**Sudan University of Science and Technology**

**College of Postgraduate Studies**

# Characterization of Some Phytochemicals from Sudanese Medicinal Plants and their Biological Activity.

# توصيف بعض الفيتوكيميائيات المستخلصة من نباتات طبية سودانية وفعاليتها البيولوجية.

A Thesis Submitted in Fulfillment of the Requirements for the

Ph.D. Degree in Chemistry

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# الإستهلال

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

**سورة العلق (الأيات1-5)**

**صدق الله العظيم**

# DEDICATION

Dedicated To,

My parents,

And my brothers and sisters,

# ACKNOWLEDGMENT

First, I would like to thank **Almighty Allah** for giving me the will and health to complete this work.

With sincere respect and gratitude I would like to thank Prof.. Mohamed Abdel karim Mohammed for his help and patience through the difficult periods. I would always remember and appreciate his valuable contributions and suggestions during the course of this research.My sincere thanks are extended to my co-supervisor Dr. Mohammed Suleiman.

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I thank my parents who provided me with invaluable support, encouragement and advice in many aspects during my academic career. My sincere thanks to those who helped me in any way during this work.

# Abstract

This study was designed to study the constituents of the oils of some Sudanese medicinal plants and to assess their antimicrobial effect. The GC-MS analysis of *Azadirachta indica* oil revealed the presence of 29 components. Major constituents are : 9-octadecenoic acid methyl ester(37.20%) ; methyl stearate(20.42%) ;hexadecanoic acid methyl ester(19.13%) and 9,12-octadecadienoic acid methyl ester(12.60%). At a concentration of 100mg/ml the oil showed moderate activity against *Escherichia coli* .The GC-MS analysis of *Gerwia tenax* seed oil showed 12 components dominated by : 9,12-octadecadienoic acid methyl ester (51.69%) ; hexadecanoic acid methyl ester(18.83%) ; 9-octadecenoic acid methyl ester(16.41%) and methyl stearate(9.33%). The oil exhibited significant antibacterial activity against *Staphylococcus aureus*.It also showed significant anticandidal activity. The GC-MS analysis of *Citrus aurantifolia* oil revealed the presence of 24 components. Major constituents are: 9,12-octadecadienoic acid methyl ester(34.23%); hexadecanoic acid methyl ester(26.24%); 9,12,15-Octadecatrienoic acid methyl ester(14.09%) and methyl stearate(10.66%). The oil showed moderate antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The GC-MS analysis of *Lawsonia mermus* seed oil showed 49 components dominated by: 9,12-octadecadienoic acid methyl ester(34.15%);

hexadecanoic acid methyl ester(14.00%);and methyl stearate(7.50%).The oil showed moderate antibacterial activity against *Escherichia coli*. The GC-MS analysis of *Senna italic* revealed the presence of 19 components. Major constituents are: 9,12-octadecadienoic acid methyl ester(40.27%); hexadecanoic acid methyl ester(20.06%); 9-octadecenoic acid methyl ester(17.32%); and methyl stearate(13.47%);The oil showed moderate antibacterial activity against *Bacillus subtilis*. In the GC-MS analysis of *Carthamus tinclorius* oil 24 components were detected, dominated by: 9,12-octadecadienoic acid methyl ester(51.13%); hexadecanoic acid methyl ester(13.49%); methyl stearate(9.05%); and 9-octadecenoic acid methyl ester(7.99%);This oil showed moderate antibacterial effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

# المستخلص

تمت دراسة الزيوت المستخلصة من بعض النباتات الطبية في السودان وهي (النيم، القضيم، الليمون، الحنة، السنا سنا، القرطم البلدي) وقد تم إستخلاص الزيوت الثابتة لهذه النباتات ثم التعرف على مكوناتها الكيميائية بإستخدام الكروماتوغرافيا الغازية – طيف الكتلة، كما تم تحديد نشاطها الحيوي تجاه بعض انواع الميكروبات باستخدام طريقة الإنتشار . وقد تم الحصول علي النتائج الآتية : نبات النيم وجد أنه يحتوي علي 29 مكون الأساسية منها: 9- حمض أوكتاديكينويك ميثيل إستر(37.20%); ستيارات الميثيل(20.42%); حمض هكساديكانويك ميثيل إستر(19.13%); و9،12- حمض أوكتاديكاداييينويك ميثيل إستر(12.60%)، عند تركيز(100ملجم/مل) أظهر هذا الزيت نشاط جيد ضد بكتريا *Escherichia coli* . نبات القضيم وجد أنه يحتوي علي 12 مكون الأساسية منها :9،12- حمض أوكتاديكاداييينويك ميثيل إستر(51.69%) ; حمض هكساديكانويك ميثيل إستر(18.83%); 9- حمض أوكتاديكينويك ميثيل إستر(16.41%); وستيارات الميثيل(9.33%)، عند تركيز(100ملجم/مل) أظهر هذا الزيت نشاط كبير ضد بكتريا *Staphylococcus aureus* . نبات الليمون وجد أنه يحتوي علي 24 مكونا الأساسية منها: 9،12- حمض أوكتاديكاداييينويك ميثيل إستر(34.23%); حمض هكساديكانويك ميثيل إستر(26.24%); 9،12،15- حمض أوكتاديكاتراييينويك ميثيل إستر(14.09%); وستيارات الميثيل(10.66%)، عند تركيز(100ملجم/مل) أظهر هذا الزيت نشاط جيد ضد *Candida albicans, Staphylococcus aureus , Pseudomonas Escherichia coli* . نبات الحنة وجد أنه يحتوي علي49 مكونا الأساسية منها:9 ،12- حمض أوكتاديكاداييينويك ميثيل إستر(34.15%) ; حمض هكساديكانويك ميثيل إستر(14.00%); وستيارات الميثيل(7.50%) أظهر هذا الزيت نشاط جيد ضد بكتريا *Escherichia coli* . نبات السنا سنا وجد أنه يحتوي علي 19 مكونا الأساسية منها : 9،12- حمض أوكتاديكاداييينويك ميثيل إستر(40.27%) ;حمض هكساديكانويك ميثيل إستر(20.06%); 9-حمض أوكتاديكينويك ميثيل إستر(17.32%); وستيارات الميثيل(13.47%)، عند تركيز(100ملجم/مل) أظهر هذا الزيت نشاط ملحوظ ضد بكتريا *Bacillus subtilis*. نبات القرطم البلدي وجد أنه يحتوي علي 24 مكونا الأساسية منها: 9،12- حمض أوكتاديكاداييينويك ميثيل إستر(51.13%); حمض هكساديكانويك ميثيل إستر(13.49%); ستيارات الميثيل(9.05%); و9- حمض أوكتاديكينويك ميثيل إستر(7.99%)، عند تركيز(100ملجم/مل) أظهر هذا الزيت نشاط جيد ضد بكتريا *Pseudomonas aeruginosa, Staphylococcus* .

.

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# Chapter One

## Introduction

# 1-Introduction

## 1.1 Natural Products

### 1.1.1 Definition

The term natural products today is quite commonly understood to refer to herbs, herbal concoctions, dietary supplements, traditional Chinese medicine, or alternative medicine. Natural products are generally either of periodic origin or originate from microbes, plants, or animal sources. As chemicals, natural products include such classes of compounds as alkaloids, terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases and phenolic compounds.[(1)]

The use of natural products as medicinal agents presumably predates the first recorded history as the earliest humans used various, but specific plants to treat illness, the treatment of diseases with pure pharmaceutical. Nevertheless the role of traditional medicine in the discovery of potent chemicals is quite crucial.[(2)]

### 1.1.2 Classification

(a) The classification based on chemical structure:

Aliphatic or non-aliphatic fatty compounds of open chain;sss ssxamples: fatty acids, sugars and a great amount of amino acids. Acyclic and cyclo aliphatic compounds as terpenoids, steroids and some alkaloids. Aromatic or benzoic compounds as phenols, quinones. Heterocyclic compounds such as alkaloids, flavonoids and nucleic acid.[(3)]

(b) The classification based on physiological activity :

Examples include: hormones, vitamins, antibiotics and mycotoxins. Approximately one half of the medicines used today are natural products.[3]

(c) The classification based on taxonomy:

This classification is based on morphological studies of plants, or plant taxonomy. In animals and some of the microorganisms, final metabolites are generally excreted outside the body, while in plant metabolites are stored inside the plant. While it was thought that some metabolites were specific of some plants we know today that they are widely distributed in the plant kingdom and many constituents of plants such as alkaloids and isoprenoides have been isolated from species, genera, families or specific plants..[3]

(d)The classification based on biogenesis:

Although "biogenesis" and "biosynthesis" are terms that are used sometimes indiscriminately, it is customary to use the first term for hypothesis, and the last for a synthetic route tested experimentally. The constituents of all plants animals are biosynthesized in organisms through enzymatic reaction.[3]

### 1.1.3 Terpenes

Terpenes is the generic name of a group of natural products, structurally based on isoprene (isopentenyl) units. The term may also refer to oxygen derivatives of these compounds that are known as the terpoenoid.[2]

Terpoenoid is a term which is used to indicate that all such substances has a common biosynthetic origin. Thus terpenoids all based on the isoprene molecule formula ($C_5H_8$) and their carbon skeletons are built up from the union of two or more of these ($C_5$) units. They are classified according to whether they contain two ($C_5$) , three ($C_5$) units as so on.This includes the volatile mono- and sesquiterpenes ($C_{10}$ and $C_{15}$), and the less volatile

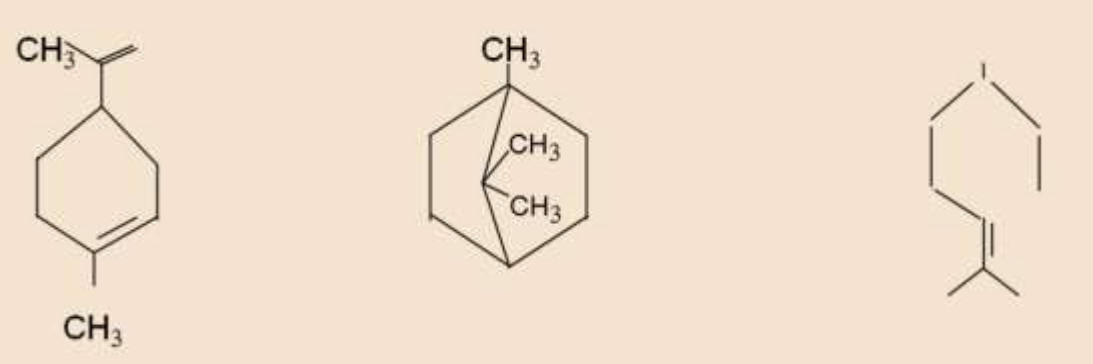
diterpenes ($C_{20}$) to the non-volatile triterpenoids and sterols ($C_{30}$) and cartonoids pigments ($C_{40}$). Each of this variable class of terpenoid( table 1-1) is of significance in either plant growth metabolism.[4]

Table (1-1): The classes of plant terpenoids

| No of isoprene units | Carbon number | Name class | Main types and occurrence |
|---|---|---|---|
| 1 | $C_5$ | Isoprene | Detected in Hamamelis japonica |
| 2 | $C_{10}$ | Monoterpenoids | Monterpenes in plant essential oils (menthol from mint) and (monoterpene lactone nepeta-lactone) tropolones (in gymnosperm wood). |
| 3 | $C_{15}$ | Sesquiterpenoids | Sesquiterpenes in essential oils<br>Sesquiterpenes lactones especially common in composite family abscisins (abscisic acid). |
| 4 | $C_{20}$ | Diterpenoids | Deterpene acids in plant resin gibberellins (gibberellic acid). |
| 6 | $C_{30}$ | Triterpenoids | Sterols (sitosterol) triterpenes (beta-amyrin) saponins (yamogenin) cardiac glycosides. |
| 8 | $C_{40}$ | Tetraterpenoids | Cartonoids (beta-cartoene). |
| N | $C_n$ | Polyisoperne | Rubber in hevea brsiliensis. |

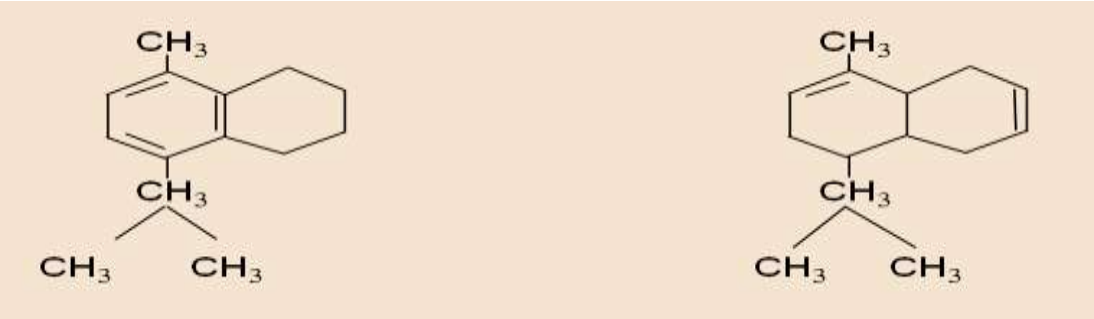
The majority of terpenes occur in plant kingdom. Some few terpenes have been obtained from other sources . Some bacteria produced the ($C_{50}$) compounds known as carotenoids. Chemically terpenoids are liquid-soluble and are located in the cytoplasm of the cell and sometimes occur in special glandular cells on leaf surface, whilst cartonoids are found with chloroplasts in the leaf and with chromoplasts in the petal.[4] Some representive groups of terpenoids are presented below:

(i) Monoterpenes



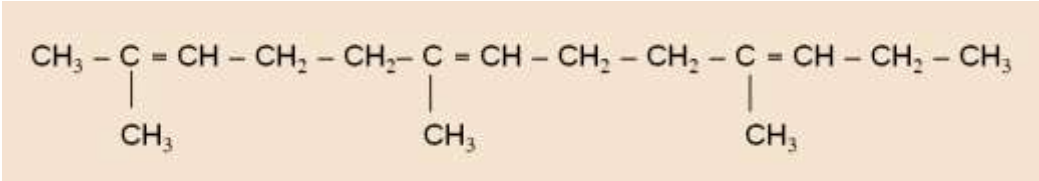

Limonene (1) Camplhor (2) Citral (3)

(ii) Sisquiterpenes



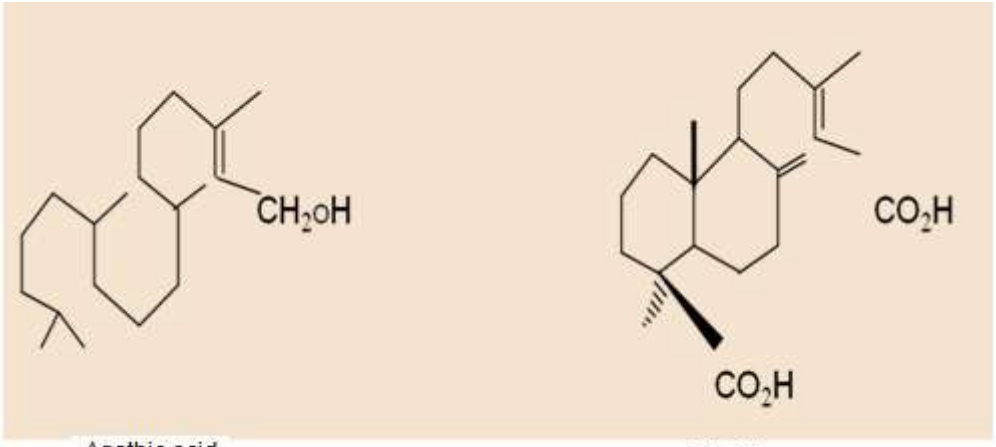

1,2-Dimethyl -1,4-Isopropyl Naphthalene (4) Codinene (5)

(iii) Triterpene




Squalene (6)

(iv) Diterpenes

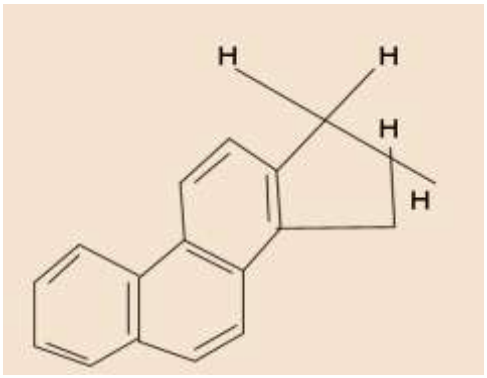



Agathic acid Phytol

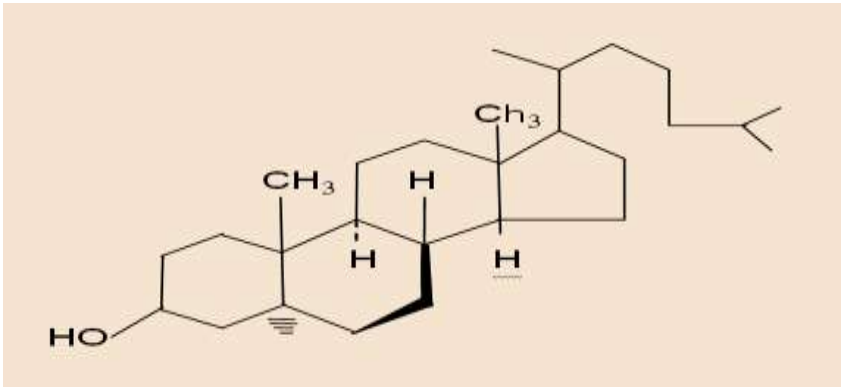
**(ii) Steroids**

The steroids from a group of structurally related compounds that are widely distributed in animals and plants. They included the most important groups such as vitamins, bile acids, sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, certain sapogenines.[4]

The steroids are a group of naturally occurring compounds that possess a lipophilic cyclopentano perhydrophenanthrene. Some derivatives of steroids are hormones (chemical messengers) that play a critical role in endocrine systems which control the regulation of metabolic processes. These hormones stimulate biological responses in a wide range of tissues to influence endocrine processes such as sexual differentiation, reproductive physiology, and maintenance of salt balance and sugar metabolism. They have also been implicated in responses to stress and behavior.[5] Structures of some steroids are shown below:




1,2-Cyclopentenophenanthrene (9)



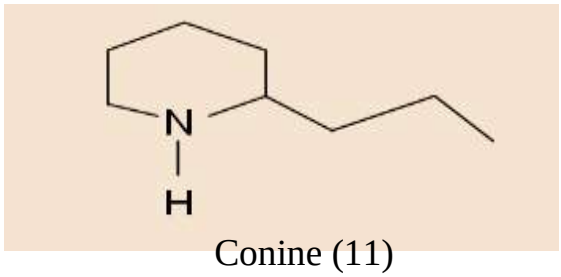

5- Alpha – cholestan – 3 – beta – ol (10)

**(iii) Alkaloids**

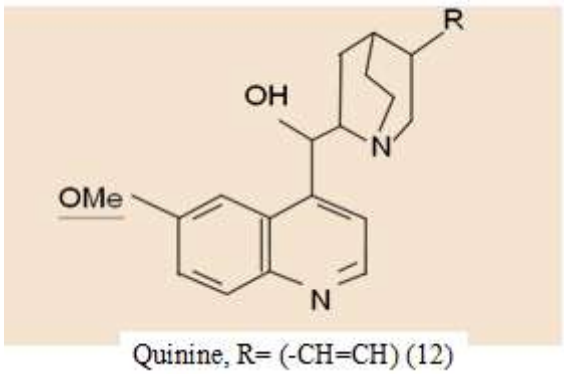
The alkaloids are heterogeneous compounds naturally occurring and it is difficult to define them accurately by a single definition. The term "alkaloid" is derived from the "vegetable alkali" used originally to describe a group of bases of botanical origin. Various authors have attempted to narrow definition, but always there is an exception; probably the best that can be said is that : most alkaloids are basic nitrogen-containing heterocyclic compounds which are isolated from the higher plants often having marked physiological properties. There are some compounds, which have been considered as alkaloids; within exception, such as: colchicines, ricinine both are not nitrogen- containing heterocyclic compounds. (6)

The amount of the alkaloid varies quantitatively in different parts of the plant (seeds, roots, leaves, bark); they occur as salts of various plant acids

such as acetic, oxalic, citric, malic, tartaric acid, etc.). Some alkaloids are very poisonous, but are used medicinally in very small quantities. Thus we find that the basic properties, complex structures, physiological action and plant origin are the main characters which define plant alkaloids. Even so, the classes of compounds known as the purines, which possess the above characters, are not usually included under the heading of alkaloids. [6] The structures of some groups of alkaloids are presented below:

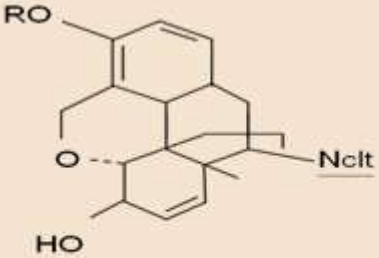



Conine (11)

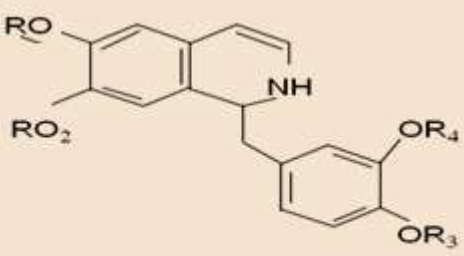



Quinine, R= (-CH=CH) (12)

(2) Isoquinoline (benzylioquinoline) groups:




Morphine, R=H (13)

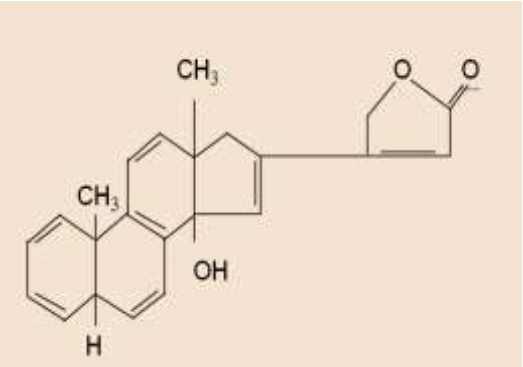



Papaverine, $R_1=R_2=R_3=R_4=CH_3$ (14)

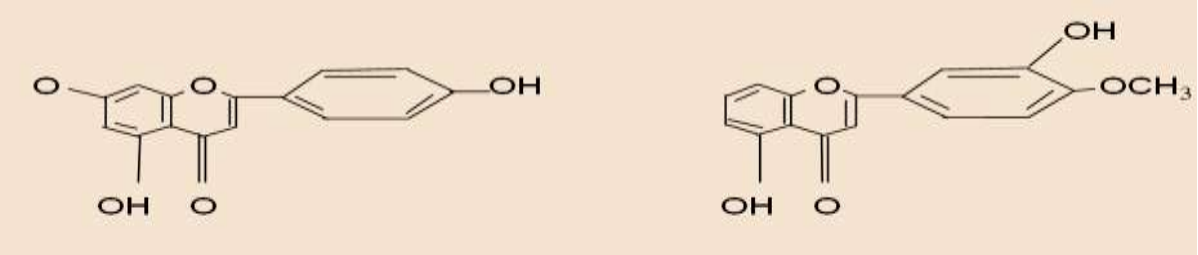
**(iv)Glycosides**

Glycosides are very important constituents of medicinal plants . Plants may contain different types of glycosides which possess different physiological effects . Glcosides are occurring naturally, and are hydrolyzed by plant acids in presence of enzymes; hence they give different types of sugars at least reducing sugar, specifically glucose and other types of sugar such as rhamnose, digitoxose, cymarose. While non-sugar substances such as aglycones or genins are structurally different in such plants.[7]

Glycosides played an important role in human life, their medicinal importance is encountered in especial groups such as steroidal glycosides (digitoxin), isolated from the digitals plant; and from other species of plants specially (leave),Digitoxin is used for heart diseases (cardiotonic glycoside . Rutin glycoside is an important glycoside which is isolated from buck wheat.[7] The structures of some groups of glycosides are presented below:




Digitoxin (Cardinolide) (15)




Apiin (16)

Hesperidin, (Citrus fruits) (17)

**(v) Flavonoids**

Flavonoids are phenolic substances which are widely distributed in all vascular plants. The flavones and the anthocyanins are sap- soluble and have a pyrone ring. Flavones are often yellow (Latin flavus, yellow). They are widely distributed in nature but are more common in higher plants and in young tissues where they occur in the cell sap. The flavonoids which occur both in the free state and as glycosides are the largest group of naturally occurring phenols. [8] More than 2000 of these compounds are now known, with nearly 500 occurring in the free state. Most are O-glycosides but a considerable number of C- glycosides flavonoids are known. Dimeric compounds are also known (biflavonyls). The glycosides are generally soluble in water and alcohols but insoluble inorganic solvents. Flavonoids generally dissolve in alkalis, giving a yellow solution (phenates) which on addition of acid becomes colorless. The flavonoids are generally yellow compounds and the intensity of their yellow color increases with the number of OH groups and with increase of the $P^H$ of the medium. [9]

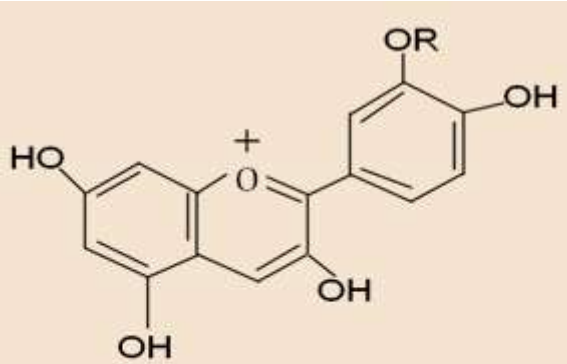
Flavonoid structures are derived from the parent substance flavones. They have similarity in their properties. The most common ten classes of flavonoids are presented in table (2). They are mainly water-soluble and are extracted with 70% ethanol and remain in the aqueous layer. They changed their color when treated with base or ammonia; thus they are easily detected on chromatograms or in solutions. They contain a conjugated aromatic system and thus show specific intensity in (UV) and visible regions of the spectrum. Finally they are present in plants bound to sugar as glycosides.[10]

Table (1-2): Types of flavonoid compounds

| Flavonoid class | Distribution | Characteristic properties |
|---|---|---|
| Anthocyanines | Scarlet, red, mauve and blue flower pigments; also in leaf, other tissues. | Ware-soluble ($\lambda_{max}$515-545 nm). Mobile in (B.A.W) on paper. (Butanol: acetic acid: water). |
| Leucoanthocynine | Colorless. Co pigments in heart woods and in leaves of woody plants. | Anthocyanidins (color extractable into amyle alcohol), in presence of 2M HCL, 0.5 hr |
| Flavonols | Colorless co pigments in both cyanic and a cyanic flower, particularly in leaves. | Acid hydrolysis, bright yellow. Spots in U.V light on forestall chromatograms; spectral max (350-386nm). |
| Flavanones | As flavonols | Acid hydrolysis, dull absorbing brown spots on forestalls chromatograms spectral max (330-350 nm). |
| Glycoflavones | As flavonols | Contain C-C linked sugar, mobile in water unlike normal flavones. |
| Bi flavonyls | Colorless; almost entirely confined to the gymnosperms. | On B.A.W chromatograms dull absorbing spots of very high Rf values. |
| Chalcones and aurones | Yellow flower pigments; occasionally found in others issues. | Give red colors with ammonia and it absorbed in visible max (370-410 nm). |
| Flavanones | Colorless, in leaf and fruits (citrus). | Give intense red color with Mg/Hcl; occasionally the bitter tests for the intensity measurements. |
| Is flavones | Colorless; often in roots. | Mobile on paper in water no specific colors tests available. |

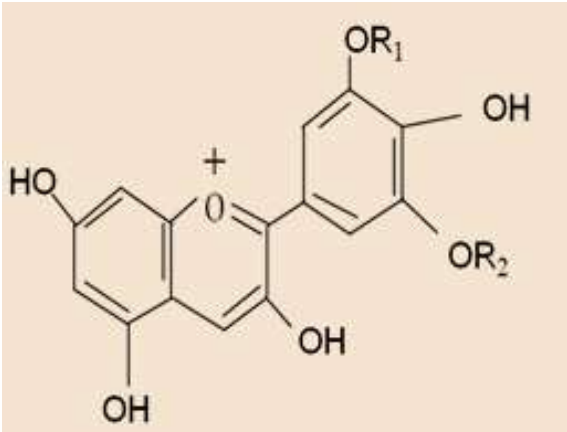
**(vi)Anthocyanidins**

Anthocyanidins are the most striking and conspicuous pigments of the plant; they are responsible for the pink-red- blue colorations in nearly all flowers, fruits and leaves. The most important groups are : catechins, flavanones, flavan-3,4-diols, flavones, anthocyanidins and flavonols.[10] Some representive examples are shown below:




Cyanidin, R=H (18)

Peonilidin, R=Me (19)



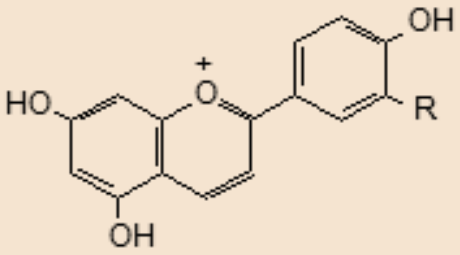

Delphinidin, $R_1$= $R_2$=H (20)

Pelunidin, $R_1$=OH, $R_2$=CH3(21)

Malvidin, $R_1$=$R_2$=$CH_3$ (22)

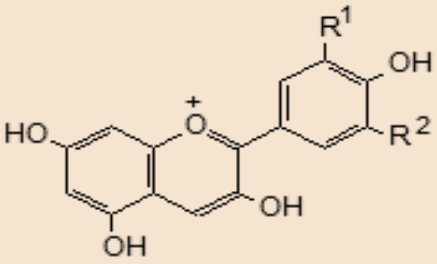
**(vii)Flavones and Flavonols**

Flavones and flavonols are widely distributed in higher plant families, they are encountered in a wide range of plants. Common types are presented below.[10]




Apigenin, R=H (23)

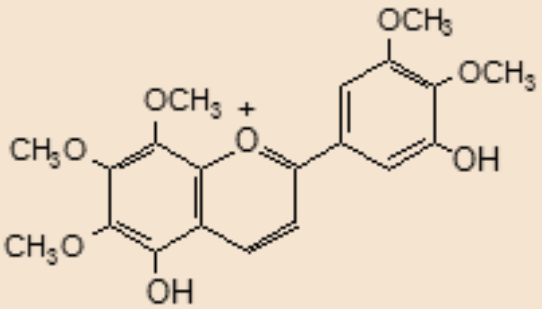
Luteolin, R=OH (24)



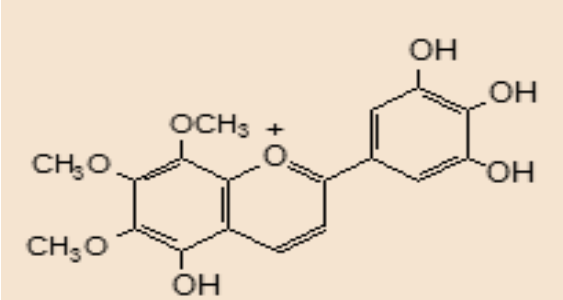

Campferol, $R_1$= $R_2$=H (25)

Quercetin, $R_1$=$R_2$=OH (26)

Myricetin, $R_1$=$R_2$=OH (27)




Digitrim (28)

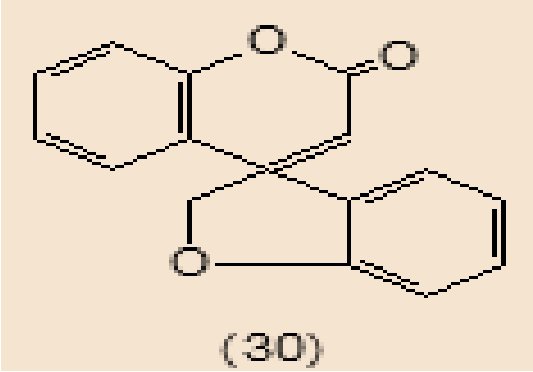


Quercetagetin(29)

**(viii)Isoflavonoids**

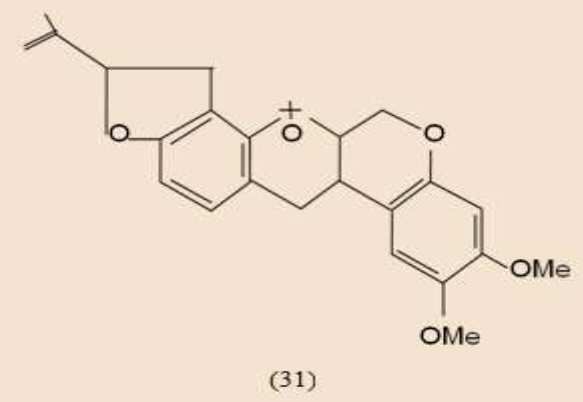
Isoflavonoids are compounds which are closely related to the flavonoids[10]. This group of flavonoids comprises the following sub-classes:

**-Coumestans (30)**



(30)

-Rotenone (31)

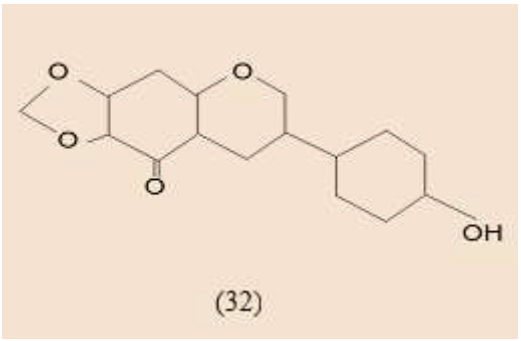


(31)

-Isoflavones

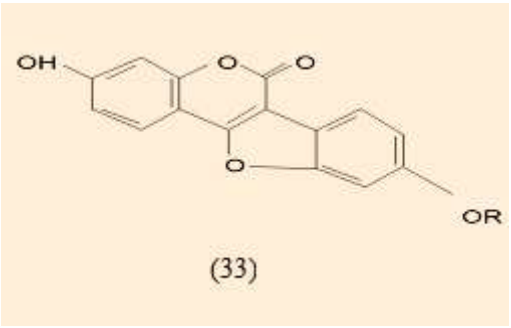
The isoflavones occur naturally, but are not as widespread as the flavones; they exist either in the free state or as glycosides. The general methods of ascertaining the structure of isoflavones is similar to that used for the flavones. Derivatives of these compounds are encountered as: [10]

Irisolone (32)




(32)

-Coumestrol, R = H (33)




(33)

## 1.2. Essential Oils

### 1.2.1 Definition of essential oils

The term essential oil dates back to the sixteenth century and derives from the drug *(Quinta essential*), named by Paracelsus von Hohenheim of Switzerland [11]. Numerous authors have attempted to provide a definition of essential oils. The French

Agency for Normalization: Agence Française de Normalisation (AFNOR) gives the following definition (NF T 75-006): “The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or “dry”” distillation. The essential oil is then separated from the aqueous phase by physical means [12].

An essential oil is a concentrated hydrophobic 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 them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits [13].

All parts of aromatic plants may contain essential oils as follows:

• Flowers, of course, 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.

### 1.2.2. Essential oils extraction methods:

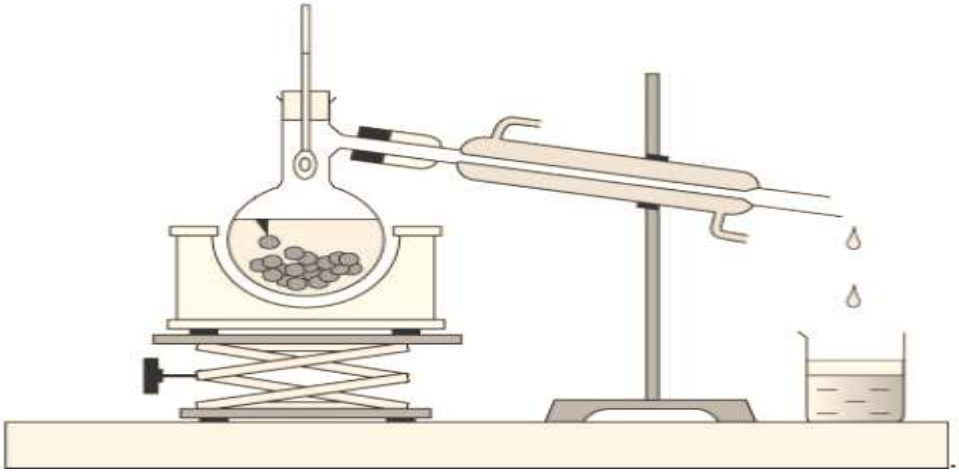
Essential oils are obtained from plant raw material by several extraction methods [14],[15]

### (i). Classical and conventional methods

There are several methods of extraction of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused in copious parts of the globe.

### a.Hydrodistillation (HD)

The conventional method for the extraction of essential oils is hydrodistillation (HD), 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(Scheme 1-1)



Scheme (1) : The schematic subsidize apparatus for hydrodistillation.

**b.Solvent extraction**

Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a 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.

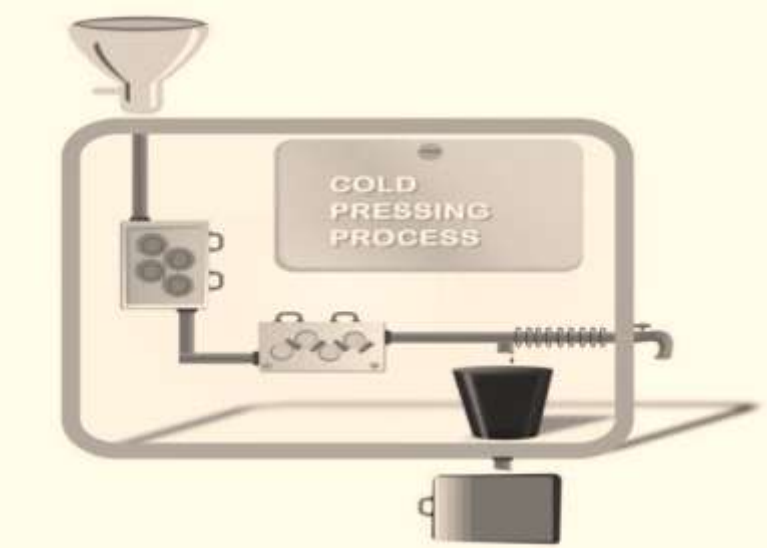
**c. Soxhlet extraction**

A Soxhlet extractor is a piece of laboratory apparatus [(16)], invented in 1879 by Franz von Soxhlet [(17)]. It was originally designed for the extraction of a lipid from a solid material . Soxhlet extraction involves 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 [18].

**d. Cold pressing method**

The term cold pressed theoretically means that the oil is expeller-pressed 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 ( Scheme 2)). Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils [19]. 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.

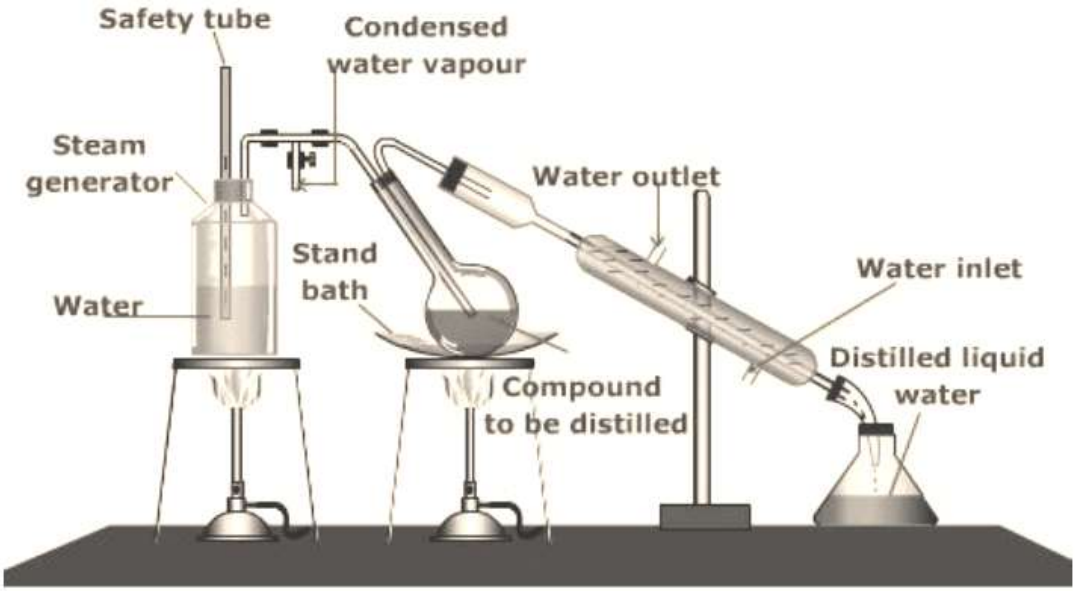



Scheme 2: Cold Pressing Method

**e. Steam Distillation**

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials 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 as shown in (Scheme 3). 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 [20]. 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.




Scheme 3: The schematic subsidize apparatus for steam distillation

## (ii). Innovative techniques of essential oils extraction (non-traditional)

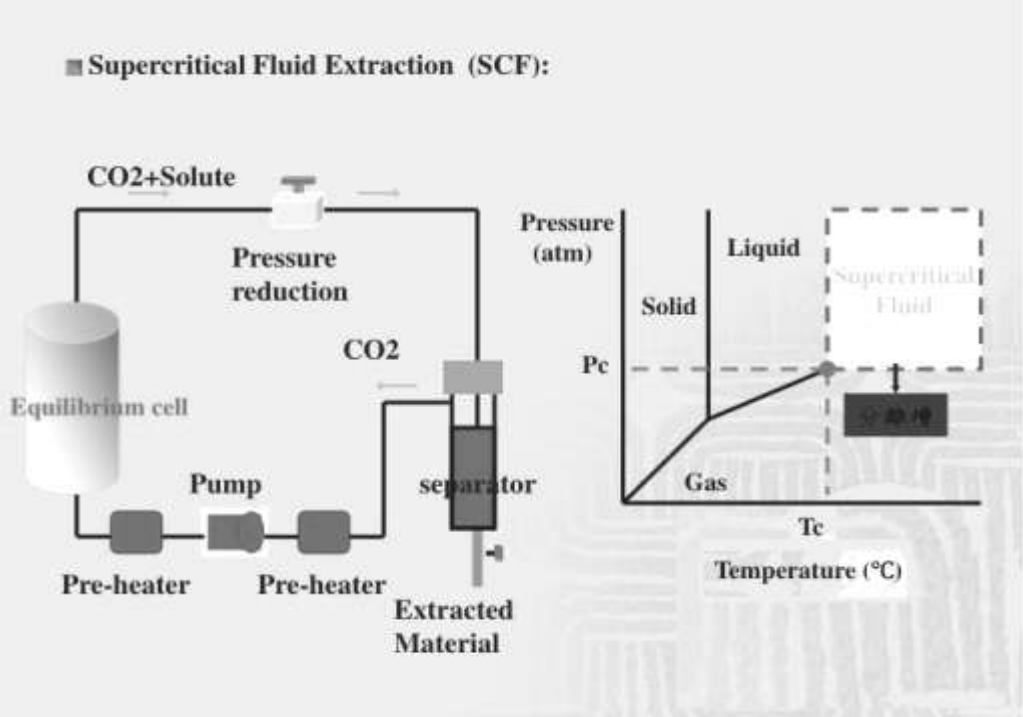
One of the disadvantages of conventional techniques is related with the thermolability of essential oils components which undergo chemical alterations (hydrolyse, isomerization, oxidation) due to the high applied temperatures. New extraction techniques must also reduce

extraction times, energy consumption, solvent use and $CO_2$ emissions.

**(i). Supercritical Fluid Extraction (SFE)**

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids 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 ( Scheme 4 ). 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 (32$^{o}$C), $CO_2$ is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [(21)]. The main drawback of $CO_2$ is its lack of polarity for the extraction of polar analytes [(22)]. 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[(23)]. 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 [24]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.



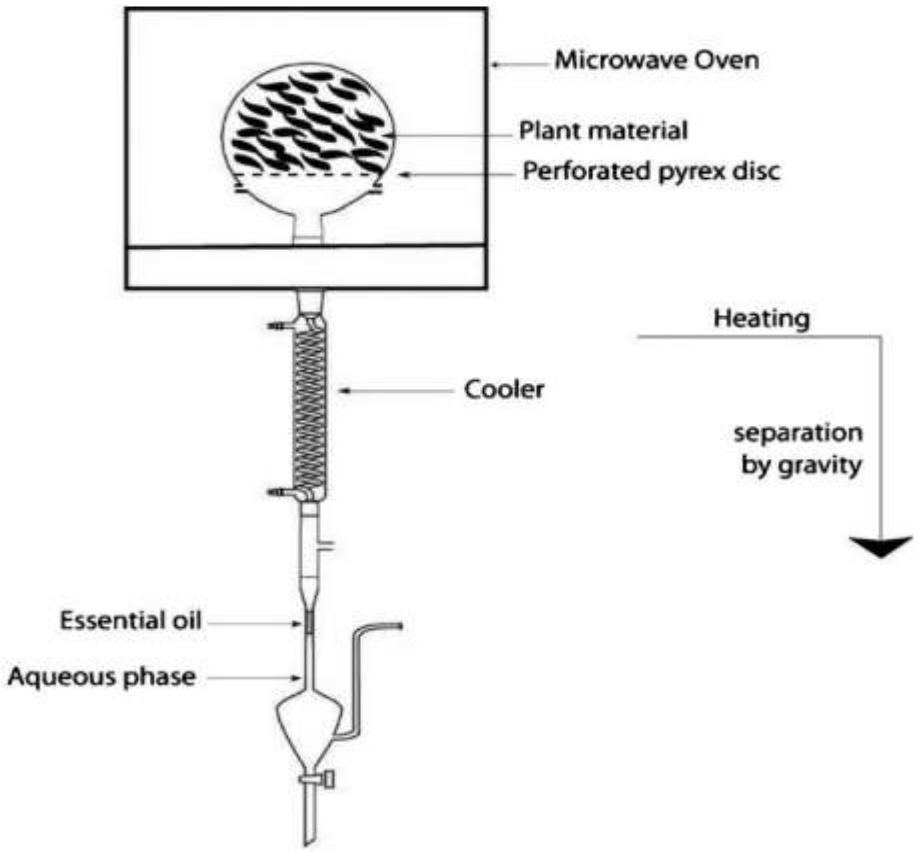

Scheme (4): Supercritical fluid extraction (SFE)

**(ii). Microwave hydro diffusion and gravity (MHG):**

(MHG) Is a new green technique for the extraction of essential oils. This green extraction technique is an original microwave blend 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 (Scheme 5)[25].

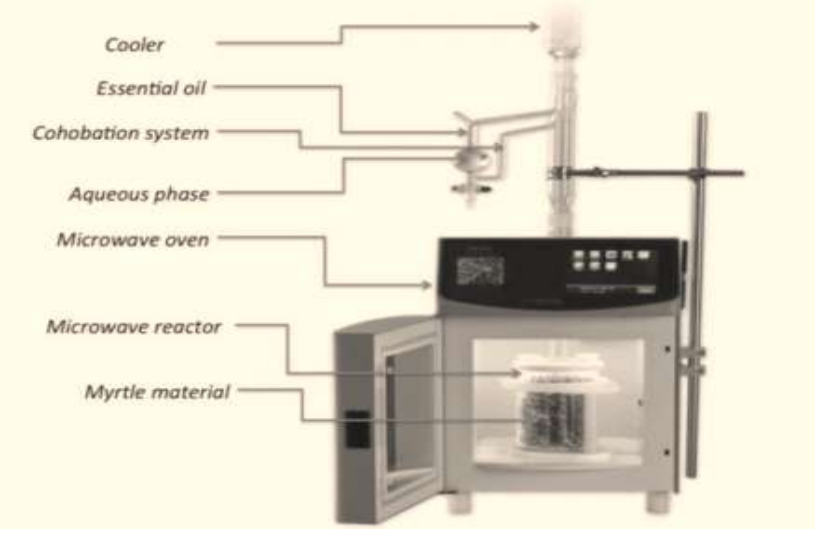
Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and as it does require less energy[26]. The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental impact and power saving [27].




Scheme 5: Microwave hydrodiffusion and gravity (MHG)

**(iii). Solvent-free microwave extraction (SFME):**

Solvent-free microwave extraction (SFME) is an extraction procedure of essential oil which is accomplished by the water of the plant material without added any solvent [28]. Based on the integration of dry distillation and microwave heating energy, (Scheme 6). It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent [29]. 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.

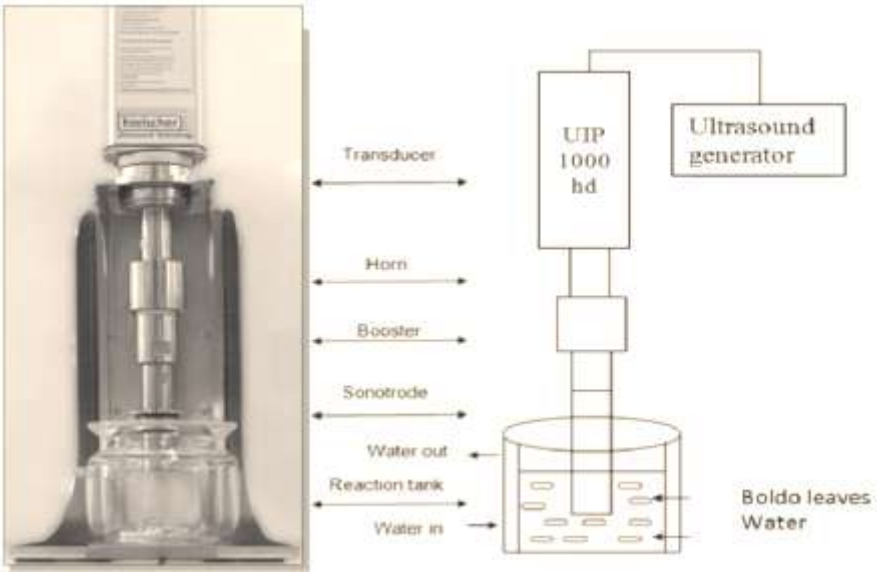



Scheme 6: Solvent-free microwave extraction (SFME)

### (iv). Ultrasonic-assisted extraction (UAE)

Ultrasonic-assisted extraction (UAE) is a good process to achieve high valuable compounds and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material [30]. 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 hydrodistillation (Scheme 7). 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[31].

The plants raw material is immersed in water or another solvent (Methanol or ethanol or anyonefromthesolvents) and at the same time, it is subjected to the work of ultrasound[32]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds [33].

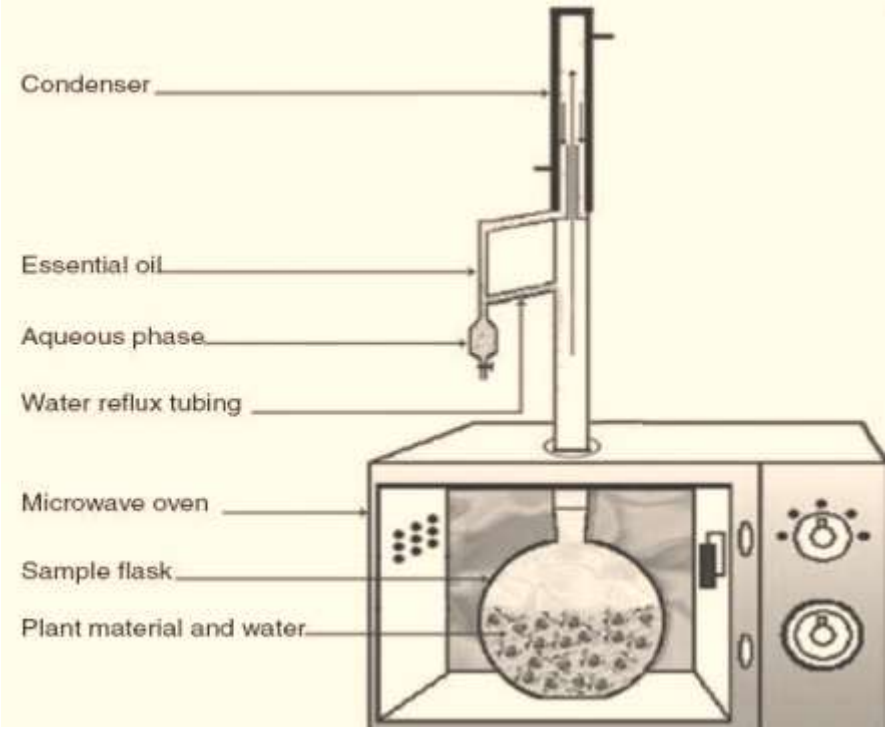



Scheme(7): Ultrasonic-assisted extraction (UAE)

**(v). Microwave-Assisted Hydrodistillation (MAHD):**

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizinga microwave oven in the extraction process. The efficiency of Microwaveassisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample [(34)]. 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 hydrodistillation technique(Scheme 8). Application of Microwave-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 [(35),(36)]and consuming only a fraction of the energy used in conventional extraction methods[(37)].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 hydrodistillation 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, volumetric heating and controllable heating process.The principle of heating using Microwave-assisted hydrodistillation 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[38].




Scheme(8): Microwave-assisted hydrodistillation

### 1.3. Chemistry of essential oils

Essential oils are localized in the cytoplasm of certain plant cell secretions, which lies in one or more organs of the plant; namely, the secretory hairs or trichomes, epidermal cells, internal secretory cells, and the secretory pockets. These oils are complex mixtures that may contain over 300 different compounds[39]. They consist of organic volatile

compounds, generally of low molecular weight below 300. Their vapor pressure at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in the vapor state [40], [41]. These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes,ketones,esters,amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohols, aldehydes, and ketones offer a wide variety of aromatic notes, such as fruity ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal (γ-selinene).

## 1.4- Biological Activities of essential oils

### 1.4.1 Antibacterial activity

An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable [42]. This can then cause leakage of ions and other cellular molecules [43] [44]. Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death[45] Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, have important antibacterial activities [46],[47],[48]. These compounds are responsible for the disruption of the cytoplasmic membrane, the driving force of protons, electron flow, active transport, and also coagulation of cell contents[42],[45],[49]. The chemical structure of essential oils affects their mode of action concerning their antibacterial activity[48]. The action of thymol against Bacillus cereus, Staphylococcus aureus, and Pseudomonas aeruginosa appears to be comparable to that of carvacrol,for example[42],[46]. However, carvacrol and thymol act differently against Gram-positive and Gram-negative species[48]. Thymol, eugenol, and carvacrol have an antimicrobial effect against a broad

spectrum of bacteria: Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus, and Helicobacter pyroli[50),(51)].

### 1.4.2. Antioxidant Activity

The antioxidant potential of an essential oil depends on its composition. The essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties[(52)]. Thymol and carvacrol are the most active compounds.Their activity is related to their phenolic structure. These phenolic compounds have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition[(53)]. The antioxidant activity of essential oils is also due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-CineoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: α-Terpinene, β-Terpinene and α-Terpinolene[(52)]. Essential oils with important scavenging capacity of free radicals may play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and immune system decline. In fact, these diseases may result from cellular damage caused by free radicals[(52),(54)].

### 1.4.3. Anti-Inflammatory Activity

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self cells) and to remove dead or damaged host cells. Linalool and linalyl acetate showed anti-inflammatory activity on oedema of paw-induced mouse carrageenan[(55)]. The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and

regulatory transcription factors, and on the expression of pro-inflammatory genes. Essential oils, therefore, represent a new option in the treatment of inflammatory diseases.

### 1.4.4. Cancer chemoprotective activity

Essential oils would act in the preventionof cancer, as well as at its removal. It is well known that certain foods, such as garlic and turmeric, aregood sources of anticancer agents[56]. Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer[57],[58]. In addition, anticancer activity of D-limonene, the main component of Citrus essential oil has been proven, especially at the level of stomach cancer and liver [59].

### 1.4.5. Cytotoxicity

Essential oil cytotoxicity mainly correlates to the presence of phenols, alcohols, and monoterpene aldehydes[60],[61]. The cytotoxic properties of essential oils are of great importance because they assume their use not only against certain human pathogens and animal parasites, but also in the preservation of agricultural and marine products against microbial attack. Indeed, some components of essential oils are effective against a variety of microorganisms as bacteria[62],viruses[63], fungi[64],[65-67],and others. In addition, α-humulene shows cytotoxicity against breast cancer cells in vitro. α-humulene was reported to be responsible for cytotoxicity (CI50 55 mM) [68].

### 1.4.6. Allelopathic Activity

According to the International Allelopathy Society (IAS), allelopathy was defined in 1996 as “The science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that

influences the growth and development of agricultural and biological systems" Allelopathic interactions derive from the production of secondary metabolites. The secondary metabolites are synthesized for a wide range defense by plant and microorganisms. The secondary metabolites involved are called allelochemicals[69].

## 1.5 Gas chromatography-Mass Spectrometry

### 1.5.1 Principle, Technique and its application

GC/MS-a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified and quantified, but it cannot readily separate them. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid-1950. Gas chromatography and mass spectrometry are, in many ways, highly compatible techniques. In both techniques, the sample is in the vapor phase, and both techniques deal with about the same amount of sample (typically less than 1 ng). This article was prepared with an aim to review different aspects GC-MS, such as principle, types, instrumentation and applications in science [70].

Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can

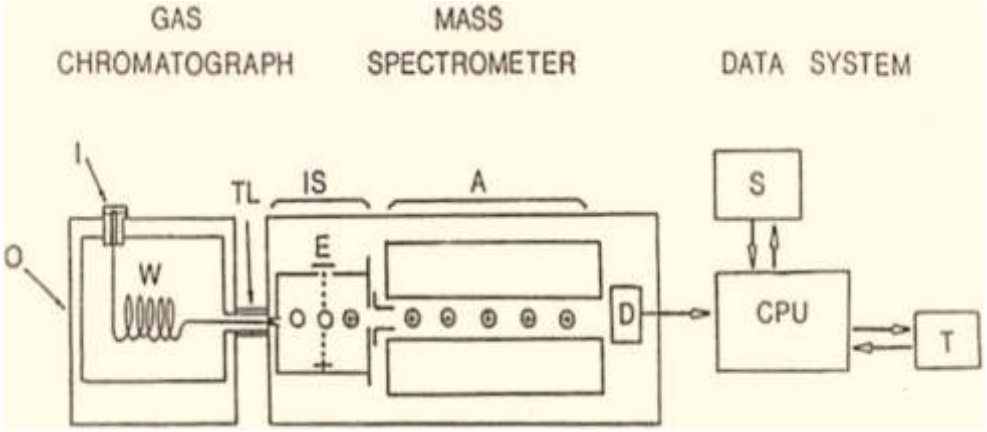
itself be the stationary phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass Spectrometry (MS), the detector for the GC is the Mass Spectrometer (MS). As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint. Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them [(71)].

### 1.5.2 Gas Chromatography

All forms of chromatography involve the distribution, or partitioning, of a compound between a mobile phase and a stationary phase. In GC, the mobile phase is a gas and the stationary phase is an immobile, high molecular weight liquid which is deposited on or chemically bonded to the inner walls of a long capillary tubing. The term GLC (gas-liquid chromatography) is also used to refer to this separation technique. The capillary tubing through which the sample moves is called the chromatographic or GC column. Presently, most GC columns used for this work are manufactured from fused silica. They are generally 30-60 m in length and have an internal diameter of about 0.2 mm[(72)]. By covering the outside surface of these capillary columns with a polymeric coating,

these flexible fused silica GC columns are made more durable. The analysis of effluents for organic compounds requires extraction of the organics from the water matrix, concentration of the extract, separation of individual components of the organic extract by a GC column and detection of the separated components as they are eluted from the GC column. Complex mixtures of organic compounds are extracted from effluents by using high-purity organic solvents. The low-volatility organic compounds extracted from an effluent sample can be concentrated to a small volume (typically, 1.0 mL or less) by removing the extraction solvent through evaporation. This concentration step is necessary in order to obtain detection limits in the low part-per-billion (ppb: 10-6 g/L). Some compounds of concern may be more volatile than the extraction solvent and would be lost in this process. Such compounds are removed from the sample by directly purging the aqueous sample using an inert gas and collecting the purged volatile compounds on an adsorbent trap designed for this purpose. In either case, organic compounds from the sample are separated from the bulk aqueous matrix and concentrated for GC analysis[73]. The organic compounds are introduced into the GC column by injecting a few microlitres (μL) of the concentrated solvent extract into an injection port (non-volatile organics) or by heating the sorbent trap (volatile organics). An inert carrier gas (He, $N_2$, $H_2$), is used to sweep the extracted organic compounds, which are now in the vapor state, through the GC column. Compounds that have different solubilities in the liquid phase of the GC column will take different times to traverse the length of the column. For a specific set of experimental conditions, the time it takes a compound to travel through a GC column is a physical property of that compound - called its retention time. Generally, higher molecular weight compounds will have greater retention times than lower molecular weight compounds. Also,

compounds that have a similar polarity to that of the liquid phase will be more soluble in the phase and will have greater retention times than compounds less soluble in the liquid phase. Therefore, organic compounds in a mixture can be separated from each other by using gas chromatography, and the retention times of these compounds can be used to assist in their identification. Some environmental samples are so complex that there are hundreds of compounds present in their concentrated organic extracts. There are currently no GC columns available that can completely separate all components of such complex mixtures from each other. However, in most cases the principal sample components can be detected individually [73].




Scheme(9). Diagram of a GC-MS System.

In another design, ions travel through a magnetic field where their momentum is affected by the magnetic field strength. Conditions can be controlled to allow the analyzer to scan across a range of m/z to form a mass spectrum. This design is called a magnetic sector mass analyzer. Other designs are described in detail in references [74].Given previously. An important concept in GC-MS is resolution. In GC, resolution refers to the Ability of the GC column to separate components in a mixture from

each other. In mass spectrometry, mass resolution refers to the ability of an analyzer to separate ions that have similar m/z [74].

### 1.5.2.1. Gas Supply

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure (Gas Supply and Pressure Control from theory and Instrumentation of GC-GC Channel) [74].

### 1.5.2.2 Injector

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.

### 1.5.2.3. Column

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal[75].

### 1.5.2.4. Oven

Gas chromatography has ovens that are temperature programmable, the temperature of the gas chromatogram.

### 1.5.2.5. Mass spectrometry

As the separated sample components elute from the GC column, they are monitored using any of a large number of detectors developed for this purpose. The most versatile of these detectors is the mass spectrometer (MS). When an MS detector is used to detect the compounds that elute from a GC column, the combined technique is called gas

chromatography-mass spectrometry (GC-MS). A schematic drawing of a GC-MS instrument is given in Figure 1. Initially, molecules enter the source chamber of the mass spectrometer maintained under high vacuum, where they are bombarded by electrons [76]. The energy transferred to molecules in this process causes them to ionize and dissociate into various fragment ions. Ions may be singly or multiply-charged. The positive ions formed are made to traverse an analyzer section, . After the ions traverse the analyzer section where they are separated according to their mass-to-charge ratio (m/z), they are detected by an extremely sensitive device called an electron multiplier. By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. The mass spectrum of a compound is like a fingerprint that can be used to identify the original organic structure. It consists of a bar graph representation of the m/z of the ions and their abundances normalized to the most abundant ion (base peak). By matching the GC retention time of a sample component and its mass spectrum with those of a standard reference compound analyzed under the same conditions, a positive identification of the sample component is obtained. Several different mass analyzers have been developed. One of the most common designs consists of a square array of four parallel metal rods. By controlling radio-frequency (RP and DC voltages to these rods, an oscillating electric field is generated and this allows ions to be filtered according to their m/z. At a specific setting of voltages, only ions of the desired m/z will have a stable trajectory and will be able to reach the electron multiplier. By changing the applied voltages in a specified manner, the mass spectrum of a compound can be generated as the ions of various m/z are scanned. The entire process is performed in about one second. This design is called (QP) a quadruple mass analyzer [77]. separated is given by the formula m/Δm, where m is the nominal mass of one ion and Am is the mass

difference between that ion and the next higher mass ion that is just resolved. For example, the integer mass (or nominal mass) of both nitrogen gas ($N_2$) and carbon monoxide (CO) is 28. However, the actual mass of CO is 27.99492 while that of $N_2$ is 28.00615. A quadruple analyzer would not be able to distinguish between these two ions (mass difference = $\Delta m = 0.01123$). By using a magnetic sector GC-MS analyzer at resolution $m/\Delta rn = 28/0.01123 = 2{,}493$, these two ions are resolved from each other. Since the m/z values of ions are not simple integers, the additional resolving power of high resolution magnetic sector analyzers is sometimes needed to improve selectivity. In addition, it is sometimes possible to establish unequivocally the molecular formula of a compound by accurate mass determinations [78].

### 1.5.2.6. Ion source

In the ion source, the products are ionized prior to analysis in the mass spectrometer

### 1.5.2.7. Mass analyzer

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

### 1.5.2.8. Vacuum system

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

### 1.5.2.9. Detector

The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element

of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges) [73-75].

## 1.6. The target Plants

### 1.6.1. *Azadirachta Indicia* L (Neem)

Neem (*Azadirachta Indica*) tree which belongs to the family of Meliaceae , is a large ever green tree growing up to 60ft high in the dry tropical forests of India , Burma , Pakistan and Africa. Neem twigs are used as tooth brushes in India from times immemorial. Neem flower are white in color , generally 5 mm long and have a peculiar fragrance and produce nectar.The flowering period is generally between the months of January and April. Buds normally open in the afternoon , emanating a strong smell during night. Flowers are used in pharmaceutical , food and cosmetic industries. During the flowering season , large quantities of flower fall on earth and go wasted . Neem flowers are used in making a traditional chutney along with mangoes , tamarind , jaggery , salt , chilly and coconut.[79]

The seed kernels constitute 50-60% of the seed weight and 25% of the fruit,The fat content of the kernels ranges from 33-45%. [80] Neem oil (40% yields) is usually opaque , bitter and inedible but can be processed into non bitter edible oil with 42-50% oleic acid and 15% linoleic acid.The neem tree provides many useful compounds that are used as pesticides and could be applied to protect storage against insects. [81] Neem seed has high nutritional potential for livestock.[82] Tignic acid is the principle component responsible for the distinctive odour of neem seed,[83] as well as sulphur- containing compounds like nimbin , nimbidin and nimbosterol[84].

**-Taxonomy**

Kingdom : Plantae

Phylum : Vascular plant

Class : Magnoliopsida

Order : Rutales

Family : Meliaceae

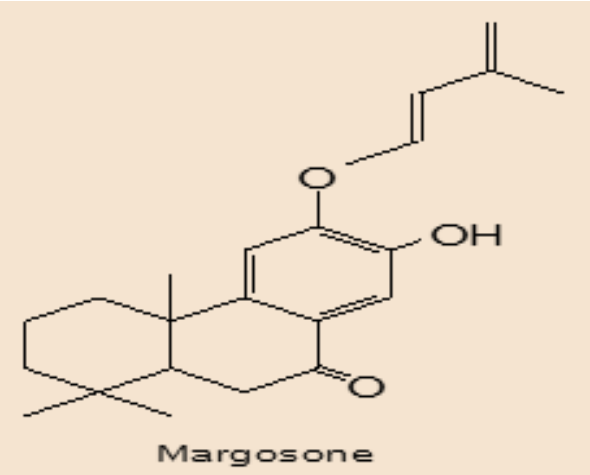
Genus : Azadirachta

Species : *Azadirachta indica*

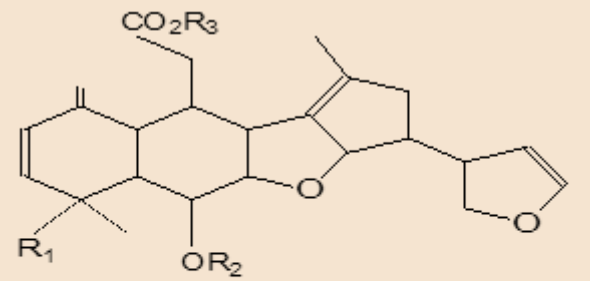
Chemical investigations of neem were undertaken by Indian pharmaceutical chemists in 1919 , whereby they isolated an acidic principle in neem oil , which they named as (margosic acid). However ,real chemical research originated in 1942 with isolation of three active constituents , viz , nimbin , nimbidin and nimbinene.In 1963 an Indian scientist extensively examined the chemistry of the active principles of neem.Following the discovery of neem kernel as a locust feeding deterrent , its chemistry has grown considerably. Several compounds have been isolated and characterized.The main feature is that most of them are chemically similar.These are also called liminoids (azadirachtin , meliantrol , salanin etc) bitter principles and occur in other botanical species as well (Rutaceae and simaroubaceae ). From the practical side these compounds also exhibit a wide variety of biological activity , for example , pesticides , cytotoxic properties .The neem constituent belonging to chemically diverse classes have been divided into two major sections viz. i)isoprenoids ,ii) non-isoprinoides.The later category

comprises glycerides, polysaccharides, sulphurones compounds flavonoids and their glycosides, amino acids , aliphatic compounds etc[92].

Structure of some constituents of Neem are given below (fig1) :




Margosone




Azidirachtin A

$R_1 = CH_3$

$R_2 = CO_2Me$

$R_3 = Ac$

In many parts of the world , the herbal remedies from medicinal plants are used traditionally , but their access to formal health care are limited.[85] *Azadirachta indica* is a very useful traditional medicinal plant in the African subcontinent. Each part of the tree has some medicinal properties which can be used to treat several diseases.[86,87] In India , the neem tree twigs are commonly used to scrub teeth.Moreover , the neem tree branches are used as one of the most effective forms of dental care in

traditional medicines , even though , it seems a little bit unpleasant for the users.[88] Interestingly, the neem trees are an excellent alternative for modern tooth care products. Besides, the leaves of the neem tree are also used as natural treatment for acne sufferers.[89] Similarly, treatment of infected eyes can be carried by the use of neem leaves. A similar infusion can also be used in the treatment of sore throats. [90] All parts of neem trees including leaves , seeds , roots , bark and the flowers of the plant are used to cure different ailments , such as stomach ulcers , jaundice and to overcome a variety of infectious and parasitic diseases , ranging from leprosy , chicken pox to malaria. Infusions and teas made from leaves are used to alleviate malaria attacks , intestinal complaint , treat dental headache , stimulating the appetite , heartburn and as insect repellent, in addition to that it was also used as a diuretic and for diabetes as well as to treat numerous skin diseases[91] The use of aqueous extracts from seeds to treat head lice is widely known. Neem oil showed good antiseptic properties. It is applied in the treatment of such skin complaints as furuncles and eczema, as well as to relieve intestinal worm infections.[92] Apart from that, neem-based products from *Azadirachta indica* are traditionally used for pest control in agriculture and gardening since long in India.[93,94 ]

## 1.6.2. *Gerwia tenax*(ForssK)Fiori

*Gerwia tenax*(Forssk.) is a small- leaved white cross berry that belongs to the Tiliacea family. This fruit-producing deciduous shrub or small tree is prevalent in African and south-east Asian countries, with the fruits known locally as guddaim in Sudan. The tree has a wide distribution in the savannh plantation area in the north and middle of Sudan[95]. *Gerwia tenax* has been used in folk medicine in several ways in different countries. The roots have

been used to treat jaundice, lungwort infections, and asthma. There is commercial potential in using the fruits in beverages, yogurt, ice cream, and baby food. The fruits are used by rural villagers as an iron supplement for anemic children. A thin porridge called "nesha" is prepared by boiling millet flour and the pulp of *Gerwia tenax* fruits and then adding custard to the mixture[96]. This porridge is given to pregnant and lactating women to improve their health and their ability to produce milk for their children[97]. *Grewia tenax* fruits have been used as a traditional treatment for irritations and skin infections in both human beings and animals in Sudan. *Gerwia tenax* fruits, which can be eaten ripe or stored for later use, consist of large quantities of carbohydrates in a liquefied form as well as a large quantity of calcium. Different parts of several species in the genus *Grewia* are used as folk medicine in various areas of the world[98].

**-Taxonomy:**

Kingdom: Plantae

Division: Angiospermae

Sub-division: Dicotyledons.

Class: Thalamiflorae

Order: Tiliales

Family: Tiliaceae

Genus: Grewia

Species: G.tenax,G.villosa.

Different parts of plant are traditionally used for treating different human and livestock ailments. Fruit pulp is used to treat swelling in the body. For this purpose seeds are extracted from ripen fruits

and remaining pulp is applied externally on the affected parts by gentle application. Seeds and green leaves used at the time of the animal delivery. Ripen fruits are important source of iron and are given to pregnant women to cure anemia caused by iron deficiency. Leaves and twigs are important components of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling[(99)].It is widely used for the treatment of various common diseases such as stomachs upset, cough, fever, diarrhea, dysentery, jaundice and rheumatism[(100)].Fruits of *Grewia tenax* significantly increased hemoglobin and are considered as a simple safeguard against iron deficiency and often used in special diets for pregnant women and anemic children[(101),(102)]. Its root and fruits are well known household remedy for the treatment of osteoporosis, tissue and wound healing. The fruit is a rich source of carbohydrates, protein, vitamins and minerals and species is nutritionally balanced[(103)].

### 1.6.3.*Citrus aurantifolia*

*Citrus aurantifolia* is a species that belongs to the family Rutaceae which has about 150 genera and 1600 species that are broadly distributed in tropical, subtropical and temperate zones around the world. In Brazil, the family Rutaceae is represented by about 29 genera and 182 species; some of them have economic importance [(104)].Citrus is a genus that comprises about 70 species of subshrubs and shrubs which may be either grown or spontaneously found in Germany, Spain, Mexico, Venezuela, Cuba, Jamaica, Ecuador and in many regions in Brazil[(104)].Species of the genus Citrus have been highlighted because they are rich in essential oils which are very versatile and often used as

flavorings in several goods, such as beverages, soaps, cosmetics and household products. Their essential oils have also been frequently used in medical treatments due to their antimicrobial, antifungal, antibacterial and antiparasitic properties[105]. Many recent studies that have attempted to determine the chemical composition of essential oils extracted from C. aurantifolia. Researchers have noted that the components of these oils display large intraspecifc chemical variation[106],[107].The species C. aurantifolia has been known because it exhibits important biological activities, such as antimicrobial activity against several pathogens – Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas spp, Aspergillus niger and Candida albicans – antiaflatoxigenic and anticancer activities[108,109,110,111].

**-Taxonomy:**

Kingdom: plantae

Division:Magnoliophyta

Class: Tracheobionta

Order: Sapindales

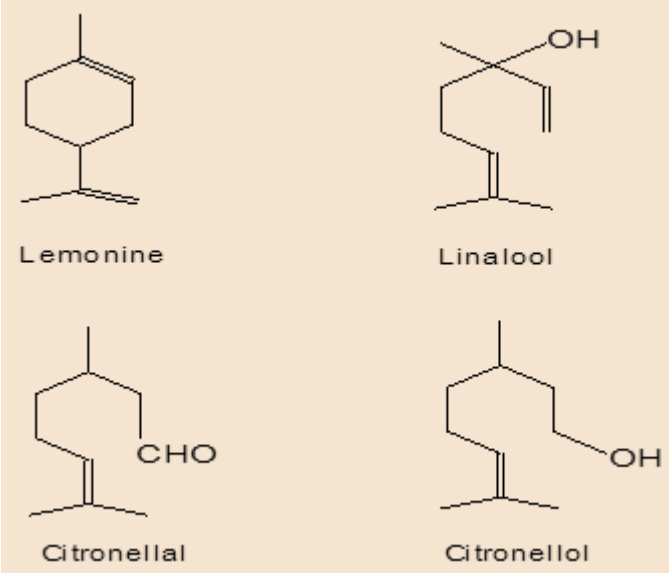
Family : Rutacea

Genus : Citrus

Species: Caurantifolia

The chemical composition of essential oils from *C. aurantifolia* leaves and fruit peel was similar to the ones from other species of Citrus, such as *C. limonia* and *C. reticulata* previously described in the literature since they all have the same major constituents[112]. Limonene was found at large amounts in *C. aurantifolia* leaves and fruit peel, besides being the major constituent of essential oils from *C. sinensis, C. latifolia* and C. l*imoni*a[107,113].Previous reports on the essential oil obtained from others

*C. aurantifolia* specimens have indicated that terpenes predominate in the oil, and that the chemical composition of the essential oil varies signifcantly depending on the origin of the plant. Samples from Italy contained limonene, β-myrcene, β-pinene, γ-terpinene, citral and β-bisabolene as the major compounds[105,114].β-pinene and limonene were also the major components in an essential oil sample collected in South Korea[108-115]. Major constituents of essential oils from *C. aurantifolia* are: limonene , linalool , citronellal and citronellol .




Robert Jacob et al., reported the potential of citrus limonoids as anticancer agent in mice. It was found that five limonoids aglycones (limonin, nomilin, obacunone, isoobacunoic acid, ichangin) induced significant amounts of glutathione-S-transferase (GST) in the liver and intestinal mucosa. GST is a major detoxifying enzyme system which catalyzes the conjugation of glutathione with many potentially carcinogenic compounds which are highly electrophilic in nature[116].

Ashok Kumar et al., reported antimicrobial activity and phytochemical analysis of Citrus fruit peels . He reported antibacterial activity of five

different solvent extracts(ethyl acetate, acetone, ethanol, petroleum ether and water) prepared by soxhlet extractor from two citrus fruit peel (Citrus sinensis and Citrus limon) . They were screened against five pathogenic bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi.* The highest antibacterial potentiality was exhibited by the acetone peel extract of Citrus sinensis followed by the ethyl acetate peel extract of Citrus limon. The peel extract of Citrus sinensis and Citrus limon can be considered to be as equally potent as the antibiotics, such as metacillin and penicillin [(117)].

Mehmet Karaca et al., reported the Investigation of anti-inflammatory activity of Bergamot oil.Essential oil [(118)].

### 1.6.4. *Lawsonia mermus*

Lawsonia is a monotypic genus represented by *Lawsonia alba* (syn. *Lawsonia inermis* Linn.). The plant belongs to the family Lythreacea and is a native of south west Asia and north Africa. It is a well known ornamental dye plant and has several names in different languages e.g. henna in Arabic, henna tree or alhenna in English, mehndi in Urdu, Hindi and Bengali, tien kao or tien deng in Thai, medi in Gujrati, mohuz in Kashmiri etc. It is cultivated in moderate to warm tropical regions as a hedge plant. In several countries it is cultivated on a large scale to obtain leaves to dye hands and hair. It is a glabrous branched shrub with greyish brown bark. Its leaves are elliptic, acute and often mucronulate with small white or rose-colored fragrant flowers. The seeds are approximately pea size with numerous pyramidal and smooth shapes[(119)]. Traditionally, a paste of leaves is used to prevent skin inflammation[(120,121)] , cure ulcers and wounds[(122)]. Its leaves have also been used as an expectorant, constipating, haematinic, febrifuge, cough,

burning sensation, hemicranias, cephalagia, diarrhoea, dysentery, leucoderma, leprosy, boils, scabies, hepatopathy, anemia, hemoptysis, fever and opthalmia[123,124,125]. Its bark has been used to prevent jaundice, spleen and liver enlargement[126,127] ,calculus affliction, and skin diseases[128]. The essential oil obtained from the flowers has been used in perfumery[129]. The flowers are also reported as refrigerant, cardiotonic, febrifuge, soporific, cephalalgia, amentia and insomnia[129].The key coloring agent present in leaves is lawsone[130]. Chemical studies undertaken on various parts of the plant have led to the isolation of various compounds such as lacoumarin[131],flavonoids[132,133],phenolic glucosides[134],naphthoquinone[135,136],saponins[137],triterpenoids[138,139],and dioxin derivatives[140].Biological studies on the extracts and purified constituents exhibited antimicrobial[141,142],anti-sickling[143,144],macrophagestimulating[145],hepatoprotective[146],antiinflammatory,antipyretic[147], anticomplementary[129],cytotoxic[148],antioxidant, protein glycation inhibitory[149],and immunomodulatory [150),(151].

**-Taxonomy**

Kingdom: Plantae

Order: Myrtales

Family: Lythraceae

Genus: Lawsonia

Species: L. inermis

A wide range of compounds has been reported from L. alba, including flavonoids/neoflavonoid[132-135,149-152,153,154],terpenoids[129,135,138,139,155-160],steroids[133,159,161,162], fatty acids[155,159,163]:

Seventeen flavonoids (Apigenin-7-O-glucoside Leaves ,Apigenin-4'- O-glucoside Leaves ,Luteolin-7-O-glucoside Leaves ,Luteolin-3'-O-

glucoside,…) were reported from the leaves and aerial parts including a neoflavonoid lawsonicin (Lawsonici).

Terpenoids are the second major class of compounds isolated from this plant. Four sesquiterpenes were reported including three degraded sesquiterpenes(α-Ionone ,β-Ionone)from the flowers, and vomifoliol from the aerial part, and an intact isocaryophyllene from the leaves. Some lupane triterpenes, five(Lupeol, Betulin,Betulinic acid Bark ,3β 3 - Dihydroxy lupine-20 (20)-ene (hennadiol),(20S)-3β, 30-Dihydroxylupane) have been reported from the bark, two (Lawsonic acid,Lupanaldehyd) from the aerial parts, and one (isoplumbagin) from the whole plant, three ursane triterpenes, two (Lawnermis acid ,Methyl ester of lawnermis acid) from the seeds, one(Lawsonin)from the aerial parts of L. alba., and four oleananetriterpenes(Lawsowaseem,Lawsoshamim,Oleanaldehyde,Oleano ilic acid ) were isolated also from the aerial parts of L. alba. β-Sitosterol was isolated from leaves and bark; two steroids (3-O-β-D-Glucoside of β-sitosterol,Stigmasterol) were obtained from the leaves and two steroids (Lawsaritol, Lawsaritol A) from the roots and a steroid glycoside balanitisin A from the fruits (Balanitisin A).

The seeds of L. alba yielded five fatty acids (Palmatic acid,Stearic acid,Arachidic acid,Behenic acid,Linoleic acid), leaves afforded two fatty acid derivatives (Ethyl hexadecanoate,Methyl linolenate), and the whole plant furnished one fatty acid derivative (n-Triacontyl-n-tridecanoate).

The ethyl acetate extract of L. alba showed antibacterial activity against two Gram-positive bacteria and two Gram-negative bacteria[(164)].Three naphthoquinones inhibited the growth of two Gram positive bacteria, three Gram-negative bacteria and five fungi[(165)]. L. alba methanolic

extract and its major flavonoidal composition showed growth inhibition against one Gram-positive and four Gramnegative bacteria in a dose dependent manner[166,167].

L. alba showed significant trypanocidal activity[168]. Also L. alba leaves extract was found to induce significant analgesic and antipyretic activities[169]. L. alba seed oil also showed significant analgesic activity[170].

It was reported that L. alba seeds powder failed to show any antifertility activity[171]. However later on in 1977 S. R. Munshi et al. communicated that leaves powder used as a suspension or incorporated into the diet, inhibited the fertility of rats[172].

L. alba leaves 70% ethanol extract showed strong hypolipidaemic and hypoglycaemic activities when administered orally at 0.8 g/kg in alloxan induced diabetic mice[173]. 95% Methanolic leaves extract showed significant in vitro antihyperglycemic effect[174].

### 1.6.5. *Carthamus tinctorius* L.

*Carthamus tinctorius* L., which is widely accepted as Safflower or false saffron, belongs to the Compositae or Asteraceae family. This thistle-like species typically thrives in an arid climate, namely southern Asia, China, India, Iran, and Egypt[175]. The genus *Carthamus* from the Compositae family comprises 16 recognized species[176]. Carthamus tinctorius is the only cultivated species of this genus, but the others are either wild or weeds. Carthamus oxyacantha as one of the wild species is widespread in Turkey, subtropical regions of western Iraq, Iran, northwest India, throughout Kazakhstan, Turkmenistan, and Uzbekistan[177].This species is important because it is assumed to be the ancient male parent of

cultivated safflower[178] and these two species have been successfully crossed with each other[179].Nevertheless, it seems that Carthamus palestinus Eig. species has the most likely affinity to cultivated safflower[180]. Therefore, this plant is used for numerous culinary and textile purposes. With the advent of synthetic aniline dyes, it has been mainly grown as an oilseed and birdseed that has some applications in medicinal fields[181]. More to the point, its oil has high nutritional value and consists of 70% polyunsaturated fatty acid (i.e., linoleic acid), 10% monounsaturated oleic acid, and mere amounts of stearic acid[182],[183].

**-Taxonomy**

Kingdom : Plantae

Order : Asterales

Family : Asteraceae

Genus : Carthamus

Species : Carthamus tinctorius

Traditionally this crop was grown for its flowers for colouring and flavouring foods. Flowers contain the water soluble yellow dye carthamidin ($C_{16}H_{20}O_{11}$) and a water insoluble red dye carthamin ($C_{21}H_{22}OH.H_2O$).These have been the source of yellow and red dye in the food and industries to colour cotton and silk[184]. Recently, these yellow and red pigments have been shown to be safe for cosmetic colourings such as face cream, shampoo, perfume or body lotion and hair cream. In Chinese medicine, flower petals have been used as a stimulant for blood circulation and phlegm, healing of fractures, contusions and strain and for various female maladies. It was used for the problem in mensuration to increase blood flow and, mixture of ground safflower seed and mustard oil has been used to reduce rheumatic pain[185].The florets of Carthamus tinctorius have been used as a remedy for stroke, gynecological disease,

coronary heart disease, angina pectoris, and hypertension in Chinese folk medicine[186].In Korea, the safflower seed extracts have traditionally been used for the treatment of blood stasis, the promotion of bone formation and the prevention of osteoporosis[187],[188].In India and Afghanistan the tea made from safflower foliage was used to prevent the abortion in women. Male sterility and dead sperm diseases have also been treated with using safflower dicotyledons[184].It was widely used as a traditional Thai herbal remedy for blood, heart and nerves tonics, blood detoxifier, lymph stimulator, menstruation enhancer, to relief menstruation pain, to control blood pressure and for various types of dyslipidemic syndromes[189].Oil is used by both food producers and industry. However, Safflower is currently grown mostly for its edible oil, considered as a favourable oil for human consumption due to high quantity (70-75%) of polyunsaturated (linoleic acid) or mono-unsaturated fatty acid (oleic acid), which play an important role in reducing cholesterol level in blood[190-193 ].

The chemical groups isolated from Carthamus tinctorius included, oils, proteins, minerals, phenolics, flavonoids, alkaloids, lignans, carboxylic acids, steroids, polysaccharides, quinochalcone C-glycosides and quinone-containing chalcones[194-202].Many factors such as genotype, ecology, morphology, physiology and agronomic practices influence the oil content and fatty acid synthesis of crops[203].Commercial safflower varieties contained 32 to 52 percent oil. The crop was divided into two categories based on oil quality: high linoleic (a polyunsaturated fatty acid) acid varieties, these contain 75 percent linoleic acid, and high oleic (monounsaturated fatty acid) acid varieties[193].Safflower seeds oil content of the four varieties of Carthamus tinctorius ranged from 28.84 to 35.38 g/100g. Safflower oils contained palmitic acid, palmitoleic acid, margaric

acid, margaroleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and behenic acid. Linoleic acid was the principal fatty acid (77.94-79.49%) followed by oleic acid as the second main fatty acid. Palmitic acid was the major saturated fatty acid (7.2-8.6%) followed by stearic acid (2-2.39%)[204],[205].However in another study, evaluation of four safflower (Carthamus tinctorius L.) genotypes for oil content and fatty acid composition showed that oil content varied from 22.16 to 34.39%. Among the detected fatty acids, linoleic acid (75.81- 77.86%) was the predominant fatty acid followed by oleic (12.57–13.75%), palmitic (6.09–7.07%) and stearic (2.17–2.62%) acid, while trace amounts of other fatty acids were presented and the values of them did not exceed 0.81%. The oil content and fatty acid composition of oil among the genotypes were significantly different ($P<0.01$), indicating that synthesis of them is influenced by genotype. The lipids of safflower rich in polyunsaturated essential fatty acids, linoleic acid makes the oil nutritionally and therapeutically valuable for human consumption[206].

### 1.6.6.*Senna Italica* Mil

*Cassia* is large genus with about 550 species that belongs to the family Caesalpiniaceae.It was split into three genera(Cassia, Senna and Chamaecrista). Leaves and seeds of Cassia species are used as laxative and in diabetese and leprosy[207].Cassia Leaf extracts were found to be effective against seed brone pathogenic fungi and also have been used as a ethnoverteringary medicine[208].Senna species have been of medicinal interest due to their good therapeutic values in folk medicine [209].Senna italica is otherwise called as Cassia italica, Senegal senna, Italian senna, Cassia obovataor.). In many region these plants are cultivated commercially and medicinally. The decoction and maceration of leave and pods of *Senna itlalica* are used for skin problems such as burns and

ulcers. The roots are used for liver complains, gallbladder disorders , nausea and dysmenorrhoea[210]. *Senna italica* is easily distinguishable through its many distinctive features.There are three subspecies in *Senna italica* based on the size of the inflorescence and the longth of the petiole. The subspecies are italic, micrantha and arachoides. Plants which contain a variety of phytocompounds –like *Senna* species- have found very important applications on the fields of agriculture ,human and veterinary medicine[211].

**-Taxonomy:**

Kingdom : Plantae

Division : Angiospermae

Class : Dicotyledoneae

Order : Fabales = Rosales

Family : Fabaceae = leguminosae

Genus : Senna= Cassia

Species : S.Senna

Leaves, pods and immature seeds are used as purgative, decoction and maceration are used to cure stomach complaints, fever, jaundice, veneral diseases and biliousness. This plant is also used as abortifacient and against intestinal worms. Leaves fresh or dried or pulverized are used for skin problems, burns and ulcers. Flowers are made into tea and used as purgative and to induce labour. Maceration of root is used to cure colic and influenza and boiled roots are used to dress wounds. Root infusion is used as eye drops for sore eyes and for the treatment of indigestion, liver complaints, nausea, vomiting and dysmenorrhoea. Young seeds are eaten as snacks or as vegetable. In

Mauritania seeds are smoked. Leaves are traded as neutral Henna, hair conditioner which impart yellow colour[212].

**Aim of this study**

This study was designed to:

-Extract oils from target species.

-Analyze the extracted oils by GC-MS .

-evaluate the antimicrobial activity of the target oils.

# Chapter Two

## Materials and Method

# 2-Materials and Methods

## 2.1-Materials

### 2.1.1- Instruments

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

### 2.1.2-Test organisms

The studied oils were screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Candida albicans*.

### 2.1.3-Plant material

Seeds of *Azadirachta Indica,* were collected from Khartoum,Sudan. Seeds of *Gerwia tenax* , *Citrus aurantifolia*, *Lawsonia mermus, Senna italic* , *Carthamus tinclorius* were collected from Kordofan, western Sudan. The target plants were authenticated by The Medicinal and Aromatic Plants Research Institute,Khartoum (Sudan).

## 2.2- Methods

### 2.2.1-Extraction of oil

Powdered plant material(300g) was exhaustively macerated with n-hexane.The solvent was removed under reduced pressure to afford the oil.

### 2.2.2- GC-MS analysis

The target oils were analyzed by the hyphenated technique 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(99% pure) was used as carrier gas. Oven temperature program and other chromatographic conditions are presented below:

Table 2.1: Oven temperature program

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

Table 2.2: Chromatographic conditions

| | |
|---|---|
| Column oven temperature | 150.0$^{o}$C |
| Injection temperature | 300.0$^{o}$C |
| Injection mode | Split |

| | |
|---|---|
| Flow control mode | Linear velocity |
| Pressure | 139.3KPa |
| Total flow | 50.0ml/ min |
| Column flow | 1.54ml/sec. |
| Linear velocity | 47.2cm/sec. |
| Purge flow | 3.0ml/min. |
| Spilt ratio | - 1.0 |

## 2.2.3-Antimicrobial assay

### (i)Bacterial suspensions

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

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about $10^8$-$10^9$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)-Fungal suspensions**

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

**iii)-Antibacterial test**

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oils. (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 test samples. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume micrometer pipette and allowed to diffuse at room temperature

for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

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

# Chapter Three

# Results and Discussion

# 3-Results and Discussion

## 3.1-*Azadirachta Indica* oil

GC-MS analysis of *Azadirachta Indica* oil was conducted and the identification of the constituents was accomplished by retention times and MS fragmentation pattern. A 90-95% match was observed when comparing the mass spectra with the database on MS library.

### 3.1.1-Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 29 constituents (Table 3.1)).The typical total ion chromatograms (TIC) is depicted in Fig (3.1)

The major constituents of the oil are:

i) 9-Octadecenoic acid methyl ester (37.20%)

ii) Methyl stearate(20.42%)

iii)Hexadecanoic acid methyl ester(19.13%)

iv) 9, 12-Octadecadienoic acid methyl ester (12.60%)

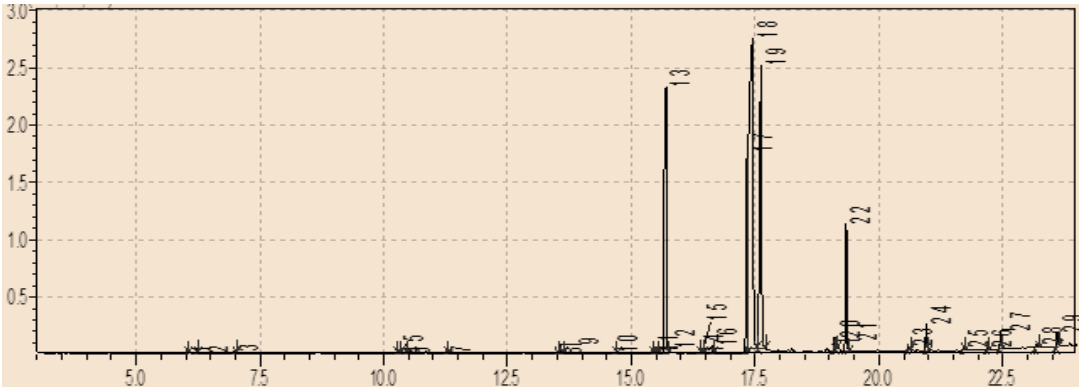


Fig (3.1): Total ions chromatograms

**Table 3.1**: Constituents of *Azadirachta Indica* oil

| No. | Name | Ret.Time | Area% |
|---|---|---|---|
| 1. | Cyclopropane, 1,2-dimethyl-1-pentyl- | 6.020 | 0.05 |
| 2. | (S)-(+)-6-Methyl-1-octanol | 6.215 | 0.05 |
| 3. | L-.alpha.-Terpineol | 6.978 | 0.11 |
| 4. | trans-.alpha.-Bergamotene | 10.250 | 0.01 |
| 5. | .alpha.-ylangene | 10.316 | 0.00 |
| 6. | 1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)- | 10.435 | 0.01 |
| 7. | Dodecanoic acid, methyl ester | 11.253 | 0.04 |
| 8. | 6,10-Dodecadienoic acid, 3,7,11-trimethyl-, methyl ester, (E)-(S)- | 13.488 | 0.02 |
| 9. | Methyl tetradecanoate | 13.563 | 0.37 |
| 10. | Pentadecanoic acid, methyl ester | 14.639 | 0.05 |
| 11. | 7-Hexadecenoic acid, methyl ester, (Z)- | 15.429 | 0.05 |
| 12. | 9-Hexadecenoic acid, methyl ester, (Z)- | 15.470 | 0.22 |
| 13. | Hexadecanoic acid, methyl ester | 15.698 | 19.13 |
| 14. | Hexadecanoic acid, 14-methyl-, methyl ester | 16.371 | 0.03 |
| 15. | cis-10-Heptadecenoic acid, methyl ester | 16.433 | 0.06 |
| 16. | Heptadecanoic acid, methyl ester | 16.641 | 0.28 |
| 17. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.341 | 12.60 |
| 18. | 9-Octadecenoic acid (Z)-, methyl ester | 17.439 | 37.23 |
| 19. | Methyl stearate | 17.621 | 20.42 |
| 20. | Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- | 19.099 | 0.61 |
| 21. | cis-11-Eicosenoic acid, methyl ester | 19.133 | 0.25 |
| 22. | Eicosanoic acid, methyl ester | 19.334 | 5.25 |
| 23. | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | 20.591 | 0.19 |
| 24. | Docosanoic acid, methyl ester | 20.950 | 1.07 |
| 25. | Tricosanoic acid, methyl ester | 21.715 | 0.12 |
| 26. | Hexatriacontane | 22.192 | 0.11 |
| 27. | Tetracosanoic acid, methyl ester | 22.454 | 0.80 |
| 28. | Squalene | 23.194 | 0.28 |
| 29. | Tetratriacontane | 23.599 | 0.59 |

Major constituents are discussed below:

**i) 9-Octadecenoic acid methyl ester (37.20%)**

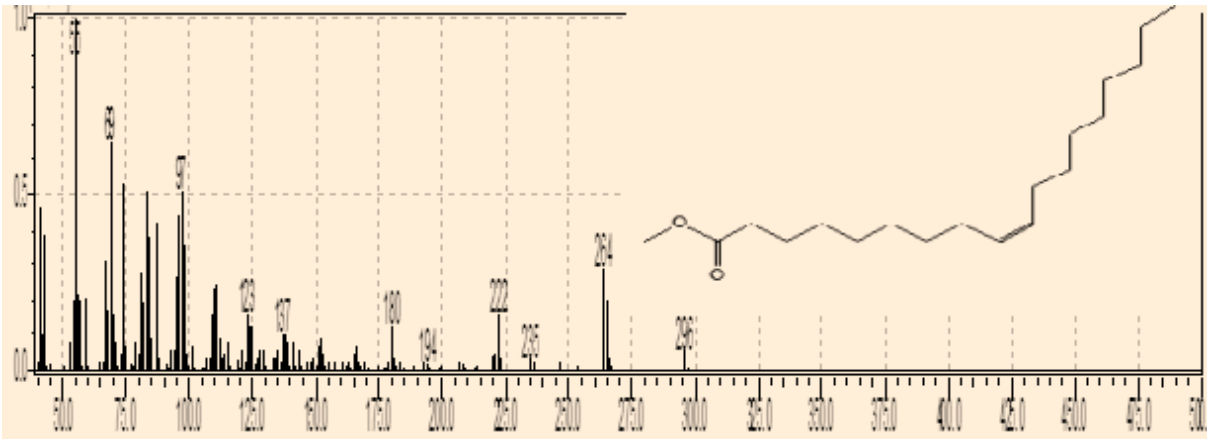


Fig (3.2): Mass spectrum of 9-octadecenoic acid methyl ester

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig(3.2).The peak at m/z 296, which appeared at R.T. 17.439 in total ion chromatogram, corresponds the molecular ion: $M^{+}[C_{19}H_{36}O_{2}]^{+}$ .The signal at m/z266 is due to loss of a methoxyl .

**ii) Methyl stearate(20.42%)**

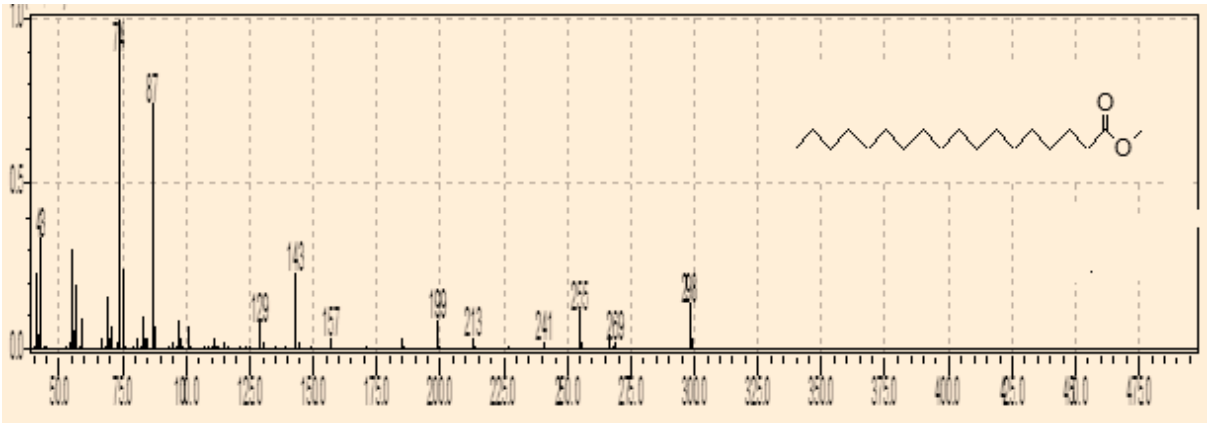


Fig (3.3): Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is displayed in Fig(3.3).The peak at m/z 298 ( R.T. 17.621) is due to $M^+[C_{19}H_{38}O_2]^+$ , while the signal at m/z267 corresponds to loss of a methoxyl group.

**iii) Hexadecanoic acid methyl ester (19.13%)**

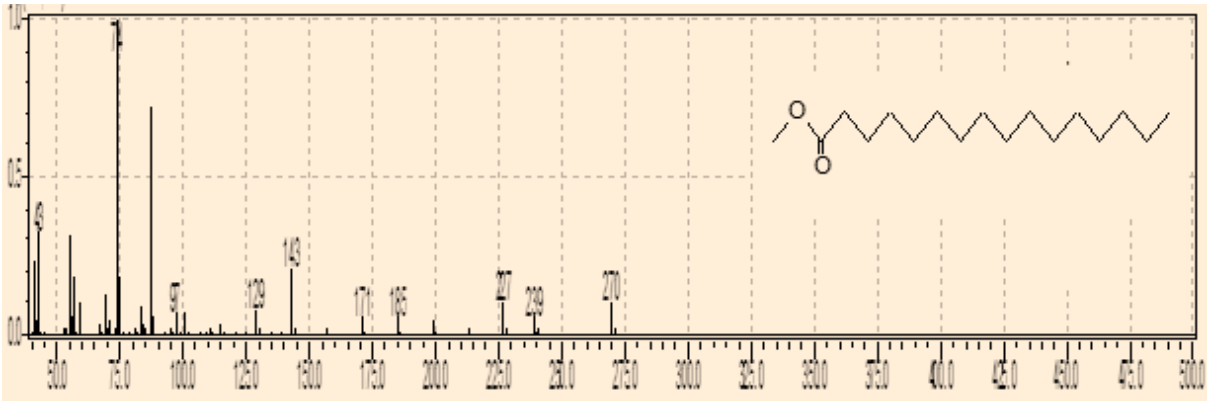


Fig (3.4): Mass spectrum of hexadecanoic acid methyl ester

Fig (3.4) shows the mass spectrum of hexadecanoic acid methyl ester.The peak m/z 270(R.T. 15.698) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 is due to loss of a methoxyl .

**iv) 9,12-Octadecadienoic acid methyl ester(12.60%)**

The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Fig(3.5).The signal which was observed at m/z294( R.T. 17.341) is due to $M^+[C_{19}H_{34}O_2]^+$ , while the signal at m/z263 corresponds to loss of a methoxyl.

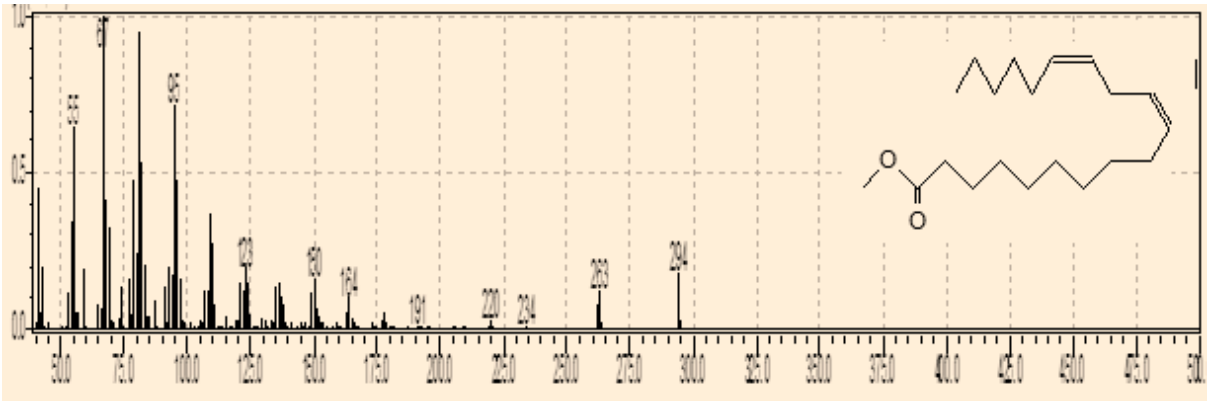


Fig (3.5): Mass spectrum of 9,12-octadecadienoic acid methyl ester

### 3.1.2-Antimicrobial activity

*Azadirachta Indica* seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table(3.2).The results were interpreted in the following manner: (<9mm: inactive;9-12mm:partially active; 13-18mm: active; > 18mm:very active) .Tables(3.3) and (3.4) represent the antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed good activity against *Escherichia coli*. However, at the same concentration, it exhibited partial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The oil failed to give any anticandidal activity.

**Table (3.2)**: Antimicrobial Activity of the *Azadirachta Indica* seed oil

| Oil | Antibacterial activity | | | | |
|---|---|---|---|---|---|
| | Gram positive | | Gram negative | | |
| mg/ml | *Bs.* | *Sa.* | *Ec.* | *Pa.* | *Ca.* |

| | | | | | |
|---|---|---|---|---|---|
| 100 | 10 | 10 | 14 | -- | -- |

Table 3.3 : Antibacterial activity of standard chemotherapeutic agents

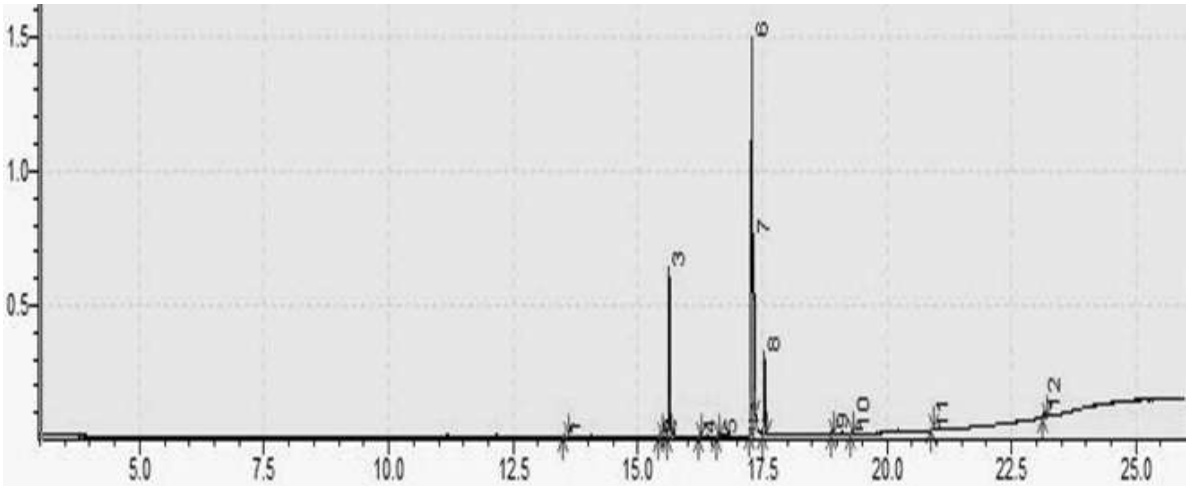
| Drug | Conc. mg/ml | Bs. | Sa. | Ec. | Ps. |
|---|---|---|---|---|---|
| Ampicillin | 40 | 15 | 30 | - | - |
| | 20 | 14 | 25 | - | - |
| | 10 | 11 | 15 | - | - |
| Gentamycin | 40 | 25 | 19 | 22 | 21 |
| | 20 | 22 | 18 | 18 | 15 |
| | 10 | 17 | 14 | 15 | 12 |

Table 3.4 : Antifungal activity of standard chemotherapeutic agent

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

## 3.2-*Gerwia tenax*

GC-MS analysis of *Gerwia tenax* oil was conducted. The GC-MS analysis revealed the presence of 12 components (Table 3.5).The typical total ion chromatograms(TIC) is depicted in Fig(3.6).




Fig(3.6):Chromatograms of *Gerwia tenax* oil

Table (3.5): Constituents of Gerwia *tenax* oil

| No | RT | Area % | Name |
|---|---|---|---|
| 1 | 13.531 | 0.29 | Methyl tetradecanoate |
| 2 | 15.437 | 0.25 | 9-Hexadecenoic acid methyl ester |
| 3 | 15.630 | 18.83 | Hexadecanoic acid methyl ester |
| 4 | 16.251 | 0.11 | 15-Methyl- Hexadecanoic acid methyl ester |
| 5 | 16.606 | 0.18 | Heptadecanoic acid methyl ester |
| 6 | 17.288 | 51.69 | 9,12-Octadecenoic acid methyl ester |
| 7 | 17.328 | 16.41 | 9-Octadecenoic acid methyl ester |
| 8 | 17.543 | 9.33 | Methyl stearate |
| 9 | 18.906 | 0.89 | 2-(2-ethylcyclohexyl)-cyclopropaneoctanoic acid |
| 10 | 19.301 | 0.73 | Eicosenoic acid methyl ester |
| 11 | 20.921 | 0.29 | Docosanoic acid methyl ester |
| 12 | 23.160 | 1.00 | Squalene |

Major constituents of the oil are :

## i) 9, 12-Octadecadienoic acid methyl ester (51.69%)

Fig. 3.7 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester .The peak at m/z294 (R.T. 17.288) 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.

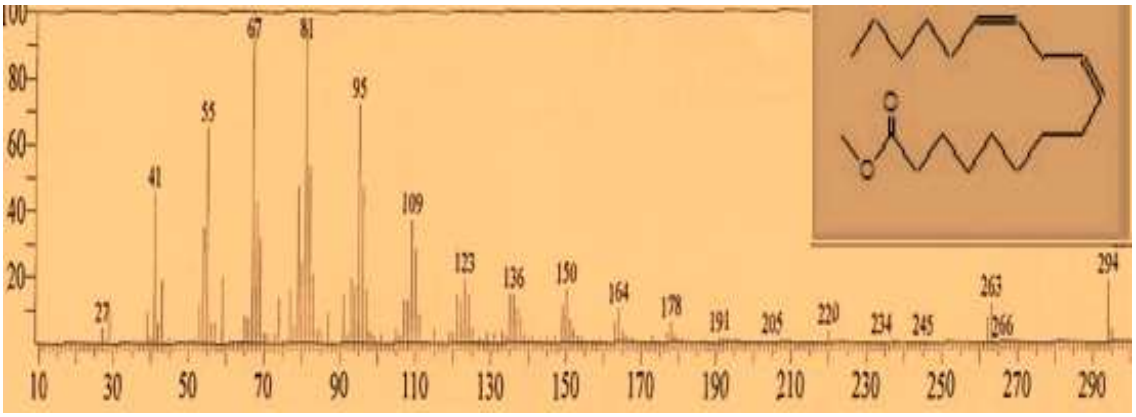



Fig (3.7): Mass spectrum of 9,12-octadecadienoic acid methyl ester

## ii) Hexadecanoic acid methyl ester (18.83%)

Figure(3.8) presents 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. 15.630 in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

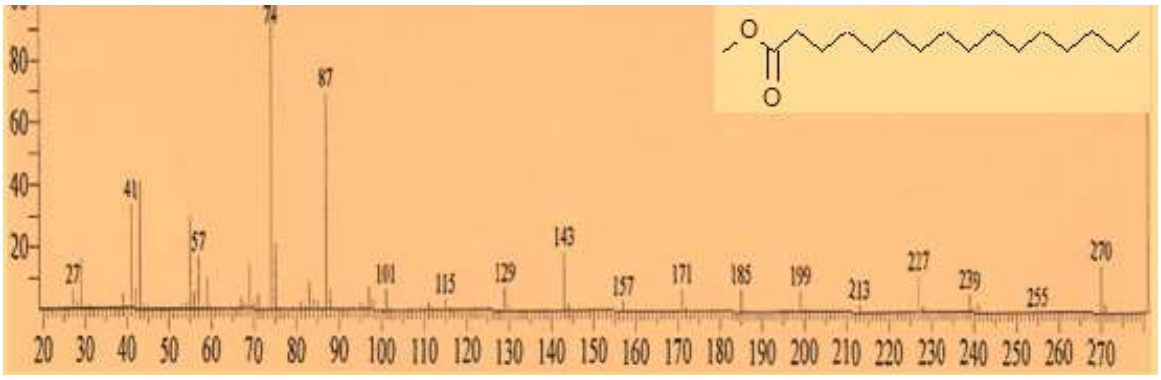


Fig (3.8): Mass spectrum of hexadecanoic acid methyl ester

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

### iii) 9-Octadecenoic acid methyl ester (16.41%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig (3.9).The peak at m/z 296 (R.T. 17.328) corresponds $M^+[C_{19}H_{36}O_2]^+$ , while the signal at m/z266 is attributed to loss of a methoxyl .

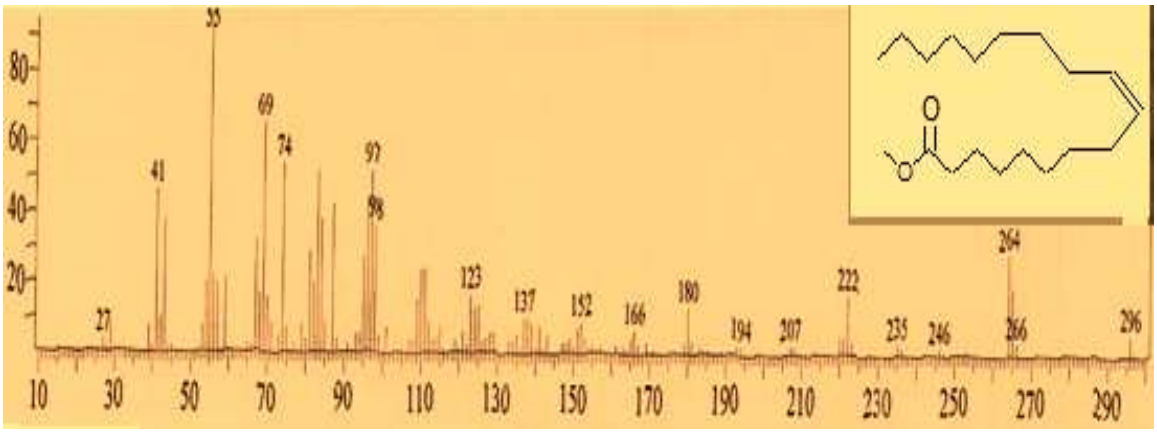



Fig (3.9): Mass spectrum of 9-octadecenoic acid methyl ester

9-Octadecenoic acid (oleic acid) is included in animal fats and vegatables , hence it is included in the normal human diet. Oleic acid is used as emollient. It is used in small amount as excipient in pharmaceutical industries. 9-Octadecenoic acid could be

responsible for the hypotensive effect of olive oil. It has been claimed that the presence of oleate in olive oil is associated with decreased risk of breast cancer.

**iv) Methyl stearate(9.33%)**

Figure(3.10) 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. 17.543).The peak at m/z267 corresponds to loss of a methoxyl .

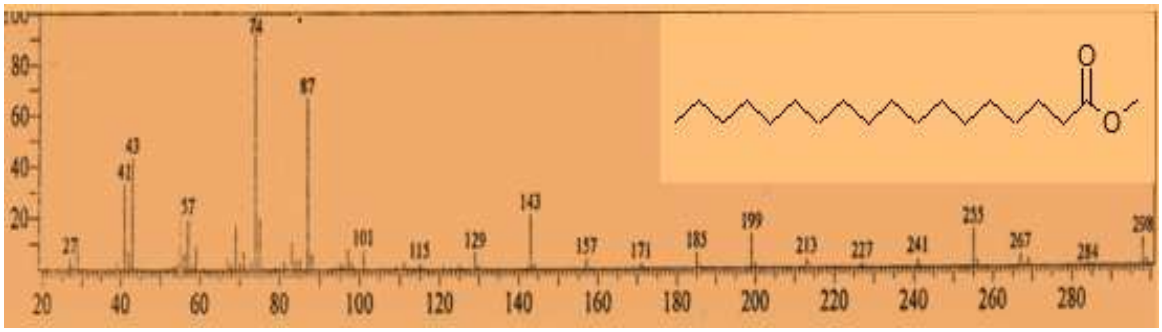



Fig (3.10): Mass spectrum of methyl stearate

## 3.2.1-Antimicrobial activity

In cup plate agar diffusion bioassay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table(3.6).

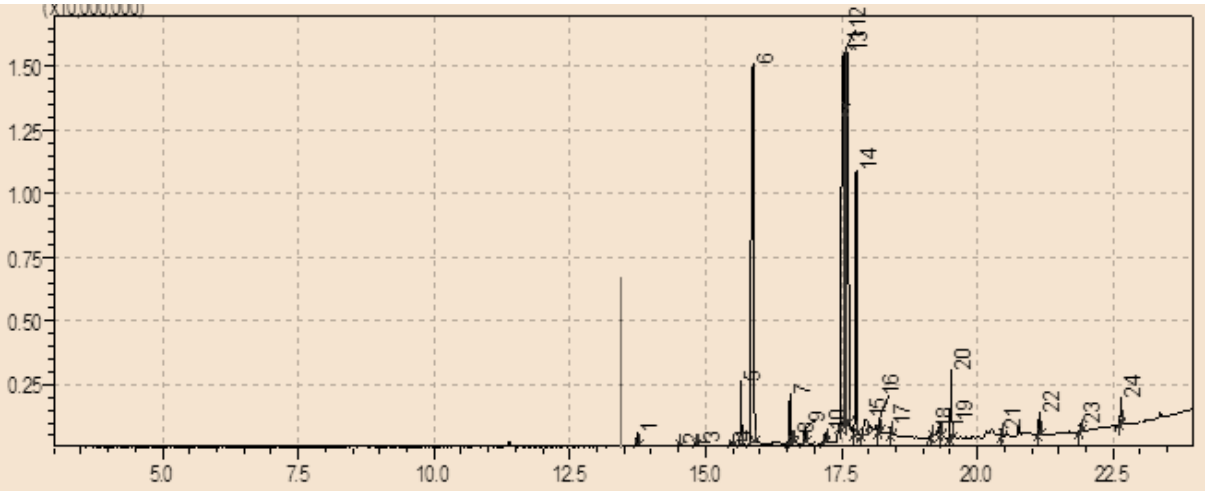
Table (3.6): Antimicrobial activity of the Gerwia tenax oil

| Sample | Ec | Pa | Sa | Bs | Ca |
|---|---|---|---|---|---|
| *Gerwia tenax* oil (100mg/ml) | 10 | 12 | 20 | - | 20 |

The oil showed significant antibacterial activity against *Staphylococcus aureus.*It also showed significant anticandidal activity. However, it exhibited partial activity against *Pseudomonas aeruginosa* and *Escherichia coli.*

## 3.3- *Citrus aurantifolia*

*Citrus aurantifolia* oil was studied by GC-MS. The GC-MS analysis revealed the presence of 24 constituents (Table 3.7).The typical total ion chromatograms (TIC) is depicted in Fig(3.11)




Fig(3.11): Total ions chromatograms

**Table (3.7)**: Constituents of *Citrus aurantifolia(christm)swing* oil

| No. | Name | Ret.Time | Area% |
|---|---|---|---|
| 1. | Methyl tetradecanoate | 13.735 | 0.44 |
| 2. | Tetradecanoic acid, 12-methyl-, methyl ester | 14.514 | 0.07 |
| 3. | Pentadecanoic acid, methyl ester | 14.814 | 0.15 |
| 4. | Pentadecanoic acid, 14-methyl-, methyl ester | 15.466 | 0.17 |

| | | | |
|---|---|---|---|
| 5. | 9-Hexadecenoic acid, methyl ester, (Z)- | 15.651 | 2.06 |
| 6. | Hexadecanoic acid, methyl ester | 15.878 | 26.24 |
| 7. | Hexadecanoic acid, 14-methyl-, methyl ester | 16.554 | 1.54 |
| 8. | cis-10-Heptadecenoic acid, methyl ester | 16.615 | 0.31 |
| 9. | Heptadecanoic acid, methyl ester | 16.822 | 0.46 |
| 10. | 8-Octadecenoic acid, methyl ester | 17.201 | 0.34 |
| 11. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.548 | 34.24 |
| 12. | 9-Octadecenoic acid (Z)-, methyl ester | 17.591 | 1.84 |
| 13. | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 17.619 | 14.09 |
| 14. | Methyl stearate | 17.773 | 10.66 |
| 15. | 9,12-Octadecadienoic acid (Z,Z)- | 17.941 | 1.71 |
| 16. | cis-10-Nonadecenoic acid, methyl ester | 18.196 | 0.43 |
| 17. | Heptadecanoic acid, 16-methyl-, methyl ester | 18.417 | 0.28 |
| 18. | Methyl (Z)-5,11,14,17-eicosatetraenoate | 19.170 | 0.26 |
| 19. | 11-Eicosenoic acid, methyl ester | 19.321 | 0.43 |
| 20. | Eicosanoic acid, methyl ester | 19.519 | 2.25 |
| 21. | Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- | 20.451 | 0.26 |
| 22. | Docosanoic acid, methyl ester | 21.142 | 0.73 |
| 23. | Tricosanoic acid, methyl ester | 21.907 | 0.26 |
| 24. | Tetracosanoic acid, methyl ester | 22.645 | 0.78 |

Major constituents are briefly discussed below:

## i) 9,12-Octadecadienoic acid methyl ester (34.23%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig(3.12).The peak at m/z294 (R.T. 17.548) coincides with $M^+[C_{19}H_{34}O_2]^+$ , while the peak at m/z263 is due to loss of a methoxyl.

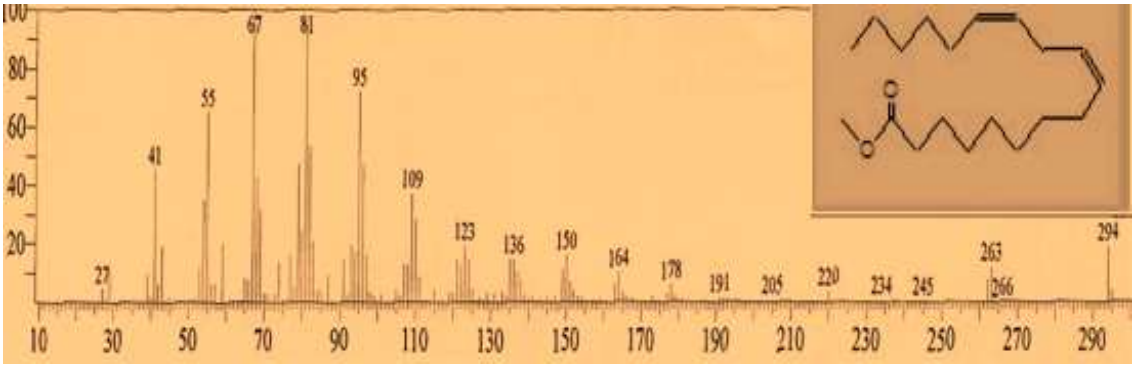



Fig (3.12): Mass spectrum of 9,12-octadecadienoic acid methyl ester

## ii) Hexadecanoic acid methyl ester(26.24%)

Figure (3-13) 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.15.878) in total ion chromatogram.The fragment at m/z239 is due to loss of a methoxyl function.

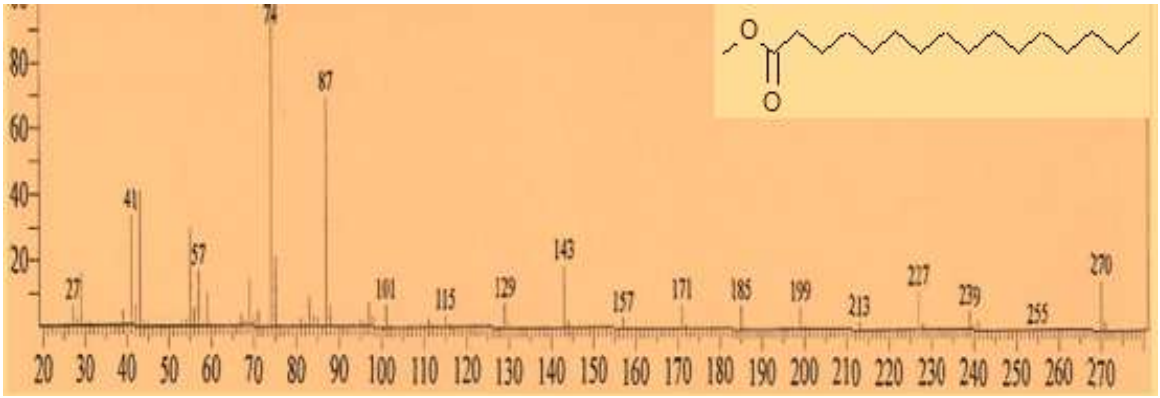



Fig (3.13): Mass spectrum of hexadecanoic acid methyl ester

## iii) 9,12,15-Octadecatrienoic acid methyl ester(14.09%)

Figure (3.14) shows the mass spectrum of 9,12,15-octadecatrienoic acid methyl ester.The molecular ion : $M^+[C_{19}H_{32}O_2]^+$ appeared at m/z 292 at (R.T.17.619) in total ion

chromatogram.The fragment at m/z261 is due to loss of a methoxyl .

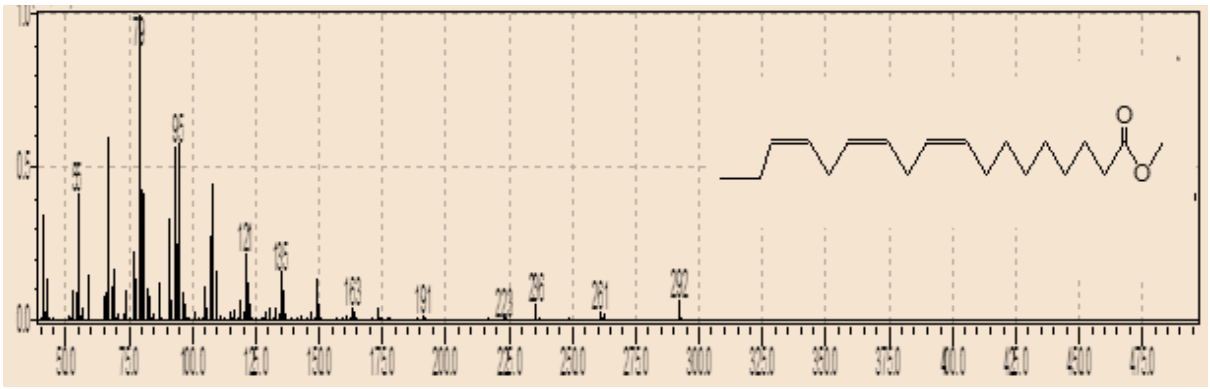


Fig (3.14): Mass spectrum of 9,12,15-Octadecatrienoic acid methyl ester

### iv) Methyl stearate(10.66%)

The mass spectrum of methyl stearate is displayed in Fig(3.15).The peak at m/z 298 with R.T. 17.773 is due to $M^{+}[C_{19}H_{38}O_2]^{+}$ , while the signal at m/z267 corresponds to loss of a methoxyl group.

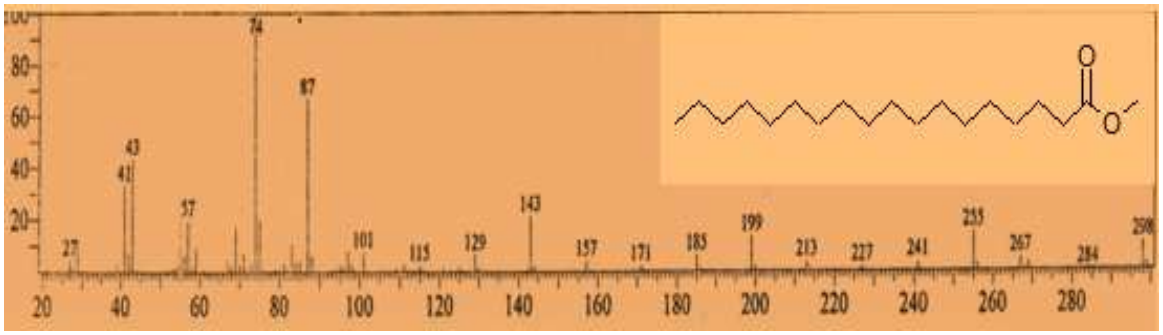



Fig (3.15): Mass spectrum of methyl stearate

## 3.3.1-Antimicrobial 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(3.8).

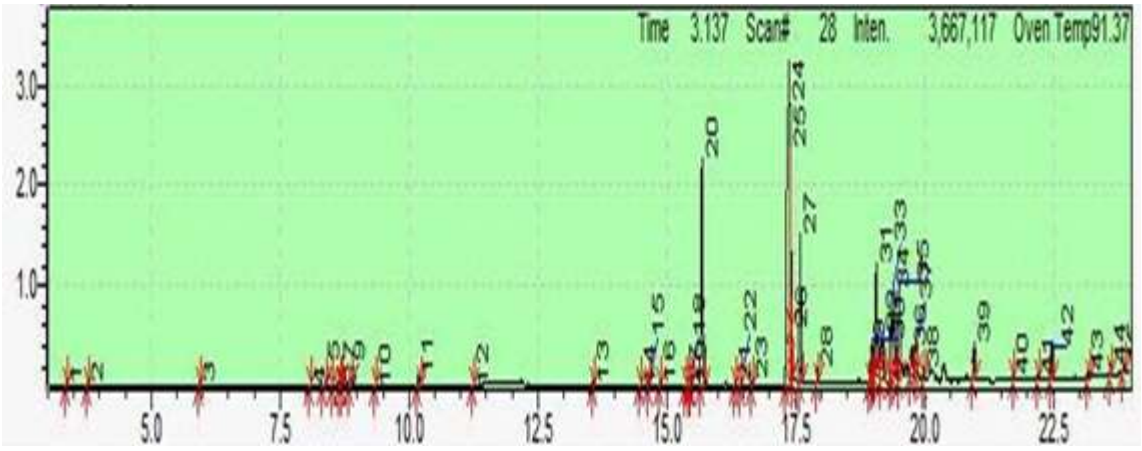
**Table (3-8)**: Antimicrobial Activity of the *Citrus aurantifolia(christm)swing* oil

| Oil | Antibacterial activity | | | | |
|---|---|---|---|---|---|
| | Gram positive | | Gram negative | | |
| mg/ml | *Bs.* | *Sa.* | *Ec.* | *Pa.* | *Ca.* |
| 100 | 12 | 17 | 16 | 15 | 15 |

The oil showed significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*,and *Pseudomonas aeruginosa*. It also showed good antifungal activity against *Candida albicans*. However, it exhibited partial activity against *Bacillus subtilis.*

## 3.4-*Lawsonia mermus* L.

GC-MS analysis of *Lawsonia mermus* L. oil was accomplished. The GC-MS analysis of the studied oil revealed the presence of 49 components (Table 3.9)).The typical total ion chromatograms (TIC) is depicted in Fig (3.16).




Fig(3.16):Chromatograms of *Lawsonia mermus* L. oil

Table(3.9): Constituents of *Lawsonia mermus L* oil

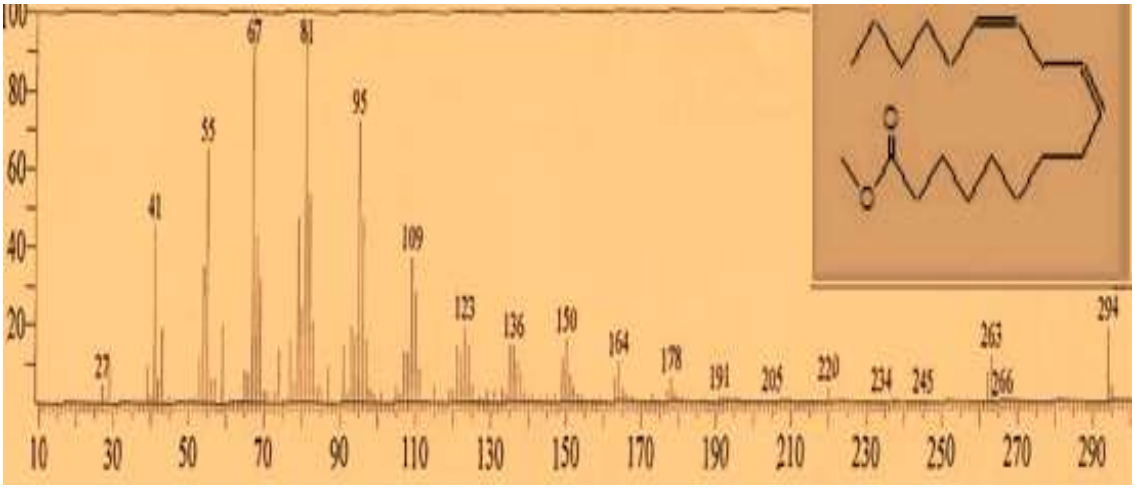
| No. | Name | Ret.Time | Area% |
|---|---|---|---|
| 1. | Hexanoic acid, methyl ester | 3.354 | 0.11 |
| 2. | 2-Heptenal, (Z)- | 3.761 | 0.08 |
| 3. | Octanoic acid, methyl ester | 5.935 | 0.12 |
| 4. | 3-Butoxy-2,4-dimethyl-1-pentene | 8.085 | 0.02 |
| 5. | 2,4-Decadienal, (E,E)- | 8.353 | 0.03 |
| 6. | 4-Decenoic acid, methyl ester | 8.508 | 0.01 |
| 7. | 2,4-Decadienal | 8.668 | 0.02 |
| 8. | Decanoic acid, methyl ester | 8.694 | 0.02 |
| 9. | Methyl 8-oxooctanoate | 8.848 | 0.08 |
| 10 | Methyl 4-oxododecanoate | 9.352 | 0.05 |
| 11 | Nonanoic acid, 9-oxo-, methyl ester | 10.184 | 0.48 |
| 12 | Dodecanoic acid, methyl ester | 11.256 | 0.04 |
| 13 | Methyl tetradecanoate | 13.571 | 0.19 |
| 14 | 6-Octadecenoic acid, methyl ester, (Z)- | 14.485 | 0.03 |
| 15 | Pentadecanoic acid, methyl ester | 14.645 | 0.05 |
| 16 | 2-Pentadecanone, 6,10,14-trimethyl- | 14.864 | 0.08 |
| 17 | 7,10-Hexadecadienoic acid, methyl ester | 15.375 | 0.03 |
| 18 | 7-Hexadecenoic acid, methyl ester, (Z)- | 15.444 | 0.05 |
| 19 | 9-Hexadecenoic acid, methyl ester, (Z)- | 15.480 | 0.07 |
| 20 | Hexadecanoic acid, methyl ester | 15.688 | 14.00 |
| 21 | Hexadecanoic acid, ethyl ester | 16.335 | 0.14 |
| 22 | cis-13,16-Docasadienoic acid, methyl ester | 16.442 | 0.10 |
| 23 | Heptadecanoic acid, methyl ester | 16.652 | 0.29 |
| 24 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.378 | 34.19 |
| 25 | 9-Octadecenoic acid (Z)-, methyl ester | 17.400 | 4.16 |
| 26 | 9-Octadecenoic acid, methyl ester, (E)- | 17.428 | 1.45 |
| 27 | Methyl stearate | 17.596 | 7.46 |
| 28 | Linoleic acid ethyl ester | 17.931 | 0.63 |
| 29 | Cyclopropaneoctanoic acid, 2-[[2-[(2- | 18.946 | 0.90 |

| | | | |
|---|---|---|---|
| | ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester | | |
| 30 | 9,12-Octadecadienoyl chloride, (Z,Z)- | 18.984 | 1.59 |
| 31 | 9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol | 19.070 | 7.18 |
| 32 | 17-Octadecynoic acid, methyl ester | 19.183 | 3.70 |
| 33 | Eicosanoic acid, methyl ester | 19.348 | 3.87 |
| 34 | Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- | 19.404 | 4.47 |
| 35 | 8,11,14-Docosatrienoic acid, methyl ester | 19.515 | 5.03 |
| 36 | Methyl 2-octylcyclopropene-1-octanoate | 19.749 | 2.03 |
| 37 | (R)-(-)-14-Methyl-8-hexadecyn-1-ol | 19.841 | 2.01 |
| 38 | 10,11-Epoxy-n-undecan-1-ol | 19.986 | 0.53 |
| 39 | Docosanoic acid, methyl ester | 20.964 | 1.72 |
| 40 | Tricosanoic acid, methyl ester | 21.728 | 0.21 |
| 41 | Hexatriacontane | 22.206 | 0.20 |
| 42 | Tetracosanoic acid, methyl ester | 22.466 | 1.46 |
| 43 | Pentacosanoic acid, methyl ester | 23.179 | 0.27 |
| 44 | Tetratriacontane | 23.611 | 0.48 |
| 45 | Hexacosanoic acid, methyl ester | 23.866 | 0.37 |

Major constituents are :

## i) 9,12-Octadecadienoic acid methyl ester (34.15%)

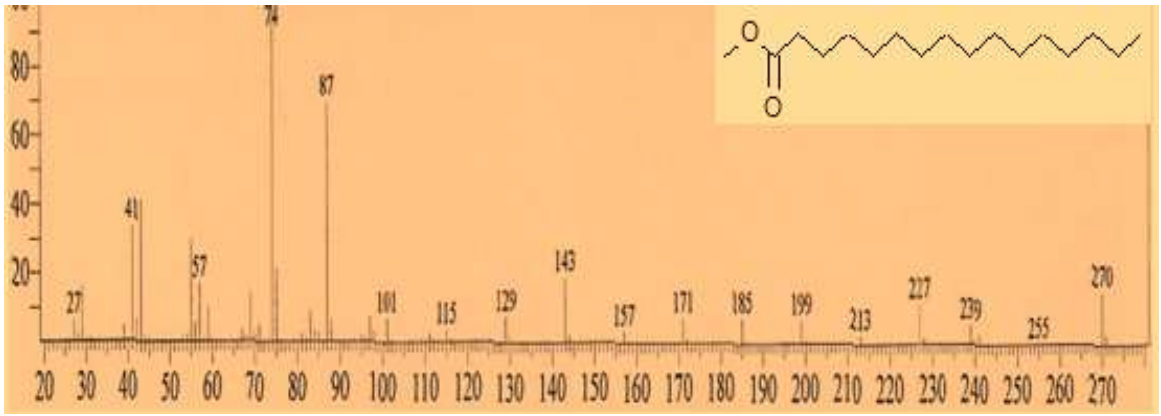
The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig(3.17).The peak at m/z294 (R.T. 17.378) coincides with $M^{+}[C_{19}H_{34}O_2]^{+}$, while the peak at m/z263 is due to loss of a methoxyl




Fig(3.17): Mass spectrum of 9,12-octadecadienoic acid methyl ester.

## ii) Hexadecanoic acid methyl ester(14.00%)

Figure(3.18) 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.

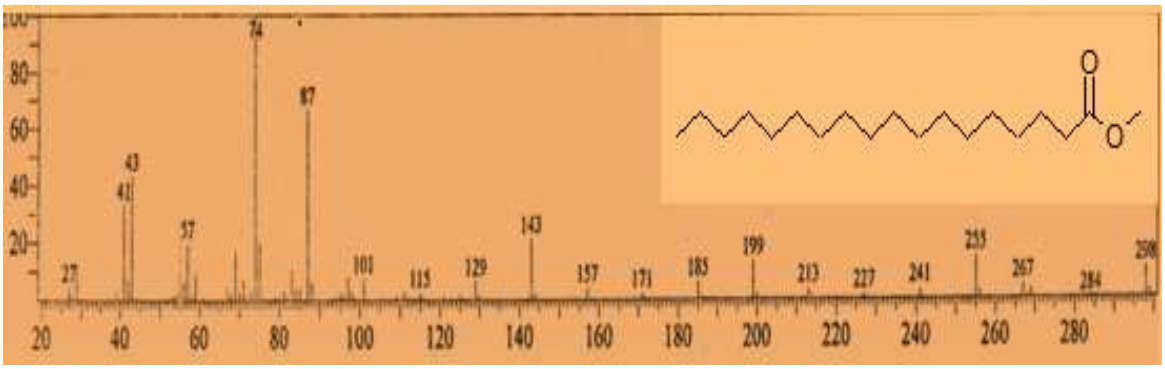



Fig(3.18): Mass spectrum of hexadecanoic acid methyl ester

## iii) Methyl stearate(7.50%)

The EI mass spectrum of methyl stearate is displayed in Fig(3.19).The peak at m/z 298 with R.T. 17.596 is due to

$M^+[C_{19}H_{38}O_2]^+$ , while the signal at m/z267 corresponds to loss of a methoxyl group.




Fig(3.19): Mass spectrum of methyl stearate

### 3.4.1-Antimicrobial 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(3.10).

**Table (3.10)**: Antimicrobial Activity of the *Lawsonia mermus* oil

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

The oil showed moderate antibacterial activity against *Escherichia coli*,and. However, it exhibited partial activity against *Pseudomonas aeruginosa.*

## 3.5- *Senna italic* Mill.

GC-MS analysis of *Senna italic* **Mill** oil was conducted **.**The GC-MS analysis of the studied oil revealed the presence of 19 constituents (Table 3.11).The typical total ion chromatograms (TIC) is depicted in Fig (3.20).

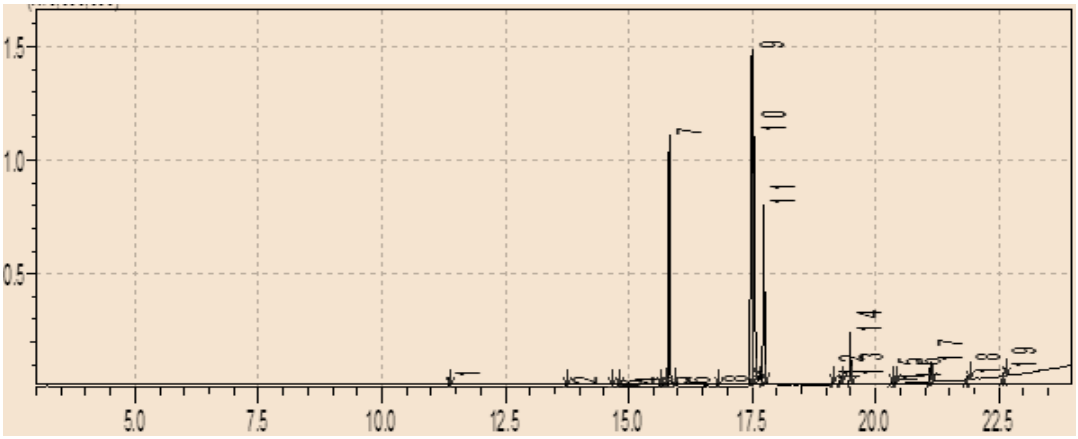



Fig (3.20): Total ions chromatograms

**Table (3.11)**: Constituents of *Senna italic* **Mill** oil

| No. | Name | Ret.Time | Area% |
|---|---|---|---|
| 1. | Butylated Hydroxytoluene | 11.362 | 0.60 |
| 2. | Methyl tetradecanoate | 13.715 | 0.18 |
| 3. | 5-Octadecenoic acid, methyl ester | 14.631 | 0.03 |
| 4. | Pentadecanoic acid, methyl ester | 14.791 | 0.07 |
| 5. | 9-Hexadecenoic acid, methyl ester, (Z)- | 15.627 | 0.13 |
| 6. | 7-Hexadecenoic acid, methyl ester, (Z)- | 15.720 | 0.17 |
| 7. | Hexadecanoic acid, methyl ester | 15.826 | 20.06 |
| 8. | Heptadecanoic acid, methyl ester | 16.797 | 0.27 |
| 9. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.498 | 40.26 |
| 10 | 9-Octadecenoic acid (Z)-, methyl ester | 17.532 | 17.32 |
| 11 | Methyl stearate | 17.737 | 13.47 |
| 12 | cis-11,14-Eicosadienoic acid, methyl ester | 19.130 | 0.47 |
| 13 | 11-Eicosenoic acid, methyl ester | 19.295 | 0.56 |
| 14 | Eicosanoic acid, methyl ester | 19.491 | 3.43 |

| | | | |
|---|---|---|---|
| 15 | Heneicosanoic acid, methyl ester | 20.320 | 0.25 |
| 16 | Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- | 20.406 | 0.24 |
| 17 | Docosanoic acid, methyl ester | 21.114 | 1.48 |
| 18 | Tricosanoic acid, methyl ester | 21.881 | 0.40 |
| 19 | Tetracosanoic acid, methyl ester | 22.620 | 0.61 |

## Major constituents are briefly discussed below:

### i) 9,12-Octadecadienoic acid methyl ester(40.27%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig(3.21).The peak at m/z294 (R.T. 17.498) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl.

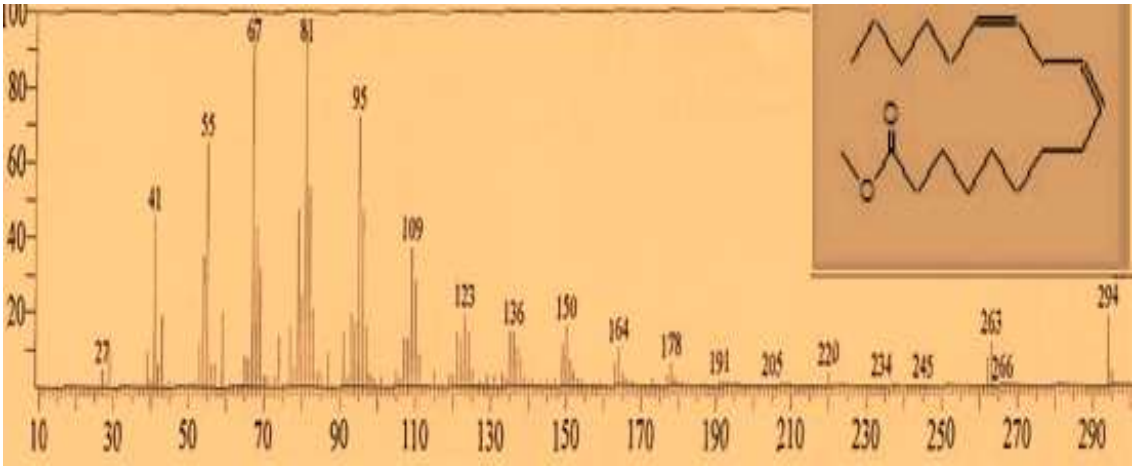



Fig (3.21): Mass spectrum of 9,12-octadecadienoic acid methyl ester

### ii) Hexadecanoic acid methyl ester (20.06%)

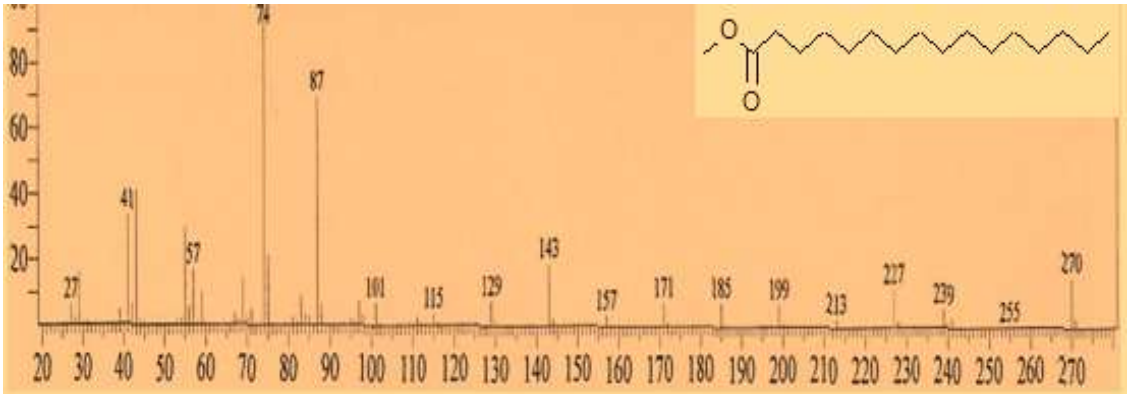



Fig (3.22): Mass spectrum of hexadecanoic acid methyl ester

Fig (3.22) shows the mass spectrum of hexadecanoic acid methyl.The peak m/z 270(R.T. 15.826) was detected in the spectrum. It corresponds $M^{+}[C_{17}H_{34}O_{2}]^{+}$ .The peak at m/z239 is due to loss of a methoxyl .

### iii) 9-Octadecenoic acid methyl ester (17.32%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig (3.23).The peak at m/z 296 (R.T. 17.532) corresponds $M^{+}$ $[C_{19}H_{36}O_{2}]^{+}$ , while the signal at m/z266 is attributed to loss of a methoxyl .

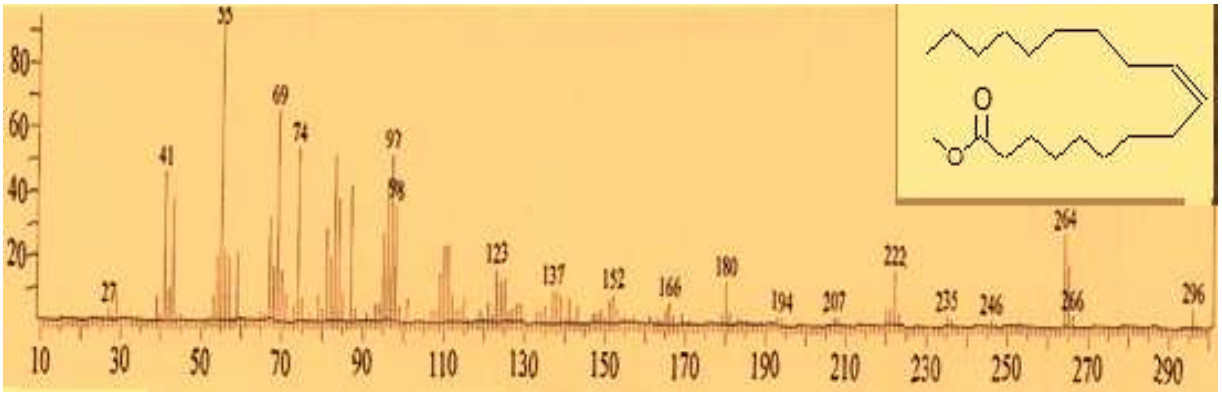



Fig (3.23): Mass spectrum of 9-octadecenoic acid methyl ester

### iv) Methyl stearate(13.47%)

The mass spectrum of methyl stearate is displayed in Fig(3.24).The peak at m/z 298 with R.T. 17.737 is due to $M^{+}[C_{19}H_{38}O_{2}]^{+}$ , while the signal at m/z267 corresponds to loss of a methoxyl group.

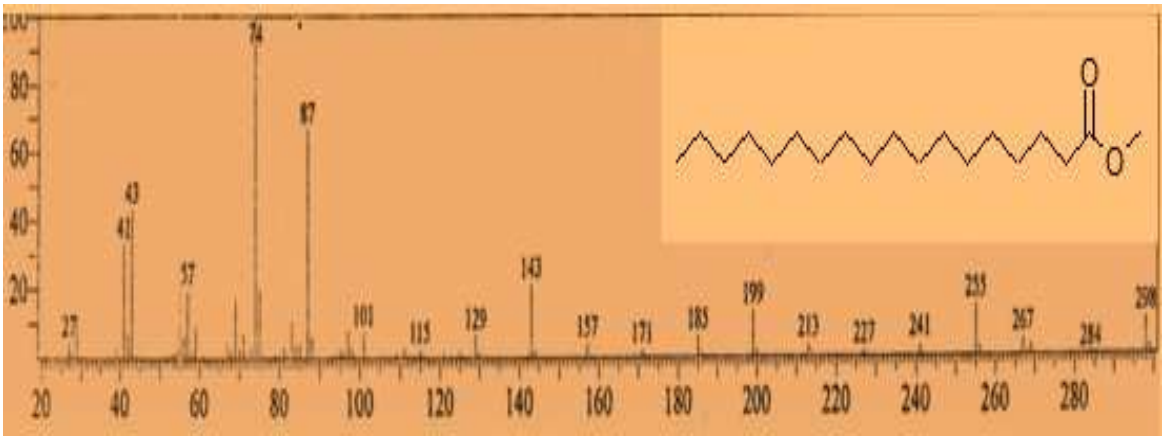



Fig (3.24): Mass spectrum of methyl stearate

## 3.5.1-Antimicrobial activity

The oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table(3.12).

**Table (3.12)**: Antimicrobial Activity of the *Senna italic* **Mill**. oil

| Sample | Ec. | Pa. | S.a | Bs. | C.a |
|---|---|---|---|---|---|
| *Senna italic Mill* oil (100mg/ml) | 10 | 12 | 12 | 14 | - |

The oil showed moderate antibacterial activity against *Bacillus subtilis*. However, it exhibited partial activity against

*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.The oil failed to give any anticandidal activity.

### 3.6- *Carthamus tinclorius*

GC-MS analysis of *Carthamus tinclorius* oil was carried out and the identification of the constituents was accomplished by comparison of the retention times and MS data (NIST-software). Excellent matching was observed when comparing the mass spectra with the database on MS library.

The GC-MS spectrum of the studied oil revealed the presence of 24 components (Table 3.13)).The typical total ion chromatograms (TIC) is depicted in Fig (3.25).

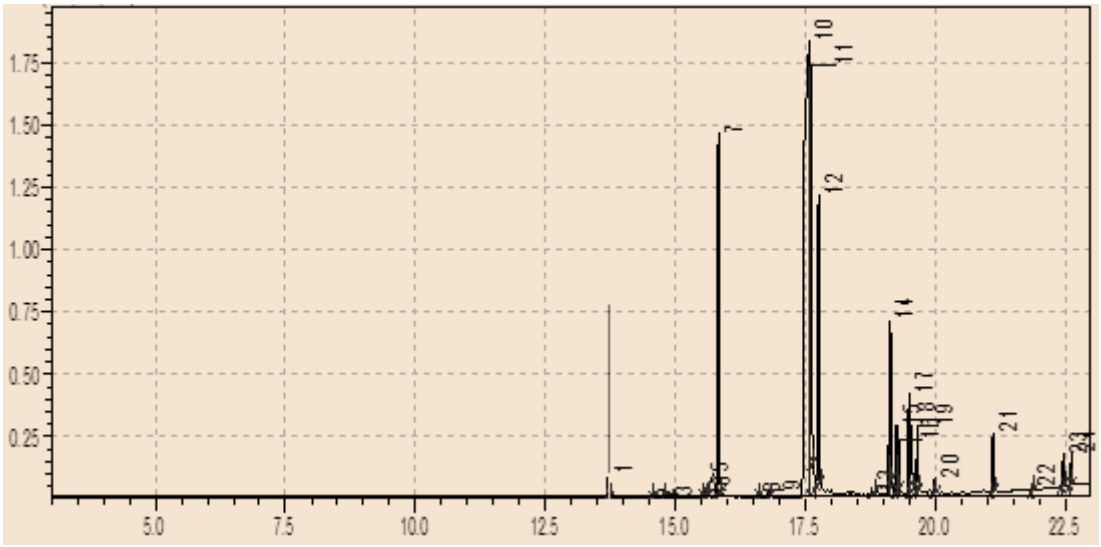



Fig (3-25): Chromatograms of *Sudanese Carthamus tinclorius* oil

Table (3.13): Constituents of *Sudanese Carthamus tinclorius* oil

| No. | Name | Ret.Time | Area% |
|---|---|---|---|
| 1. | Methyl tetradecanoate | 13.702 | 0.59 |

| | | | |
|---|---|---|---|
| 2. | 5-Octadecenoic acid, methyl ester | 14.516 | 0.01 |
| 3. | Pentadecanoic acid, methyl ester | 14.779 | 0.08 |
| 4. | 7,10-Hexadecadienoic acid, methyl ester | 15.511 | 0.03 |
| 5. | 7-Hexadecenoic acid, methyl ester, (Z)- | 15.569 | 0.10 |
| 6. | 9-Hexadecenoic acid, methyl ester, (Z)- | 15.615 | 0.21 |
| 7. | Hexadecanoic acid, methyl ester | 15.826 | 13.49 |
| 8. | cis-11,14-Eicosadienoic acid, methyl ester | 16.577 | 0.16 |
| 9. | Heptadecanoic acid, methyl ester | 16.787 | 0.20 |
| 10 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.575 | 51.14 |
| 11 | 9-Octadecenoic acid (Z)-, methyl ester | 17.595 | 7.99 |
| 12 | Methyl stearate | 17.749 | 9.05 |
| 13 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 18.763 | 0.15 |
| 14 | Tridecanedial | 19.125 | 4.26 |
| 15 | Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- | 19.246 | 1.81 |
| 16 | cis-11-Eicosenoic acid, methyl ester | 19.283 | 1.00 |
| 17 | Eicosanoic acid, methyl ester | 19.482 | 2.60 |
| 18 | PGH1, methyl ester | 19.534 | 1.69 |
| 19 | 1-Naphthalenol, decahydro-4a-methyl- | 19.644 | 1.68 |
| 20 | Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- | 19.982 | 0.52 |
| 21 | Docosanoic acid, methyl ester | 21.100 | 1.51 |
| 22 | Tricosanoic acid, methyl ester | 21.864 | 0.10 |
| 23 | 15-Tetracosenoic acid, methyl ester, (Z)- | 22.450 | 0.82 |
| 24 | Tetracosanoic acid, methyl ester | 22.602 | 0.81 |

## Major constituents are as follows:

## i) 9,12-Octadecadienoic acid methyl ester(51.13%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Fig(3.26).The signal which was observed at

m/z294( R.T. 17.575) is due to $M^{+}[C_{19}H_{34}O_2]^{+}$ , while the signal at m/z263 corresponds to loss of a methoxyl.

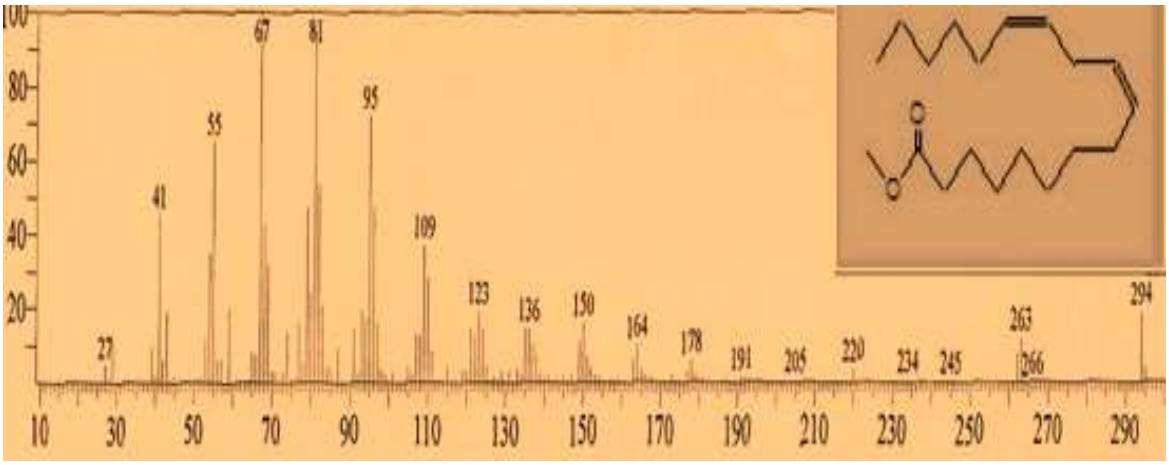



Fig (3.26): Mass spectrum of 9,12-octadecadienoic acid methyl ester

## ii) Hexadecanoic acid methyl ester (13.49%)

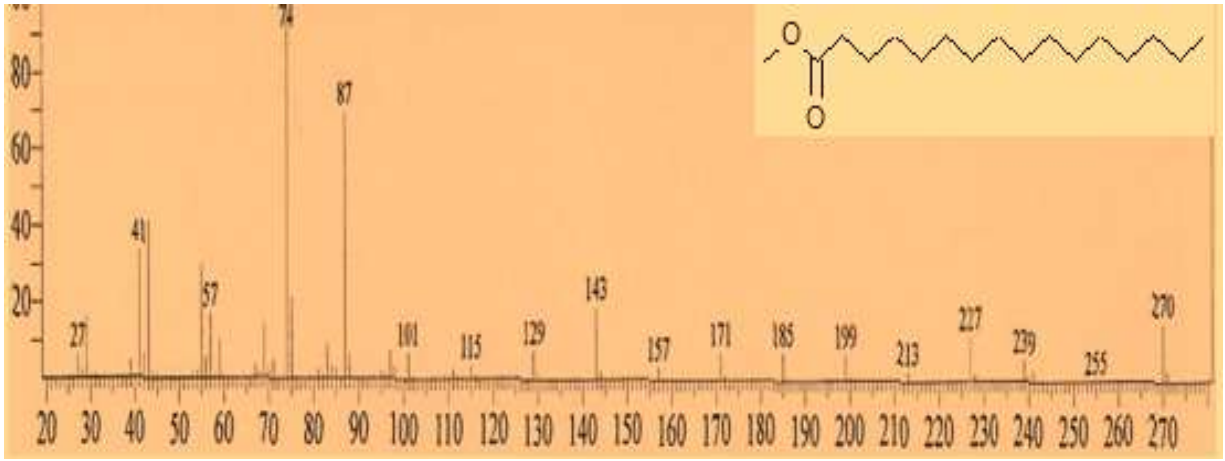



Fig (3.27): Mass spectrum of hexadecanoic acid methyl ester

Fig. (3.27) shows the mass spectrum of hexadecanoic acid methyl ester.The peak m/z 270(R.T. 15.826) was detected in the spectrum. It corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$ .The peak at m/z239 is due to loss of a methoxyl .

## iii) Methyl stearate(9.05%)

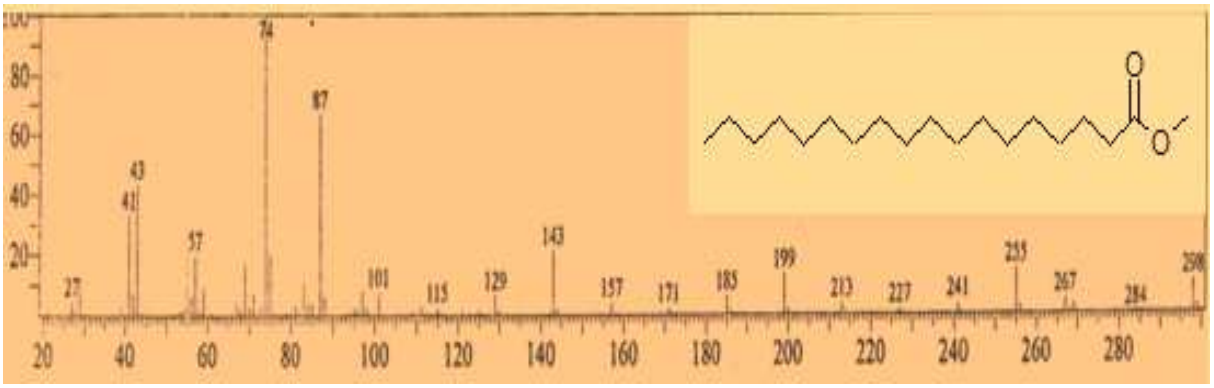



Fig (3.28): Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is displayed in Fig(3.28).The peak at m/z 298 with R.T. 17.749 is due to $M^+[C_{19}H_{38}O_2]^+$ , while the signal at m/z267 corresponds to loss of a methoxyl group.

**iv) 9-Octadecenoic acid methyl ester (7.99%)**

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig (3.29).The peak at m/z 296 (R.T. 17.595) corresponds $M^+$ $[C_{19}H_{36}O_2]^+$ , while the signal at m/z266 is attributed to loss of a methoxyl .

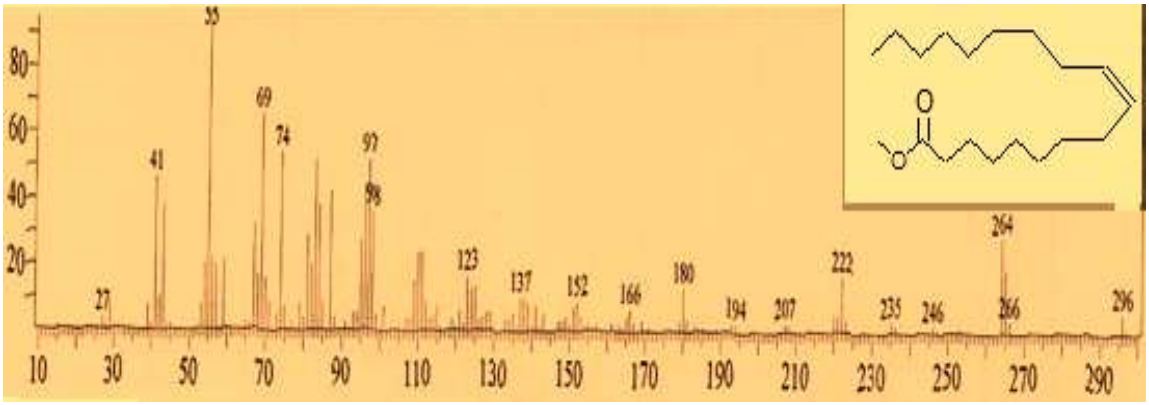



Fig (3.29): Mass spectrum of 9-octadecenoic acid methyl ester

### 3.6.1-Antimicrobial 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(3.14).

**Table (3.14)**: Antimicrobial Activity of the *Carthamus tinclorius* oil

| Sample | Ec | Pa | Sa | Bs | Ca |
|---|---|---|---|---|---|
| *Sudanese Carthamus tinclorius* (100mg/ml) | - | 15 | 15 | 7 | 16 |

The oil exhibited significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.It also showed significant anticandidal activity. However, it failed to give antibacterial activity against *Escherichia coli* and *Bacillus subtilis*.

# Conclusion

This study was designed to study the constituents of the oil of some Sudanese Medicinal Plants and to assess their antimicrobial effect. These species are: *Azadirachta Indica*, *Gerwia tenax*, *Citrus aurantifolia*, *Lawsonia mermus*, *Senna Italica* and *Carthamus tinctorius*. The constituents of the oil were unmasked by a GC-MS analysis. The GC-MS analysis of *Azadirachta Indica*, *Gerwia tenax*, *Citrus aurantifolia*, *Lawsonia mermus*, *Senna Italica*, *Carthamus tinctorius* oils showed: 29, 12, 24, 49, 19 and 24 components respectively.

The oil were screened for their antimicrobial potential and significant to moderate responses were detected.

# Recommendations

The following is highly recommended:

1- Other phytochemicals of the target species may be isolated and their structures may be elucidated and the biological activity could be screened.
2- The isolated oils may be subjected to *in vivo* antimicrobial potency.

3- The extracted oils may be screened for other biological effects such as anti-inflammatory, anti-diabetic, anti-viral, anti-lashmenial..etc.

# References

**References:**


1- Charles.B., "Natural products", 2005 Spainhour publishers, Medrid.

2- Hailemichael.T., "Isolation and structure Elucidation of Natural Products from Plants" ,2005, Marc publishers, Hamburg.

3- Stearn.T.,"Botanical Latin" 4th Edition,1992, Timber Press, Portland.

4- Finar.J., "Organic Chemistry", vol.(2), 5th edition,2003, Cardinal Newman, Londonm UK.

5- Anna.R.,"Analogues: Synthesis and Receptor/Enzyme interactions",2006, University of Canterbury, UK.

6- Lawrence.M., "Phytochemistry Organic Metabolites", vol.(11),1973, Chapman and Hall, London.

7- Suni,K.T and Bani,T., "Chemistry of Natural Products",2015,Springer,New York.

8- Taleb.C, and Silvia.H," Antimicrobial Activity of Flavonoids and Steroids Isolated from Two Chromolaena Species" Revista Brasileira de Ciências Farmacêuticas, 39.4:403-408 (2003).

9- Okwou.D.,"Isolation and Characterization of Flavonoids Chalcones and Anthocyidines from Bridelia Ferruginea Benth",*Der.Chemica.Sinica*.,**1(2):**21-28( 2010).

10 – Butler.G, Bailely,R.,"Chemistry and Biochemistry of Herbage", Vol.(2);1973 Chapman and Hall. Ltd, London.

11 – Guenther.E., "The Essential Oils",1984, Van Nostrand Company Inc.NewYork.

12 - Association Française de Normalisation (AFNOR). Huiles Essentielles, Tome 2, Monographies Relatives Aux

Huiles Essentielles, 6th Edition,2000, AFNOR, Association Française de Normalisation: Paris, France.

13 – 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).

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

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

16 – Laurence.M, Moody.J, "Experimental organic chemistry: Principles and Practice" , 1989, Wiley-Blackwell,Hoboken, New Jjersey.

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

18 – 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)

19 - Taylor.W.E.,"Aromatherapy for the Whole Person" 1981, Stanley Thornes, UK.

20 – Sujay.R, and Suresh.B., "In-vitro antioxidant properties of Indian traditional paan and its ingredients", *Indian Journal of Traditional Knowledge(IJTK),* **3(2):** 187-191(2004).

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

22 – Pourmortazavi.S, Hajimirsadeghi.S.,"Supercritical fluid extraction in plant essential andvolatile oil analysis" *Journal of Chromatography A*, **1163(1-2):** 2-24(2007)

23 – Fadel.H, Marx.A, El-Gorab.A., "Effect of extraction techniques on the chemical composition and antioxidant activity of Eucalyptus camaldulensis var. brevirostris leaf oils*", Zeitschrift für Lebensmitteluntersuchung und-Forschung* **208(3):** 212-216(1999) .

24 – Capuzzo.A, Maffei.M, Occhipinti.A.,"Supercritical fluid extraction of plant flavors and fragrances", *Molecules*,**18(6):**7194-7238(2013).

25 – 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)..

26 – Chemat.F, Lucchesi.M, Smadja.J.,"Extraction sans solvant assistée par micro-ondes de produits naturels" *EP Pat* **1.439**: 218(2004)

27 – 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)

28 – 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).

29 – 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).

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

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

32 - 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).

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

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

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

36 – 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).

37 - 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).

38 – 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).

39 – Sell.S., "The Chemistry of Fragrance. From Perfumer to Consumer", 2nd Edition,2006, The Royal Society of Chemistry: Cambridge, UK.

40 - Vainstein.A, Lewinsohn.E, Pichersky.E, Weiss.D., "Floral Fragrance. New Inroads into an Old Commodity*",.Plant Physio,***l27:** 1383-1389(2001).

41 – Pophof.B, Stange.G, Abrell.L., "Volatile Organic Compounds as Signals in a Plant-Herbivore System: Electrophysiological Responses in Olfactory Sensilla of the Moth Cactoblastis cactorum" *Chem. Senses* ,**30:**51-68(2005).

42 - Sikkema.J, Poolman.B., "Interactions of cyclic hydrocarbons with biological membranes*". J. Biol. Chem*.**269:**8022-8028(1994).

43 – Gustafson.J, Liew.Y, Chew.S, Markham.J., "Effects of tea tree oil on Escherichia coli" *Appl. Microbiol*.**26:**194-198(1998).

44 – Ultee.A, Moezelaar.R., "The phenolic hydroxyl group of carvacrol in essential for action against the food-borne pathogen Bacillus cereus" *Appl. Environ.Microbiol*.**68:**1561-1568(2002).

45 – Denyer.S, Hugo.W., "Biocide-induced damage to the bacterial cytoplasmic membrane. In Mechanisms of Action of Chemical Biocides, the Society for Applied Bacteriology", 1991, Blackwell Scientific Publication:Oxford,UK.

46 – Lambert.R, Skandamis.P, Coote.P., "A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol" *J. Appl. Microbiol*. **91:**453-462(2001).

47 – Dorman.D, Deans.G., "Antimicrobial agents from plants: Antibacterial activity of plant volatile oils" *J.Appl.Microbiol.* **88:**308-316(2000).

48 – Knobloch.K, Weis.N, Schwarm.H., "Action of terpenoids on energy metabolism. In Progress in Essential Oil Research: 16th International Symposium on Essential Oils", 1986, Brunke, E.J., Ed.; De Walter de Gruyter*:* Berlin, Germany.

49 – Pauli.A., “Antimicrobial properties of essential oil constituents”. *International Journal of Aromatherapy*,**11(3):**126-133(2001).

50 – Marino.M, Bersani.C, Comi.G., “Antimicrobial activity of the essential oils of Thymus vulgaris L. measured using a bioimpedometric method” *J. Food Prot*.**62:**1017-1023(1999).

51 – Senatore.F, Napolitano.F, Ozcan.M., “Composition and antibacterial activity of the essential oil from Crithmum maritimum L”. *Flav. Frag. J*. **15:**186-189(2000).

52 – Aruoma.I., “Free radicals, oxidative stress, and antioxidants in human health and disease” *J.Am. Oil Chem.Soc*.**75:**199-212(1998).

53 – Burt.S., “Essential oils: Their antibacterial properties and potential applications in foods” *J.Food Microbiol*.**94:**223-253(2004).

54 – Kamatou.G, Viljoen.A., “A review of the application and pharmacological properties of α-Bisabolol and α-Bisabolol-rich oils” *J. Am. Oil Chem. Soc*, **87:**1-7(2010).

55 – Sharififar.F, Mirtajadini.M, Azampour.M, Zamani.E “Essential Oil and Methanolic Extract of Zataria multiflora Boiss with Anticholinesterase Effect”. *Pak. J. Biol. Sci*.**15:**49-53(2012).

56 – Pyun.S, Shin.S., “Antifungal effects of the volatile oils from Asiium plants against Trichophyton species and synergism of the oils wih ketoconazole”. *Phytomedicine* ,**13:**394-400(2006).

57 – Milner.J., “A historical perspective on garlic and cancer. Recent advances on the nutritional effects associated with the use of garlic as a supplement” *J. Nutr*,**131:**1027-1031(2001).

58 – Milner.J., “Preclinical perspectives on garlic and cancer. Signifiance of garlic and its constituents in cancer and cardiovascular disease” *J. Nutr*. **136:**827-831(2006).

59 – Legault.J, Dahl.W, Pichette.A., "Antitumor activity of balsam fir oil: Production of reactive oxygen species induced by α-Humulene as possible mechanism of action". *Planta Med.***69:**402-407(2003).

60 – Santoro.G, Guimaraes.G, Salgado.A, Soares.M., "Effect of Oregano (Origanum vulgare L.) and Thyme (Thymus vulgaris L.) essential oils on Trypanosoma cruzi (Protozoa: Kinetoplastida) growth and ultrastructure" *Parasitol. Res.***100:**783-790(2007).

61 – Schnitzler.P, Koch.C, Reichling.J., "Susceptibility of drugresistant clinical HSV-1 strains to essential oils of Ginger, Thyme, Hyssop and Sandalwood", *Antimicrob. Agents Chemother*.**51:**1859-1862(2007).

62 – Parveen.M, Hasan.K, Takahashi.J, Murata.Y, Kitagawa.E., "Response of Saccharomyces cerevisiae to a monoterpene: Evaluation of antifungal potential by DNA microarray analysis".*J. Antimicrob. Chemother***, 54:**46-55(2004).

63 – Hong.E, Choi.G, Choi.K, Jeung.E., "Antibacterial and antifungal effects of essential oils from coniferous trees".*Biol.Pharm. Bull*.**27:**863-866(2004).

64 –Santoro.G, Cardoso.M, Guimaraes.L, Mendonca.Z., "Activity of essential oils from Achillea millefolium L., Syzygiumaromaticum L. and OcimumbasilicumL.on epimastigotes and trypomastigotes".*Exp. Parasitol*. **116:** 283-290(2007).

65 –Rota.C, Carraminana.J, Burillo.J, Herrera.A., "In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens". *J. Food Prot.***67:**1252-1256(2004).

66 – Si.W, Gong.J, Tsao.R, Zhou.T, Yu.H, Poppe.C, Johnson.R., "Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria". *J. Appl. Microbiol*.**100:**296-305(2006).

67 – Sonboli.A, Babakhani.B, Mehrabian.A., "Antimicrobial activity of six constituents of essential oil from Salvia". *Z.Naturforschung*.**61:**160-164(2006).

68 - El Hadri.A, Sanz.J, Idaomar.M.,"Cytotoxic activity of α-humulene and transcaryophyllene from Salvia officinalis in animal and human tumor cells". *An. Real Acad. Nac. Farm.* **76(3):** 343-356(2010).

69 - Moon.T, Wilkinson.J.,"Antiparasitic activity of two Lavandula essential oils against Giardia duodenalis, Trichomonas vaginalis and Hexamitainflata".*Parasitol.Res*.**99:**722-728(2006).

70 - Syed.Z, Khushnuma.M., "GC-MS Principle, Technique and its application in Food Science",2014, University of Agricultural Science and Technology of Kashmir ,India.

71 - Clement.R.E, Taguchi.VY., "Techniques for the gas chromatography-mass spectrometry identification of organic compounds in effluents",1989,Queen's Prenter for Ontario,Canada.

72 – Karasek.F, Clement.R., "Gas Chromatography–Mass Spectrometry: Principles and Techniques",1988, Elsevier Publishers, Amsterdam.

73 – Robert.A, Meyrs.J., SonsLtd. "CEncyclopedia of Analytical Chemistry". ISBN0471976709(2014)

74 – Watson.J, Throck., "Introduction to Mass Spectrometry"2nd Edition,1985, Raven Press,New York.

75 - Christman.R., "Guidelines for GC/MS Identification",1982, Environ. Sci. Technol.

76 - Christman.R.F., "GC/MS Criteria Revisited", 1982, Environ. Sci.Technol.

77 - Christman.R., "Editorial Policy Changes", 1984, Environ. Sci. Technol .

78 - Watson, J. Throck., “Introduction to Mass Spectrometry”, 2nd Edition,1985, Raven Press, New York.

79 – Nursing.R, Prabhakara.R., “Chemical fatty acid , volatile oil composition and antioxidant activity of shade dried neem (Azasirachta Indical.) flower powder” international *food Research Journal*,**21(2)**:807-813(2014).

80 – Eckey.E., “Vegetable fats and oils”,1954, Reinhold publishing, New York .

81 – Rahim.M., “Biological activity of azadiractinenriched neem kernel extracts against Rhyzopertha dominiea (f) (coleopteran: Bostrichidae) in stored wheat” *Journal of stored product Research* **34:**123-128(1998).

82 – Udayashekhara.P., “Chemieal composition and biological evaluation of debitterised and defatted neem (azadirachtaindica) seed kernel cake”, *Journal of American oil chemists society* **64:**1348-1352(1987).

83 – Iwu.M., “Handbook African Medicinal Plants”, 1993, CRC Press London.

84 – KarKar.C., “Utilization of neem (A.indica) and its by-products: Report of the modified neem cake manorial project 1969-1976”.Directorate of non-edible oils soap industry khadi and village Industries Commossion,Bombay, India(1976).

85 - (W.H.O) W.H.O“General guidelines for methodologies on research and evaluation of traditional medicine” (2001).

86 - Shahed.S, Ahmed.F, Saha.S, Bachar ,S., “Evolution of Brine on some drug resistance bacteria in Bangladesh” *pharmacognosy J*,**3(20):**66-7(2011).

87 - Velusamy.P, Das.J, pachaiappon.R, Pandian.K., “Greener approach for synthesis of antibacterial silver nanoparticles using

aqueous , solution of neem gum (Azadirachta Indica L) ", *Ind crops prod*.**66:**103-109(2015).

88 - Prashant.G, Chandu.G, Chandu.G, Shafiulla.M., "The effect of mango and neem extract on four organisms causing dental caries: streptococcus mutans . streptococcus salivavius , streptococcus mitis , and Streptococcus sanguis: An in vitro study*" Indian J Dent Res* .**18(4):**148-151(2007).

89 - Bhowmik.D, Yadav.J, Tripathi.K, Kumar.K., "Herbal remedies of Azadirachta Indica and its medicinal application" *J chem pharm Res*.**2(1):** 62-72(2010).

90 - Bis was.K, Chattopadhyay.I, Banerjee.R., "Biological activities and medicinal properties of neem (Azadirachta Indica)" *Curr sci*.**82(11):**1336-1345(2002).

91 - Sujarwo.W, Keim.P, Caneva.G, Nicoletti.M., "Ethnobotanical uses of neem (Azadirachta Indica A.juss, Meliaceae) leaves in Bali(Indonesia) and the Indian subeontinent in relation with historical background and photochemical properties".*JEthnopharmacol*.**189:**18693(2016).

92 - Forster.P, Moser.G., "Status report on global neem usage : Deutsche Gesellschaft fur Technische Zusammenarbeit (G T Z) ", 2000, Eschborn Publishers, Germany.

93 - Habluetzel.A, Esposito,F., "Azadirachta Indica as apublic health tool for the control of malaria and other vector-borne diseases". *Indian JMed Res*.**130(2):** 112-114(2009).

94 - Vietmeger,N., "Neem: a tree for solving global problems". Report of an ad hoe panel of the Board on science and Technology for International development (1992).

95 - FAO. "Traditional Food Plants: A Resource Book for Promoting the Exploration Consumption of Food Plant in Arid, Semi-arid and

Sub-humid lands of Eastern Africa. Rome: FAO Food and Nutritionpaper":42(1988).

96 – Abdualrahman.M, Ali.A, Suliman.A., "Nutritional of Guddaim Fruits (Grewia tenax) and its Utilization in Ice Cream Production".*J.Sc.Tech*.**12:**03(2011)

97 – Aboagarib.E, Yang.R, Hua.X, Siddeeg.A., "Chemical Compositions, Nutritional properties and volatile compounds of Guddaim(Grewia Tenax.Forssk) Fiori Fruits".*J Food Nutr Res*.187–192(2014).

98 – Aboagarib.E, Yang.R, Hua.X., "Physicochemical, Nutritional, and Functional Characteristics of seed, Peel and Pulp of Grewia tenax(Forssk)Fiori Fruits". *Trop J Pharm Res*.**14(12):**2247(2015).

99 - El Ghazali.G, El Tohami.M, El Egami.A., " Medicinal Plants of the Sudan, Medicinal Plants of the White Nile Provinces",(1994). Khartoum University Press, Khartoum.

100- El Ghazali.G, El Tohami.M, El Egami.A, Abdalla.W., "Medicinal Plants of Sudan , Part(III), Medicinal Plants of the eastern Nuba Mountains",(1997),Khartoum University Press, Khartoum.

101- El Siddig.K, Gebauer.J, Inanaga.S, Ludders.P., "Auxin effects on rooting of stem Cuttings of Grewia tenax: A potential alleviator of Iron-deficiency Anaemia in the Sudan, In: Deutschr Tropentag Conference on The Global Food and Product Chain-Dynamics, Innovations,Conflicts,Strategies"(Hohenheim)(2005).

102- Gebauer.J., Patzelt.A., Buerkert.A., "First record of Grewia tenax(Forssk.) Fiori in northern Oman, a valuable fruit producing shrub".*Genet Resour Crop Evol* , **54:**1153-1158(2007).

103- Abdelmutti.O., "Biochemical of Nutritional Evaluation of Famine Food of Sudan",1991,University of Khartoum, Sudan.

104- Campelo.L, Feitosa.C, Sousa.G., "Constituintes químicos e estudos toxicológicos do óleo essencial extraído das folhas de Citrus limon Burn(Rutaceae)",*Rev Bras Pl Med*.**15:**708-716(2013).

105- Estevam.E., "Composição química e atividades biológicas dos óleos essenciais das folhas frescas de Citrus limonia Osbeck e Citrus latifolia Tanaka (Rutaceae) ".*Rev Virtual Quim* **8:** 1842-1854(2016).

106- Tundis.R, Loizzo.M, Bonesi.M, Colica.C., "Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of Citrus aurantifolia Swingle, C. aurantium L., and C. bergamia Risso and Poit. peel essential oils". *J Food Sci* **77:** 40-46(2012).

107- Amorim.J, Simas.D, Pinheiro.M, Moreno.D, Alviano.C., "Anti-inflammatory properties and chemical characterization of the essential oils of four Citrus species".PLoS ONE.11 (2016).

108- Aibinu.I, Ogunsanya.T, Odugbemi.T., "Evaluation of the antimicrobial properties of different parts of Citrusaurantifolia (Lime Fruit) as used locally". *Afr J trad CAM* , **4:** 185-190(2007).

109- Abyaneh.R, Rezaee.B, Jaimand.K, Saberi.R.,"Chemical composition and antiaflatoxigenic activity of Carum carvi L., Thymus vulgaris and Citrus aurantifolia essential oils",*Food Control,* **20:** 1018-1024(2009).

110- Pathan.R, Gali.P, Gowtham.T, Pasupuleti.S., "In vitro antimicrobial activity of Citrus aurantifolia and its phytochemical screening",*Asian Pac J Trop Dis,* **2:** 328-331(2012).

111- Narang.N, Jiraungkoorskul.W., "Anticancer activity of Key Lime, Citrus aurantifolia". *Pharmacogn Rev* **10:** 118-122(2016).

112- Martins.M., "Schistosomicidal effects of the essential oils of Citrus limonia and Citrus reticulata against Schistosoma mansoni". Chemistry & biodiversity,14(1):e1600194(2017).

113- Eldahshan.O, Halim.A., "Comparison of the composition and antimicrobial activities of the essential oils of green branches and leaves of Egyptian navel orange (Citrus sinensis (L.) Osbeck var. malesy" *Chem Biodivers* **13:** 681-685(2016)..

114- Spadaro.F, Costa.R, Circosta.C, Occhiuto.F., "Volatile composition and biological activity of key lime Citrus aurantifolia essential oil". *Nat Prod Commun* **7:** 1523-1526(2012).

115- Khan.N, Jamila.N, Hong.Y, Choi.J, Lee.C, Kim.K., "Determination of volatile flavour profiles of Citrus spp. fruits by SDE-GCMS and enantiomeric composition of chiral compounds by MDGC-MS". *Phytochem Anal* **28:** 392-403(2017).

116- Jacob.R, Gary.M., "The potential of Citrus limonoids as anticancer agents".Perishables Handling Quarterly;102(2000).

117- Kumar.A, Narayani.M, Subanthini.A, Jayakumar.M., "Antimicrobial activity and phytochemical analysis of citrus fruits peel utilization of fruit waste". *International Journal of Engineering Science and Technology* .**3:**6; 5414-5421(2011).

118- Mehmet.K, Hanefi.O, Aydın.H, Mehmet.T, Hasan.A, Veysel.K., "Investigation of antiinflammatory activity of Bergamot oil".European Journal of General Medicines.4: 4; 176-179(2007).

119- Sastri.N., "The Wealth of India:Council of Scientific and Industrial Research", New Delhi, p. 47 (1962).

120 - Chopra.R, Nayar.L, ChopraC., "Glossary of Indian Medicinal Plants National Institute of Science and Communication",1956, SCIR Publication,New Delhi, India.

121-Jain.S., "editors Medicinal plants",1968, National Book Trust, New Delhi, India.

122- Nadkarni, KMm, and A. K. Nadkarni. “Indian Materia Medica, Popular Prakashan Pvt”,1976, Ltd., Bombay.

123-Warrier.K, Nambiar.P, Ramankutty.C., “Indian Medicinal Plants: A Compendium of 500 Species,Orient longman pvt”,2004,ltd., Chennai, India.

124– Bich.D, Chung.B, Chuong.N, Dong.D, Dam.P., “The medicinal plants and animals in Vietnam”,2004, Science and Technology Publishing House, Hanoi, Vietnam.

125 - Reddy.R.**,** “Folk Medicine from Chittoor District, Andhra Pradesh, India, Used in the Treatment of Jaundice”, Pharmaceutical Biology, *International Journal of Crude Drug Research*, **26:**3, 137-140(1988).

126- Kirtikar.R, Basu.D., “Indian Medicinal Plants”,1956, Lalit Mohan Basu, Allahabad, India.

127- Dymock.W**,** Warden.H, Hooper.D., “Pharmacographia indica” ,1976, Periodical Experts, Delhi, India.

128- Gupta.A, Tandon.N., “Medicinal plants of India”,1987,Indian Council for Medical Research ,New Delhi, India.

129-Handa.G, Kapil.A, Sharma.S., “Organic Chemistry Including Medicinal Chemistry”,*Indian Journal of Chemistry*,**36**:252 (1997).

**130**-Cox.E., “Hair dyes. I. The chemistry and analysis of henna”, The Analyst 63, 397 (1938).

**131-**Bhardwaj.K, Murari.R., “Lacoumarin from Lawsonia inermis” *Phytochemistry*,**15(11):**1789 (1976).

132-Afzal.M, Al-Oriquat.G., “Flavone glycosides from Lawsonia inermis” , *Heterocycles*,**14:**1973-1976 (1980).

133- Mahmoud, Z. F., NA Abdel Salam, and S. M. Khafagy. "Constituents of henna leaves (Lawsonia inermis L.) growing in Egypt." **Fitoterapia**,**51(3):** 153-155(1980).

134 -Takeda.Y, Fatope.O., "New phenolic glucosides from Lawsonia inermis", *Journal of Natural Products*, **51:**725-729(1988).

135 - Siddiqui.B, Ali.T., "Two new and a known compound from Lawsonia inermis ", *Helvetica Chimica Acta*,**86(6):**2164-169 (2003).

136- Gupta.S, Ali.M.,"A naphthoquinone from Lawsonia inermis stem bark". *Phytochemistry*,**33(3):**723-724 (1993).

137 - Khan.M, . Singh,N and Dhawan.N., "Occurrence and identification of a new antiviral saponin from Lawsonia alba Lam fruits",*National Academy Science Letters (India)*,**19(7-8):** 145-148 (1996).

138 - Siddiqui.S, Kardar.N.,"Triterpenoids from Lawsonia alba", *Phytochemistry*, **58(8):** 1195-1198 (2001).

139-Siddiqui.S, Kardar.N, Khan.S., "Two New Triterpenoids from Lawsonia alba",*Zeitschrift für Naturforschung B*,**60(1):**37-40, (2005).

140- Uddin.N, Begum.S.,"Two new dioxin derivatives from the aerial parts of Lawsonia alba",*Natural Product Research* **23(18):**1740-1745(2009).

141-Malekzadeh.F., "Antimicrobial activity of Lawsonia inermis L" *Applied and Environmental Microbiology*, **16(4):** 663 (1968).

142-Tripathi.D, Srivastava.H, Dixit.N., "A fungitoxic principle from the leaves ofLawsonia inermis Lam" *Experientia*,**34(1):**51-52 (1978). .

143-Clarke.D, Jones.R, Martin.M., "The anti-sickling drug lawsone (2-OH-1, 4-naphthoquinone) protects sickled cells against membrane damage" *Biochemical and Biophysical Research Communications*, **139(2):**780-786 (1986).

144-Chang.H, Suzuka.E., "Lawsone (2-OH-1, 4-naphthoquinone) derived from the henna plant increases the oxygen affinity of sickle cell blood", *Biochemical and Biophysical Research Communications*,**107(2):** 602-608 (1982).

145- Wagner.H, Kreher.B., "In vitro stimulation of human granulocytes and lymphocytes by pico-and femtogram quantities of cytostatic agents" *Arzneimittel Forschung*, **38(2):** 273-275(1988).

146- Anand.K, Singh.B, Chand.D., "An evaluation of Lawsonia alba extract as hepatoprotective agent" *Planta Medica*, **58(1):**22-25 (1992).

147- Alia.B, Bashir.A, Tanira.M., "Anti-inflammatory, antipyretic, and analgesic effects of Lawsonia inermis L.(henna) in rats", *Pharmacology*, **51(6):** 356-363 (1995).

148-Ali.M, Grever.R., "A cytotoxic naphthoquinone from Lawsonia inermis".*Fitoterapia*.**69(2):**181-183 (1998).

149-Sultana.N, Choudhary.I, and A. Khan, "Protein glycation inhibitory activities of Lawsonia inermis and its active principles", *Journal of Enzyme Inhibition and Medicinal Chemistry*, **24(1):**257-261(2009).

150- Mikhaeil.B, Badria.A, Maatooq.G.,"Antioxidant and immunomodulatory constituents of henna leaves", *Zeitschrift für Naturforschung* , **59(7-8):**, 468-476(2004)..

151-Hsouna.A, Culioli.G., "Antioxidant constituents from Lawsonia inermis leaves: isolation, structure elucidation and antioxidative capacity" , *Food Chemistry*, **125(1):**193-200(2011).

152- Uddin.N.,"Studies on the Bioactive Constituents of Lawsonia alba (Henna) and analysis of salicylic acid and its derivatives using MALDI-MS and HPLC-ESI-MS",2011,University of Karachi, Pakistan.

153- Meng.J, Jiang.T, Bhatti.H, Siddiqui.B., "Synthesis of dihydrodehydrodiconiferyl alcohol: the revised structure of lawsonicin", *Organic and Biomolecular Chemistry*, **8(1):**107-113 (2010).

154-Uddin.N, Siddiqui.B, Begum.S, Bhatti.A., "Bioactive flavonoids from the leaves of Lawsonia alba (Henna) ", *Phytochemistry Letters*, **4(4):** 454-458 (2011)..

155-Oyedeji.A, Ekundayo.O, Koenig.W., "Essential oil composition of Lawsonia inermis L. leaves from Nigeria", *Journal of Essential Oil Research*,**17(4):**403-404(2005).

156– Wong.K.C, and Teng.Y.," Volatile components of Lawsonia inermis L. flowers ",*Journal of Essential Oil Research*,**7(4):**425-428 (1995).

157-Kardar.M., "Studies on the Chemical Constituents of the Aerials Parts of Lawsonia alba",2005, University of Karachi,Pakistan.

158- Afzal.M, Al-Oriquat.G.,"Isolation of 1, 2-dihydroxy-4-glucosyloxynaphthalene from Lawsonia innermis", *Heterocycles*, **22(4):** 813-816(1984)..

159- Antia.M, Kaushal.R., "Essential oil from the flowers of camphire or henna plant", *Current Science*,**19(9):**284 (1950).

160-Chakrabartty.T, Poddar.G.,"Triterpenoids and other constituents of Lawsonia inermis" *Indian Journal of Chemistry Section B Organic Chemistry Including Medicinal Chemistry*, **15:**96-97 (1977).

161- Chakrabartty.T, Poddar.G., "Isolation of dihydroxy lupene and dihydroxy lupane from the bark of Lawsonia inermis" *Phytochemistry (Oxford)*, 21, 814 (1982).

162- Gupta.S, Ali.M, Niwa.M., Alam, M. Sarwar, "24β-Ethylcholest-4-en-3β-ol from the roots of Lawsonia inermis" *Phytochemistry*, **31(7):**2558-2560(1992).

163- Gupta.S, Ali.M,Niwa.M.,"Isolation and characterization of a dihydroxysterol from Lawsonia inermis"*Natural Product Letters*,**4(3):**195-201(1994).

164- Agarwal.R, Ghatak.N, Dhingra.R., "Chemical examination of the seed oil of Lawsonia alba",*Indian Oil Soap Journal*,**25:**145, (1959).

165- Ali.A, Jülich.D, Kusnick.C., "Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities", *Journal of Ethnopharmacology*, **74(2):**173-179 (2001).

166- Dama.L, Poul.N, Jadhav.V., "Antimicrobial activity of naphthoquinonic compounds", *Journal of Ecotoxicology and Environmental Monitoring*, **8(3):** 213-215 (1998)..

167- Arun.P, Purushotham.K, Jayarani.J, Kumari.V.," Arun, P., et al. "In vitro antibacterial activity and flavonoid contents of Lawsonia inermis (Henna)" *International Journal of PharmTech Research*, **2(2):**1178-1181(2010).

168-Makhija.I, Kumar.V, Devkar.R, Khamar.D, Manglani.N.," Lawsonia inermis-from traditional use to scientific assessment", *African Journal of Pharmaceutical Sciences and Pharmacy*, **2(1):**145 (2011).

169- Okpekon.T, Yolou.S, Gleye.C, Roblot.F, Loiseau.P, Frappier.F., "Antiparasitic activities of medicinal plants used in Ivory Coast", *Journal of Ethnopharmacology*, **90(1):** 91-97(2004).

170- Mohsin.A, Shah.H, Tariq.M., "Analgesic, antipyretic activity and phytochemical screening of some plants used in traditional Arab system of medicine" , *Fitoterapia***, 60(2):**174-177 (1989).

171- Bagi.M, Kakrani.H, Kalyani.G., "Fitoterapia59, 49 (1988)..

172- Bodhankar.S, Garg.K, Mathur.V.,"Antifertility screening of plants. Part IX. Effect of five indigenous plants on early pregnancy in female albino rats", *The Indian Journal of Medical Research*", **62(6):** 831 (1974).

173- Munshi.S, Shetye.T., "Antifertility activity of three indigenous plant preparations", *Planta Medica*, **31(1):** 73-75 (1977).

174-Sawah.J, Jagakarsa.M., "The effect of Inai (Lawsonia inermis Linn) leaves extract on blood sugar level: an experimental study", *Journal of Pharmacology Research*,**2:** 20(2008).

175–Khan.S., "Medicinal plants for the management of post menopausal osteoporosis".*A review. Open Bone.J*; **2:**1–13(2010).

176–Robbelen.G., "Mutation breeding for quality improvement: acase study for oilseed crops. In: Kitto PH (ed) Plant mutation breeding for crop improvement" ,IAEA, Vienna, pp 3–30(1991).

177–Ohlrogge.J., "Design of new plant products: engineering of fatty acid metabolism". *Plant Physiol*, **104:**821–826(1994).

178–Hirsinger.F., "New annual oil crops. In: Robbelen G, Downey RK, Ashri A (eds) Oil crops of the world". McGraw Hill, New York,518–532(1989).

179–Downey.R, Robbelen.G., "Brassica species. In: Robbelen G, Downey RK, Ashri A (eds) Oil crops of the world". McGraw Hill, New York,339–362(1989).

180–Green.A., "A mutant genotype of flax (Linum usitatissimum L.) containing very low levels of linolenic acid in its seed oil". *Can J Plant Sci*, **66:**499–503(1986).

181–Rowland.G., "An EMS-induced low linolenic acid mutant in McGregor flax (Linum usitatissimum L.) ".*Can J Plant Sci* **71:**393–396(1991).

182–Vilatersana.R, Susanna.A, Garcia-Jacas.N, Garnatje.T., "Generic delimitation and phylogeny of the Carduncellus–Carthamus complex (Asteraceae) based on ITS sequences". *Plant Syst Evol*, **221:** 89–105(2000)

183–Knowles.P, Ashri.A., "Safflower: Carthamus tinctorius (Compositae). In: Smartt J, Simmonds NW (eds) Evolution of crop plants". Longman, Harlow, pp 47–50(1995).

184–Dajue.L, Munde.H., " (Eds.). Safflower. Carthamus tinctorius", international plant Genetic Resources Institute, Rom, Italy:10(1996).

185–Emongor.V., "Safflower (Carthamus tinctorius L.) the underutilized and neglected crop", *Asian Journal of Plant Science*, **9:** 299-306(2010).

186–Zhang.H, Dong.Z, She.J., "Modern study of traditional Chinese medicine",Vol 3. Xue Yuan Press, Beijing, China, 2057(1998).

187–Huh.S, Kang.H, Yoo.J, Kim.S, Cho.K., "The effect of safflower seed fraction extract on human periodontal ligament fibroblast and MC3T3-E1 cell in vitro". *Journal of Korean Academy of Periodontology*, **31:** 833–846(2001).

188–Kim.J, Bae.C, Park.R, Choi.W, Cho.H, Choi.S., "Bone protecting effect of safflower seeds in ovariectomized rats".*Calcified Tissue International*,**71:**88-94(2002).

189–Boonyaprapas.N, Chokchaijareonporn.O., "Samoon prai maipeunban (Herbal plants, in Thai)",1996, Faculty of Pharmacy, Mahidol University.

190–Quiroga.R, Buschiazzo.D., "Safflower productivity as related to soil water storage and management practices in semiarid regions". *Communications in Soil Science and Plant Analysis*.**32:**2851-2862(2001).

191–Nimbkar.N, Singh.V., " Safflower (Carthamus tinctorrius L.) ", CRC Press, (2005).

192-PDR for Herbal Medicines. Medical Economics Company, Inc. at Montvale, 652(2000).

193– Armah.G, Loiland.J, Karow.R., "Safflower. Extension Research Associate", 2002, Department of Crop and Soil Science, Oregon State University.

194– Hattori.M, Huang.L, Kawata.Y, Tezuka.Y, Kikuchi.T.,"6-hydroxykaempferol and its glycosides from Carthamus tinctorius petals". *Phytochemistry,* **31:** 4001-4004(1992).

195– Li.F, He.S, Ye.Y., "Flavonoids from Carthamus tinctorius", Zhongguo Huaxue, 20: 699-702(2002).

196–Roh.S, Han.Y, Kim.H., "Inhibitory effects of active compounds isolated from safflower (Carthamus tinctorius L.) seeds for melanogeneis". *Biol Pharm Bull*, **27:** 1976-1978(2004).

197–Sato.H, Kawagishi.H, Nishimura.T, Yoneyama.S, Yoshimot .Y., "Serotobenine, a novel phenolic amide from safflower sedds (Carthamus tinctorius L.) "*Agric Biol Chem*, **49:** 2969-2974(1985).

198–Sadao.S, Yoshihiko.T, Satomi.K, Akitami.I., "Conjugated serotonins and phenolic constituents in safflower seed (Carthamus tinctorius L.) ". *Agric Biol Chem*, **44:** 2951-2954(1980).

199–Nagatsu.A, Zhang.L, Watanake.T, Taniauchi.N, Hatano.K., "New steroid and matairesinol glycosides from safflower (Carthamus tinctorius L.) oil cake". *Chem Pharm Bull*, **46:** 1044-1047(1998).

200–Chang.T, Han.X, Tu.F., "Chemical constituents and pharmacological effects of safflower". *Xiandai Yaowu Yu Linchuang*, **14:** 201-203(1999).

201–Cosge.B, Gurbuz.B, Kiralan.M., "Oil content and fatty acid composition of some (Carthamus tinctorius L.) varieties sown in spring and winter". *Int J Nat Eng Sci*, **1:**11-15(2007).

202–Ben.A, Zraibi.L, Sindic.M, El amrani.A, Caid.S., "Comparative study of four safflower oils (Carthamus tinctorius) varieties grown in eastern of Morocco". Inside Food Symposium(2013).

203–Mohammed.J., "The effect of environmental factors on the physiology, yield and oil composition of safflower (Carthamus tinctorius L.) ". 2013, University of Plymouth.

204–Vosoughkia.M, Ghareaghag.H, Ghavami.M, Delkhosh.B., "Evaluation of oil content and fatty acid composition in seeds of different genotypes of safflower". *International Journal of Agricultural Science and Research*, **2(1):** 59-66(2011).

205–Rajesham.C, Narasinga.N, Anitha.U., "Studies on the medicinal plant biodiversity in forest ecosystem of Mahadevpur forest of Karimnagar (A.P)India". *Bioscience discovery*, **4(1):**81-88(2013).

206–Umesh.P, Sanjay .R.,"Effect of some plant extracts against seed borne infection of Collectotricum destructivum on Vinga uniguculata L", *Bioscience Discovery*, **3(2):** 266- 269(2012).

207–Mitra.P, Parcha.V, Mamta.F, Aman.U., "Cassia:A wonder gift to medicinal sciences".*International Journal of community Pharmacy*, **1(2):** 16-38(2008).

208–Debela.H, Zemode.A, Ensermu.K., "Use of traditional plants by people of'Boosat' sub district central eastern Ethiopia". *Ethiop. J.Health Sci*, **16(2):**141-156(2006).

209–Sajal.K, Kulkarni.K, Deo.D, Pande.B, Bhagat.L., "Use of ethno veterinary medicines (EVM) from Vidarbha region(MS) India".*Bioscience Discovery*, **5(2):**180- 186(2014).

210–Schmelzer.G., “Medicinal plants volume 1.Plant Resource of tropical Africa PORTA foundation.Backhuys publications” *Netherland*, **11(1):** 508-511(2008).

211- Food and Agriculture Organization (FAO)“Traditional food plants”, Food and Nutrition Paper, FAO, pp 150–154(1988).

212- Hoover.R, Hughes.T, Chung.H., “Composition, molecular structure, properties, and modification of pulse starches”. *Food Res Int*, **43:** 399–413(2010).