,12-octadecadienoate	20.265	3.97
17	n-Propyl9,12-octadecadienoate	20.638	6.91
18	Oleicacid,butylester	20.660	2.62
19	Butyl9,12,15-octadecatrienoate	20.715	0.74
20	Octadecanoicacid,butylester	20.864	0.65
21	Tetratetracontane	23.363	1.73
22	Hexatriacontane	24.901	4.17
23	Tetracontane	26.763	3.53

Thefallowingcompoundsweredetectedinthechromatogramasmajorconstituents:

i)cis-13,16-Docasadienoicacid(21.00%)

ii)n-Hexdecanoicacid(15.00%)
iii)Oleicacid(9-Octadecenoicacid)-(9.61%)

Fig.3.2showsthemassspectrumofcis-13,16-docasadienoicacid.Thepeakatm/z336(RT.18.741)correspondsM$^+$[$C_{22}H_{40}O_2$].ThemassspectrumofhexdecanoicacidispresentedinFig.3.3.Thepeakatm/z256whichappearedat(RT.16.

933)isduetoM^{+}[$C_{16}H_{32}O_2$].Fig.3.4illustratesthemassspectrumofoleicacid(9-octadecenoicacid).Thesignalatm/z282whichappearedatRT18.743accountsforthemolecularion:M^{+}[$C_{18}H_{34}O_2$].

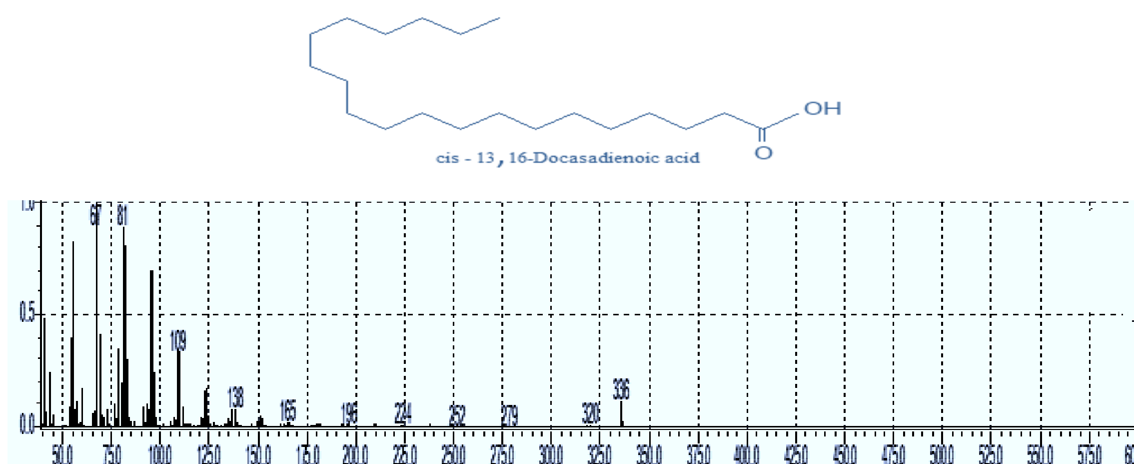


Fig.3.2:Massspectrumofcis-13,16-docasadienoicacid

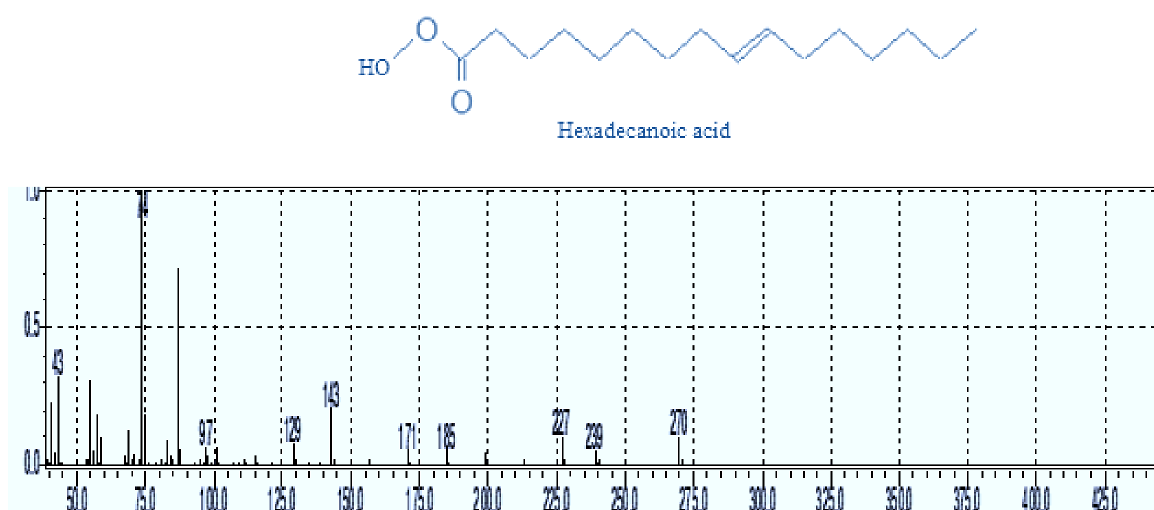
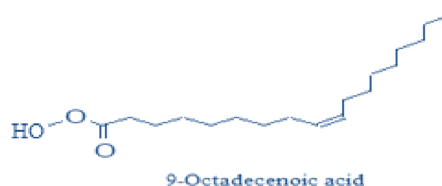


Fig.3.3:Massspectrumofhexadecanoicacid



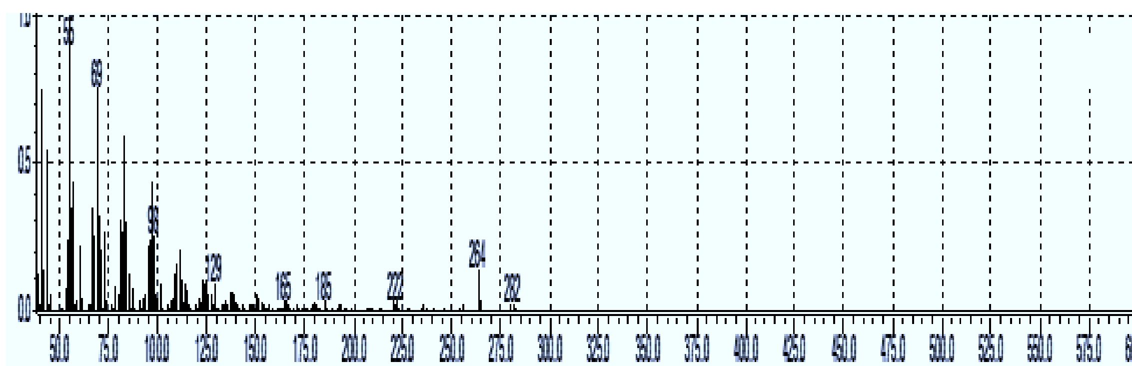


Fig.3.4:Massspectrumof(9-ocatadecenoicacid)oleicacid

3.1.2-Antimicrobialactivity

*Echinochloacolona*oilwasscreenedforantimicrobialactivityagainstfivestandardorganisms .TheinhibitionzonesarepresentedinTable3.2.Theoilshowedgoodactivityagainst*Escherichiacoli*,*Pseudomonasaeroginosa*and*Candidaalbicans*.Tables3.3and3.4illustratetheantimicrobialactivityofstandarddrugs.

Table3.2:Inhibtionzones(mm)of*Echinochloacolona*oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil100mg/mL	--	---	15	15	14

Sa.:Staphylococcusaureus.
Bs.:Bacillussubtilis.
Ec.:Escherichiacoli.
Pa.:Pseudomonasaeroginosa.
Ca.:Candidaalbicans.

Table3.3:Inhibitionzonesofstandardantibacterialagents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-

Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table3.4:Inhibitionzone(mm)sofstandardantifungalagent

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

3.2-*Ipomoeasinensis*HantoodShH

3.2.1-GasChromatography-MassSpectraanalysis

Gaschromatography-massspectrometryhasbeenusedfortheidentificationandquantificationofthestudiedoil.Theanalysisrevealedthepresenceof20components-Table(3.5).ThetotalionchromatogramispresentedinFig.3.5.

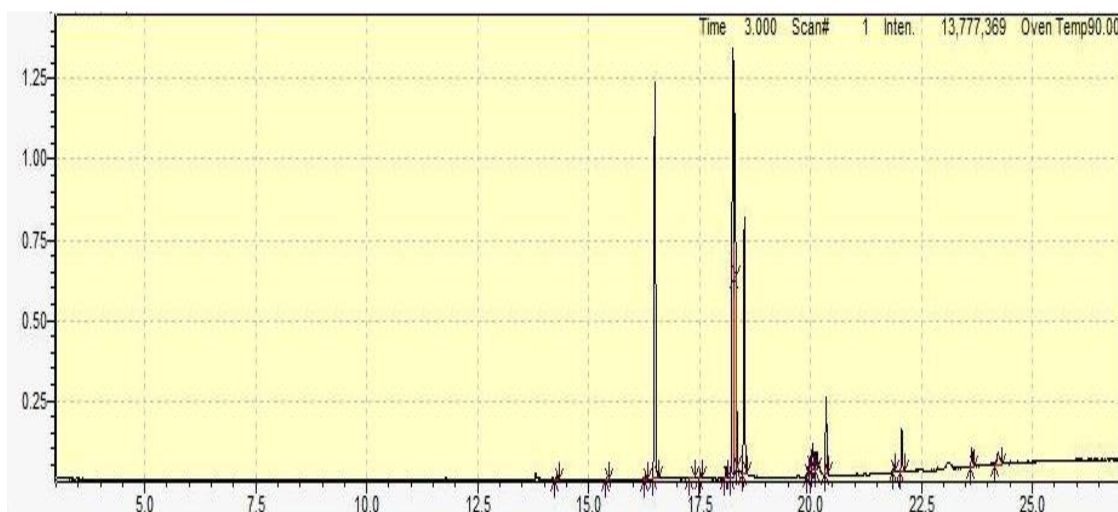


Fig.3.5:Totalionschromatograms

Table3.5:Constituentsoftheoil

No.	Name	Ret.Time	Area%
1.	Methyltetradecanoate	14.275	0.20
2.	Pentadecanoicacid,methylester	15.407	0.03
3.	9-Hexadecenoicacid,methylester,(Z)-	16.288	0.33
4.	Hexadecanoicacid,methylester	16.497	25.27
5.	cis-10-Heptadecenoicacid,methylester	17.306	0.09
6.	Heptadecanoicacid,methylester	17.519	0.22
7.	4,7,10-Hexadecatrienoicacid,methylester	18.097	0.62
8.	9,12-Octadecadienoicacid(Z,Z)-,methylester	18.259	32.29
9.	9-Octadecenoicacid(Z)-,methylester	18.302	7.32
10	9,12,15-Octadecatrienoicacid,methylester,(Z,Z,Z)-	18.325	6.03
11	Methylstearate	18.511	14.64
12	Cyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-,methylester	19.957	0.45
13	11,14-Eicosadienoicacid,methylester	19.995	0.44
14	Heptadecanedioicacid,9-oxo-,dimethylester	20.049	1.36
15	Oxiraneoctanoicacid,3-octyl-,methylester,cis-	20.121	1.04
16	Eicosanoicacid,methylester	20.356	3.91
17	13-Docosenoicacid,methylester,(Z)-	21.882	0.31
18	Docosanoicacid,methylester	22.060	2.47
19	Heneicosanoicacid,methylester	23.640	1.15
20	.gamma.-Sitosterol	24.236	1.83

Thefallowingcompoundsweredetectedinthechromatogramasmajorconstituents:

i)9,12-octadecadienoicacidmethylester(32.29%)

ii)Hexdecanoicacidmethylester(25.27%)
iii)methylstearate(14.64%)

iv)9-Octadecnoicacidmethylester(7.32%)

Fig.3.6showsthemassspectrumof9,12-octadecadienoicacidmethylester.Thepeakatm/z294(RT.18.259)correspondsM^+[$C_{19}H_{34}O_2$

]$^+$.ThemassspectrumofhexdecanoicacidmethylesterispresentedinFig.3.7.Thepeakatm/z270whichappearedat(RT.16.497)isduetoM$^+$[$C_{17}H_{32}O_2$]$^+$.Fig.3.8illustratesthemassspectrumofmethylstearate.Thesignalatm/z298whichappearedatRT.18.511accountsforthemolecularion:M$^+$[$C_{19}H_{38}O_2$]$^+$.Themassspectrumof9-ctadecenoicacidmethylesterisshowninFig.3.9.Thepeakatm/z296whichappearedat(RT.18.302)isduetoM$^+$[$C_{19}H_{36}O_2$].

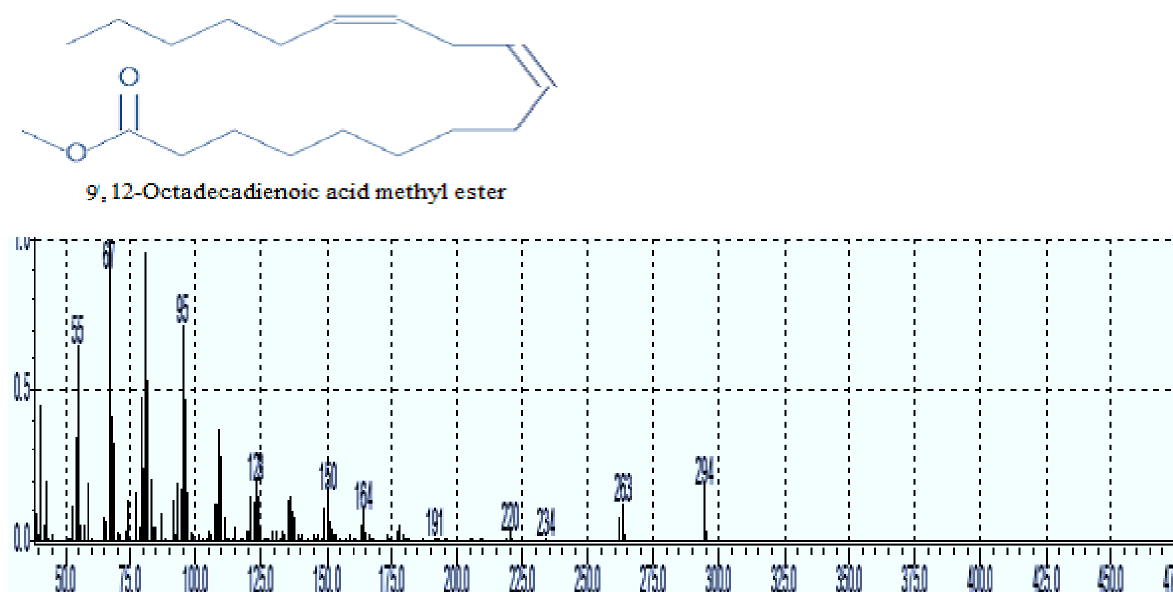
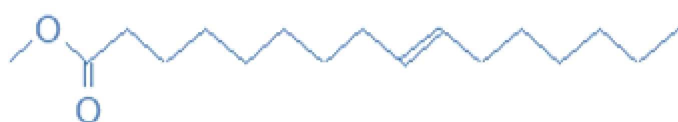


Fig.3.6:Massspectrumof9,12-octadecadienoicacid(Z,Z)-,methylester



hexadecanoicacid,methylester

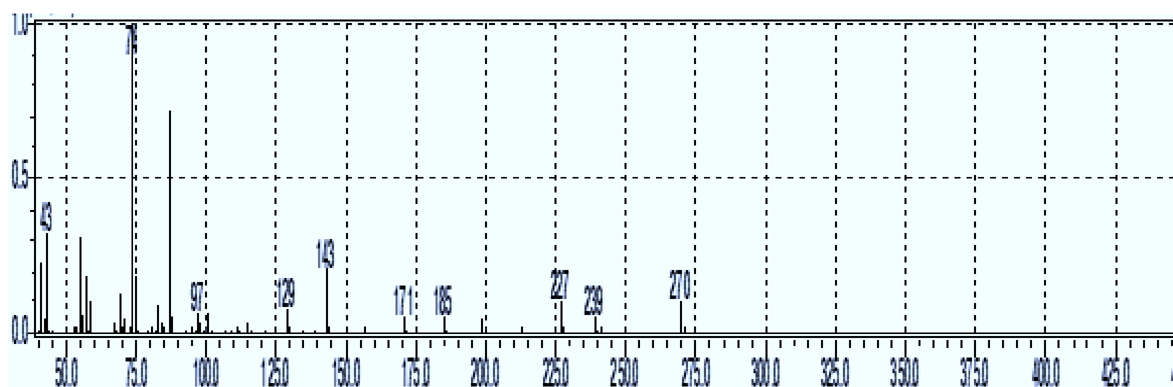


Fig.3.7:Massspectrumofhexadecanoicacid,methylester

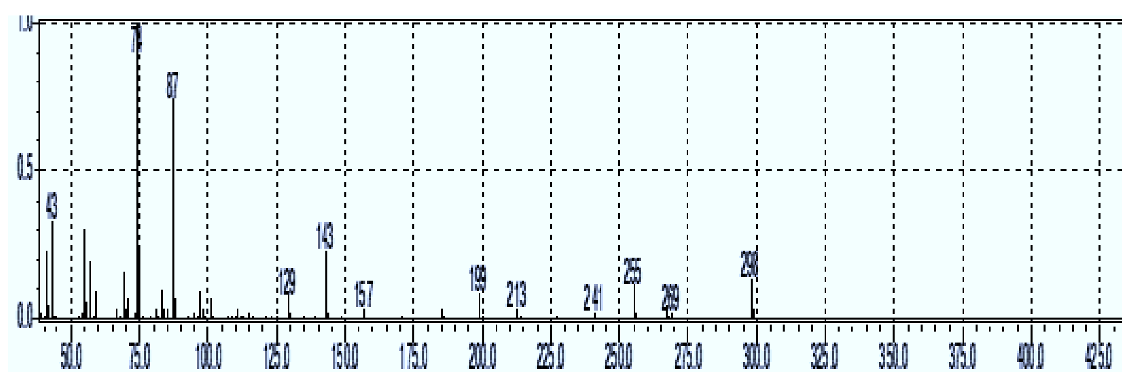
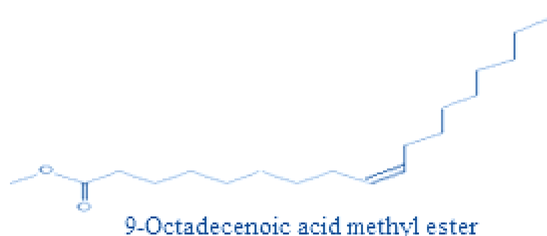


Fig.3.8:Massspectrumofmethylstearate



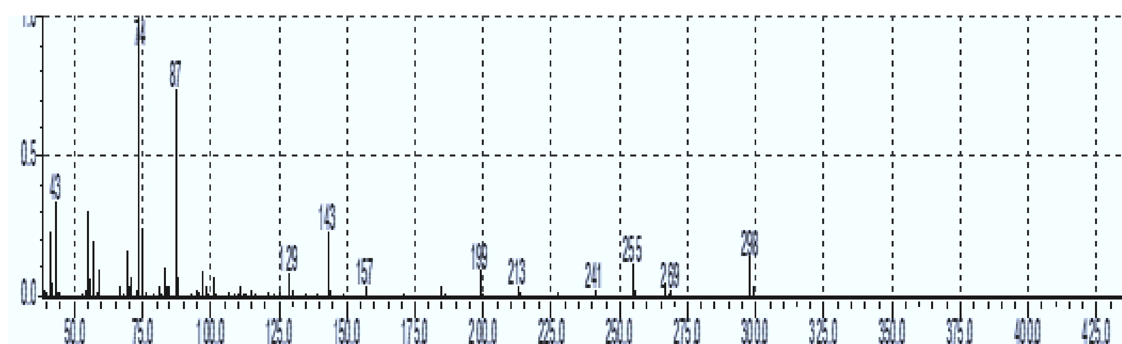


Fig:3.9:Massspectrumof9-octadecenoicacid(Z)-,methylester

3.2.2-Antimicrobialactivity

*Ipomoeasinensis*oilwasscreenedforantimicrobialactivityagainstfivestandardhumanpathogens.TheinhibitionzonesarepresentedinTable3.6.Theoilshowedgoodanticandidalactivityandgoodactivityagainst*Staphylococcusaureus*and*Escherichiacoli*..

Table3.6:Inhibtionzones(mm)of*Ipomoeasinensis*oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil100mg/mL	15	---	16	13	16

Sa.:Staphylococcusaureus.
Bs.:Bacillussubtilis.
Ec.:Escherichiacoli.
Pa.:Pseudomonasaeroginosa.
Ca.:Candidaalbicans.

3.3-*Dmebraretrafexa*

3.3.1-GasChromatography-MassSpectraanalysis

GC/MSwasconductedfor*Dmebraretrafexa*oil.Theanalysisrevealedthepresenceof17components-Table(3.7).ThetotalionchromatogramispresentedinFig.3.10.

Fig.3.10:Totalionschromatograms

Table3.7:Constituentsoftheoil

No.	Name	Ret.Time	Area%
1.	Methyltetradecanoate	14.270	0.17
2.	Pentadecanoicacid,methylester	15.405	0.06
3.	7-Hexadecenoicacid,methylester,(Z)-	16.246	0.07
4.	9-Hexadecenoicacid,methylester,(Z)-	16.285	0.34
5.	Hexadecanoicacid,methylester	16.494	30.93
6.	cis-10-Heptadecenoicacid,methylester	17.305	0.10
7.	Heptadecanoicacid,methylester	17.516	0.23
8.	9,12-Octadecadienoicacid(Z,Z)-	18.253	32.37

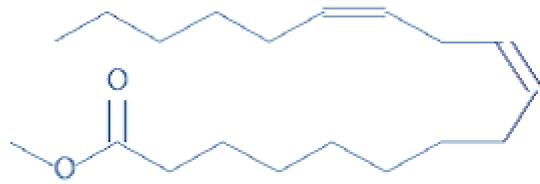
	,methylester		
9.	9-Octadecenoicacid(Z)-,methylester	18.296	19.13
10	Methylstearate	18.506	7.53
11	17-Octadecynoicacid,methylester	20.196	2.76
12	Eicosanoicacid,methylester	20.354	2.73
13	8,11,14-Docosatrienoicacid,methylester	20.549	0.63
14	Octadecanoicacid,9,10-dihydroxy-,methylester	21.040	0.87
15	Heneicosanoicacid,methylester	21.218	0.44
16	Docosanoicacid,methylester	22.057	1.11
17	Tetracosanoicacid,methylester	23.636	0.53

Thefallowingcompoundsweredetectedinthechromatogramasmajorconstituents:

i)912-octadecadienoicacidmethylester(32.37%)

ii)Hexdecanoicacid(30.93%)
iii)9-Octadecenoicacidmethylester(19.13%)
iv)methylstearate(7.53%)

Fig.3.11showsthemassspectrumof912-octadecadienoicacidmethylester.Thepeakatm/z294(RT.18.259)corresponds$M^+[C_{19}H_{34}O_2]^+$.ThemassspectrumofhexdecanoicacidmethylesterispresentedinFig.3.12.Thepeakatm/z270whichappearedat(RT.16.494)isdueto$M^+[C_{17}H_{32}O_2]^+$.Themassspectrumof9-ctadecenoicacidmethylesterisshowninFig.3.13.Thepeakatm/z296whichappearedat(RT.18.296)isdueto$M^+[C_{19}H_{36}O_2]$.Fig.3.14illustratesthemassspectrumofmethylstearate.Thesignalatm/z298whichappearedatRT.18.506accountsforthemolecularion:$M^+[C_{19}H_{38}O_2]^+$.



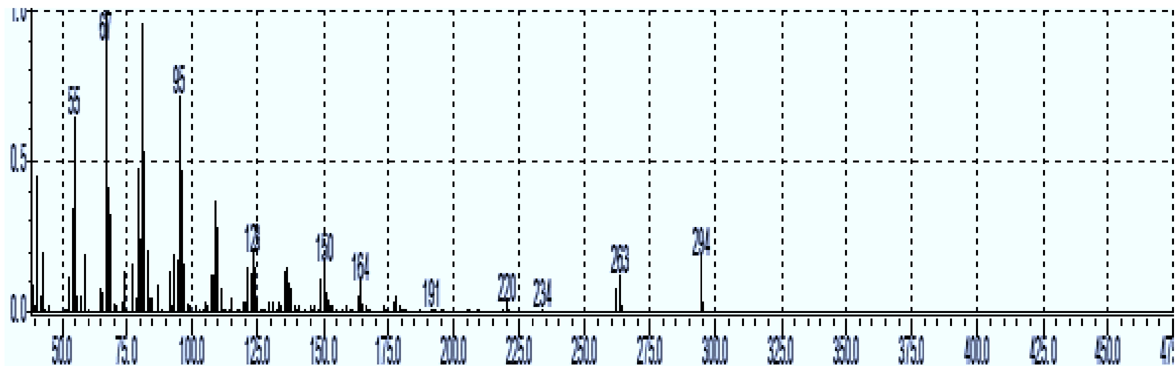
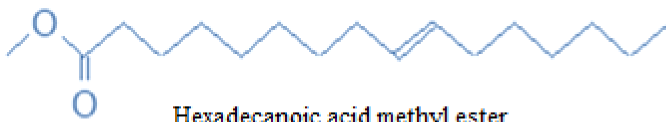


Fig.3.11:Massspectrumof9,12-octadecadienoicacid(Z,Z)-,methylester



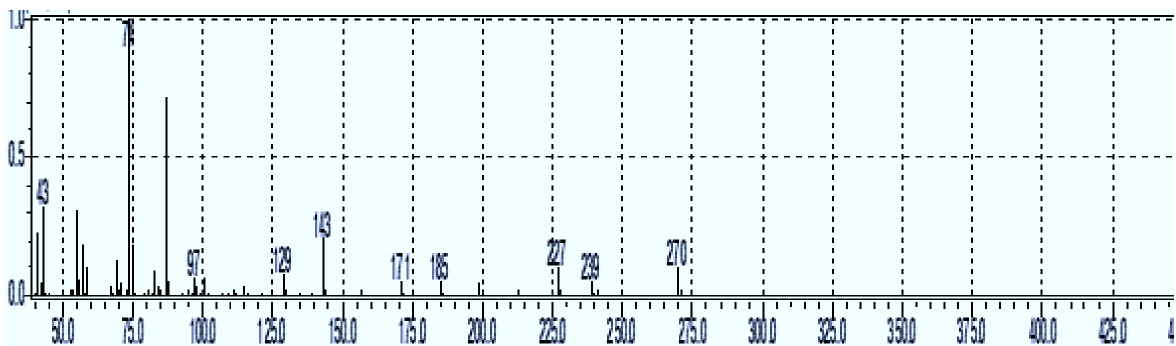
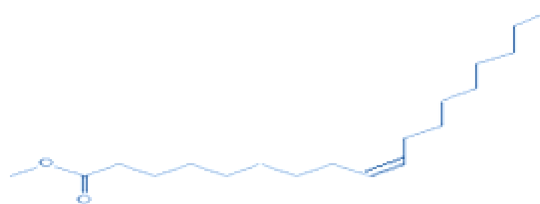


Fig.3.12:Massspectrumofhexadecanoicacid,methylester



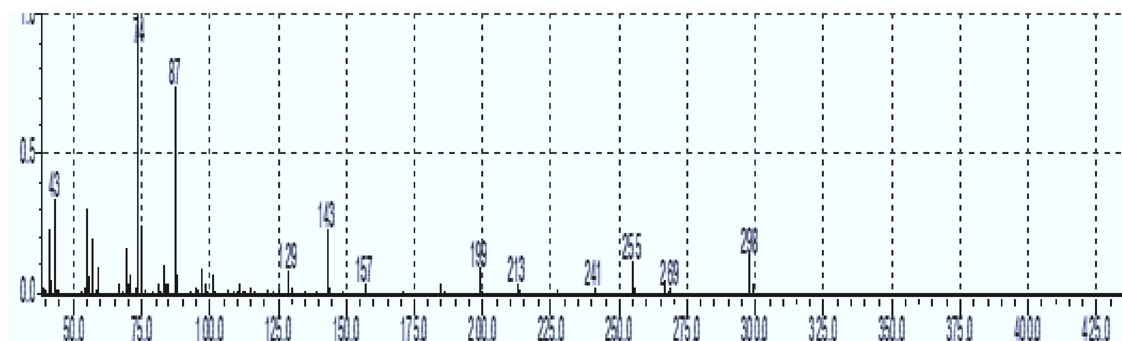
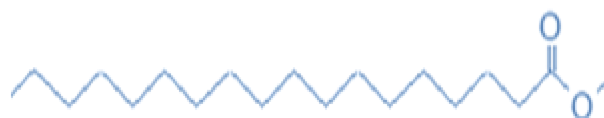


Fig:3.13:Massspectrumof9-octadecenoicacid(Z)-,methylester



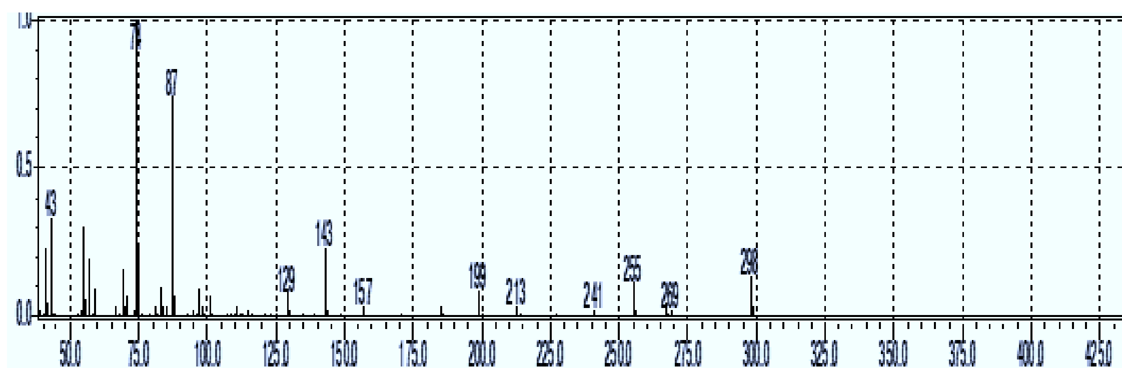


Fig.3.14:Massspectrumofmethylstearate

3.3.2-Antioxidantactivity

*Dmebraretrafexa*oilshowedweakradicalscavengingactivityagainststableDPPHradicals(Table3.8).

Table3.8:Antioxidantactivityof*Dmebraretrafexa*oil

Sample	%RSA±SD(DPPH)
*Dmebraretrafexa*oil	6±0.01
Standard(propylgallate)	92.2±0.01

3.4-*Hibscuscannabinus*

3.4.1-GasChromatography/MassSpectraanalysis

GC/MSwasconductedforthestudiedoil.Theanalysisrevealedthepresenceof26components-Table(3.9).ThetotalionchromatogramispresentedinFig.3.15.

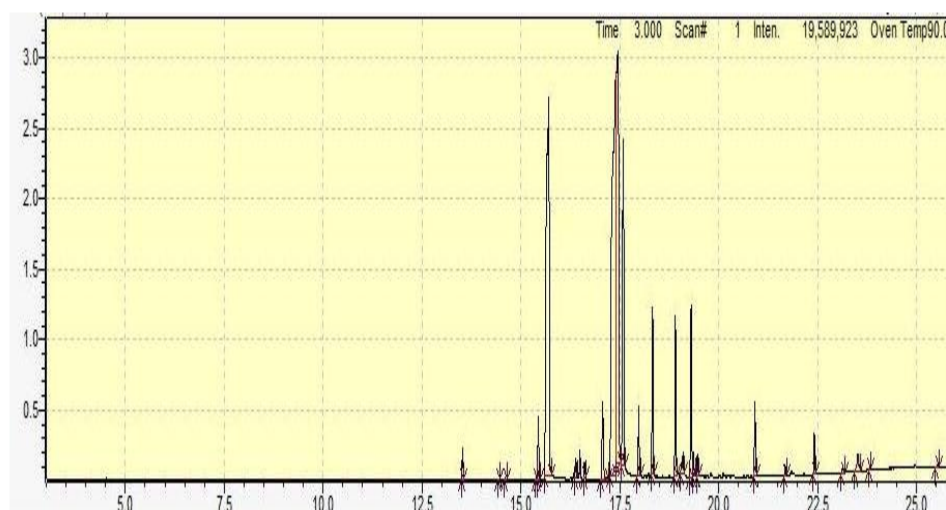


Fig.3.15:Totalionschromatograms

Table3.9:Constituentsoftheoil

ID#	Name	Ret.Time	Area%
1.	Methyltetradecanoate	13.528	0.54
2.	6-Octadecenoicacid,methylester,(Z)-	14.443	0.01
3.	Pentadecanoicacid,methylester	14.603	0.04
4.	7-Hexadecenoicacid,methylester,(Z)-	15.395	0.07
5.	9-Hexadecenoicacid,methylester,(Z)-	15.440	1.21
6.	Hexadecanoicacid,methylester	15.708	21.72
7.	cis-10-Heptadecenoicacid,methylester	16.389	0.42
8.	Heptadecanoicacid,methylester	16.610	0.32
9.	Methyl2-octylcyclopropene-1-heptanoate	17.067	1.96
10	9,12-Octadecadienoicacid(Z,Z)-,methylester	17.405	29.48
11	9-Octadecenoicacid(Z)-,methylester	17.458	18.54
12	Methylstearate	17.592	9.33
13	cis-11,14-Eicosadienoicacid,methylester	17.979	1.23
14	cis-10-Nonadecenoicacid,methylester	18.328	3.30

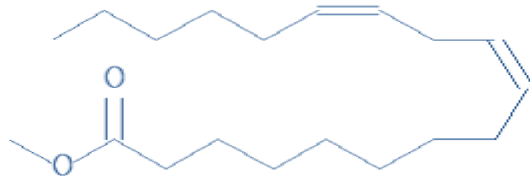
15	Cyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-,methylester	18.908	3.33
16	cis-11-Eicosenoicacid,methylester	19.099	0.67
17	Eicosanoicacid,methylester	19.304	3.58
18	6,9,12,15-Docosatetraenoicacid,methylester	19.360	0.40
19	9,12,15-Octadecatrienoicacid,methylester,(Z,Z,Z)-	19.468	0.41
20	Docosanoicacid,methylester	20.918	1.43
21	Tricosanoicacid,methylester	21.682	0.21
22	Tetracosanoicacid,methylester	22.420	0.84
23	Pentacosanoicacid,methylester	23.134	0.11
24	Lupeol	23.521	0.72
25	Hexacosanoicacid,methylester	23.821	0.08
26	VitaminE	25.534	0.05

Thefallowingcompoundsweredetectedinthechromatogramasmajorconstituents:

i)9,12-octadecadienoicacidmethylester(29.48%)

ii)Hexdecanoicacidmethylester(21.72%)
iii)9-Octadecenoicacidmethylester(18.54%)
iv)methylstearate(9.33%)

Fig.3.16showsthemassspectrumof9,12-octadecadienoicacidmethylester.Thepeakatm/z294(RT.17.405)corresponds$M^+[C_{19}H_{34}O_2]^+$.ThemassspectrumofhexdecanoicacidispresentedinFig.3.17.Thepeakatm/z270whichappearedat(RT.15.708)isdueto$M^+[C_{17}H_{32}O_2]^+$.Themassspectrumof9-ctadecenoicacid methylesterisshowninFig.3.18.Thepeakatm/z296whichappearedat(RT.17.458)isdueto$M^+[C_{19}H_{36}O_2]^+$.Fig.3.19illustratesthemassspectrumofmethylstearate.Thesignalatm/z298whichappearedatRT.17.592accountsforthemolecularion:$M^+[C_{19}H_{38}O_2]^+$.



9,12-octadecadienoicacid(Z,Z)-,methylester

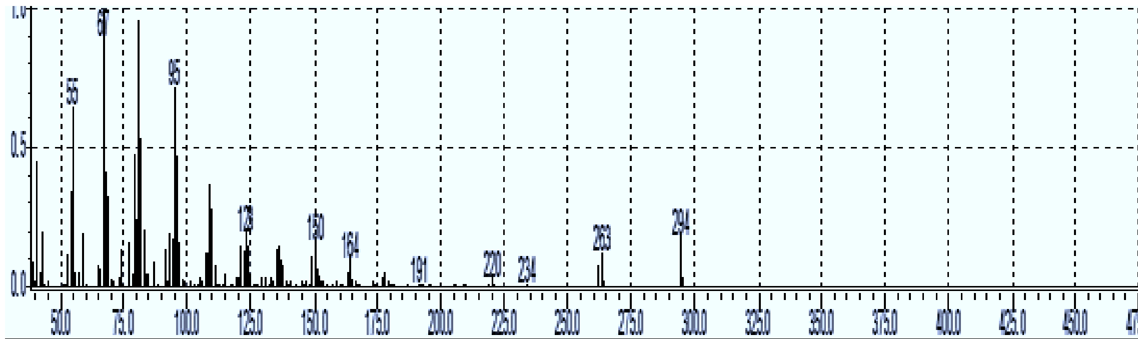
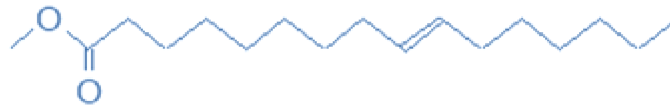


Fig.3.16:Massspectrumof9,12-octadecadienoicacid(Z,Z)-,methylester



hexadecanoicacid,methylester

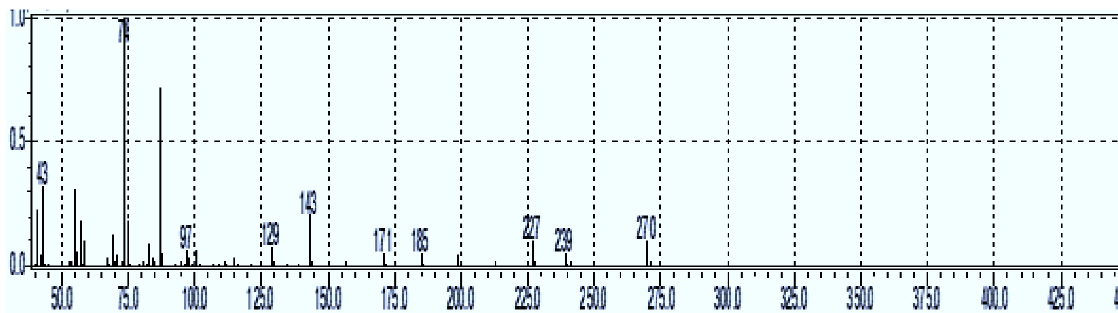
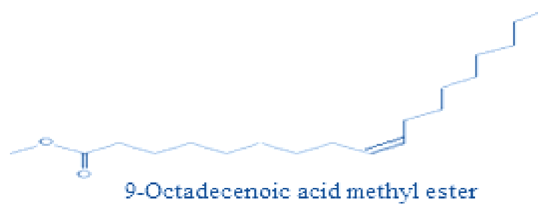


Fig.3.17:Massspectrumofhexadecanoicacid,methylester



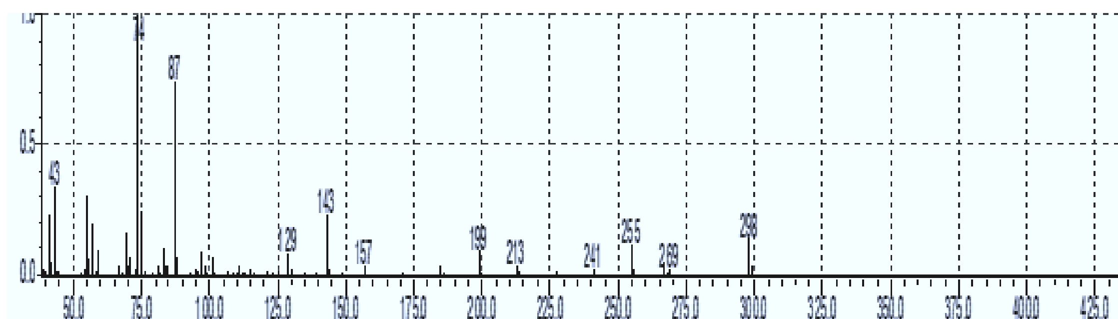
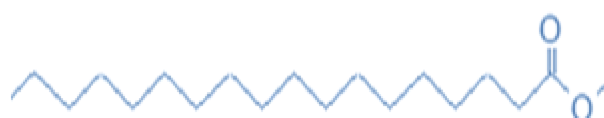


Fig:3.18:Massspectrumof9-octadecenoicacid(Z)-,methylester



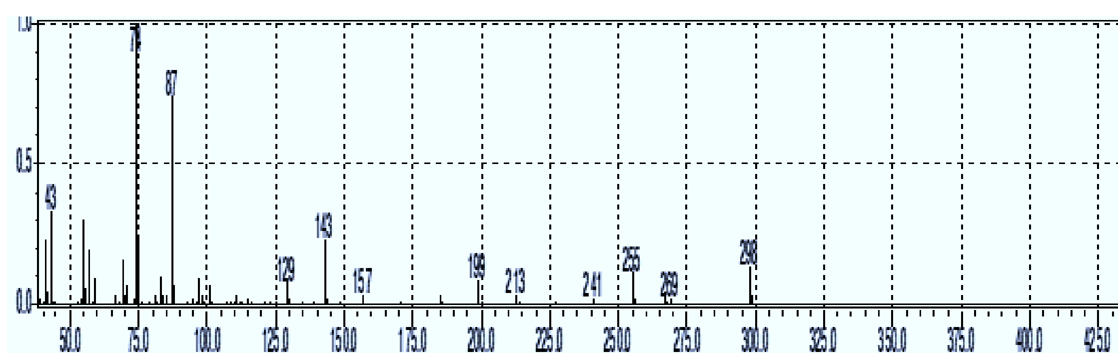


Fig.3.19:Massspectrumofmethylstearate

3.4.2-Antioxidantactivity

*Hibscuscannabinus*oilshowedmoderateradicalscavengingactivityagainststableDPPHradicals(Table3.10).

Table3.10:Antioxidantactivityofoil

Sample	%RSA±SD(DPPH)
*Hibscuscannabinus*oil	38±0.07
Standard(propylgallate)	93.2±0.01

3.5-*Proboscidealouisianica*

3.5.1-GasChromatography/MassSpectraanalysis

*Proboscidealouisianica*oilwasanalyzedbyGC/MS.15componentshavebeendetected.Totalionschromatogramsispresentedin Fig.3.20,whileconstiruentsareillustratedinTable3.11.

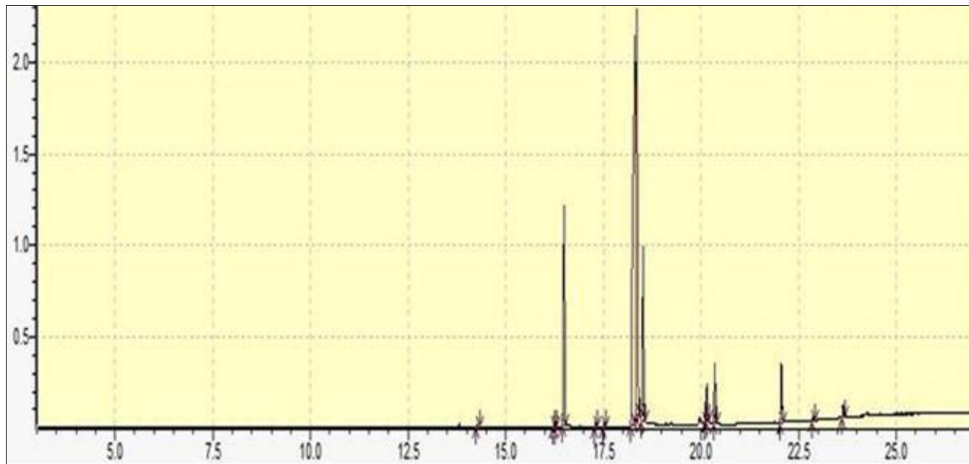


Fig.3.20:Totalionchromatograms

Table3.11:Constituentsoftheoil

No	Name	Ret.Time	Area%
1.	Methyltetradecanoate	14.278	0.08
2.	7-Hexadecenoicacid,methylester,(Z)-	16.242	0.08
3.	9-Hexadecenoicacid,methylester,(Z)-	16.289	0.19
4.	Hexadecanoicacid,methylester	16.499	12.01
5.	cis-10-Heptadecenoicacid,methylester	17.308	0.12
6.	Heptadecanoicacid,methylester	17.521	0.14
7.	9,12-Octadecadienoicacid(Z,Z)-,methylester	18.310	43.99
8.	9-Octadecenoicacid(Z)-,methylester	18.360	25.40
9.	Methylstearate	18.520	8.89
10.	9-Octadecenoicacid,12-hydroxy-,methylester,[R-(Z)]-	20.130	0.84
11.	cis-13-Eicosenoicacid,methylester	20.154	1.78
12.	Eicosanoicacid,methylester	20.356	2.66
13.	Docosanoicacid,methylester	22.062	2.98
14.	Tricosanoicacid,methylester	22.867	0.16
15.	Tetracosanoicacid,methylester	23.641	0.68

Majorconstituentsoftheoilarediscussedbelow:

-9,12-Octadecadienoicacidmethylester(43.99%)

TheEImassspectrumof9,12-octadecadienoicacidmethylesterisshowninFig.3.21.Thepeakatm/z294,whichappearedatR.T.18.310intotalionchromatogram,correspondsM^{+}[$C_{19}H_{34}O_2$]$^{+}$.Thepeakatm/z263isduetolossofamethoxyl,whilethefragmentm/z59isduetolossof(CH_3-O-C=O).

-9-Octadecenoicacidmethylester(25.40%)

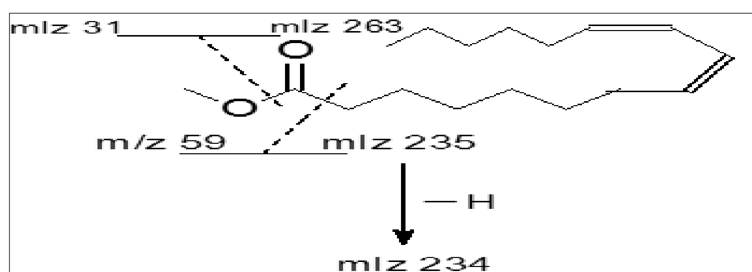
Themassspectrumof9-octadecenoicacidmethylesterisdisplayedinFig.3.22.Thepeakatm/z296,whichappearedatR.T.18.360correspondsM^{+}[$C_{19}H_{36}O_2$]$^{+}$.Thesignalatm/z265accountsforlossofamethoxylfunction.

-Hexadecanoicacidmethylester(12.01%)

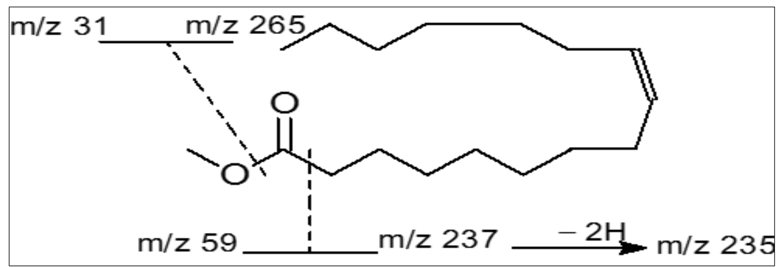
TheEImassspectrumofhexadecanoicacidmethylesterisshowninFig.3.23.Thepeakatm/z270,whichappearedatR.T.16.499intotalionchromatogram,correspondstoM^{+}[C17H34O2]$^{+}$.Thepeakatm/z239correspondstolossofamethoxyl.

-Methylstereate(8.89%)

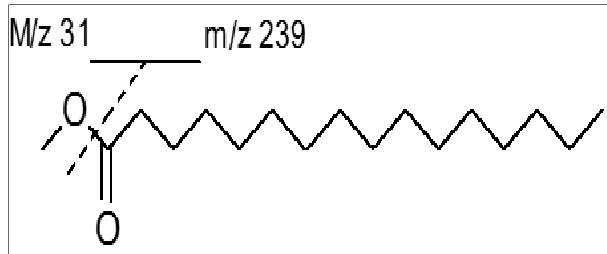
ThemassspectrumofmethylstereateispresentedinFig.3.24.Thesignalatm/z298(RT.18.520)isduetothemolecularion:M^{+}[$C_{19}H_{38}O_2$],whilethesignalatm/z267accountsforlossofamethoxyl.



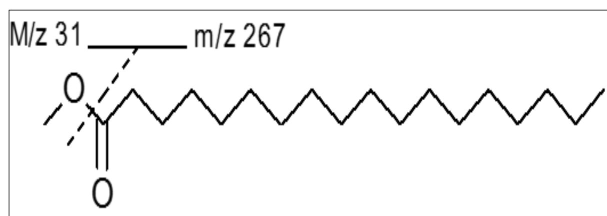
Majorfragmentsof9,12-octadecadienoicacidmethylester



Majorfragmentsof9-octadecenoicacidmethylester



Majorfragmentsofhexadecanoicacidmethylester



Majorfragmentationofmethylstearate

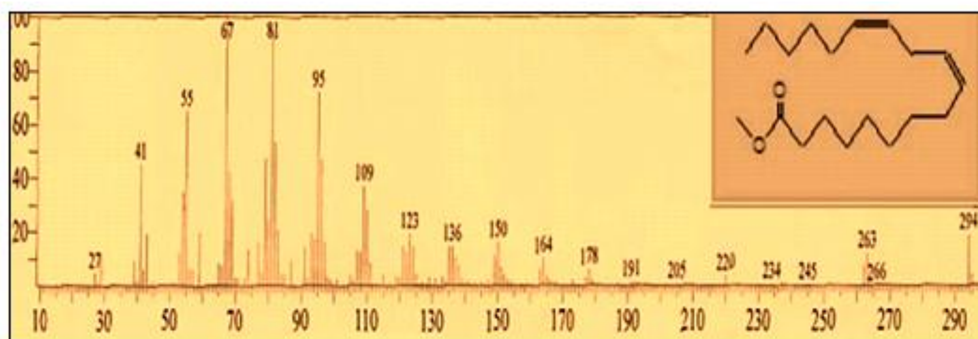


Fig.3.21:Massspectrumof912-octadecdienoicacidmethylester

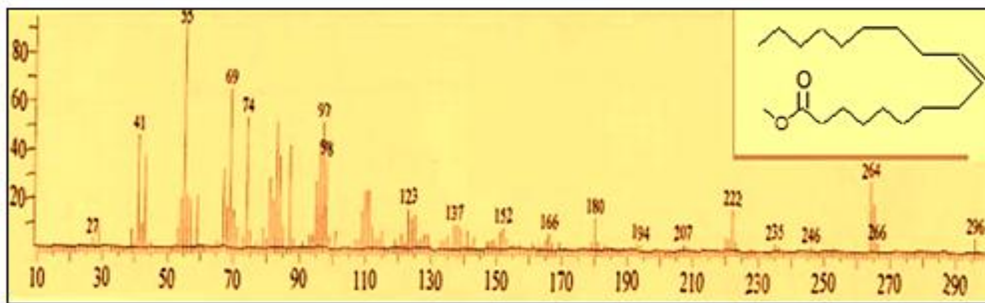


Fig.3.22:Massspectrumof9-octadecanoicacidmethylester

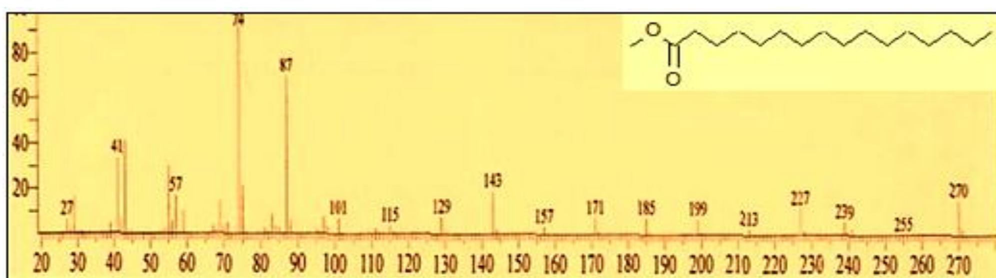


Fig.3.23:Massspectrumofhexadecanoicacidmethylester

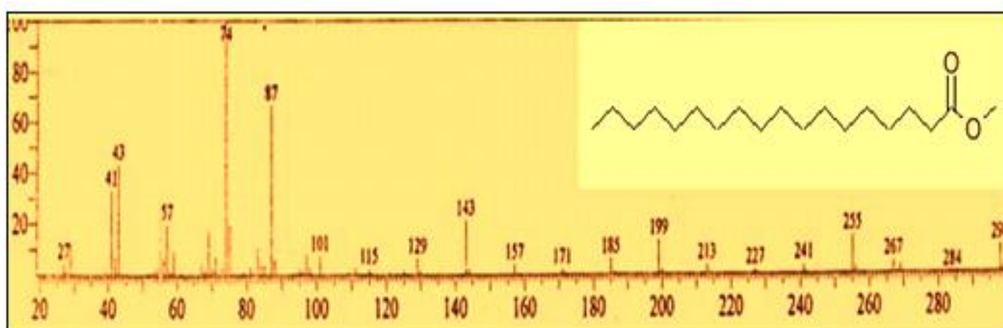


Fig.3.24:Massspectrumofmethylstearate

3.5.2-Antimicrobialassay

Thediscdiffusionbioassaywasusedtoscreentheantimicrobialactivityoftheoilagainstfivestandardhumanpathogens.Theaverageofthediametersofthegrowthofinhibitionzonesareshowni nTable3.12.*Proboscidealouisianica*oilshowedmoderateanticandidalactivity.Italsoexhibitedweakactivityagainst*Staphylococcusaureus*.However,itfailedtoexhibitactivityagainstothertestorganisms.

Table3.12: Inhibitionzones(mm)of*Proboscidealouisianica*oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil100mg/mL	11	---	--	--	14

Sa.:Staphylococcusaureus.
Bs.:Bacillussubtilis.
Ec.:Escherichiacoli.
Pa.:Pseudomonasaeroginosa.
Ca.:Candidaalbicans.

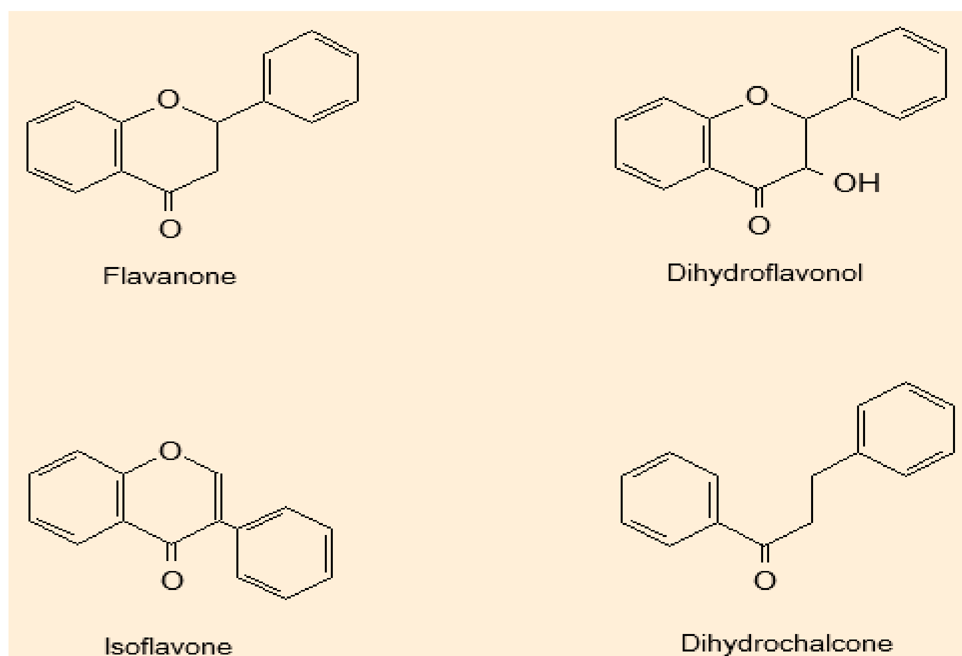
3.6-*Punicagranatum*L

Amongthestudiedplantspeciesthephytochemicalscreeningforflavonoidsrevealedthatthesp ecies:*Punicagranatum*istherichestinflavonoids.Henceitwasdecidedtoinvestigatethemajor flavonoidofthisspecies.

3.6.1-Majorflavonoidof*Punicagranatum*

Themajorflavonoidof*Punicagranatum*-compoundI-hasbeenisolatedbyTLC.APartialstructurehasbeenproposedonthebasisofitsUVand ^{1}HNMR data.

IntheUVcompoundIabsorbsatλ_{max}270nm(Fig.3.25).Suchabsorptionischaracteristicof:isof lavonesflavanonesdihydrochalconesanddihydroflavonols.Alltheseclassesofflavonoidsgiv eonlybandIpeakintherange230-290nmandthisisduetolossofconjugationbetweenringBandthecarbonylfunctiononC-4.



TheisolatedcompoundisnotanisoflavonesincetheUVspectrum(Fig.3.25)didnotrevealanyshouldercharacteristicofisoflavonesintherange300-340nm.Alsothecharacteristic3-OHfunctionofdihydroflavonolswasnotrevealedbythesodiummethoxidespectrumofcompoundI(Fig.3.26).Thisspectrumdidnotshowanybathochromicshiftcharacteristicofa3-OHgroup.Ontheotherhandflavanonesshowdoublemultipletscenteredatδ2.8ppmandδ5.2ppm.ThesemultipletsareduetomutualsplittingofthetwomagneticallyunequivalentprotonsatC3splittingeachotherintoadoubledoubletandthesesignalsarefurthersplitintoamultipletbytheprotononC-2.Theprotononcarbon2andbysimilarsplittingbytheprotonsonC-2givesanothermultipletcenteredatδ5.2ppm.Howeversuchmutipletswerenotdetectedinthe^{1}HNMRspectrum(Fig.3.30)ofcompoundI.ConsequentlycompoundIisadihydrochalcone:

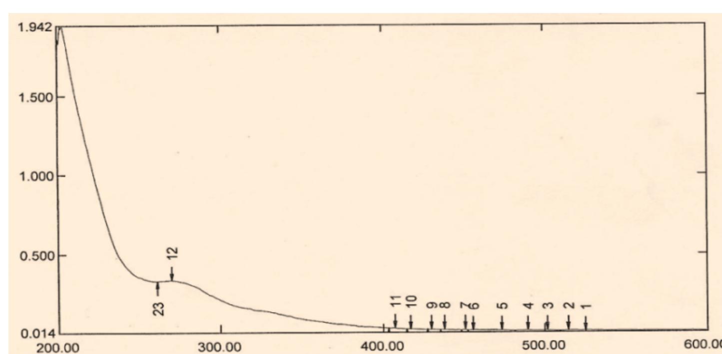


Fig.3.25:UVspectrumofcompoundI

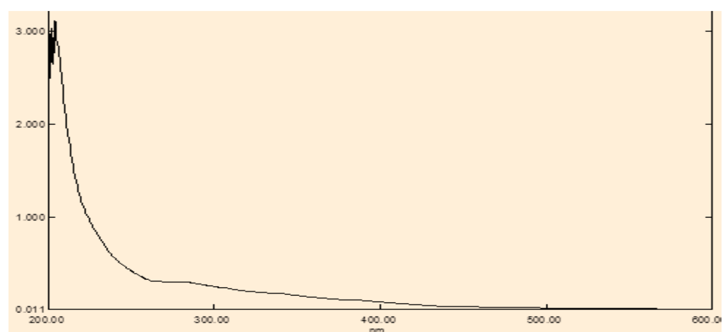


Fig.3.26:SodiummethoxidespectrumofcompoundI

Thenthehydroxylationpatternonthenucleusofthedihydrochalconehasbeeninvestigatedbys omeUVshiftreagents.Ausefulshiftreagentissodiumacetatewhichgivesbathochromicshifts diagnosticofa7-
OHgroup.ThesodiumacetatespectrumofcompoundI(Fig.3.27)didnotrevealanybathochro micshiftindicatinabsenceofa7-OHfunction.

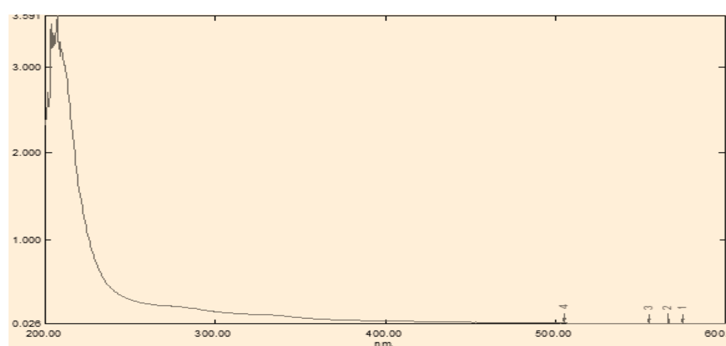


Fig.3.27:SodiumacetatespectrumofcompoundI

TheUVshiftreagent–aluminiumchloride-inducesbathochromicshiftdiagnosticof3-and5-
OHaswellascatecholsystems.Thisreagentcomplexeswiththe4-
ketofunctionandahydroxylgrouplocatedeitheratposition3or5.Italsocomplexeswithcatecho lssystems.Thecatecholcomplex-unlikethe4-keto–3-OHorthe4-keto-5-
OHcomplexesdecomposesinacidicmediahencetheadditionofacidtothealuminiumchloride complexmaydistinguishbetweenthesetypesofcomplexes.

ThealuminiumchloridespectrumofcompoundIshowedabathochromicshiftdiagnosticofaca techolsystem(Fig.3.28).ThespectrumdecomposedinpresenceofHCl(Fig.3.29).

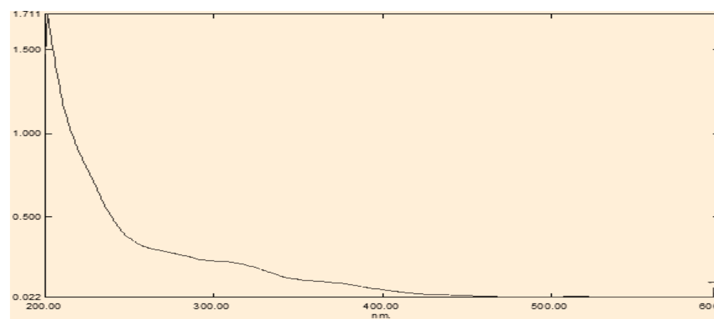


Fig.3.28:AluminiumchloridespectrumofcompoundI

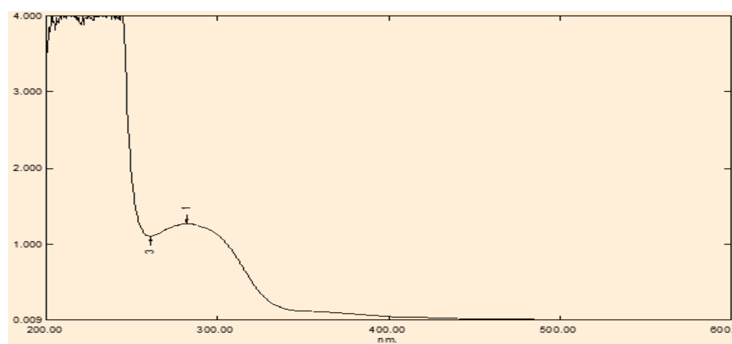
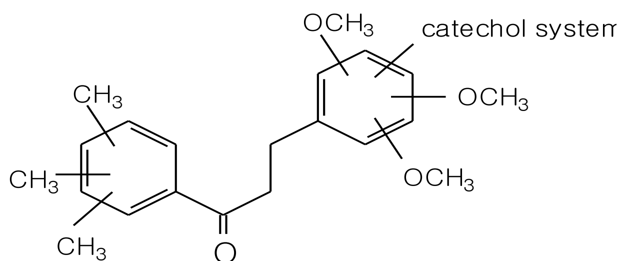


Fig.3.29:Aluminiumchloride/HClspectrumofcompoundI

^{1}HNMRspectrum(Fig.3.30)ofcompoundIshowedδ(ppm):1.24(assignedfor3methylgroups);3.47(accountingfor3methoxylfunctions).Anaromaticprotonappearedatδ6.85ppm.

Onthebasisoftheabovespectraldatathefollowingpartialstructurewasproposedforcompound I:



TentativestructureofcompoundI

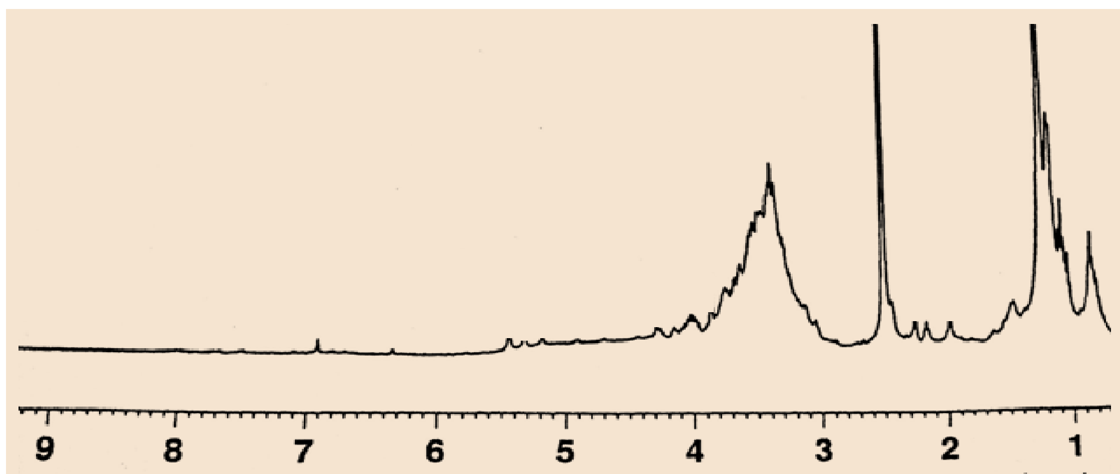


Fig.3.30:^{1}HNMRapectrumofcompoundI

Conclusion

Theoilsoffiveplants(Echinochioacolona,Ipomoeasinensis,Dmebraretrafexa,Hibiscuscannabinusandproboscidealouisianica)werestudiedbygaschromatography-massspectraandthenthebiologicalactivitywasevaluated.Inadditionthemajorflavonoidofpomegranate(compound1)wasisolatedbypaperchromatographyandidentifiedbyNMRandUV-Visibledata.

Recommendations

Thefollowingfutureworkishighlyrecommended:

1- Otherbiologicalactivitiesofextractedoils(likeantiviral,antimalairial,…..etc)maybeevaluated.
2- Thestructureoftheisolatedflavonoidmaybefullyelucidatedthrough2DNMRexperiments.
3- Otherphytochemicalsinthestudiedplantsmaybeinvestigated.

REFERENCES

1-Alexopoulou, E., Papatheohari, Y. Christou, M., Monti, M. (2013). Origin, Description, Importance, and Cultivation Area of Kenaf, pp 1- 12. In: Monti, A., Alexopoulou, A (Eds). Kenaf: A Multi-Purpose Crop for Several Industrial Applications. Springer-Verlag, London.

2-AOCS (2003). Official methods and recommended practice of the American oil chemists, society 5th edition by chapman, IL.

3-Ayadi, R., Hanana, M., Mzid. R., Khouja, M. I., Hanachi, A. S. (2016). *Hibiscus Cannabinus* L. Kenaf a Review Paper. Journal of Natural Fibers, 14: 1-19.

4-Black, M., Bewley, J. D. (2000). Seed technology and its biological basis. (1st ed). pp. 147. UK: CRC Press.

5-Cheng, W., Jahurul, J. M. H., Nyam, K. (2016). Kenaf Seed Oil: A Potential New Source of Edible Oil. Trends in Food Science and Technology, 52: 57 – 65.

6-Webber, C. L., Bledsoe, V. K. (2002). Kenaf yield components and plant composition. Trends in New Crops and New Uses, pp. 350-357.

7-Riffle, M.S. (Oklahoma State University, Stillwater, OK); Waller, G.R.; Murray, D.S.(1991). Composition of Essential Oil from Proboscidea louisianica (Martyniaceae) From the journal Proceedings of the Oklahoma Academy of Science (USA) ISSN : 0032-0889.

8-Brooks, R.E., and Weedon, R.R., Flora of the Great Plains. Great Plains Flora Association. University Press of Kansas, Lawrence, KS (1986) p. 503.

9-Martin, W.C., and Hutchins, C.R., A Flora of New Mexico. Vol. 2. J. Cramer, Vaduz, Germany (1980) pp.1856-1859 .

10-Naban G., Whiting, A., Dobyns, H., Hevly, R., and Euler, R., Devil's Claw Domestication: Evidence from South western Indian Fields. J. Ethnobiol. 1, 135-164 (1981).

11-Eastern Africa weeds control. Oxford University press, Nairobi. 39p Galinato I, Moody K, Piggin. CM. 1999. Upland rice weeds of South and Southeast Asia. Manila (Philippines): International Rice Research Institute.

12-Akobundu, O Agyakwa C.W. (1998). A handbook of West African Weeds.International Institute of Tropical Agriculture, Ibadan, Nigeria 476p Johnson, D.E., 1997.

13-Weeds of rice in West Africa. WARDA, Bouaké. Moody K. 1989.

14-Weeds reported in rice in South and Southeast Asia. Manila(Philippines):International Rice Reseach Institute. 442 p.

15-Moody K, Munroe CE, Lubigan RT, Paller Jr. EC. 1984. Major weeds of the Philippines. College, Laguna (Philippines): University of the Philippines at Los Baños.

16-Chauhan BS, Johnson DE, 2009. Seed germination ecology of Junglerice (*Echinochloa colona*): A major weed of rice.Weed Sci 57: 235–240 Hegazy AK, Fahmy GM, Ali MI, Gomaa NH, 2005.

17-Growthand phenology of eight common weed species. J Arid Environ 61: 171–175.

18-www.africanplants.senckenberg.de/root/index.php.83.

19-Austin DF (1997). Dissolution of *Ipomoea* series Anisomerae(Convolvulaceae).Taxon 28: 359–361

20-Monica R. Loizzo, ... Rosa Tundis, in Nonvitamin and Nonmineral Nutritional Supplements*, 2019.*

21-Participants of the FFI/IUCN SSC Central Asian regional tree Red Listing workshop, Bishkek, Kyrgyzstan (11-13 July 2006) (2020). "Punica granatum". IUCN Red List of Threatened Species. ***2020****: e.T63531A173543609*. Retrieved 16 November 2020.

22-"*Punica granatum L.*, The Plant List, Version 1". *Royal Botanic Gardens, Kew and Missouri Botanical Garden. 2010.* Archived *from the original on 11 August 2013*. Retrieved 26 June 2015.

23-Pengelly,A. " The Constituents of Medicinal Plants " , 2nd Ed. , Cabi Publishing, London , UK(1996).

24-Publications of the Dept. of Pharmacognosy and Natural Products, Faculty of Chemistry, University of Oriental, Republic of Uruguay(2004).

25- Association Française de Normalisation (AFNOR). Huiles Essentielles, Tome 2, Monographies Relatives Aux Huiles Essentielles, 6th ed.; AFNOR, Association Française de Normalisation: Paris, France, (2000).

26– Somesh.S, Rupali.S, Swati.M., *“In-vitro Comparative Study on Antimicrobial Activity of five Extract of Few Citrus Fruit*”,Peel & Pulp vs Gentamicin. *Australian Journal of Basic and Applied Sciences*, 9(1): 165-173(2015).

27– Wang.L.,“Recent advances in extraction of nutraceuticals from plants”. *Trends Food Sci.Technol*., 17: 300-312(2006).

28– Dick.A.,“Extraction of secondary metabolites from plant material”a review. *Trends Food Sci. Technol*.,191-197(1996).

29– Laurence.M, Moody.J.,“*Experimental organic chemistry: Principles and Practice*” Wiley-Blackwell,(1989).

30– Soxhlet.F., “Die gewichtsanalytische Bestimmung des Milchfettes”. *Dingler's Polytechnisches Journal,German,* 232: 461-465(1879).

31– Hesham.A, abdurahman.N, Rosli.Y., *“Techniques For Extraction of Essential Oils From Plants*”.*Australian Journal of Basic and Applied Sciences* , 10(16):117-127(2016)

32- Taylor.E.,“Aromatherapy for the Whole Person” UK: Stanley Thornes, pp: 22-26. (1981)

33– Rai.R, Suresh.B., *Indian Journal of Traditional Knowledge* , 3(2): 187-191(2004).

34– Rozzi.N, Phippen.J.,“Supercritical fluid extraction of essential oil components from lemon-scented botanicals” *Lebensm.- Wiss.U.Technol*., 35: 319-324(2002)

35– Pourmortazavi.S, Hajimirsadeghi.S.,“Supercritical fluid extraction in plant essential andvolatile oil analysis” *Journal of Chromatography A*, 1163: 2-24(2007)

36– Fadel.F, Marx.A, El-Gorab.A., "Effect of extraction techniques on the chemical composition and antioxidant activity of Eucalyptus camaldulensis var. *brevirostris leaf oils*"208: 212-216(1999) .

37– Capuzzo.A, Maffei.M, Occhipinti.A*., "Supercritical fluid extraction of plant flavors and fragrances Molecules"*18:7194-7238(2013).

38– Abert.V, Fernandez.F, Visinoni.F, Chemat.F., *"Microwave hydrodiffusion and gravity, a new technique for extraction of essential oils"Journal of Chromatography A*, 1190: 14-17(2008).

39– Chemat.F, Lucchesi.M, Smadja.*J.,*"Extraction sans solvant assistée par micro-ondes de produits naturels" 439: 218(2004)

40– Vian.M, Fernandez.X, Visinoni.F, Chemat.F.,"Microwave hydrodiffusion and gravity, a new technique for extraction of essential oils*" Journal of Chromatography A*, 1190: 14-17(2008)

41– Lucchesi.M, Smadja.J, Bradshaw.S, Louw.W, Chemat.F., "Solvent-free microwave extraction of Elletaria cardamomum L: A multivariate study of a new technique for the extraction of essential oil" *Journal of Food Engineering*, 79: 1079-1086(2007).

42– Filly.X, Minuti.M, Chemat.F., "Solvent- free microwave extraction of essential oil from aromatic herbs: from laboratory to pilot and industrial scale" *Food Chem,*150:193-198(2014).

43– Kentish.S, Ashokkumar.M., "Selected applications of ultrasonics in food processing" *Food Eng Rev*.,1: 31-49(2009).

44- Garcı´a-Pe´rez.V, Ca´rcel.J., "Ultrasonic drying of foodstuff in a fluidized bed: parametric study". *Ultrasonics*, 44: 539-543(2006).

45- Karim Assami.D., "Ultrasound-induced intensification and selective extraction of essential oil from Carum carvi L. seeds". *Chem. Eng. Process. Process Intensif,* 62: 99-105(2012).

46– Sereshti.H, Bakhtiari.S., "Bifunctional ultrasonics assisted extraction and determination of Elettaria cardamomum Maton essential oil" *Journal of Chromatography* A,1238:46–53(2012).

47– Brachet.A, Christen.P, Veuthey.J., “Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves”. *Phytochemical Analysis*,13: 162-169(2002).

48– Lucchesi.M, Chemat.F, Smadja.J., “Original solvent free microwave extraction of essential oils from spices”*Flavor and Fragrance Journal*., 19: 134-138(2004).

49– Ferhat.M, Meklati.J, Chemat.F., “An improved microwave Clevenger apparatus for distillation of essential oils from orange peel”, *Journal of Chromatography A*, 1112(1-2): 121-126(2006).

50 - Farhat.A, Ginies.C, Romdhane.M, Chemat.F., “Eco-friendly and cleaner process for isolation of essential oil using microwave energy: Experimental and theoretical study”, *Journal of Chromatography* A,1216(26): 5077-5085(2009).

51– Letellier.M, Budzinski.H, Charrier.L, Dorthe.A., “ Optimization by factorial design of focused microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediment”,*J.Anal.Chem*.,364: 228-37(1999).

52– Sell.S., “The Chemistry of Fragrance. From Perfumer to Consumer”, 2nd ed.; The Royal Society of Chemistry: Cambridge, UK, p. 329(2006).

53– Guenther.E., “The Essential Oils”Van Nostrand Company Inc.NewYork(1948).

54. Mirheydar, H., Herbal Information: Usage of Plants in Prevention and Treatment of Diseases, Islamic Culture Press Center, Tehran, Iran, (2001).

55. Markham, K.R."Techniques of Flavonoids Identification", 1982. Academic Press, London.

56. Harborne, J.B. "Phytochemical Methods: A guide to Modern Techniques of Plant Analysis", Chapman and Hall, London, 1973; 1–74.

57. Nuutila, A.M., Kammiovirta, K.M., OksmanCaldentey, J. Food Chem, 2002; 76: 519.

58. Argaez, B., Flowers, B.A., Gimenez-Turba, A., Ruiz, G., Waterman, P.G., Pena-Rodriguez, L.M., J. Phytomed, 2007; 11: 214.

59. Skibola, C.F. and Smith, M.T., Free Radical Biology and Medicine, 2000; 29: 375-383.

60. Messina, M. and Messina, V., Journal of the American Dietetic Association, 1991; 91: 836-840. 7. Dajas, F., Brazilian Journal of Medical and Biological Research, 2003; 36: 1613.

61- " Plant Extracts : A Basis for a Sector Development " , Alfonso Martín Escudero Foundation. Madrid (1999).