Sudan University of Science and Technology

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The GC-MS Analysis and Antimicrobial Activity of Oils from Potential Medicinal Herbs

تحليل الكروموتوغرافيا الغازيه وطيف الكتله والنشاط المضاد للميكروبات

لزيوت بعض الأعشاب الواعدة الطبيه

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By

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الاستـهلال

بسم الله الرحمن الرحيم

قال تعالى :

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَاعَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العظيم

سورة البقرة – الآية (32).

Dedication

To

My parents

My wife and daughters

My brothers and sisters

Acknowledgment

Thanks at first and last for **Almighty Allah** who gave me the strength while doing this project.

Then I would like to express my special and deep gratitude to Prof. Mohamed Abedel Karim for his guidance and efforts in supervising and leading this research.

Also I thank the technical staff- Dept. of Chemistry- Sudan University of Science and Technology for all facilities.

Thanks are extended to the staff of the Medicinal and Aromatic Plants Research Institute for their help and support.

Finally thanks to my family for their infinite support.

Abstract

This research was carried out to study the oils from six potential medicinal plants used in Sudanese system of medicine. The oils from the targeted plants have been studied by gas chromatography- mass spectrometry and then the antimicrobial potential has been evaluated.

The GC-MS analysis of Haplophyllum *tuberculatum* seed oil showed 22 components the major constituents are: 9, 12-octadecadienoic acid methyl ester (49.60%); 9, 12, 15-octadecatrienoic acid methyl ester (17.91%); hexadecanoic acid methyl ester (11.70%) and methyl stereate (8.07%).

Brassica juncea oil showed 15 components. Major constituents of the oil are: -13-Docosenic acid methyl ester(43.61%); 9, 12-Octadecadienoic acid methyl ester(17.50%) ; 9 ,12, 15-Octadectrienoic acid methyl ester(12.49%) and cis-13-eicosenoic acid methyl ester(7.83%).

Momordica chantia oil gave 21 components dominated by: 9,12-octadecadienoic acid methyl ester (47.64%) ; hexadecanoic acid, methyl ester (17.06%) and 9,12,15-octadecatrienoic acid methyl ester(13.29%).

Cordia myxa oil exhibited 17 components the major are: 9,12-octadecadienoic acid methyl ester (28.81%); hexadecanoic acid, methyl ester (23.24%);11-octadecenoic acidmethyl ester(16.89%) and 11-octadecenoic acidmethyl ester(16.89%). Thirty one components were

detected in *Helianthus annuus* oil with major constituents: 9,12-octadecadienoic acid methyl ester (44.67%) ;9-octadecenoic acid methyl ester(19.71%) and hexadecanoic acid , methyl ester (17.16 %).

Securidaca longipedum oil showed 22 components dominated by: 9 12-octadecadienoic acid methyl ester(29.04%) ; hexadecanoic acid methyl ester(22.90%) ; methyl stearate(15.30%) and 9 12 15-Octadecatrienoic acid methyl ester(7.10%).

The oils have been evaluated for their antimicrobial activity against five human pathogens(*Bacillus subtilis*, *Escherchia coli, Staphylococcus aureus Pseudomonas aeruginosa, Candida albicans*) using the cup plate agar diffusion method. The oils showed various antimicrobial responses against the test organisms

المستخلص

أَجري هذا البحث بغرض دراسة لستة نباتات لها أثر طبي تستخدم فى الطب الشعبى السودانى . تمت دراسة الزيوت لهذه النباتات بواسطة كروماتوغرافيا الغاز - طيف الكتلي وتم التعرف على نشاطها كمضاد ميكروبات .

إحتوي زيت الحزا علي 22 مكونا أهمها:

9,12-octadecadienoic acid methyl ester (49.60%); 9,12,15-octadecatrienoic acid methyl ester (17.91%); hexadecanoic acid methyl ester (11.70%) and methyl stereate (8.07%).

كما إحتوي زيت الخردل الهندى علي 15 مكون اهمها :

13-Docosenic acid methyl ester(43.61%) ; 9 12-octadecadienoic acid methyl ester(17.50%) ; 9 12 15-octadectrienoic acid methyl ester(12.49%) ; cis-13-eicosenoic acid methyl ester(7.83%).

وتضمن زيت الكلارا على 21 مكونات أهمها :

9,12-octadecadienoic acid methyl ester (47.64%) ; hexadecanoic acid, methyl ester (17.06%) and 9,12,15-octadecatrienoic acid methyl ester(13.29%).

بينما تضمن زيت المخيط 17 مكون أهمها: 9,12-octadecadienoic acid methyl ester (28.81%); hexadecanoic acid, methyl ester (23.24%);11-octadecenoic acidmethyl ester(16.89%) and 11-octadecenoic acidmethyl ester(16.89%).

إحتوى زيت شمس المعروف علي المركبات الرئيسه التالية :

9,12-octadecadienoic acid methyl ester (44.67%) ;9-octadecenoic acid methyl ester(19.71%) and hexadecanoic acid , methyl ester (17.16 %).

وتضمن زيت الوارطاب على 22 مكون أهمها :

9 ,12-octadecadienoic acid methyl ester(29.04%) ; hexadecanoic acid methyl ester(22.90%) ; methyl stearate(15.30%) and 9, 12 ,15-octadecatrienoic acid methyl ester(7.10%).

فى إختبار مضاد الميكروبات اعطت هذه الزيوت نتائج متفاوته ضد الميكروبات قيد الإختبار.

Table of contents

Subject	Page No
الإستهلال	i
Dedication	ii
Acknowledgement	iii
Abstract	iv
ملخص البحث	vi
Table of Contents	viii

Chapter One

1	Introduction	1
1.1	General overvew	1
1.2	Essential oils	1
1.3	Terpene hydrocarbons	5
1.4	Oxygenated compounds	6
1.5	Extraction of essential oil	7
1.5.1	Steam distillation	8
1.5.2	Hydrodistillation	9
1.5.3	Hydrodiffusion	11
1.5.4	Solvent extraction	12
1.5.5	Soxhlet extraction	13
1.5.6	Cold pressing method	15

1.6	Common innovative extraction techniques	17
1.6.1	Supercritical fluid extraction	17
1.6.2	Microwave-assisted hydrodistillation	17
1.6.3	Ultrasound-assisted extraction	19
1.6.4	Solvent-free microwave extraction	20
1.6.5	Microwave hydro diffusion and gravity (MHG)	22
1.7	Analysis of essential oils	23
1.7.1	Gas chromatography analysis	23
1.7.2	Gas chromatography-Mass spectrometry (GC/MS)	24
1.8	Biological activity of essential oils	28
1.8.1	Antibacterial activity of essential oil	29
1.8.2	Antioxidant activity of essential oil	32
1.8.3	Antifungal activity of essential oil	34
1.9	Uses of essential oil	35
1.10	The targeted plant species	36
1.10.1	Momordica charantia	36
1.10.2	Cardia myxa	37
1.10.3	Helianthus annuus	39
1.10.4	Securidaca longepedunculata	41
1.10.5	Haplophyllum tuberculatum	41
1.10.6	Brassica juncea	43
	Aim of this study	45

Chapter two

2	Materials and Methods	46
2.1	Materials	46
2.1.1	Plant material	46
2.1.2	Instruments	46
2.1.3	Test organisms	46
2.2	Methods	47
2.2.1	Extraction of oil	47
2.2.2	GC-MS Analysis	47
2.2.3	Antimicrobial assay	48

Chapter three

3	Results and Discussion	51
3.1	Haplophyllum tuberculatum	51
3.1.1	GC-MS analysis	51
3.1.2	Antimicrobial activity	55
3.2	Brassica juncea	56
3.2.1	GC-MS analysis	56
3.2.2	Antimicrobial activity	59
3.3	Momordica charantia	60
3.3.1	Gas chromatography – mass spectroscopy	60
3.3.2	Antimicrobial activity	63
3.4	Cardia myxa	64
3.4.1	Gas chromatography – mass spectroscopy	64

3.4.2	Antimicrobial activity	68
3.5	Helianthus annuus	68
3.5.1	Gas chromatography – mass spectroscopy	68
3.5.2	Antimicrobial activity	72
3.6	Securidaca longepedunculata	72
3.6.1	GC-MS analysis	72
3.6.2	Antimicrobial activity	76
	Conclusion	78
	Recommendations	78
	References	

List of Figures

Figure NO	Description	Page NO
1	The schematic subsidize apparatus for Steam distillation	9
2	The schematic subsidize apparatus for hydrodistillation	10
3	The schematic subsidize apparatus for hydrodifusion	11
4	Solvent extraction	13
5	Soxhlet extraction	14
6	Cold pressing method	15
7	Microwave-assisted hydrodistillation	19
8	Ultrasound-assisted extraction (UAE)	20
9	Solvent-free microwave extraction (SFME)	21
10	Microwave hydro diffusion and gravity (MHG)	22
3.1	Total ions chromatogram of Haplophyllum tuberculatum	51
3.2	Mass spectrum of 9,12-octadecadienoic acid	54
3.3	Mass spectrum of 9,12,15-octadecatrienoic acid	54
3.4	Mass spectrum of hexadecanoic acid methyl ester	55
3.5	Mass spectrum of methyl stearate	55
3.6	Mass spectrum of 13- docosenoic acid, methyl ester.	57
3.7	Mass spectrum of 9,12-octadecanoic acid methyl	58

	ester.	
3.8	Mass spectrum of 9,12,15-Octadecatrienoic acid methyl ester	58
3.9	Mass spectrum of Cis-11-Eicosenoic acid methyl ester	59
3.10	Total ions chromatograms of Momordica chantia	61
3.11	Mass spectrum of 9,12-octadecanoic acid methyl ester	62
3.12	Mass spectrum of hexadecanoic acid methyl ester	62
3.13	9,12,15-octadecatrienoic acid methyl ester	62
3.14	Total ions chromatograms of Cardia myxa	64
3.15	Mass spectrum of 9,12-octadecanoic acid methyl ester	66
3.16	Mass spectrum of hexadecanoic acid methyl ester	67
3.17	9-octadecenoic acid methyl ester	67
3.18	11-octadecenoic acid methyl ester	67
3.19	Total ions chromatograms of Helianthus annuus	69
3.20	Mass spectrum of 9,12-octadecanoic acid methyl ester	71
3.21	9-octadecenoic acid methyl ester	71
3.22	Mass spectrum of hexadecanoic acid methyl ester	71
2.23	Total ions chromatograms of Securidaca longipedum	73
3.24	Mass spectrum of 9,12-octadecanoic acid methyl ester	75

3.25	Mass spectrum of hexadecanoic acid methyl ester	75
3.26	Mass spectrum of methyl stearate	76
3.27	Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester	76

List of Tables

Figure NO	Description	Page NO
2.1	Oven temperature program	47
2.2	Chromatographic conditions	47
3.1	Constituents of Haplophyllum tuberculatum seed oil	53
3.2	Inhibition zones (mm/mg sample)	56
3.3	Constituents of Brasica juncea oil	56
3.4	Inhibition zones (mm/mg sample) of Brasica juncea oil	60
3.5	Constituents of Momordica chantia oil	63
3.6	Inhibition zones (mm/mg sample)	64
3.7	Constituents of Cardia myxa oil	65
3.8	Inhibition zones (mm/mg sample) of Cardia myxa oil	68
3.9	Constituents of Helianthus annuus oil	70
3.10	Inhibition zones (mm/mg sample) of Helianthus annuus	72
3.11	Constituents of Securidaca longipedum oil	74
3.12	Inhibition zones (mm/mg sample) of Securidaca longipedum oil	

1. Introduction

1.1 General overview

Oils are extracted from the leaves, petals, stems, seed, and even the roots of the plants which generally contain volatile oil in all their proportions in defferent concentrations. Each volatile oil is unique, being different even within the same plant, and has wonderful applications and as varied as nature itself.

The complex combination of organic substances that form volatile oil gives its specific note which depend on the species, the harvest period, the climate, as well as the part of the plants form which it is extracted.

Numerous compounds that make up essential oils have been identified. Only the mint has more than 200 compounds. Many compounds are structural isomers[1]. Among these active principles of plants, substances such as fenchone, limonine, menthol, pinene and sabonene. Some of these are present in infinitesimal quantities[2].

1.2. Essential oils

Essential oils are natural volatile complex plant compounds, oily or lipiud-like in nature and frequently characterized by strong fragrance[3,4], they have low solubility in water but are soluble in fats, alcohol, organic solvents and other hydrophobic substances and are generally liquid at room temperature. They are stored in specialized

plant cells, usually oil cell or ducts, resin ducts, glands or trichomes (glandular hairs)[5,6] and many be extracted from the leaves, flowers, buds, fruits, roots wood or bark of plants by variety of methods including solvent and supercritical fluid extraction, expression under pressure, fermentation or enfleurage[3]**.** Steam or hydro-distillation are used predominantly for commercial production[4,7]. Essential oil make up only a small proportion of the wet weight of plant material, usually approximately 1% or less [6,8]. The presence, yield and composition of essential oils may be influenced by many factors, including climate, plant nutrition and stress[9]. In commercial production settings, selection and breeding programs are often instigated to improve yields and faster desired compositions[10]. Essential oils are also called ethereal oils, volatile oils, plant oils, or aetheroleum. The essential part of the term 'essential oil' is thought to be derived from a phrase attributed to Phillippus Aureolus Theophrastus Bombastus von Hohenheim (1491-1541), or Paracelsus as he became known as a Swiss physician who named the active compound of a drug preparation 'quinta essential [4,11,12]. The term 'essential oil' groups together a wide range of chemical compound on the basis of their historic use and method of extraction, usually steam distillation, and belies the varies and complexity of compounds found within them[13]. Essential oils are often described as secondary plant metabolites. Traditionally, secondary plant metabolites have been all those compounds synthesized by the plant which do not appear to be

essential for plant growth and development and/or those compounds uyhwithout an obvious function[14]. They are also not universally synthesized in all plants, in contrast primary metabolites are produced by plants, usually to have an obvious function and are part of the essential metabolic processes of respiration and photosynthesis[15].

Essential oils are found in many different plants, especially the aromatic plants and they vary in odor and flavour, which are governed by the type and amount of constituents present in oils. Additionally, the amount of essential oil from different plants is different and this determines the price of essential oil. Essential oils are considered the chemical weapons of the plant world. Plants use essential oils for deter insects or protection from pathogens such as fungus, pests, even other invasive plants or animals. They produce essential oils as part of their stress response to changes in climate or to protect themselves from harsh environmental conditions. They also acts as 'plant pheromones' in an effort to attract and seduce their pollinators, the oxygenated molecules of essential oils serves as chemical messengers to the cells. They bring life to the plants, destroying infestation, aiding growth and stimulating healings. Their ethereal nature concentrated as scents, through which plants communicate with their surrounding world[16]. Apart from aromatic compounds, indigenous pigments contribute to varying colours of essential oil. This can affect the applications as the ingredient in some particular foods. Essential oils have been known to possess

antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved. Almost any part of a plant may be the source of the oil, which could be extracted and fully exploited for food applications or others. Modern technologies have been continuously developed to conquer the limitation of conventional methods, and to enhance the extraction efficacy. Due to the increasing attention in natural additives, essential oils from several plants have been used more widely, especially in conjunction with other preservations under concept of "hurdle technology." Thus, essential oils can serve as the alternative additives or processing aid as green technology[17].

Essential oils are not simple compounds or simple mixtures of several individual compounds. They may contain up to approximately 100 compounds, although many contain about 20-60. Usually the chemical characterization of many essential oils reveals the presence of only 2-3 major components at a fairly high concentration (20–70%) compared to other components present in trace amounts[8,18,19]. The compound found in essential oils are from variety of chemical classes[20], in general the constituent of essential oils are terpenes[3,21]. Most essential oils are composed of terpenes, terpenoids, and other aromatic and aliphatic constituents with low molecular weights. Terpenes or

terpenoids are synthesized within the cytoplasm of the cell through the mevalonic acid pathway[22]. Terpenes are composed of isoprene units and are generally represented by the chemical formula $(C_5H_8)_n$. Terpenes can be acyclic, monocyclic, bicyclic, or tricyclic[23]. Owing to the diversity in their chemical structures, terpenes are classified into several groups such as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), and triterpenes ($C_{30}H_{40}$). The major component (~90%) of bioactive essential oils is constituted of monoterpenes[3]. In general, the constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids)[3,21]. Compounds and aroma of essential oils can be divided into two major groups: terpene hydrocarbons and oxygenated hydrocarbons.

1.3. Terpene hydrocarbons

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C5), which are the combinations of two isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$. The

diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) exist in essential oils at low concentration[3]. Terpenoids (a terpene containing oxygen) is also found in essential oils. Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$ (MW 136 amu) and $C_{15}H_{24}$ (MW 204 amu), respectively. Although sesquiterpenes are larger in molecules, structure and functional properties of sesquiterpenes are similar to the monoterpenes[24]. For diterpenes, triterpenes, and tetraterpenes, they have larger molecule than monoterpenes and sesquiterpenes, but they are present at very low concentration in essential oils[3].

1.4. Oxygenated compounds

These compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from the terpenes and termed "terpenoids." Some oxygenated compounds prevalent in plant essential oils are as follows:

- Phenols: thymol, eugenol, carvacrol, chavicol, and so on.

- Alcohols: Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, and so on.

- Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, ands o on.

- Aldehydes: citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, and so on.

- Ketones: carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, and so on.

- Esters: bornyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, and so on.

- Oxides: 1,8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, and so on.

- Lactones: bergaptene, nepetalactone, psoralen, aesculatine, citroptene,and so on.

- Ethers: 1,8-cineole, anethole, elemicin, myristicin, and so on.

1.5. Extraction of essential oils

Essential oils can be extracted from several plants with different parts by various extraction methods. The manufacturing of essential oils, and the method used for essential oil extraction are normally dependent on botanical material used. State and form of material is another factor used for consideration. Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss of bioactivity and natural characteristics. For severe cases: discoloration, off-odor/flavor as well as physical change such as the increased viscosity can occur. Those changes in extracted essential oil must be avoided. Essential oils are

obtained from plant raw material by several extraction methods[25,26].

There are several numbers methods of extraction behavior of essential oils. The Common Conventional techniques used for the extraction of essential oils are. Steam distillation (SD), hydrodistillation (HD), solvent extraction, enfleurage and maceration.

1.5.1. Steam distillation

Steam distillation is the most widely used method for plant essential oil extraction[26]. The proportion of essential oils extracted by steam distillation is 93% and the remaining 7% can be further extracted by other methods[27]. Basically, the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and breakdown of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released[27,28]. The temperature of heating must be enough to break down the plant material and release aromatic compounds or essential oils. A new process design and operation for steam distillation of essential oils to increase oil yield and reduce the loss of polar compounds in waste water was developed. The system consists of a packed bed of the plant materials, which sits above the steam source. Only steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the

distillate is reduced. Also, water-soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent [29].

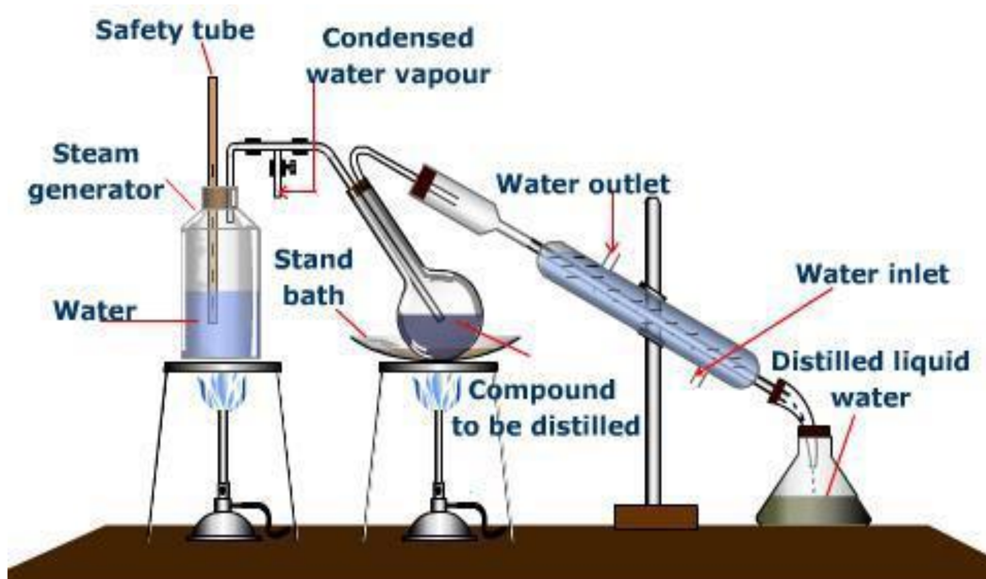


Fig. 1: The schematic subsidize apparatus for steam distillation

1.5.2. Hydrodistillation

Hydrodistillation (HD) is a traditional method for removal of essential oils. Water or hydrodistillation is one of the oldest and easiest methods[30]. Being used for the extraction of essential oils. Hydrodistillation normally is used for isolation essential oils from the aromatic and medicinal plants.

(HD) has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate non-water soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling. This method protects the

oils extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The steam and essential oil vapour are condensed to an aqueous fraction. The advantage of this technique is that the required material can be distilled at a temperature below 100 °C[31]. Ohmic-assisted HD (OAHD) is another advanced HD technique[32]. OAHD method had the extraction time of 24-75 min, while HD took 1 h for extraction of essential oil from thyme. No changes in the compounds of the essential oils obtained by OAHD were found in comparison with HD.

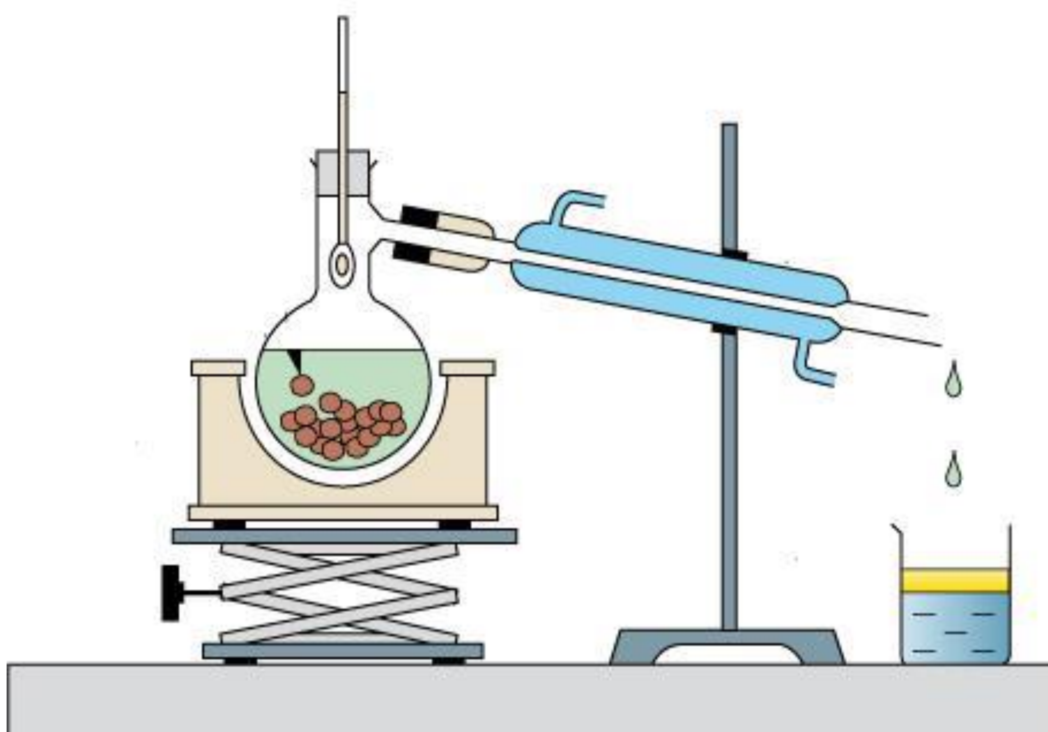


Fig. 2: The schematic subsidize apparatus for hydrodistillation.

1.5.3. Hydrodiffusion

Hydrodiffusion extraction is a type of steam distillation, which is only different in the inlet way of steam into the container of still. This method is used when the plant material has been dried and is not damaged at boiling temperature[33,34]. For hydrodiffusion, steam is applied from the top of plant material, whereas steam is entered from the bottom for steam distillation method. The process can also be operated under low pressure or vacuum and reduces the steam temperature to below 100 °C. Hydrodiffusion method is superior to steam distillation because of a shorter processing time and a higher oil yield with less steam is used[35].

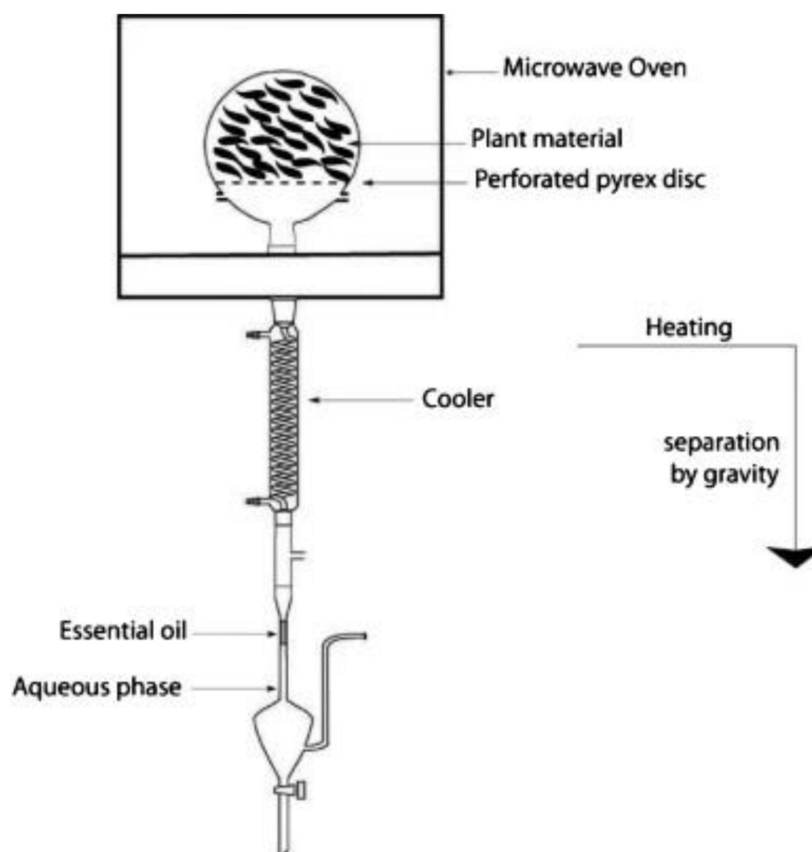


Fig. 3: The schematic subsidize apparatus for hydrodifusion.

1.5.4. Solvent extraction

Conventional solvent extraction has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane, petroleum ether, methanol, or ethanol can be used for extraction[36]. 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 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. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost[37]. The most frequently applied sample preparation procedure in plant material analysis. The quality and quantity of extracted mixture are determined by the type of extra heat applied because of the method is limited by the compound solubility in the specific solvent used. Although the method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility[38].

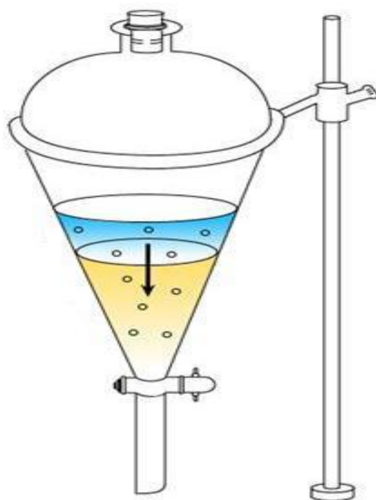


Fig. 4: Solvent Extraction

1.5.5. Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus[39], invented in 1879 by Franz von Soxhlet[40]. It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of 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, this procedure is repeated until virtually complete extraction is achieved. There are several advantages of Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed.

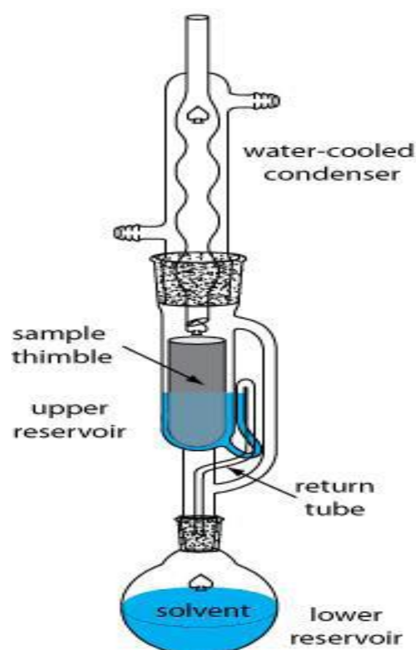


Fig. 5: Soxhlet Extraction

1.5.6. 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 approximately 100% pure and retains all the properties of the plant. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method, cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils[41]. 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.

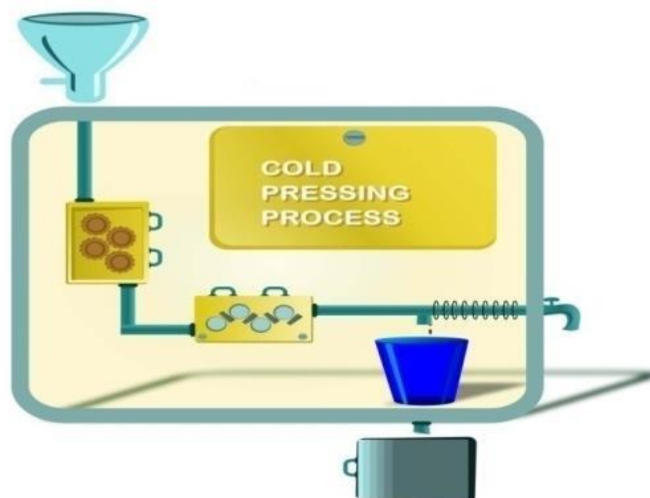


Fig. 6: Cold Pressing Method

One of the disadvantages of conventional techniques is related with the thermolability of Essential oils components which undergo chemical alterations (hydrolyses, isomerization, oxidation) due to the high applied temperatures. The quality of extracted Essential oils is therefore extremely damaged particularly if the extraction time is long. It is important that extraction methods could maintain Essential oils chemical composition and natural proportion at its original state. Since economy, competitiveness, eco-friendly, sustainability, high efficiency and good quality become keywords of the modern industrial production, the development of essential oils extraction techniques has never been interrupted. Strictly speaking, conventional techniques are not the only way for the extraction of essential oils. Novel techniques, for example, abide by green extraction concept and principles have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of official methods. New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO_2 emissions. With technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as the production of costly essential oils in a natural state without any alteration of their thermosensitive components or the extraction of essential oils for micro-analysis. Common Innovative Techniques used for

the extraction of essential oils are:

1.6. Common innovative extraction techniques

1.6.1. Supercritical fluid extraction

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. Supercritical fluids have been considered as an alternative medium for essential oil extraction. Carbon dioxide (CO_2) is the most commonly used supercritical fluid because of its modest critical conditions[42,43]. Under high-pressure condition, CO_2 turns into liquid, which can be used as a very inert and safe medium to extract the aromatic molecules from raw material. No solvent residue remains in the final finished product since the liquid CO_2 simply reverts to a gas and evaporates under normal atmospheric pressure and temperature[42].

1.6.2. Microwave-assisted hydrodistillation

Microwave-assisted hydrodistillation (MAHD) is an advanced hydrodistillation technique utilizing a microwave oven in the extraction process[44]. The efficiency of Microwave assisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample[45]. Conventional techniques for the extraction of active constituents are time and solvent consuming,

thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step[46]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of microwave-assisted hydrodistillation technique. 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[47,48]. and consuming only a fraction of the energy used in conventional extraction methods[49]. 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[50].

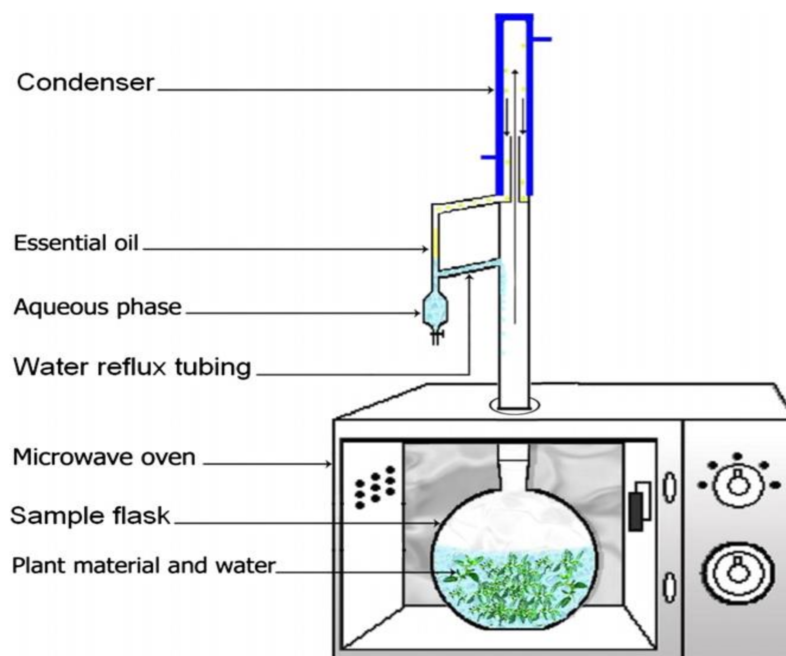


Fig. 7: Microwave-assisted hydrodistillation

1.6.3. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could involve to the increase in the estimate of some food by-products when used as sources of natural compounds or plant material. The major importance will be a more effective extraction, saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. This technique was developed in 1950 at laboratory apparatus[51]. 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. Ultrasound technology has been featured as a valuable method in food engineering processes and plants[52], and become this field from

the techniques active. In these applications the power ultrasound 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[53]. The plants raw material is immersed in water or another solvent (Methanol or ethanol or anyone from the solvents) and at the same time, it is subjected to the work of ultrasound[54]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds[55].

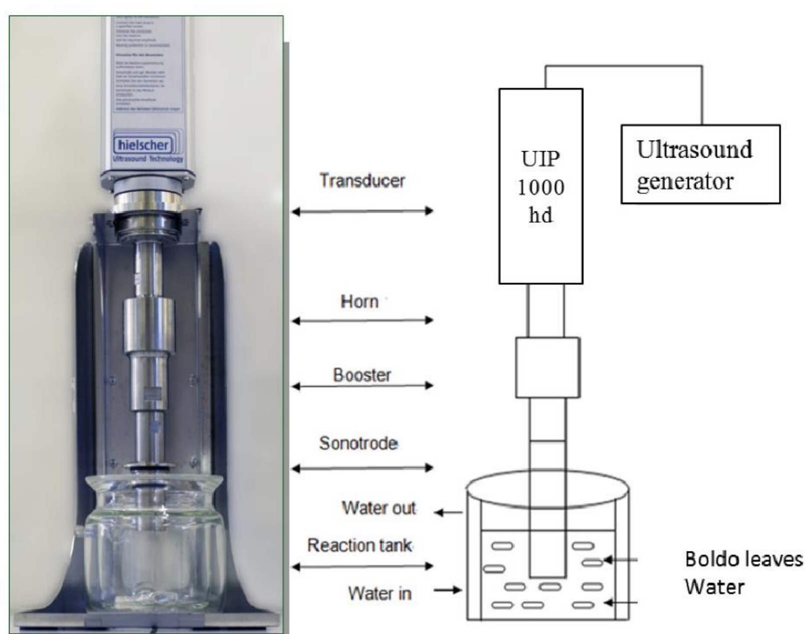


Fig. 8: Ultrasound-assisted extraction (UAE)

1.6.4. Solvent-free microwave extraction

Solvent-free microwave extraction (SFME) is the extraction procedure of essential oil which is cloaca by the in site water of the plant material without added any solvent[56]. Developed this method by Cheat and co-workers[57]. Based on the integration of

dry distillation and microwave heating energy, it consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent[58]. 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. The separated essential oil was dried over anhydrous sodium sulfate and stored at 4 ^{0}C in the dark. The extraction yield of essential oil was calculated as follows: Extraction yield (ml/kg) = V/M where V is the volume of essential oil in herb samples (ml), and M is the mass of the herb samples (kg).

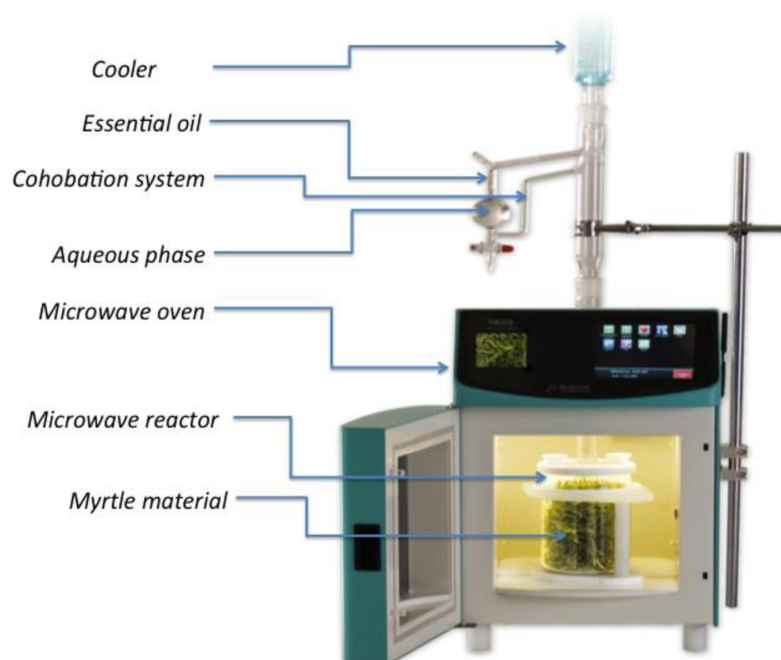


Fig. 9: Solvent-free microwave extraction (SFME)

1.6.5. Microwave hydro diffusion and gravity (MHG)

Microwave hydro diffusion and gravity (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, it was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of material plants. Microwave hydro diffusion 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[59]. 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[60].

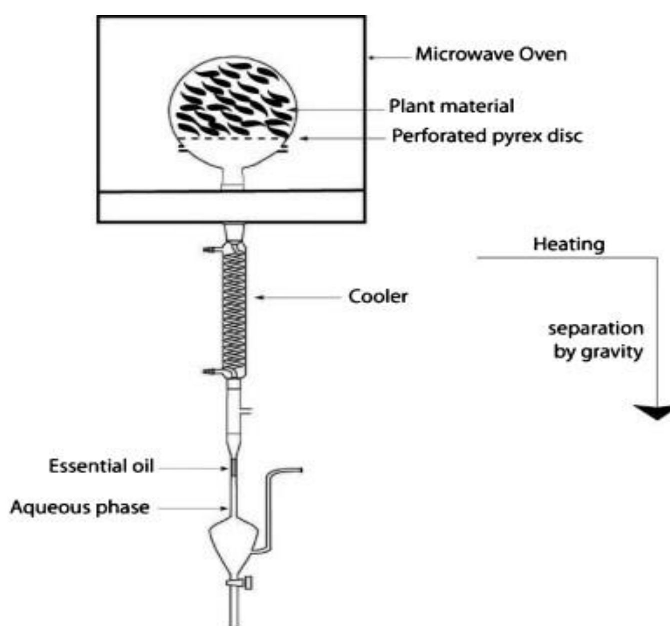


Fig. 10: Microwave hydro diffusion and gravity (MHG)

1.7. Analysis of essential oils

The two main purposes of essential oils analysis are:

(i) To identify and quantify as many constituents as possible.

(ii) To evaluate the quality of the oils and detect any possible adulteration that may affect their usage.

Analysis of essential oils is generally performed using Gas chromatography (qualitative analysis) and Gas chromatography-mass spectroscopy (qualitative analysis)[61]. Gas chromatography analysis is a common confirmation test.

1.7.1. Gas chromatography analysis

Gas chromatography **(GC)** is a chemical instrument used for separating chemicals in a complex sample and provides a representative spectral output. In a gas chromatographic system, the sample to be analyzed may be a liquid solution or a collection of molecules adsorbed on a surface, e.g., the solid-phase micro extraction (SPME) system. During the transfer into the GC, the sample is volatilized by rapid exposure to a zone kept at relatively high temperature (200-300°C) and mixed with a stream of carrier gas (Ar, He, N_2, or H_2). The resulting gaseous mixture enters the separation section, a chromatographic column, which in its current version is a fused-silica tubular capillary coated internally with a thin polymer film. The gas chromatography instrument vaporizes the sample and then separates and analyzes the various components.

Each component ideally produces a specific spectral peak. The time elapsed between injection and evaluation is called “Retention time”. The sample is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separates before eluting from the column end. The detector measure different compounds as they emerge from the column.

1.7.2. Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC/MS) analysis is a method which combines the features of gas, liquid chromatography and mass spectrometry to identify different substances within a test sample. The gas chromatography-mass spectrometry instrument is made of two parts: The gas chromatography (GC) portion separates the chemical mixture into pulses of pure chemicals and mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed through the GC[62], the chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions. This is important because the particles must be

charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. This information is sent to the computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that travelled through the filter. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent. Knowledge of the identity and relative amounts of the essential oils extracted from plants has great importance to several fields of basic and applied research in chemistry, biology and many other disciplines. Obtaining this knowledge requires overcoming many analytical challenges posed by these complex mixtures, because they normally present large variations in component amounts, chemical structures and functionalities. Gas chromatography (GC) is recognized as the most suitable technique to find out how many components and in what proportion there are in a complex matrix of volatile compounds. When it is coupled to mass spectrometry (GC-MS), additional information arises about each separated compound: molecular mass, elemental composition (when high resolution mass spectrometry is used), functional groups, and in certain cases, molecular geometry and spatial isomerism.

There are two basic strategies in GC-MS, for the identification of compounds. The first is the use of standard substances (certified reference material). However, not always all standards are available, many of them are not easily accessible for a large number of analytes. The second strategy is the combination of several approaches, among which are the following: (a) retention indices (RI), in conjunction with (b) experimental mass spectra (EI, 70 eV) and (c) their comparison with those of databases of retention indices obtained in columns of orthogonal polarity (polar and nonpolar) and of standard mass spectra (EI, 70 eV). The combination of several experimental parameters and data, i.e., retention times measured in both columns and mass spectra is mandatory for the structural identification of components in a mixture. The identification can be tentative (preliminary, presumptive) or confirmatory. Confirmation (positive or unambiguous) requires, in many cases, the use of a certified standard compound. Multiple analytes from complex mixtures, however, can have similar retention times or their mass spectra seem alike or have only very small quantitative differences (ion intensities). Limonene epoxides, xylenes, and many structurally similar terpenes, are examples of this situation. However, the possibility of simultaneous coincidence of both the retention indices calculated in both columns (polar and nonpolar) and of mass spectra for two different substances, in fact, is very remote, almost unlikely. For some cases, such as those that may have legal implications, i.e., environmental, forensic cases or disputes, control of doping agents in

sports competitions, it is absolutely mandatory to use certified standard substances for identification and confirmation. The analysis of an essential oil, perfume, aroma, and fragrance fractions (or any other complex mixture), in order to quantify and identify its components, done by one-dimensional chromatography, should comply with the following conditions[63]:

(i) Using preferably long capillary columns (50, 60 m)

(ii) Performing the analysis in two capillary columns with orthogonal phase (e.g., DB-1 or DB-5 and DB-WAX)

(iii) Obtaining experimental mass spectra EI (70 eV) and doing a comparative search, preferably, on various mass spectra databases (e.g., NIST, Wiley, Adams)

(iv) Calculating linear retention indices in two columns, polar and nonpolar.

(v) Using standard compounds for further structural confirmation.

The combination of all these parameters allows confirmatory identification of the mixture components. In GC- MS analysis it is very important to ensure that the chromatographic peaks are "homogeneous", as the co-elution of various substances can lead to structural miss -assignments.

The large number of fragment ions (cations and cation-radicals) in the mass spectrum is due, firstly, to their formation from molecular ions which have very different excesses of internal energy. All molecular ions with internal energies lower than the potential energy of appearance of an ion fragment with the lowest formation

activation energy will be recorded in the mass spectrum as not dissociated molecular ions. Their intensity in the spectrum depends on the molecular structure and, particularly, their ability to delocalize (stabilize) the positive charge, which allows the ion M+• to exist for longer time than is required for its detection (ca. > 10-5 s) in a mass spectrometer. One of the major limitations of the electron ionization technique lies in the fact that molecules that are ionized, should be in the vapor phase, i.e. being volatilize without undergoing thermal decomposition, prior to their ionization. Even so, many of the volatilize and thermostable molecules do not exhibit molecular ions in their mass spectra, only fragment ions, something that limits obtaining information on molecular weight. In fact, less than 10 % of all existing molecules are suitable for analysis by mass spectrometry by electron ionization. The excluded cases are highly polar species (e.g., salts, amino acids), those of high-molecular weight (e.g., proteins, nucleic acids, polymers), and thermolabile compounds (e.g., sugars). In some cases, chemical derivatization of the molecule permits to increase its volatility and thermal stability and decrease its polarity[63].

1.8. Biological activity of essential oils

Microorganisms such as bacteria, fungi, viruses and protozoa are an etiological agents of many infectious diseases, and compounds with specific activity against these microorganisms, that is antimicrobial activity, are our best weapon for treating these diseases. Even before the role of microorganisms in disease pathogenesis was appreciated

or understood, attempts at treating such illnesses often utilized plant-based medicines that contained compounds with antimicrobial activity. These plant-based medicines included essential oils[64]. Essential oils and their components have specific biological functions, many of which lend themselves to commercial exploitation. Given the range and complexity of the compounds present in essential oils it is hardly surprising that they have the capacity to affect many biological systems. The medicinal properties of plants from Apiaceae family are known from ancient times due to their essential oils[65]. According to survey (1993) of World Health Organisation (WHO), 80% of patients in India, 85% in Burma and 90% in Bangladesh are treated from these traditional medicines[66].

Antimicrobial activity of essential oils can be attributed largely to the major groups of compounds found in them: monoterpenes, sesquiterpenes and nonterpenaceous components such as phenylpropanoids. Where they are present in significant proportions, sulfur compounds such as those found in Allium are often the main antimicrobial compounds.

1.8.1. Antibacterial activity of essential oil

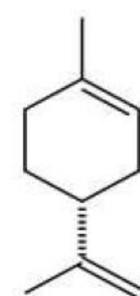
Bacterial infections remain a major causative agent of human death, even today. In addition, the use of several antibacterial agents at higher doses may cause toxicity in humans, this has prompted researchers to explore alternative new key molecules against bacterial strains[67]. In this regard, plant essential oils and their major chemical constituents are potential candidates as antibacterial agents,

several types of essential oils and their major chemical constituents have been reported to possess a wide range of bacterial inhibitory potentials.

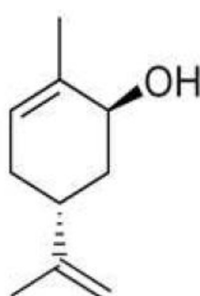
The effect of antibacterial activity of essential oils may inhibit the growth of bacteria (bacteriostatic) or destroy bacterial cells (bactericidal). Nevertheless, it is difficult to distinguish these actions. In relation to this, antibacterial activity is more frequently measured as the minimum bactericidal concentration (MBC) or the minimum inhibitory concentration (MIC)[4]. Rapid antibacterial screening of essential oils is usually conducted using the agar diffusion technique, where essential oils are added to filter paper discs or holes, which are put in agar that has been uniformly inoculated with a bacterial strain. After incubating, the inhibition zone represents the antimicrobial action[68]. The effectiveness of essential oils differs from one type to another as well as against different target bacteria depending on their structure (Gram-positive and Gram-negative bacteria). For instance, sandalwood and vetiver oils exhibit higher inhibitory activity against Gram-positive bacteria; however, they fail to inhibit Gram-negative bacterial strains[69,70]. Hydrophobicity of essential oils allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable[71]. This can then cause leakage of ions and other cellular molecules[72]. The essential oils of cinnamon, clove, pimento, thyme, oregano, and rosemary were shown to possess strong antibacterial activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*[73]. Clove

oil was found to be the most effective among all the tested essential oils. The antimicrobial effect of these oils was correlated to the occurrence of the major compounds such as carvacrol, thymol, cinnamic aldehyde, eugenol, and *p*-cymene[74]. 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[75,76]. The chemical structure of essential oils affects their mode of action concerning their antibacterial activity[77]. The action of thymol against Bacillus cereus, Staphylococcus aureus, and Pseudomonas aeruginosa appears to be comparable to that of carvacrol, for example. However, carvacrol and thymol act differently against Gram-positive and Gram-negative species[78]. 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[79, 80].

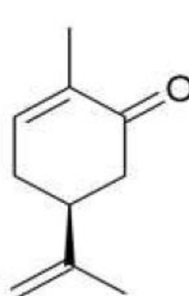
Some Major constituents of essential oil are given below:



Limonene

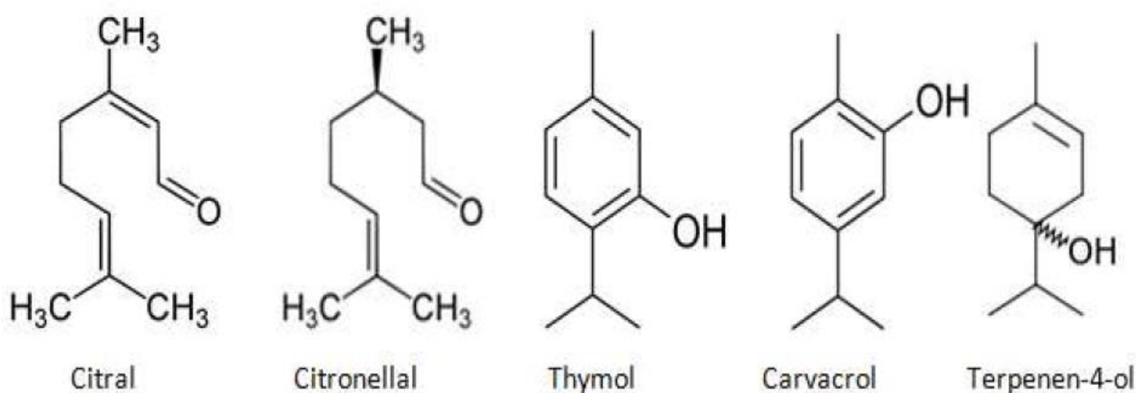


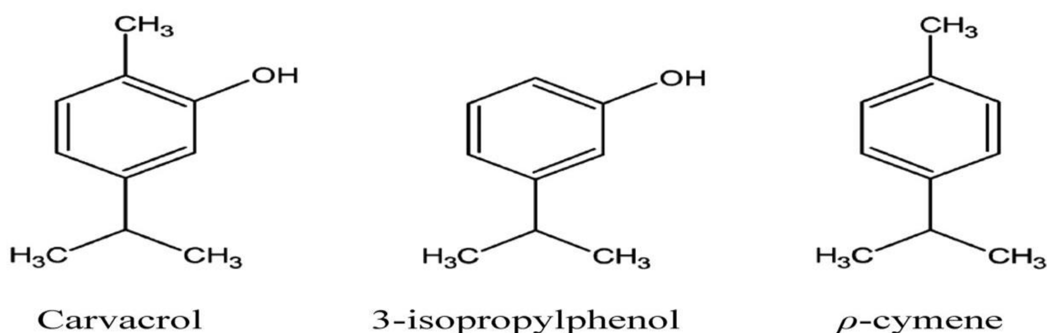
Carveol

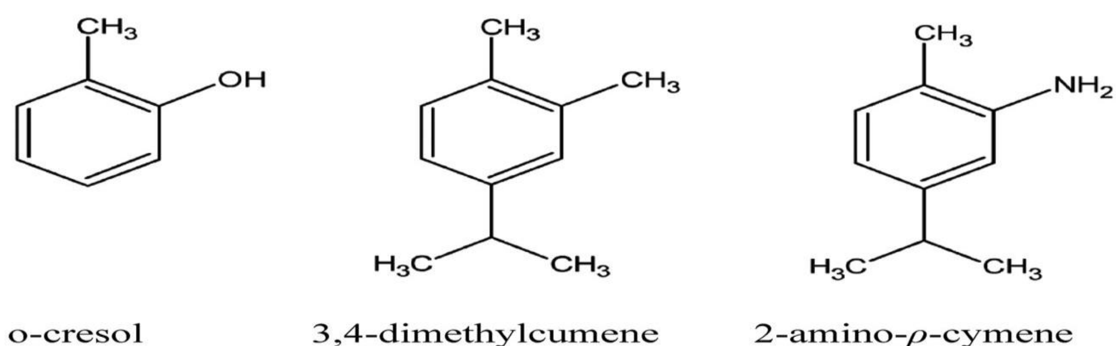


Carvone

1,8- cineole







1.8.2. Antioxidant activity of essential oil

Essential oils have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts[81]. With those functions, essential oils can serve as the potential natural

antioxidants, which can be used to prevent lipid oxidation in food systems. The antioxidant activity is generally related with the major active compounds in essential oils. 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[24]. The antioxidant activity of essential oils is also due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-CineoIe, 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[82,83]. Antioxidant activity varies with source of essential oils, the differences in antioxidant activity of different essential oils were mostly due to the differences in types and amounts of antioxidant components present in essential oils[24,84]. Antioxidant activity of essential oil is also affected by extraction method or solvents used. Nonpolar extracts showed less effective activities than polar extracts. Therefore, antioxidant activity of essential oil is strictly related with the polarities of their phytochemicals[85]. Non-phenolic antioxidants of plant extracts might also contribute to the antioxidant activity[86]. Additionally

the harvesting period of plant also determines the concentration of the major oil components such as phenolic compounds, which are directly related with the antioxidant activity of essential oil[87].

1.8.3. Antifungal activity of essential oils

Essential oils can represent one of the most promising natural products for fungal inhibition[88.89]. Many essential oils obtained from different plants or herbs exhibited intense antifungal properties[90,91]. Fungi are very difficult to target because of cellular and molecular levels, human pathogenic fungi, and eukaryotes which are very similar with their host. However, eukaryotes and human pathogenic fungi and their hosts have similarities at molecular and cellular levels. [92].

Essential oils of plants, such as coriander, anise, and fennel show differences in their antifungal activity against *Candida albicans* with MICs (minimum inhibitory concentrations) of 0.25%, 0.5%, and 1%, respectively. Among the essential oils, Japanese mint, ginger grass, cinnamon, lemongrass, clove, anise, and geranium oils are particularly encouraging against *Candida albicans* and the essential concentration range between 0.01% and 0.15% [93,94]. Essential oils can rapidly inhibit growth of dermatophytes and their spores. The antifungal activity of essential oil might be caused by the properties of terpenes/terpenoids, that due to their highly lipophilic nature and low molecular weight are capable of

disrupting the cell membrane, causing cell death or inhibiting the sporulation and germination of food spoilage fungi[95,96].

1.9. Uses of essential oils

Essential oils have been used for thousands of years for medicinal and health purposes[97]. Essential oils are produced by the immune intelligence of the plants, people can use essential oils to support their wellness and healthy stress response. The amount of essential oil from different plants is different and this determines the price of essential oil. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products and have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes[97,98]. Another major utilization of essential oils is in the agro food business, both for creating refreshments and for enhancing sensorial properties of food items[99].
There are many ways to use essential oils, their use is extremely diversified depending on the source, the quality, the extraction method.

1.10. The targeted plant species

1.10.1. *Momordica charantia*

Momordica charantia is a very vigorous, annual climbing plant producing stems 5 metres or more long that scramble over the ground or climb up into the surrounding vegetation, supporting itself by means of tendrils.The plant is an important market vegetable in southern and eastern Asia, it is also cultivated on a small scale in tropical America and in the southern part of the United States.The plant is also grown for medicinal purpose. *Momordica charantia* was traditionally used by people in tropical regions. Infections, cancer, leukemia, and diabetes are among the most common conditions it was believed to improve. The leaves and fruit have both been used occasionally to make teas and beer or to season soups in the Western world.

The fruit of the plant, which is known as the bitter melon, has an oblong shape with a warty exterior and is dark green in color. At least three different groups of constituents in bitter melon have been reported to have blood-sugar lowering actions of potential benefit in diabetes mellitus. These include a mixture of steroidal saponins known as charantin, insulinlike peptides, and alkaloids.

Balsam pear

1.10.2. *Cordia myxa*

Cordia myxa (Sapistan plum) is a large evergreen shrub or tree with a dense crown, that can grow up to 12 metres tall. The plant is a multi-purpose tree, it is often harvested from the wild for local use as a food, medicine and source of materials. It has been cultivated for its many uses since the time of the ancient Egyptians, and is still sometimes cultivated nowadays for its edible and medicinal fruit in many areas of the tropics and subtropics

Cordia myxa

The fruit has been reported to be rich in polysaccharide. Ripe fruit produces a jelly-like, sticky mass. Unani system of drug medicine uses this plant as antibacterial, antiviral and antitussive. Joshandah, polyherbal formulations, are extensively used by the masses in India for the treatment of common cold, catarrh, cough, respiratory distress, fevers of which C. dichotoma as chief ingredient[104,105]. From the ancient time, leaves and stem bark are used in the treatment of dyspepsia, fever, diarrhea, leprosy, gonorrhoea and burning sensation. Leaf of plant traditionally shows the therapeutic uses and actions such as anthelmintic, astringent, diuretic, demulcent, purgative, expectorant, tonic, ulcer and cough[106-110].

The bark, leaves and fruit have medicinal properties, they are used variously as diuretics, demulcents and in the treatment of stomach aches, coughs and chest complaints.The juice of the bark is taken internally in the treatment of fevers. Combined with coconut oil, it is taken to treat colic.The powdered bark is applied to the skin in cases of broken bones before a plaster is applied, to improve healing. Bark powder is used externally in the treatment of skin diseases. A macerate of the leaves is used as a treatment for sleeping-sickness taken internally, and applied externally as a lotion to the fly-bites.The juice of the leaves is applied to the forehead to relieve headaches. The leaves are applied to wounds, sores and ulcers. The sticky, mucilaginous pulp of the fruit is the source of a well-known medicine in the Near and Middle East. Called 'sapistan', it is useful in the treatment of coughs, sore throats and chest-complaints on

account of its demulcent property. The fruit is also used externally as an emollient plaster to maturate abscesses, to calm rheumatic pain and as an antiparasitic on ringworm. Stereols and a gum have been recorded in the leaves.The leaves and the fruits contain pyrrolizidine alkaloids, coumarins, flavonoids, saponins, terpenes and sterols[110].

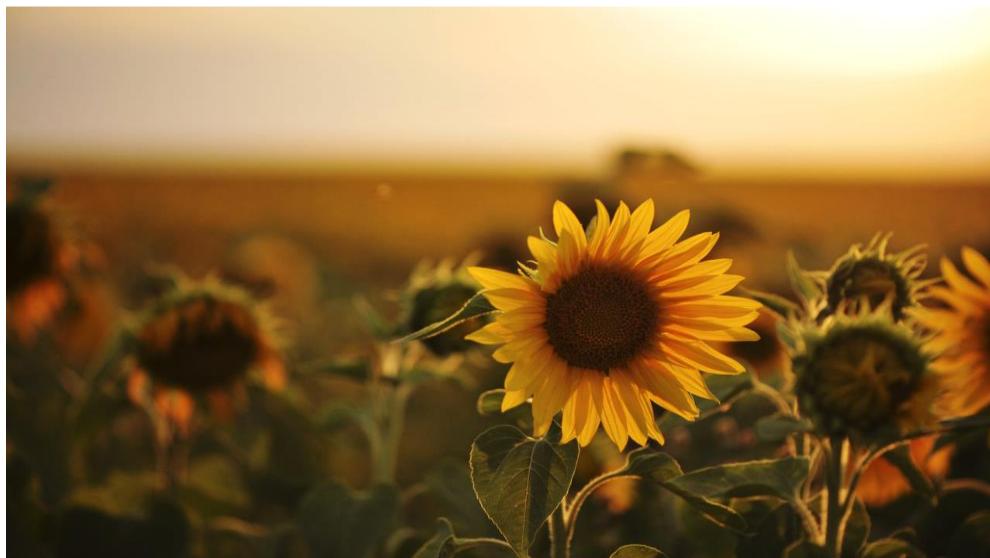
1.10.3. *Helianthus annuus*

Helianthus [111] is a genus comprising about 70 species of annual and perennial flowering plants in the daisy family Asteraceae. Except for three South American species, the species of *Helianthus* are native to North America and Central America. The common names "sunflower" and "common sunflower" typically refer to the popular annual species *Helianthus annuus*, whose round flower heads in combination with the ligules look like the sun[111]. This and other species, notably Jerusalem artichoke (*H. tuberosus*), are cultivated in temperate regions and some tropical regions as food crops for humans, cattle, and poultry, and as ornamental plants. The species *H. annuus* typically grows during the summer and into early fall, with the peak growth season being mid-summer[111].

H. annuus being rich in nutrients such as calcium, iron, magnesium, zinc and vitamins A, K and E, it's used effectively against inflammation and general irritations of the skin. Being very high in vitamin E, it acts as an emollient which traps moisture and keeps skin well hydrated. It also helps prevent damage to cells by

ultraviolet light and aids against premature aging of skin by protecting the collagen and elastin content[112].

The plant is a source for beta-carotene which is converted to vitamin A with benefits of protection from sun damage and free radicals. Omega-6 (linoleic acid) which is found in sunflower oil, assists with cell development and treating disorders such as eczema, acne and scarring[112].



Helianthus annuus

A number of characteristics make the oil of this plant also beneficial for hair. The linoleic acid it contains assists with slowing hair loss. The menthol of this plant exhibits dandruff control while its antibacterial properties help keep the scalp microbial free. Its emollient properties keep hair soft, moist and assist with untangling[112].

1.10.4. *Securidaca longepedunculata*

Securidaca longepedunculata **Fres** is a semi deciduous shrub used as a traditional medicine in many parts of Africa against a number of invertebrate pests, including insects infesting stored grain[113].

Securidaca longepedunculata

Securidaca longepedunculata Fres roots is characterized by the occurrence of 2-hydroxy-benzoic acid methyl ester (methyl salicylate, I) which accounts for about 90% of the plant material and a saponin which contains presenegenine[114].

1.10.5. *Haplophyllum tuberculatum*

Haplophyllum tuberculatum A. Juss is a perennial plant in the family Rutaceae. The plant is native to North Africa and some Middle East countries. Many reviews[115-124] reported on the traditional uses of *Haplophyllum tuberculatum*. All parts of this plants and especially leaves find some applications in ethnomedicine[125-130] .

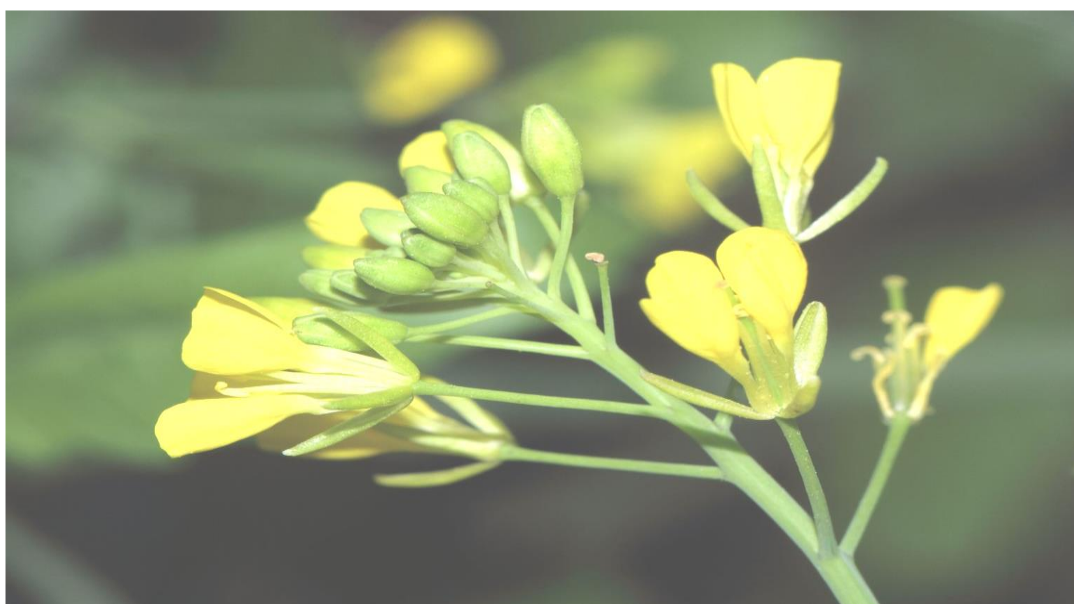
Haplophyllum tuberculatum

Haplophyllum tuberculatum contains some phytochemicals like alkaloids, flavonoids, coumarins and volatile oil known for their potential bioactivity[131,132]. In Sudanese system of medicine *Haplophyllum tuberculatum* is used against gynecological disorders, asthma, rheumatic pain and allergic rhinitis[127-129]. In Iraq folklore the plant is used for stomach-ache, convulsions and nervous disorders[133]. In Oman leaves of *Haplophyllum tuberculatum* are used traditionally against arthritis and headache, while in Saudi Arabia the plant is a natural remedy for skin discoloration, infections and parasitic deseases[127-129]. In Algeria, local healers use this plant for ulcers, injury, diabetes, hypertension, fever, constipation, menstrual pain, tonsillitis, cough and rheumatism[126] . It has been reported that the plant has antioxidant and cytoprotective potential[134]. The ethanol extract of the aerial parts exhibited efficient antimicrobial potency[135]. An alkaloid isolated from this plant species (tuberine) showed

significant antimicrobial activity against some miroorganisms[136]. The antimicrobial activity of *Haplophyllum tuberculatum* polyphenols has also been investigated[137,138].

1.10.6. *Brassica juncea*

Brassica is a genus comprising more than 150 species in the family (Brassicaceae). Different Brassica species are cultivated worldwide for their economic value[139]. Leaves of these plant species are edible and diverse medicinal uses of seeds are known in many communities.



Brassica juncea

Brassica juncea **L. Czern. Coss.** Is a palnt in the mustard family.The plant is widely cultivated in many countries for its nutritive and medicinal values. For centuries *Brassica juncea* hs benn used as a natural remedy[140]. Seeds are traditionally used against muscular rheumatism, vomiting and jaundice[141-143].Seeds mixed with moringa

olefera is a remedy for spleen and liver diseases[144] .Some *Brassica juncea* preparations have been used as diuretic, liver –bile stimulators and laxative[145] .Seeds are used by local healers against abscesses, cold, rheumatism and lumbago[146]. A Paste made from seds is used as treatment for arthritis, backache, styes, paralysis and edema of lungs and live. Seeds are also used as stimulant and emmenagogue[147].

Diverse pharmacological activities have been associated with seeds including hypoglycemic[148], anxiolytic[149], antidiabetic[150] ,goiterogenic[151], antioxidant[152] and hepatoprotective[141] activities. It has been reported that the seeds, leaves and stem may reduce the risk of heart attachand migraine diabetic heart diseases[153,154]. The biological activity of some phytochemicals isolated from *Brassica juncea* has been explored. The antioxidant activity of some isorhamnetin and kaempferol conjugates has been reported . Two constituents of *Brassica juncea* – sinapine and sinapic acid – exhibited antioxidant,anxiolytic and cognition – improving activities[155-157].The antifungal activity of some isothiocyanates isolated from *Brassica juncea* has been documented[158-161].

Aim of this study

This study was designed to:

-Extract the oils from six medicinal plant used extensively in traditional medicine.

-Analyze the oil by GC-MS to determine the constituents.

-Evaluate the oil for its antimicrobial activity.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

Seeds of *Brassica juncea*, *Haplophyllum tuberculatum*, *Securidaca longepedunculata*, *Helianthus annuus*, *Cordia myxa* and *Momordica charantia* were purchased from the local market-Khartoum and identified by direct comparison with a reference herbarium sample.

2.1.2. Instruments

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

2.1.3. Test organisms

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

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. The GC-MS analysis

The studied oils were analyzed by GC-MS. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)was used.. Oven temperature program and other chromatographic conditions are presented below:

Table 2: Oven temperature program

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

Table 3: Chromatographic conditions

Parameter	Value
Column oven temperature	150.0ºC
Injection temperature	300.0ºC
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0mL/min
Column flow	1.54mL/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/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 10^8-10^9colony 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 compounds. Separate

Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamicin).

The agar discs were removed, alternate cup were filled with 0.1 ml of test sample 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.

After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

3. Results and Discussion

In this study six potential medicinal plants have been investigated.The oils from these plant species have been extracted and the constituents have been characterized by GC-MS. Furthermore the antimicrobial potential has been evaluated.

3.1. *Haplophyllum tuberculatum*

3.1.1. The GC-MS analysis

Haplophyllum tuberculatum seed oil was studied by GC-MS. The analysis showed 22 constituents (Table 3.1). The total ions chromatograms is depicted in Figure 3.1.

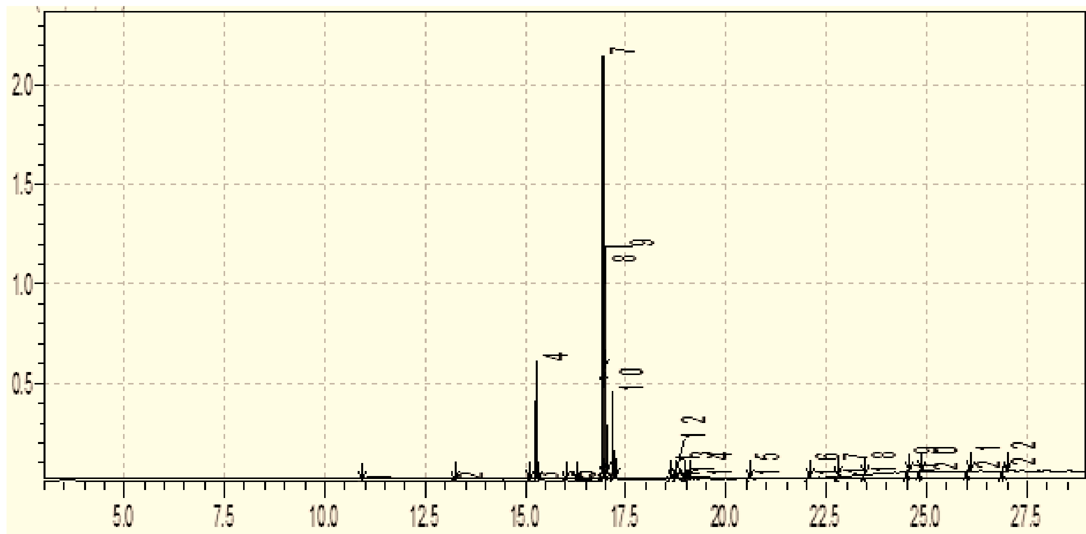


Fig.3.1 : Total ions chromatograms

Major constituents of the oil are:

9,12-octadecadienoic acid methyl ester (49.60%); 9,12,15-octadecatrienoic acid methyl ester (17.91%); hexadecanoic acid methyl ester (11.70%) and methyl stereate (8.07%).

i) 9,12-Octadecadienoic acid-(ZZ)-, methyl ester (49.60 %)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3. 2. The peak at m/z 294 (R.T. 16.927) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z 263 is due to loss of a methoxyl. 9,12-Octadecadienoic (linoleic acid) belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human bodies and are available through diet. Linoleic acid which exists in lipids of cell membrane is used in the biosynthesis of arachidonic acid .

ii) 9,12,15-octadecatrienoic acid methyl ester (17.91 %)

Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester is depicted in Fig.3.3. The peak at m/z 292, which appeared at R.T. 16.993 corresponds to M + $[C19H32O2]^+$, while the peak at m\z 261 is attributed to loss of methoxyl.

iii) Hexadecanoic acid , methyl ester (11.70 %)

Fig. 3.4 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. 15.263 in total ion chromatogram. The fragment at m/z239 is due to

loss of a methoxyl function. Hexadecanoic acid (palmitic acid) is a C16 saturated fatty acid. It is the most common fatty acid in plants and animals. This acid is the precursor of long chain fatty acids. Pamitic acid is a lipid constituent of human breast milk.

Table 3.1: Constituents of *Haplophyllum tuberculatum* seed oil

No.	Name	Ret. Ti	Area %
1.	Dodecanoic acid, methyl ester	10.871	0.12
2.	Methyl tetradecanoate	13.179	0.22
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.071	0.09
4.	Hexadecanoic acid, methyl ester	15.263	11.70
5.	Hexadecanoic acid, 14-methyl-, methyl	15.969	0.31
6.	Heptadecanoic acid, methyl ester	16.239	0.14
7.	9,12-Octadecadienoic acid (Z,Z)-,	16.927	49.60
8.	9-Octadecenoic acid (Z)-, methyl ester	16.970	7.89
9.	9,12,15-Octadecatrienoic acid, methyl	16.993	17.91
10.	Methyl stearate	17.174	8.07
11.	Methyl 8,11,14,17-eicosatetraenoate	18.587	0.11
12.	cis-11-Eicosenoic acid, methyl ester	18.721	0.24
13.	Eicosanoic acid, methyl ester	18.922	0.52
14.	2(1H)-Quinolinone, 3-(3-methyl-2-butenyl)-4-[(3- methyl-2-	19.005	0.32
15.	butenyl)oxy]-	20.541	0.27
16.	Tetracosanoic acid, methyl ester	22.041	0.24
17.	Squalene	22.766	0.13
18.	Hexacosanoic acid, methyl ester	23.442	0.09
19.	Tetratetracontane	24.522	0.22
20.	Urs-12-ene	24.833	0.17
21.	Ergost-5-en-3-ol, (3.beta.)-	26.033	0.45
22.	.gamma.-Sitosterol	26.923	1.19

iv) Methyl stearate (8.07 %)

Fig. 3.5 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T. 17.174) corresponds $M^{+}[C19H38O2]^{+}$, while the peak at m/z 267 accounts for loss of a methoxyl.

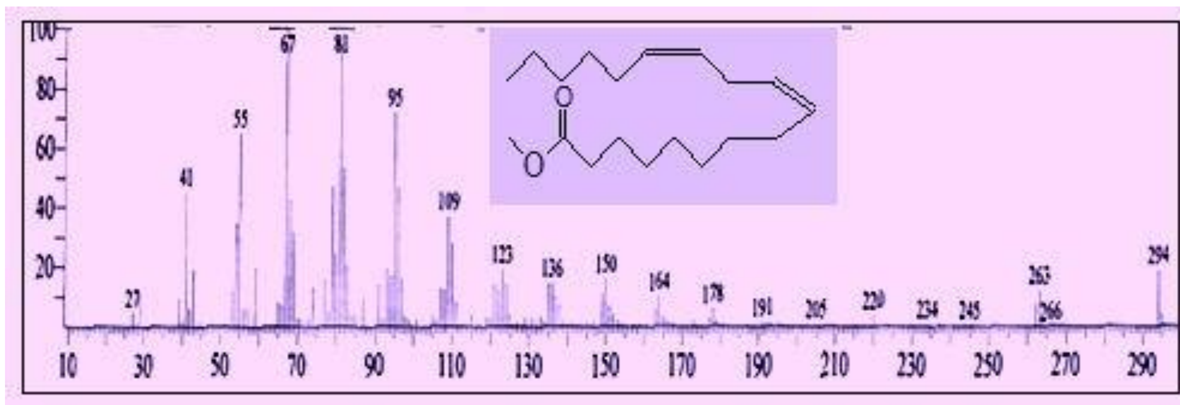


Fig.3.2: Mass spectrum of 9,12-octadecadienoic acid

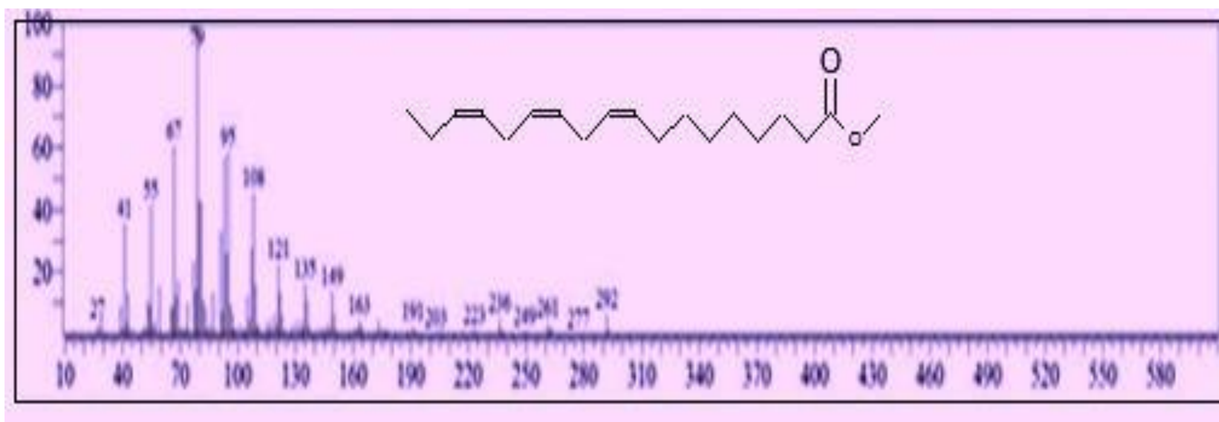


Fig. 3.3: Mass spectrum of 9,12,15-octadecatrienoic acid

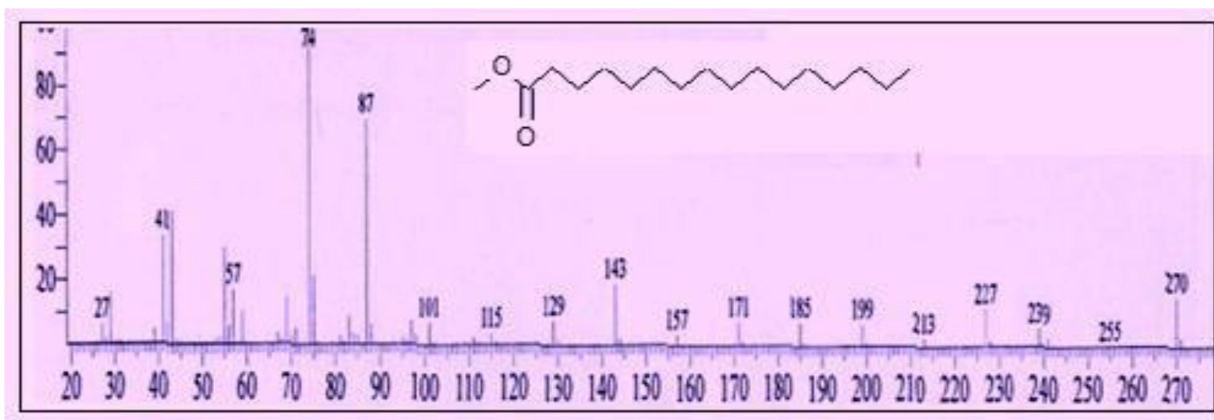


Fig. 3.4: Mass spectrum of hexadecanoic acid
methyl ester

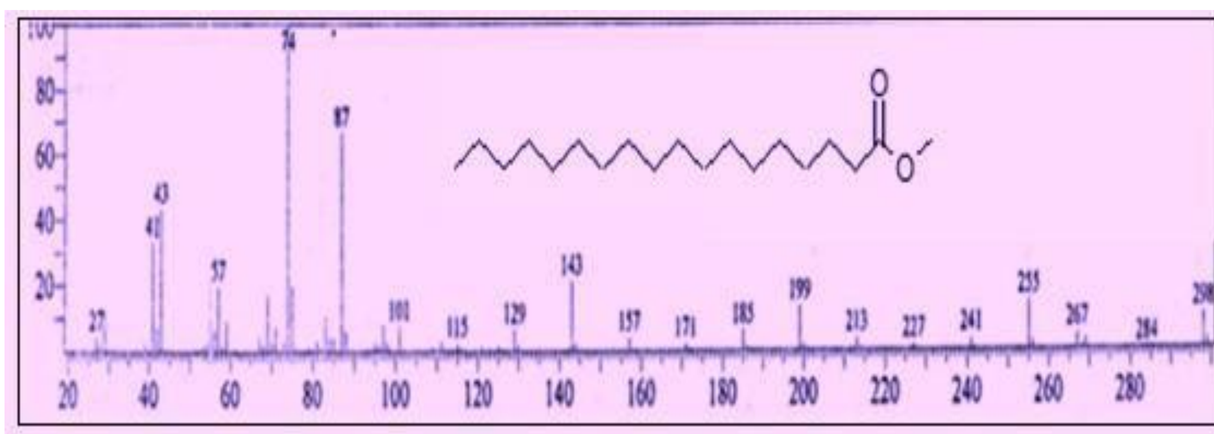


Fig.3. 5: Mass spectrum of methyl stearate

3.1.2. Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria *Bacillus subtilis,* and *staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseud*om*onas aeruginose* and fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.2, the oil exhibited good activity against *Bacillus subtilis.*

Table 3.2: Inhibition zones (mm/mg sample)

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

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.2. *Brassica juncea*

3.2.1. The GC-MS analysis

Brassica juncea oil was analyzed by GC-MS. The analysis showed 15 constituents (Table 3.3).

Table 3.3: Constituents of *Brassica juncea* oil

No.	Name	RT	Area %
1	9-Hexadecenoic acid methyls ester(Z)	15.120	0.06
2	Hexadecanoic acid methyls ester	15.309	2.43
3	9 12-Octadecadienoic acid methyl ester	16.952	17.50
4	9-Hexadecenoic acid methyls ester(Z Z)	7.012	8.11
5	9 12 15-Octadectrienoic acid methyl ester	17.033	12.49
6	Methyl stearate	17.222	0.92
7	11 14 17-Eicosatrienoic acid methyl ester	18.634	0.38
8	Cis-13-Eicosenoic acid methyl ester	18.776	7.83
9	Cis-11-Eicosenoic acid methyl ester	18.829	1.40
10	Eicosanoic acid methyl ester	18.975	0.71
11	13-Docosenic acid methyl ester	20.433	43.61
12	8 11 14-Docosatrienoic acid methyl ester	20.474	0.96
13	Docosanoic acid methyl ester	20.595	0.65
14	15 –Tetracosenoic acid methyl ester	21.940	2.33
15	Tetracosanoic acid methyl ester	22.096	0.61

Major constituents are:

-13-Docosenic acid methyl ester(43.61%)

-.9 12-Octadecadienoic acid methyl ester(17.50%).

-9 12 15-Octadectrienoic acid methyl ester(12.49%)

- cis-13-Eicosenoic acid methyl ester(7.83%).

i) 13-Docosenoic acid methyl ester(43.61%)

The mass spectrum of 13- docosenoic acid, methyl ester is shown in Fig 3.6. The peak at m/z 352, which appeared at R.T. 20.433 in total ion chromatogram, corresponds M $^{+}$ $[C_{23}H_{44}O_2]^{+}$. The peak at m/z 322 corresponds to loss of methoxyl function.

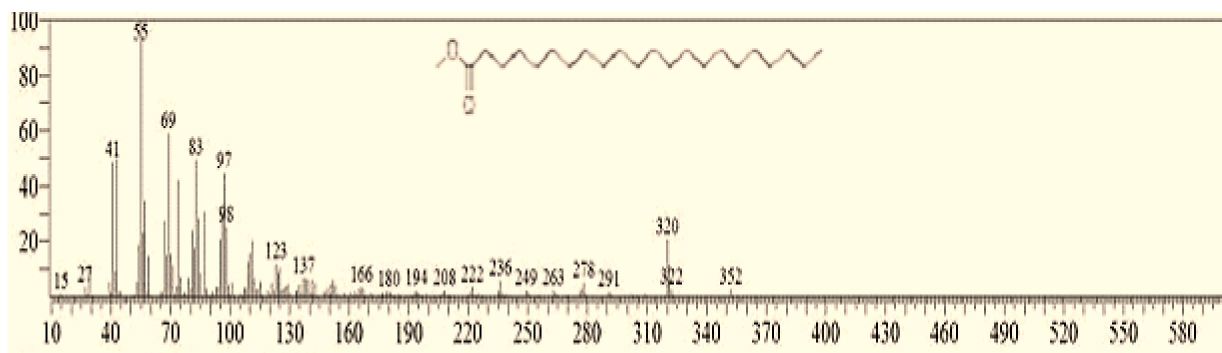


Fig. 3.6: mass spectrum of 13- docosenoic acid, methyl ester

ii) 9,12-Octadecadienoic acid methyl ester(17.50%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.7.The peak at m/z 294, which appeared at R.T. 16.952 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

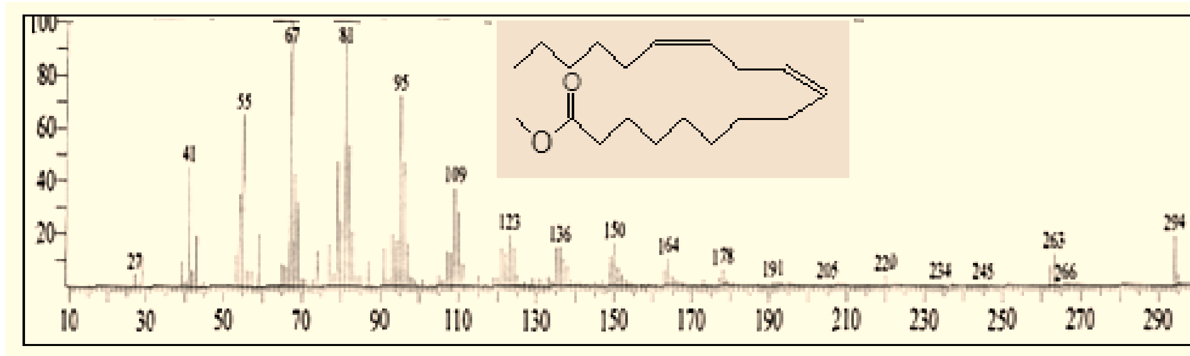


Fig. 3.7: Mass spectrum of 9,12-octadecanoic acid methyl ester

iii) 9,12,15-Octadecatrienoic acid methyl ester(17.03%)

Fig.3.8 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.17.033) in total ion chromatogram.The fragment at m/z261 is due to loss of a methoxyl .

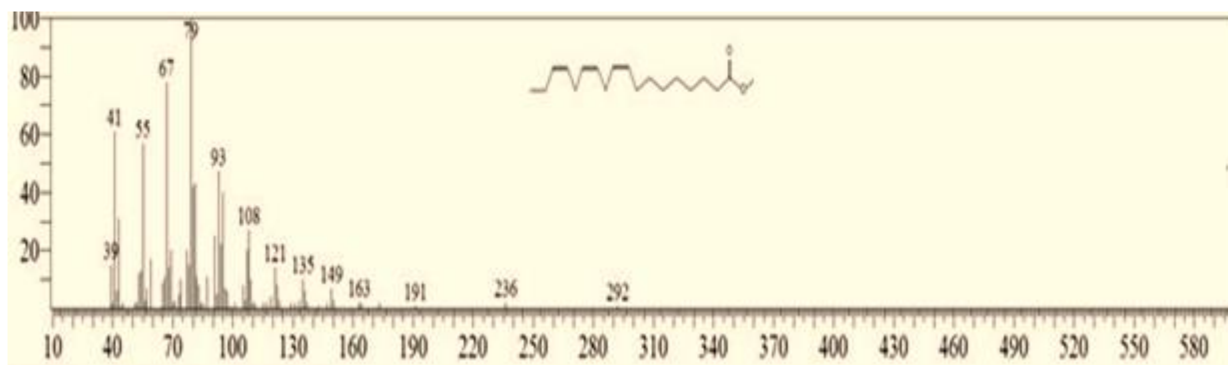


Fig.3.8: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

iv) Cis-11-Eicosenoic acid methyl ester(7.83%)

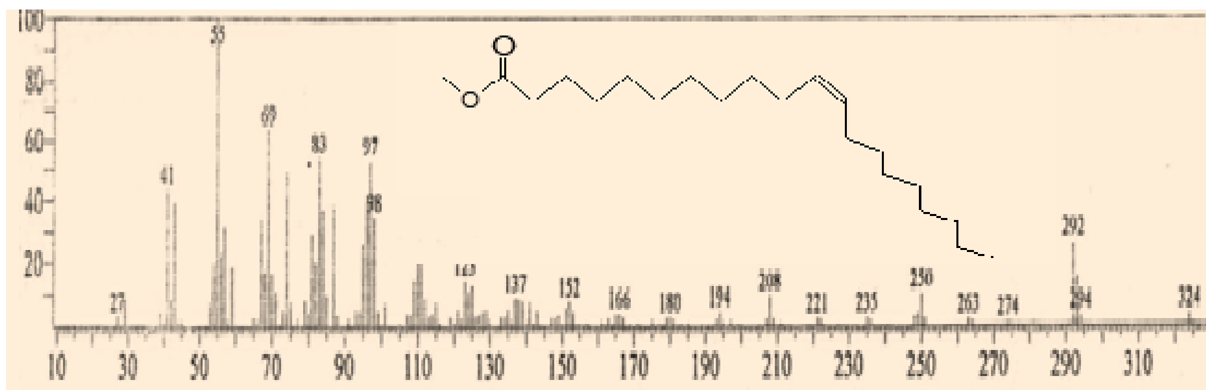


Fig. 3.9: Mass spectrum of Cis-11-Eicosenoic acid methyl ester

The EI mass spectrum of cis-11-eicosenoic acid methyl ester is shown in Fig. 3.9.The peak at m/z324, which appeared at R.T. 18.776 in total ion chromatogram, corresponds to $M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z293 corresponds to loss of a methoxyl function.

3.2.2. Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria *Bacillus subtilis,* and *staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseud*om*onas aeruginose* and fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.4, the oil exhibited partial activity against *Bacillus subtilis,Escherichia coli* and *Pseudomonas aeruginosa*.

Table 3.4: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/mL)	--	10	10	9	--
Ampicilin (40mg/mL)	30	15	--	--	--
Gentamicin (40mg/mL)	19	25	22	21	--
Clotrimazole (30mg/mL)	--	--	--	--	38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.3. *Momordica chantia*

3.3.1. The Gas chromatography – mass spectrometry

The GC-MS analysis of *Momordica chantia* oil showed 21 constituents (Table 3.5).Fig. 3.10 shows the total ions chromatograms.

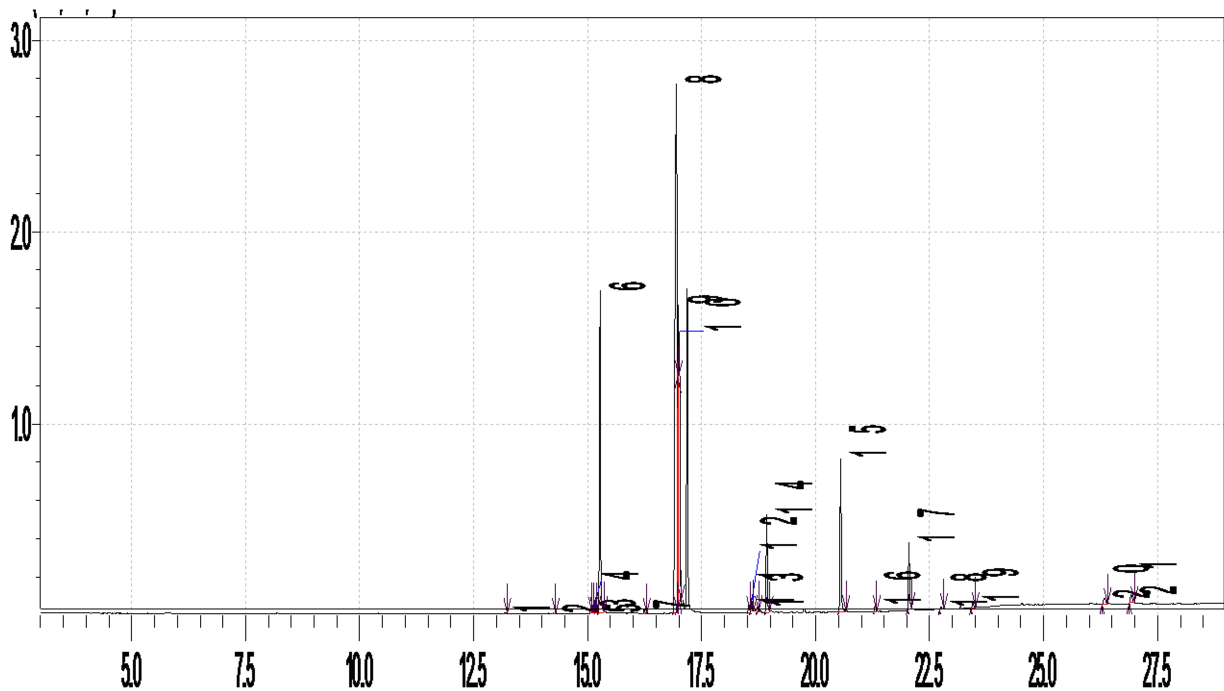


Fig.3.10: Total ions chromatograms

Major components of the oil are:

i) 9,12-Octadecadienoic acid methyl ester(47.64%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.11.The peak at m/z 294, which appeared at R.T. 16.948 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii) Hexadecanoic acid , methyl ester (17.06 %)

Figure 3.12 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. 15.272 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl function.

iii) 9,12,15-Octadecatrienoic acid methyl ester(13.29%)

Figure 3.13 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.17.010) in total ion chromatogram.The fragment at m/z261 is due to loss of a methoxyl .

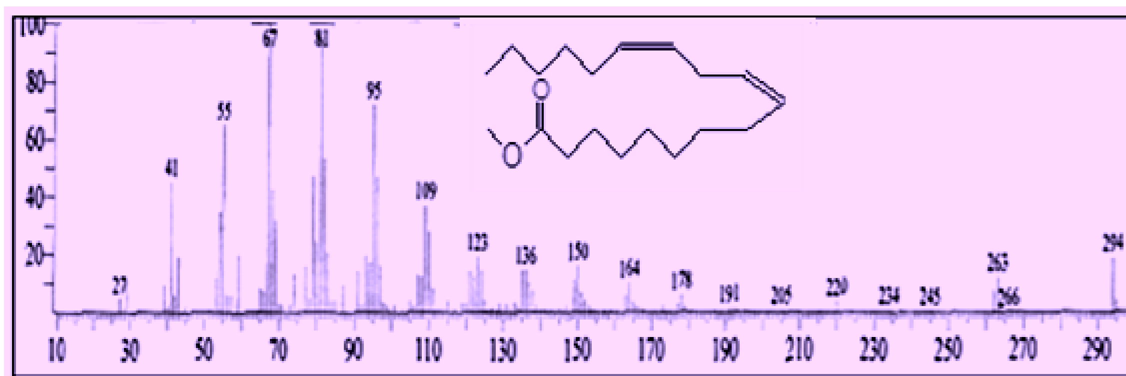


Fig. 11: Mass spectrum of 9,12-octadecanoic acid methyl ester

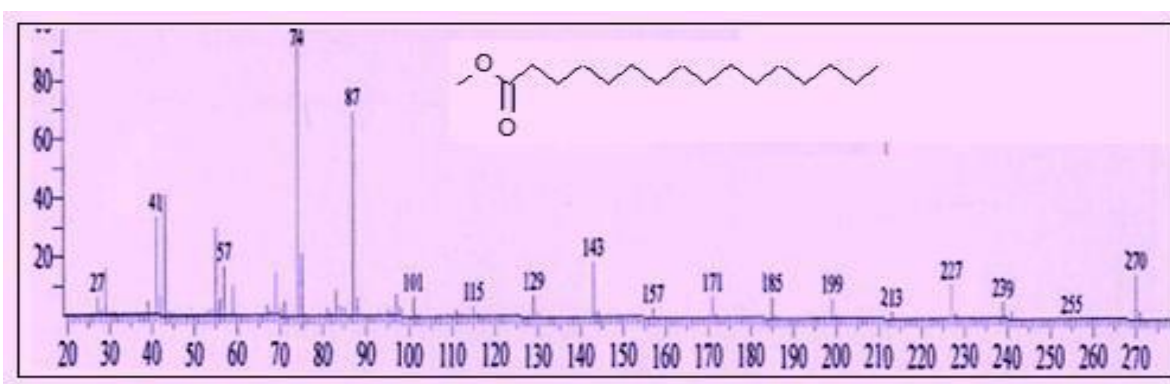


Fig.3.12: Mass spectrum of hexadecanoic acid
methyl ester

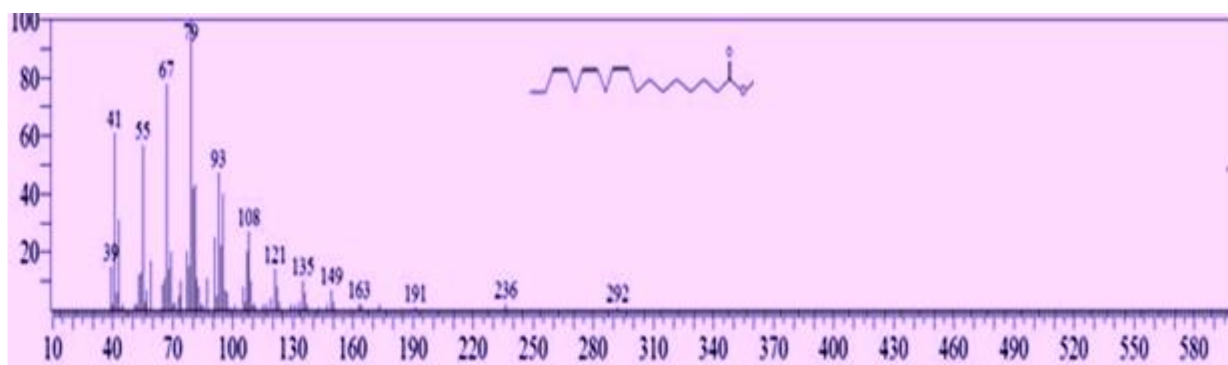


Fig .3.13: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

Table 3.5: Constituents of *Momordica chantia* oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.179	0.05
2.	Pentadecanoic acid, methyl ester	14.245	0.03
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.070	0.27
4.	9,12-Hexadecadienoic acid, methyl ester	15.111	0.04
5.	trans-13-Octadecenoic acid, methyl ester	15.163	0.03
6.	Hexadecanoic acid, methyl ester	15.272	17.06
7.	Heptadecanoic acid, methyl ester	16.240	0.21
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.948	47.64
9.	9-Octadecenoic acid (Z)-, methyl ester	16.980	1.79
10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.010	13.29
11.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.534	0.45
12.	11,14,17-Eicosatrienoic acid, methyl ester	18.589	0.46
13.	cis-11-Eicosenoic acid, methyl ester	18.724	0.20
14.	Eicosanoic acid, methyl ester	18.926	4.68
15.	Docosanoic acid, methyl ester	20.547	8.18
16.	Tricosanoic acid, methyl ester	21.307	0.36
17.	Tetracosanoic acid, methyl ester	22.044	3.36
18.	Pentacosanoic acid, methyl ester	22.756	0.16
19.	Hexacosanoic acid, methyl ester	23.444	0.38
20.	Stigmasterol	26.331	0.60
21.	.gamma.-Sitosterol	26.920	0.76

3.3.2. Antimicrobial Activity

The antimicrobial activity of the ethanol extract of ripe seeds was examined against Gram positive bacteria :*staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseudomonas aeruginose* and the fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As presented in Table 3.6, the oil exhibited good activity against *Escherichia coli.*

Table 3.6: Inhibition zones (mm/mg sample)

Type	Sa	Ec	Ps	Ca
Oil (100mg/ml)	11	14	12	12
Ampicilin (40mg/ml)	30	--	--	--
Gentamicin	19	22	21	--
Clotrimazole	--	--	--	38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.4. *Cordia myxa*

3.4.1. Gas chromatography-mass spectrometry

The GC-MS analysis of *Cordia myxa* oil exhibited the presence of 17 (Table 3.7) .

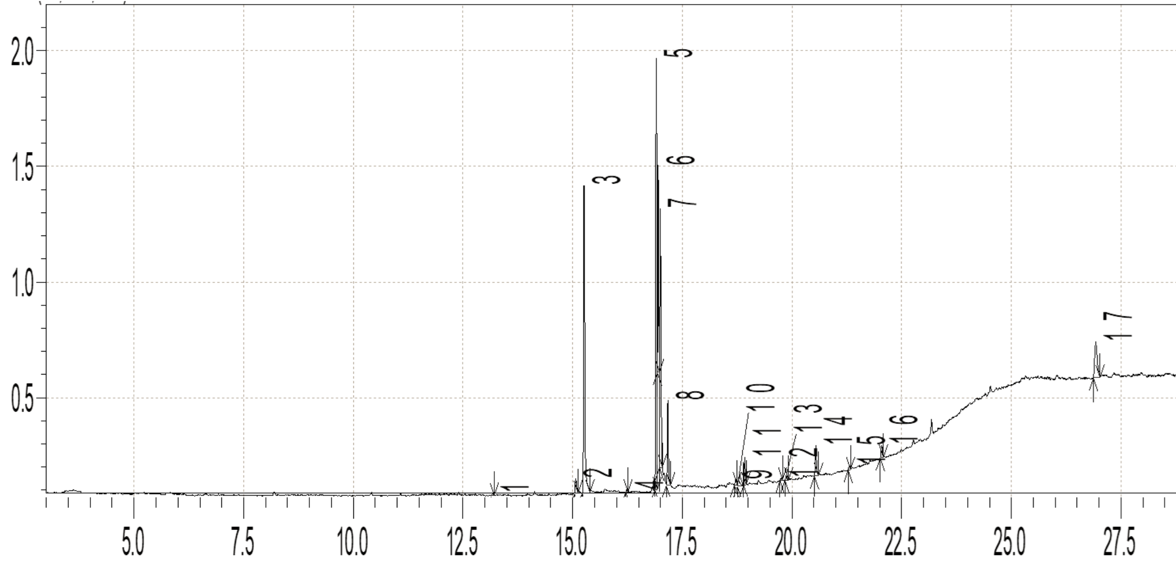


Fig.3.14 : Total ions chromatograms

Table3.7 : Constituents of *Cordia myxa* oil

No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	13.176	0.18
2	9-Hexadecenoic acid, methyl ester, (Z)-	15.071	1.31
3	Hexadecanoic acid, methyl ester	15.262	23.24
4	Heptadecanoic acid, methyl ester	16.236	0.14
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.907	28.81
6	9-Octadecenoic acid (Z)-, methyl ester	16.950	11.03
7	11-Octadecenoic acid, methyl ester	17.000	16.89
8	Methyl stearate	17.170	5.72
9	cis-11-Eicosenoic acid, methyl ester	18.721	0.37
10	11-Eicosenoic acid, methyl ester	18.780	0.82
11	Eicosanoic acid, methyl ester	18.923	1.85
12	Heneicosanoic acid, methyl ester	19.749	0.16
13	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	19.868	0.33
14	Docosanoic acid, methyl ester	20.542	2.18
15	Tricosanoic acid, methyl ester	21.309	0.37
16	Tetracosanoic acid, methyl ester	22.043	1.02
17	.gamma.-Sitosterol	26.925	5.58

Major components of the oil are:

i) 9,12-Octadecadienoic acid methyl ester(28.81%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.15.The peak at m/z 294, which appeared at R.T. 16.907 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii) Hexadecanoic acid , methyl ester (23.24 %)

Figure 3.16 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. 15.262 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl .

iii) 11-Octadecenoic acidmethyl ester(16.89%)

The EI mass spectrum of 11-octadecadienoic acid methyl ester is shown in Fig. 3.17.The peak at m/z 296, which appeared at R.T. 17.000 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_{2}]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl group.

iv) 9-Octadecenoic acid methyl ester(11.03%)

The EI mass spectrum of 9-octadecadienoic acid methyl ester is shown in Fig. 3.18.The peak at m/z 296, which appeared at R.T. 16.950 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_{2}]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl .

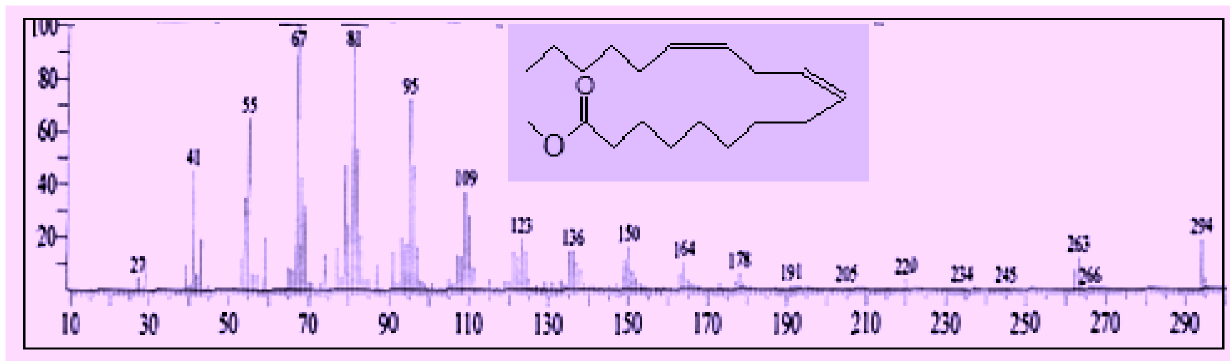


Fig. 3.15: Mass spectrum of 9,12-octadecanoic acid methyl ester

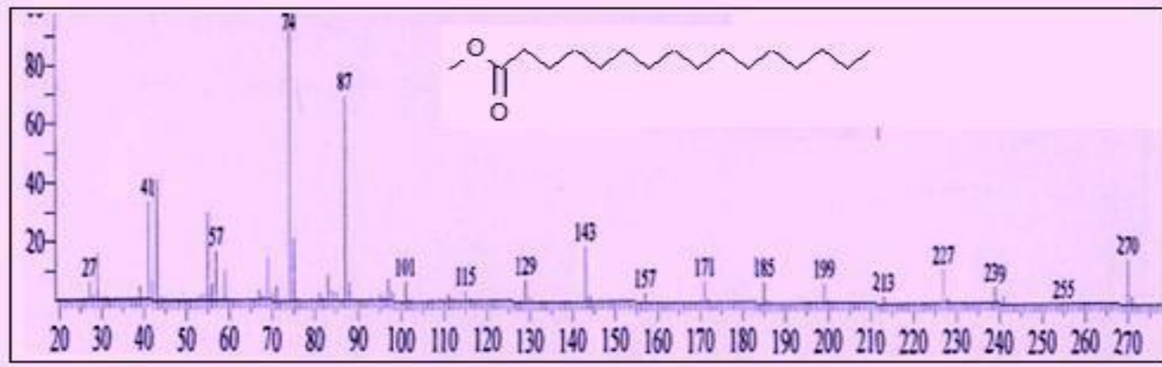


Figure 3.16: Mass spectrum of hexadecanoic acid methyl ester

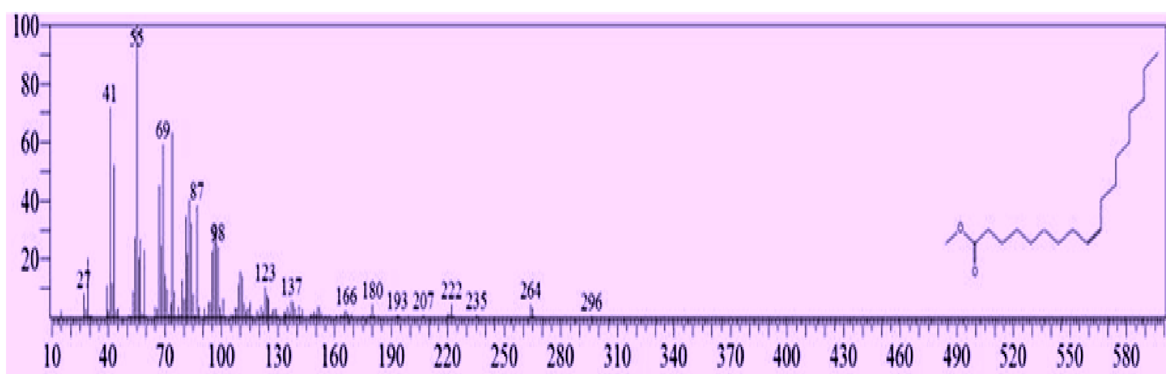


Fig. 3.17 : 9-octadecenoic acid methyl ester

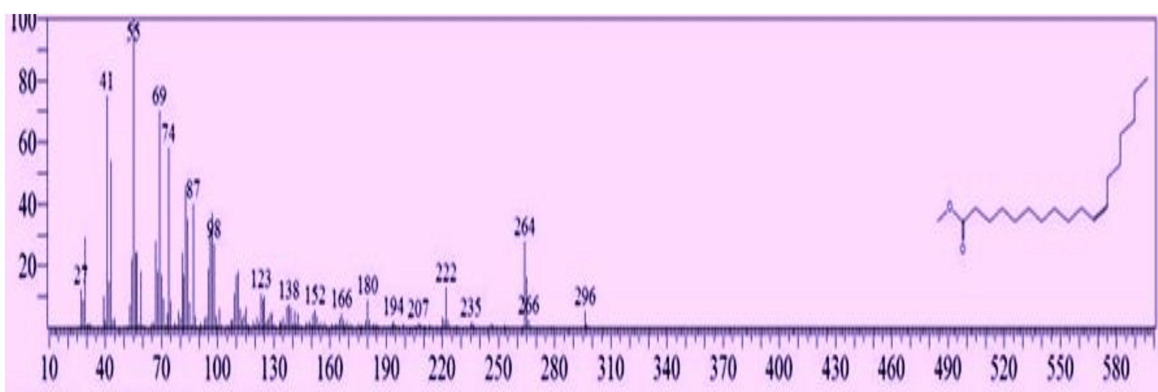


Fig. 3.18 : 11-octadecenoic acid methyl ester

3.4.2. Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria :*staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseudomonas aeruginose* and the fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.8, the oil exhibited good activity against *Bacillus subtilis* , *staphylococcus aureus*, *Pseudomonas aeruginose* beside significant anticandidal activity .

Table 3.8: Inhibition zones (mm/mg sample)

Type	Sa	Ec	Ps	Bs	Ca
Oil (100mg/ml)	15	--	14	16	18
Ampicilin (40mg/mL)	30	--	--	15	--
Gentamicin (40mg/mL)	19	22	21	25	--
Clotrimazole (30mg/mL)	--	--	--		38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.5. *Helianthus annuus*

3.5.1. The Gas chromatography-mass spectrometry

The GC-MS analysis of *Helianthus annuus* oil exhibited the presence of 31constituents (Table 3.9) .

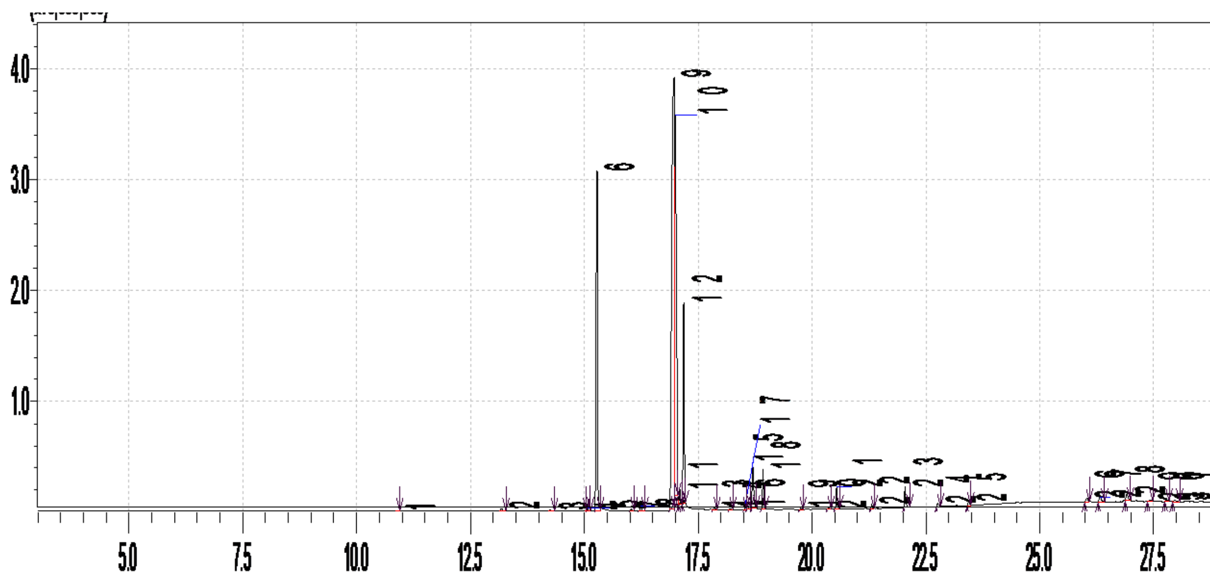


Fig.3.19 : Total ions chromatograms

Major components of the oil are:

i) 9,12-Octadecadienoic acid methyl ester(44.67%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.20.The peak at m/z 294, which appeared at R.T. 16.968 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii) 9-Octadecenoic acid methyl ester(19.71%)

The EI mass spectrum of 9-octadecadienoic acid methyl ester is shown in Fig. 3.21.The peak at m/z 296, which appeared at R.T. 17.001 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl .

Table3.9 : Constituents of *Helianthus annuus* oil

No.	Name	Ret.Time	Area%
1.	Dodecanoic acid, methyl ester	10.896	0.01
2.	Methyl tetradecanoate	13.183	0.14
3.	Pentadecanoic acid, methyl ester	14.249	0.07
4.	7-Hexadecenoic acid, methyl ester, (Z)-	15.045	0.03
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.070	0.14
6.	Hexadecanoic acid, methyl ester	15.277	17.16
7.	cis-10-Heptadecenoic acid, methyl ester	16.036	0.09
8.	Heptadecanoic acid, methyl ester	16.241	0.23
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.968	44.67
10.	9-Octadecenoic acid (Z)-, methyl ester	17.001	19.71
11.	Phytol	17.095	0.67
12.	Methyl stearate	17.183	8.18
13.	Farnesol isomer a	17.850	0.13
14.	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	18.194	0.07
15.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.534	0.28
16.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.586	0.17
17.	11,13-Eicosadienoic acid, methyl ester	18.695	2.31
18.	Eicosanoic acid, methyl ester	18.924	1.62
19.	Heneicosanoic acid, methyl ester	19.751	0.07
20.	13-Docosenoic acid, methyl ester, (Z)-	20.364	0.11
21.	Docosanoic acid, methyl ester	20.541	1.06
22.	Tricosanoic acid, methyl ester	21.306	0.18
23.	Tetracosanoic acid, methyl ester	22.043	1.04
24.	Pentacosanoic acid, methyl ester	22.760	0.20
25.	Hexacosanoic acid, methyl ester	23.441	0.22
26.	Ergost-5-en-3-ol, (3.beta.)-	26.037	0.29
27.	Stigmasterol	26.323	0.36
28.	.gamma.-Sitosterol	26.913	0.48
29.	.alpha.-Amyrin	27.415	0.10
30.	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	27.790	0.11
31.	.beta.-Amyrin	27.963	0.10

iii) Hexadecanoic acid , methyl ester (17.16 %)

Figure 3.22 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. 15.277 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl .

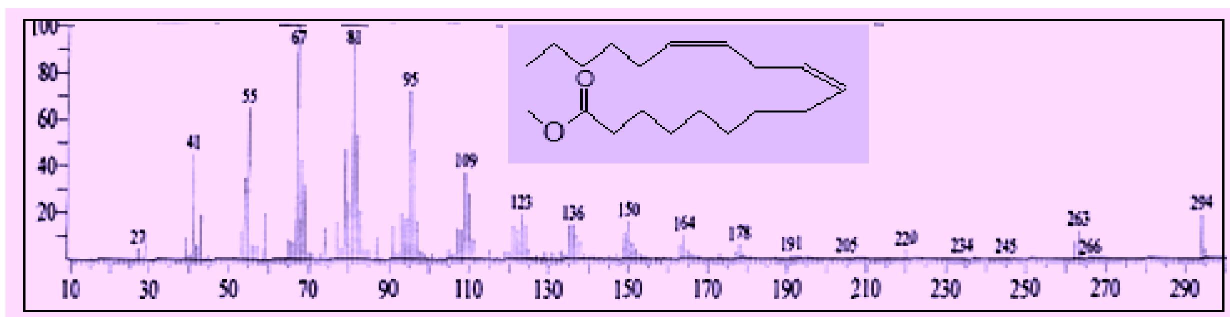


Fig. 3.20: Mass spectrum of 9,12-octadecanoic acid methyl ester

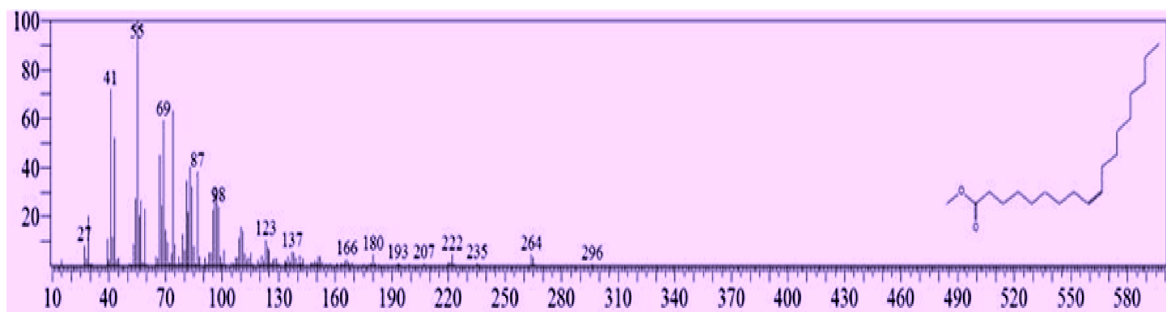


Fig. 3.21 : 9-octadecenoic acid methyl ester

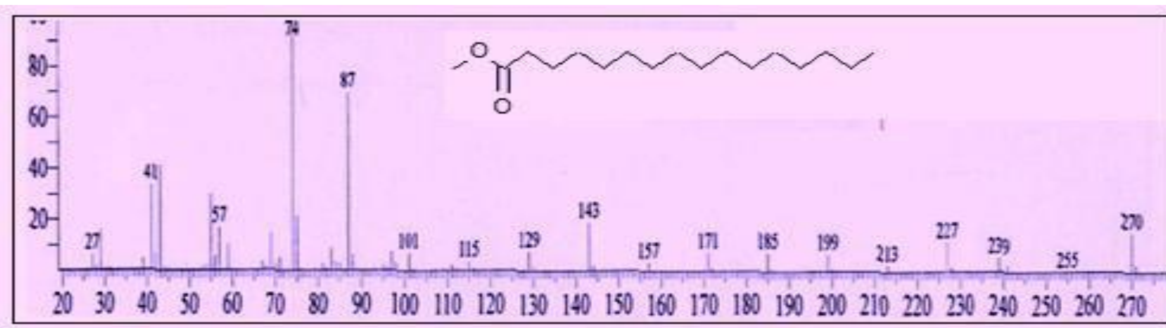


Fig.3.22: Mass spectrum of hexadecanoic acid methyl ester

3.5.2. Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria :*staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseudomonas aeruginose* and the fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.10, the oil exhibited good activity against all test organisms.

Table 3.10: Inhibition zones (mm/mg sample)

Type	Sa	Ec	Ps	Bs	Ca
Oil (100mg/mL)	15	14	14	15	14
Ampicilin (40mg/mL)	30	--	--	15	--
Gentamicin (40mg/mL)	19	22	21	25	--
Clotrimazole (30mg/mL)	--	--	--		38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.6. *Securidaca longipedum*

3.6.1. The GC-MS analysis

Securidaca longipedum oil was analyzed by GC-MS. The analysis showed 22 constituents (Table 3.11 and Fig.3.23).

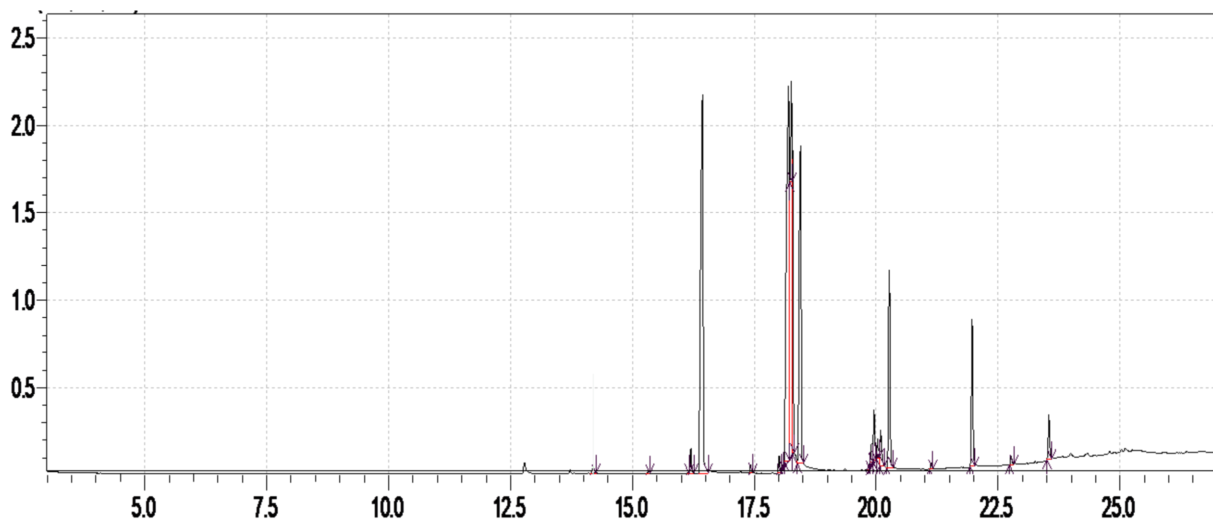


Fig.3.23 Total ions chromatograms

The GC-MS analysis showed the following major components:

i)9 12-Octadecadienoic acid methyl ester(29.04%)

ii)Hexadecanoic acid methyl ester(22.90%).

iii)Methyl stearate(15.30%).

iv)9 12 15-Octadecatrienoic acid methyl ester(7.10%).

i) 9,12-Octadecadienoic acid methyl ester(44.67%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.24.The peak at m/z 294, which appeared at R.T. 18.202 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii) Hexadecanoic acid , methyl ester (17.16 %)

Figure 3.25 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. 16.435 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl

Table 3.11: Constituents of *Securidaca longipedum* oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.183	0.20
2.	Pentadecanoic acid, methyl ester	15.314	0.06
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.150	0.05
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.196	0.81
5.	Hexadecanoic acid, methyl ester	16.435	22.90
6.	Heptadecanoic acid, methyl ester	17.426	0.24
7.	.gamma.-Linolenic acid, methyl ester	18.008	0.65
8.	Methyl stearidonate	18.085	0.18
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.202	29.04
10	9-Octadecenoic acid (Z)-, methyl ester	18.258	2.91
11	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.284	7.10
12	Methyl stearate	18.445	15.30
13	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	19.875	0.23
14	10,13-Octadecadienoic acid, methyl ester	19.906	0.87
15	Heptadecanedioic acid, 9-oxo-, dimethyl ester	19.955	1.81
16	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	20.026	0.68
17	10-Octadecynoic acid, methyl ester	20.090	1.53
18	Eicosanoic acid, methyl ester	20.268	7.65
19	Heneicosanoic acid, methyl ester	21.126	0.20
20	Docosanoic acid, methyl ester	21.966	5.56
21	Tricosanoic acid, methyl ester	22.764	0.34
22	Tetracosanoic acid, methyl ester	23.539	1.69

iii) Methyl stearate (15.30 %)

Fig. 3.26 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.18.445) corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$, while the peak at m/z 267 accounts for loss of a methoxyl.

iv) 9,12,15-Octadecatrienoic acid methyl ester(7.10%)

Figure 3.27 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.18.284) in total ion chromatogram.The fragment at m/z261 is due to loss of a methoxyl .

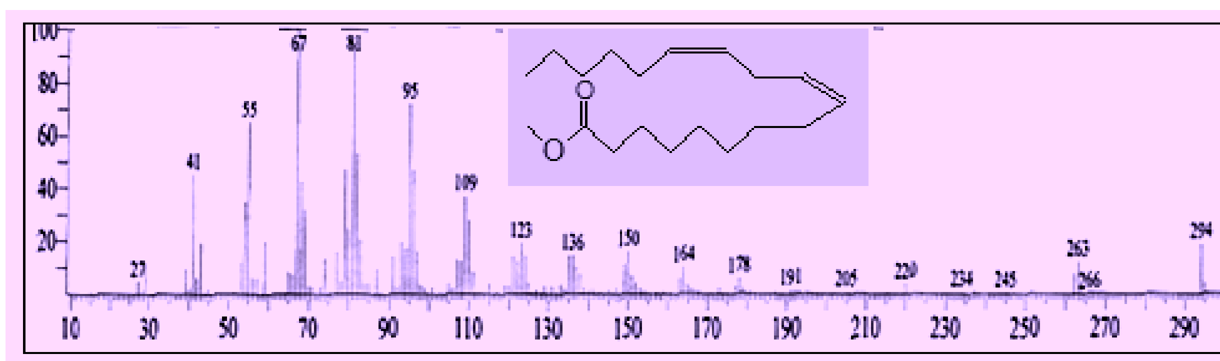


Fig. 3.24: Mass spectrum of 9,12-octadecanoic acid methyl ester

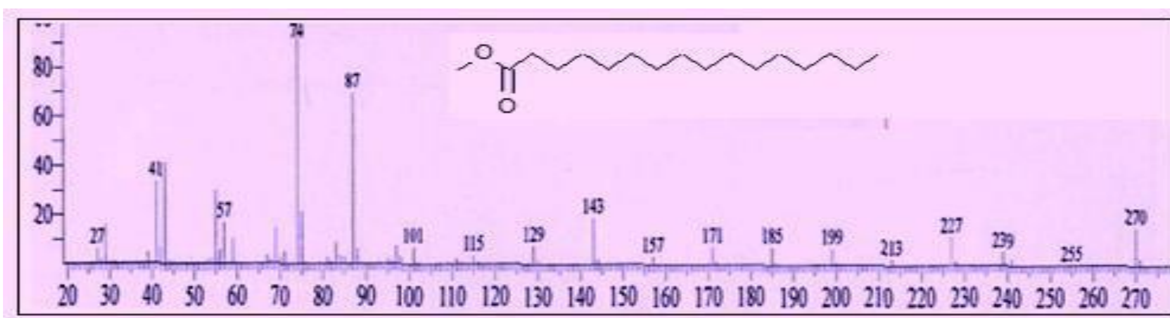


Fig.3.25: Mass spectrum of hexadecanoic acid methyl ester

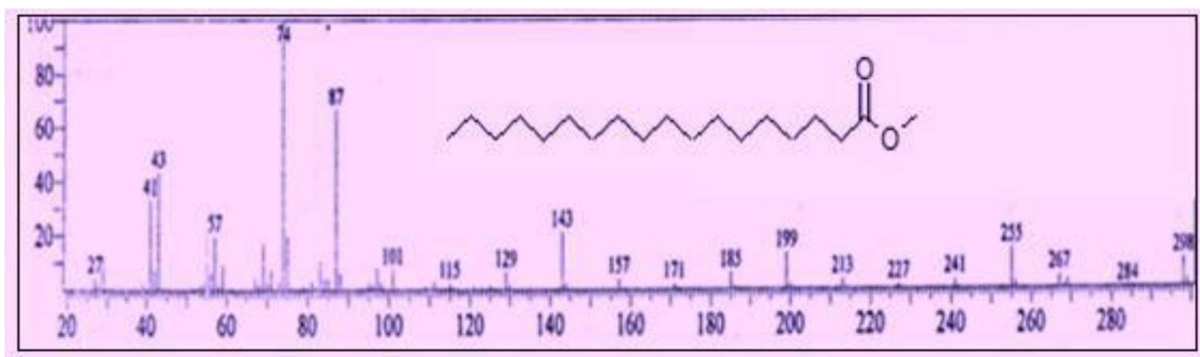


Fig.3.26: Mass spectrum of methyl stearate

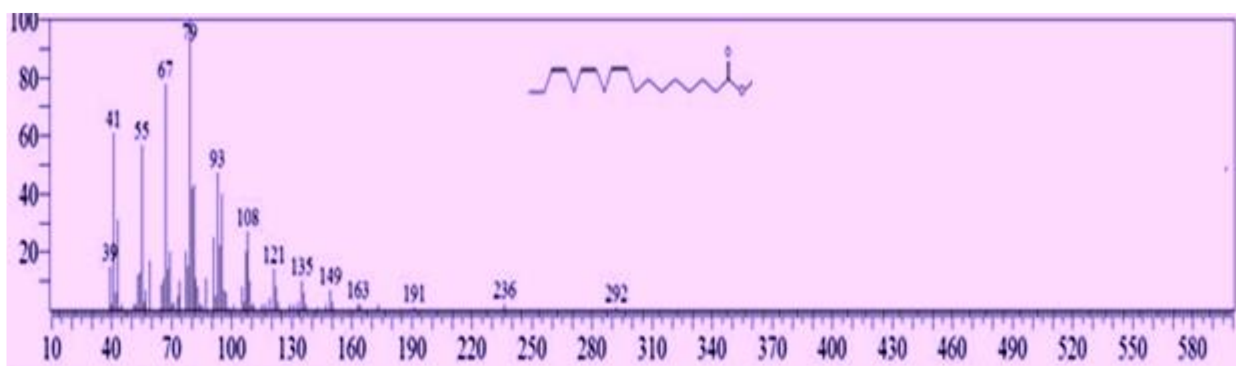


Fig .3.27: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

3.6.2. Antimicrobial Activity

The antimicrobial activity of the oil was examined against Gram positive bacteria *Bacillus subtilis,* and *staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseudomonas aeruginose* and fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.12, the oil exhibited good activity against *Staphylococcus aureus* It was also active against *Pseudomonas aeruginosa* and *Candida albicans*.However the oil exhibited weak activity against *Bacillus subtilis*.

Table 3.12: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/mL)	16	10	--	13	13
Ampicilin (40mg/mL)	30	15	--	--	--
Gentamicin (40mg/mL)	19	25	22	21	--
Clotrimazole (30mg/mL)	--	--	--	--	38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

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