Sudan University of Science and Technology

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Identification of Constituents and Antimicrobial Activity of Oils from Selected Medicinal Plants

التعرف على المكونات ونشاط المضادات الميكروبية لزيوت بعض النباتات الطبية المختارة

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

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الاستـهلال

قال تعالى:

اللّهُ لاَ إِلَـهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ.

صدق الله العظيم

سورة البقرة:الاية (255)

Dedication

To:

My parents ,

My brother and sister

Acknowledgment

First of all I would like to thank Almighty Allah, Most Merciful for giving me health, to complete this work.

I would like to thank my supervisor Prof. Mohamed Abdel Karim, for his close supervision, continuous and valuable assistance and close guidance.

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Abstract

In this study the oils from six plants of medicinal attributes (*Indigofera arrect*, *Indigofera hirsutal*, *Lagenaria siceraria*, *Fallopia conuolulus* , *Dicoma tomentoza and Carica papya*) have been investigated by GC.MS and the antimicrobial activity has been screened. The fatty acid contents of the oils was determined by retention times and the observed fragmentation pattern . Sixteen components of *Indigofera arrect*, 19 components of *Indigofera hirsutal,* 9 components of *Lagenaria siceraria* oil, 23 components of *Fallopia conuolulus* oil, 20 components of *Carica papaya* oil and18 components of *Dicoma tomentoza* oil were detected in total ion chromatograms being dominated by : Hexadecanoic acid methyl ester presence in *Indigofera arrect*, *Lagenaria siceraria*, *Fallopia conuolulus* and *Dicoma tomentoza* (34.14 %, 10.15%, 22.67 %, 16.57 %). 9,12-Octadecadienoic acid (Z,Z)-, methyl ester presence in *Indigofera arrect* and *Lagenaria siceraria* (31.76 %, 19.56 %). 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-found in *Indigofera arrect* and *Indigofera hirsutal* (15.56 %, 25.63 %). 9-Octadecnoic acid methyl ester presence in *Indigofera hirsutal, Fallopia conuolulus*, *Carica papya* and *Dicoma tomentoza* (22.49%, 25.77%, 18.182 %, 39.78 %). 9, 12-octadecadienoic acid methyl ester precence in *Indigofera hirsutal*, *Fallopia conuolulus* and *Dicoma tomentoza* (32.40 %, 32.34 %, 14.50%). Linoleic acid ethyl ester precence in *Lagenaria siceraria* (57.96 %). Methyl stearate precence in *Fallopia conuolulus* (11.17%). Oleic acid, Ethyl oleate, Stigmasterol, Dodecen-1-yl(-)succenic acid anhydride and gamma-Sitosterol precence in *Carica papya* (12.961 %, 11.52 %, 5.85%, 5.40%). The six oils was screened for antimicrobial activity against five standard organisms. The oils of *Indigofera arrect*, *Carica papya* and

Dicoma tomentoza showed significant activity against *Escherichia coli* The *Indigofera hirsutal,* oil showed significant activity against *Staphylococcus aureus* and *Bacillus subtilis.* The oil of *Lagenaria siceraria* showed moderate activity against *Pseudomonas aeroginosa.*While, the oil of *Fallopia conuolulus* showed moderate activity against *Staphylococcus aureus* beside moderate anticandidal activity. The oil of *Dicoma tomentoza* moderate activity against *Staphylococcus aureus* , *Pseudomonas aeroginosa* and *Candida albicans*. Also the oil of *Carica papya* exhibited moderate activity against Bacillus subtilis and Staphylococcus aureus beside weak anticandidal activity.

المستخلص

في هذه الدراسة زيوت ستة من النباتات الطبية (البابون الاخضر، البابون البني، القرع الحلو،اللبلاب، البابااي وعسل الطير) تم الكشف عنها بكروموتوغرافيا الغاز- مطياف الكتلة واحضعت للكشف عن نشاطها الميكروبي . تم تحديد مكونات الاحماض الدهنية بزمن الاستبقاء ونمط التكسيرحيث وجد ان البابون الاخضر يحوي 16 مكون ، البابون البني 19 مكون ، القرع الحلو 9 مكونات ، اللبلاب 23 مكون ، البابااي 20 مكون وعسل الطير 18 مكون.

وكانت مكونات الاحماض الدهنية كاتالي :

Hexadecanoic acid methyl ester ظهر في البابون الاخضر ، القرع الحلو، اللبلاب وعسل الطير بنسب (,22.49%، 10.15%، 25.77%، 16.57 %). 9,12-Octadecadienoic acid (Z,Z)-, methyl ظهرفي البابون البني والقرع الحلو بنسب (31.76 %، 19.56 %). 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- وجد في البابون البني والبابون الاخضر بنسب (15.56 %، 25.63 %). 9-Octadecnoic acid methyl ester ظهر في البابون الاخضر، اللبلاب، البابااي وعسل الطير بنسب (22.49%، 25.77%، 18.182 %، 39.78 %). 9, 12-octadecadienoic acid methyl ester ظهر في البابون الاخضر، اللبلاب وعسل الطير بنسب (32.40 %، 32.34 %، 14.50%). Linoleic acid ethyl ester ظهر في القرع الحلو بنسبة (57.96 %). Methyl stearate ظهر في اللبلاب بنسبة (11.17%). اما مركبات Oleic acid, Ethyl oleate, Stigmasterol, Dodecen-1-yl(-)succenic acid anhydride، gamma-Sitosterol في البابااي بنسب (12.961 %، 11.52 %، 5.85%، 5.40%).

اخضعت زيوت الست نباتات لاختبارات النشاط الميكروبي ضد خمس من الكائنات القياسية حيث ابدى البابون الاخضر، البابااي وعسل الطير فعالية كبيرة ضد البكتريا القولونية .زيت البابون البني

ابدى فعالية كبيرة ضد المكورات العنقودية والبكتريا العصوية. زيت القرع الحلو ابدى فعالية متوسطة ضد البكتريا الزائفة بينما زيت اللبلاب وعسل الطير اعطيا فعالية متوسطة ضد المكورات العنقودية الذهبية والمبيضات، اما زيت الباباي فابدى فعالية متوسطة البكتريا المسببة للمرض والمكورات العنقودية وفعالية ضعيفة ضد المبيضات.

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1-Introduction

1.1 General approach

Feed efficiency is a large factor in determining feedlot profitability[1] Thus, nutritionists have long looked for methods to improve efficiency by manipulating rumen fermentation. One approach has been to use antibiotics and ionophores in diets to modify rumen fermentation. Due to negative perceptions of antibiotic use however, nutritionists have begun searching for alternative rumen modifiers. This has resulted in increased interest in using plant extracts, like essential oils (**EO**), to alter rumen fermentation[2] .

EO are bioactive plant compounds found in many plants that can be obtained via steam distillation or chemical extraction[3,4,5] Previous studies have reported improved gain and efficiency in swine and poultry due to dietary EO inclusion[6,7,8.9]. EO possess antimicrobial properties that are effective against Gram positive and Gram-negative bacteria suggesting they could also be beneficial when included in ruminant diets[10,11,12]. However, their effects and the mode of action in the rumen are still unclear. Before EO inclusion in feedlot diets can become common practice, it is necessary to understand how EO affect rumen fermentation and nutrient digestibility.

1.2 Essential oil background

Many plants produce bioactive compounds like saponins, tannins, essential oils (**EO**), and phenolic compounds that have antimicrobial properties and have been shown to alter rumen fermentation. In the plant, these compounds often help protect the plant from bacterial, insect, and fungal attacks and typically contribute to the plant" s flavor or smell, i.e. its "essence"13,14,2,5,16 . EO were originally researched to determine their role in reducing palatability in some plant species[17]. For many years humans have used EO for their flavoring, scents, and preservative properties[12] .

Although EO have an oily appearance they are considered volatile or ethereal oils rather than true lipids and are commonly extracted via steam distillation or solvent extraction[3]. EO can be extracted from many parts of the plant (leaves, stem, roots, seed, flower, etc.) but composition can vary greatly among different segments[11]. EO chemical composition can also be influenced by plant growth stage, plant health, and external factors like temperature, light, and moisture[18]. Due to many influencing factors, EO chemical composition varies making it even more difficult to determine consistent effects due to EO inclusion. EO are secondary plant metabolites that are alcohol, ester, or aldehyde derivatives of terpenoids and phenylpropanoids[4,18] .

Each group is synthesized through separate metabolic pathways using different primary metabolites as precursors[4]. Terpenoids are synthesized using acetyl-CoA via the deoxyxylulose or mevalonate metabolic pathway[4,18]. Terpenoids are more numerous, diverse, and well documented than phenylpropanoids and are characterized by the basic five carbon, isoperene, (C_5H_8) structure that makes up their skeleton. Terpenoids can be further divided into subcategories based on the number of isoprene units in the skeleton: monoterpenoids and sesquiterpenoids. Monoterpenoids are the most common and contain two isoprene units ($C_{10}H_{16}$), while sesquiterpenoids contain three isoprene units ($C_{15}H_{24}$) and are less common[19]. Phenylpropanoids contain a three carbon chain bound to a six carbon aromatic ring[4,18]. Phenylpropanoids are typically derived from phenylalanine via the shikimate metabolic pathway that is only functional in plants and microorganisms[20,18]. These compounds are less common, but can be found in large percentages in some plants. Reportedly there are over 1,000 monoterpenes and roughly 50 phenylpropanoids occur naturally in plants[1]. EO have been shown to work against bacteria, protozoa, and fungi but their mode of action isn‟ t clear21,14,12,18 .

1.3- Mode of action

Several theories have been suggested to explain the antimicrobial properties possessed by EO. The most widely

accepted theory is that EO interact with bacterial cell membranes[22,11,4]. Many EO are hydrophobic and lipophilic in nature and are able to interact with lipid cell membranes, fuse with fatty acid chains comprising the membrane, and accumulate in the lipid bilayer of bacterial cells[23,24,4]. EO accumulation between the fatty acid chains causes conformational changes in the cell membrane resulting in increased membrane instability and fluidity[22]. Thus, ion leakage occurs and ion gradients across the membrane are diminished. Bacteria counteract this by using ionic pumps to facilitate transport across the membrane, but this process diverts a great deal of energy and causes bacterial growth to decrease[22,24,25]. This decreases bacterial populations and alters fermentation profile in the rumen. This method of action should be more effective against Gram-positive bacteria because they lack the protective, hydroliphic outer layer possessed by Gram-negative bacteria and EO can interact directly with the cell membrane12,25. However, EO are able to exert their antimicrobial properties on both Gram-negative and Gram-positive bacteria10,11,12,27. Due to low molecular weights, EO compounds are able cross the protective cell wall possessed by Gram negative bacteria by slowly diffusing through the outer lipopolysaccharide layer or through membrane proteins and reach the inner lipid bilayer22,11,25. EO beside interaction with the cell membrane they also interferes with protein and electron

transport, phosphorylation, and some membrane-bound enzyme dependent reactions[11,2]. It has been suggested that[28] some EO compounds are able to interact with some enzymes and other biologically active compounds present in the cell. Both phenolic and non-phenolic compounds typically interact with proteins via hydrogen bridges and ionic interactions; however, nonphenolic compounds react via various functional groups (alcohol, aldehyde, ester, etc.) they possess rather than a phenolic ring. Aldehyde compounds are thought to deactivate proteins and enzymes using alkylation and cross bridges[29]. Cinnamaldehyde (**CIN**; Figure 1.2) specific mode of action is unknown, but its antimicrobial activity is thought to be linked to the reactivity of its carbonyl group[30,31] . Other EO, CIN had no effect on membrane stability, but interacted with membrane proteins. This interaction is thought to denature membrane proteins, increase membrane permeability, and cause cell constituents to coagulate[28,32]. This coagulation eventually causes the cell to lyse and die[12]. EO may also work by inhibiting hyper-ammonia producing (**HAP**) bacteria in the rumen. HAP bacteria are not present in large quantities in the rumen, but have a very high deamination activity and generate much of the ammonia produced in the rumen[33,34,35]. Thus a reduction in HAP bacteria could cause decreased amino acid (**AA**) deamination, decreased ammonia concentrations, and increased protein utilization efficiency in the rumen[34].

Garlic oil (**GAR**) is thought to function differently than most other EO because it is a complex mixture of compounds, including diallyl disulfide (Figure 1.3) found in the plant or produced due to changes that occur during extraction and processing[25].It has been proposed[3] that its antimicrobial properties is achieved by inhibiting protein synthesis in the cell. It has also been reported[37] that GAR inhibits hydroxymethylglutaryl-CoA reductase activity which reduces the production of the cholesterol and other isoprenoids responsible for membrane stability. By inhibiting isoprenoid production cells become unstable and eventually die. It has been suggested[38] that this is how GAR and its active compounds directly inhibit *Archaea* microorganisms in the rumen and reduce methane production. However, several other studies suggest its ability to interact with sulfhydryl groups found in other active compounds responsible for its antimicrobial activity[39,40,38].

Multiple theories have been suggested to explain EO antimicrobial activities. However, since many compounds and functional groups comprise EO it is likely that multiple modes of action, rather than a single method, are exploited to exert their antimicrobial properties on rumen microbes.

1.4-Essential oils and digestibility

Organic Matter (**OM**) digestibility appears to be largely unaffected by EO inclusion. Blended EO inclusion had no effect

on true OM digestibility *in vitro*[41] or total tract digestibility[27,42]. Diallyl disulfide and GAR inclusion had no effect on apparent total tract digestibility[43]. GAR and CIN have also been reported to increase true rumen digestibility, but have no effect on total tract OM digestibility[8,2,9].

1.5- Nitrogen Digestibility

There have been mixed results when evaluating how N digestibility is affected by EO inclusion. Some studies support the theory that EO reduce protein digestibility, but others report EO had no effect or actually increased N degradation. When beef heifers were fed CIN at 0, 400, 800, and 1,600 mg·hd-1·d-1, true rumen and total tract digestibility decreased linearly[9]. GAR and diallyl disulfide fed at 312 mg·L-1 also decreased protein degradation *in vitro*, while low levels had no effect[44].

It has been shown that[9] and[38] EO had antimicrobial properties because increasing EO concentration decreased fermentation activity. Other *in vitro* studies report N degradation in the rumen increased with blended EO inclusion[41]. *In vivo,* CIN had no effect on rumen or total tract digestibility[2]. In dairy cows fed GAR or juniper berry extract rumen digestibility increased, but had no effect on total tract digestibility[8]. Peppermint[45] and mixed EO[27] also had no effect on total tract digestibility .

1.6-Fiber digestibility

NDF digestibility *in vitro* was decreased by GAR and diallyl disulfide inclusion[44], but was not affected by blended EO inclusion[41]. *In vivo,* rumen and total tract digestibility linearly decreased in heifers fed 0, 400, 800, and 1,600 mg·hd-1·d-1 CIN. Heifers fed 1,600 mg·hd-1·d-1 CIN had a greater than 12% reduction in rumen digestibility and total tract digestibility was reduced by over 10%[46]. Conversely, there was no difference in rumen and total tract NDF digestibility observed when GAR and juniper berry extracts[47] or CIN[2] were included in dairy cow diets.

1.7-Essential oils in food

It is well accepted that in the majority of instances, greater concentrations of essential oils are required for inhibition of microbial growth in food products than is evident in liquid or agar mediums[48]. Intrinsic characteristics of food matrices, including protein, fat, water activity, or pH all influence the efficiency of antimicrobial additives. For example, fresh meats, poultry, and fish not only receive minimal processing prior to marketing, but their high lipid and protein content decrease the activity of phenolics. As essential oils are hydrophobic in nature, when applied to the surface of a meat product, the oils often aggregate into the lipid portion of the meat, thus leaving the microbially contaminated surface untreated. They may also

be chemically altered, losing antimicrobial capabilities as they come in contact with intrinsic meat components[4]. These products also have pH values near neutral and high water activities, providing an ideal substrate on which microorganisms can flourish[49]. For these reasons, the use of oil incorporated films is becoming an increasingly popular area of study[50,51,52].

Fruit and vegetable products are likely candidates for essential oil application, due in large part to the virtual absence of proteins and lipids in the majority of herbaceous species. However, carbohydrates have demonstrated interference with essential oils within a food system, so further research in this area is still crucial to evaluate their interactions[53]. Additionally, the surface of most produce is covered with crevices into which microorganisms are able to insert, making uniform application of essential oils to plant surfaces difficult. In juice form, the potential is much more promising as greater homogeneity of the product is achieved, and the majority of fruit and even some vegetable juice cocktails tend to have high acidities. Low pH in juices not only selects for acid tolerant microbial species, but has also been shown to enhance to the antimicrobial properties of many essential oils. For example, it has been found[54] that essential oils applied in a liquid medium at pH 5 increased lag phase and reduced growth of *L. monocytogenes* more effectively than when administered in broth of pH 6 or 7. Additionally, juices are ideal substrates to which essential oils may be

incorporated because many plant sources of essential oils are traditionally used with commonly consumed juice beverages, such as cinnamon with apple juice or cider.

The use of spices and spice blends in many food products that already contain high levels of similar seasonings has also been examined. Ideally, reengineering of food formulations that contain high levels of spices such as oregano or thyme seasonings could take advantage of the already present sources of essential oils. Unfortunately, some work has shown that spices stimulate the growth and acid production of LAB[55]. In a study[56] seasoned dry sausages were inoculated with three strains of *Lactobacillus plantarum*. Acid production and glucose consumption within sausages was augmented in those utilizing a natural spice blend. However, seasoning with oleoresins derived from the same spices resulted in no change in acid production, indicating that oleoresins had no effect on cellular growth Spices themselves are not likely suitable replacements for concentrated volatile oils. The quantity of seasoning used is typically far too small to contain sufficient quantities of essential oil to confer antimicrobial activity. Effective dosages are likely only realistic for highly seasoned foods like fermented meat or vegetable products, which are coincidentally foods in which viable cultures are desirable. Bacterial contamination of spices and seasonings is a common occurrence as well; as the majority of essential oils are contained within the plant matter

and not on the surface, contaminants may be present on the surface of leaves or stems in spice blends and serve as an inadvertent source of contamination[11]. Utilizing oil components instead of spice blends would circumvent these issues.

1.8- Additives and combinations that enhance essential oil activity

The insoluble nature of essential oils makes delivery of phenolic compounds to the liquid fraction of food products difficult[57]. When essential oils are added to a food matrix, they typically partition off into the lipid portion of the food, leaving the aqueous segment devoid of phenolics. While viable microorganisms can be present in lipid dense zones, growth occurs within the aqueous regions; in a food product, it is feasible that microbial spoilage occurs unchecked despite a high essential oil concentration as the microorganisms are unlikely to come into contact with the lipid-bound phenolic compounds. Adding essential oils to high fat foods generally results in high phenol concentrations in the lipid portion with little present in the aqueous segment of a food. Attempts to solubilize essential oils have been generally unsuccessful, often with the solubilizing agent interfering with the antimicrobial activity of the oils[57]. Inclusion of other additives or processes, however, has been highly successful in improving the activity and efficiency of essential oils within food products. The addition of

humectants, such as salt and sugar, has been shown to improve the antimicrobial action of cinnamon or clove against mold[55].It has been reported[58] that 500 µg/mL cinnamaldehyde was required for inhibition of the food-borne organisms at 30°C, but at 20°C and 25°C the MIC dropped to 400 µg/mL[57] . In another study[57] , eugenol was combined with monolaurin, a GRAS status additive, and sodium citrate for testing against two *Lactobacillus* species and *Leuconostoc mesenteroides* for four days in MRS broth. Monolaurin also exhibits antimicrobial activity against Gram-positive bacteria as well as fungi. When evaluated alone, *L. mesenteroides* proved to be more sensitive than the lactobacilli to the antimicrobial activity of eugenol, especially at a lower temperature of 7°C. Both *Lactobacillus* species were relatively unhindered by the eugenol, although inhibition did appear to increase for one of the species at 7°C. The authors concluded that the most effective inhibition came with the use of all three additives simultaneously; a combination of eugenol at 1000 ppm in conjunction with 0.4% sodium citrate and 250 ppm monolaurin completely inhibited growth of *L. curvatus*. Additionally, sodium citrate at 0.2 or 0.4% significantly inhibited *L. sake* growth with 250 ppm monolaurin at either 500 or 1000 ppm eugenol, while *L. mesenteroides* growth was greatly hindered with 500 ppm eugenol and 100 ppm monolaurin[57].

By understanding and manipulating additive or synergistic relationships among essential oils and other food additives, it is possible that reduced concentrations may be necessary for food preservation, resulting in fewer negative sensory changes occurring in food products. The effect of oregano oil (carvacrol 57.7% and thymol 2.8%) on salted rainbow trout under modified atmosphere conditions has been investigated. Oil was added to salted fish at 0.2% and 0.4% (w/v), then placed under modified atmosphere conditions (45% CO2/5% O2/50% N2) and stored for 21 days under refrigerated conditions. The study found that LAB were significantly inhibited by the essential oil/salt/MAP conditions with a log reduction of approximately 2.6 CFU/g at both concentrations. Unfortunately, a trained sensory panel scored the odor of the treated, cooked samples as unacceptable because of off-odors.

The chemical composition and physical characteristics of meat makes it a suitable environment for bacterial growth, which includes species such as LAB, *Pseudomonas*, and a host of foodborne pathogens. LAB spoilage in meats is a relevant problem as they are facultative anaerobes that can grow and continue to spoil foods under chilled conditions[59,60] . It has been shown that the more complex a food matrix is, especially in items like meats that are high in fat and protein, the less effective essential oil activity is against resident bacteria[55,61,62] . Fresh strips of chicken breast meat have been treated[59] with an

agar slurry solution containing 0.5% thyme and balm essential oils for 15 min. Samples were stored for 21 days at 4°C. Thyme was incredibly effective at controlling LAB growth for the duration of the study; 21 day counts were only 0.8 x 103 CFU/mL, which was consistent throughout the entire 3 weeks. The antibacterial effect of balm oil was much less evident until the day 21, with balm oil closely matching the untreated control up until that point. *Salmonella* on the treated chicken was very sensitive to the activity of balm oil, while thyme oil very effectively reduced growth of *E. coli*.

The use of plant essential oils is predominantly restricted by sensory acceptability as essential oils have „Generally Recognized as Safe‟ (GRAS) status63,64,65,61. To circumvent this issue, which may include changes in flavor, astringency or

appearance, packaging materials and films may be utilized to aid in the impartation of essential oil antimicrobial benefits without negative flavor profile modification[61]. One study[61] attempted varied levels of oregano oil (which is naturally high in carvacrol) into whey protein films prior to covering beef cuts. In a 12 day trial, lactic acid bacteria on product covered by films containing no oregano oil increased nearly 5 log CFU/cm2, while organisms on beef covered by films containing 1.5% oregano oil increased less than 1 log CFU/cm2 . Essential oils have also been considered for potential application in modified atmosphere conditions. Modified

atmosphere packaged (MAP) and vacuum-packaged (VP) products are often selective for *Lactobacillus* or *Leuconostoc* species, making these organisms the primary source of spoilage in such conditions In one study[66] a broad spectrum of yeast and bacteria under the volatilized gas phases of cinnamon and clove oils have been incubated. The oils, at a 1:1 ratio, were effective against all microorganisms, especially *Pediococcus halophilus* (recently reclassified as *Tetragenococcus halophilus*). The inhibitory period increased when oxygen was reduced, and further increased when CO2 levels rose. The most significant inhibition was evident when low oxygen was used in conjunction with high CO2 levels. In fact, under <0.05% oxygen and 40% CO2, *P. halophilus* growth was delayed for 38 days on MRS agar[67,68]. Work with oregano oil in modified atmosphere packaged and vacuum-packaged food showed that organism selectivity as well as essential oil inhibition occurred[69]. Oregano oil was found to have less inhibitory potential in aerobically packaged products, as well as in MAP or VP foods with highly oxygen permeable films. However, lactic acid bacteria along with *Salmonella Typhimurium* were found to be highly susceptible to an oil concentration of 0.8% w/v regardless of atmosphere or film permeability[69].

1.9 -Extraction of oils

Essential oils have high liquor segments. Thus, it has a higher instability and a quick vanishing rate. Keeping in mind the end goal to get the best quality and amount of essential oils, extraction methodology appears to hold the key controlling step. Elements worth considering in the extraction of essential oils are sorts of plant, compound constituents of oils, area of oils inside of the plant i.e. root, bark, wood, branch, leaf, blossom, foods grown from the ground and picking the right extraction strategy[70]. Some plants like rose and jasmine contain minute essential oil. Their significant sweet-smelling properties are separated utilizing a compound dissolvable. The deciding item, known as a flat out, contains essential oil alongside other plant constituents[70]. The estimation of the fresher handling strategies depends significantly on the experience of the distiller. Every strategy is vital, and has its place in aromatherapy grade of essential oils[71].

1.9.1 -Solvent Extraction

A hydrocarbon dissolvable is added to the plant material to disintegrate the fundamental oil. At the point when the mixture is sieved and thought by refining, a substance contains gum (resinoid), or a blend of wax and key oil known as solid remains. From the concentrate, immaculate liquor is utilized to remove the oil. At the point when the liquor vanishes, the oil is

deserted. This is not viewed as the best strategy for extraction as the solvents can leave a little measure of buildup behind which could cause allergies and affect the immune system[71].

1.9.2- Maceration Method

Maceration really makes a greater amount of imbued oil instead of an essential Oil. This straightforward broadly utilized method includes leaving the pounded plant to absorb a suitable dissolvable in a shut compartment. Basic maceration is performed at room temperature by blending the ground plant with the dissolvable and leaving the blend for a few days with periodic shaking or mixing. The procedure is rehashed for more than one occasion with new dissolvable. Ultimately the last deposit of concentrate is squeezed out of the plant particles utilizing a mechanical press or an axis. The technique is suitable for both introductory and mass extraction. The fundamental hindrance of maceration is that the procedure can be very tedious, taking from a couple of hours up to a few weeks and some of the time the likelihood of changing the structure of the oil[72] .

1.9.3 -Cold Pressing

This strategy is utilized to remove the Essential Oils from citrus peels, for example, orange, lemon, grapefruit and bergamot. This strategy includes the straightforward squeezing of the peels at around 49 °C to extricate the oil. The peels are isolated from

the organic product, ground or hacked and after that squeezed. The outcome is a watery blend of crucial oil. The outcome will separate given time by virtue of differences in densities. Little adjustment from the oil" s unique state happens and these citrus oils hold their brilliant, crisp, inspiring fragrances. The downside of this technique is that, the oils removed have a moderately short shelf-life[72].

1.9.4 --Effleurage Method

This is one of the conventional methods for separating oil from blooms. The procedure includes layering fat over the blossom petals. After the fat has assimilated the key oils, liquor is utilized to discrete and removes the oils from the fat. The liquor is then vanished and the Essential Oil is gathered[72].

1.9.5- Super critical CO_2 extraction

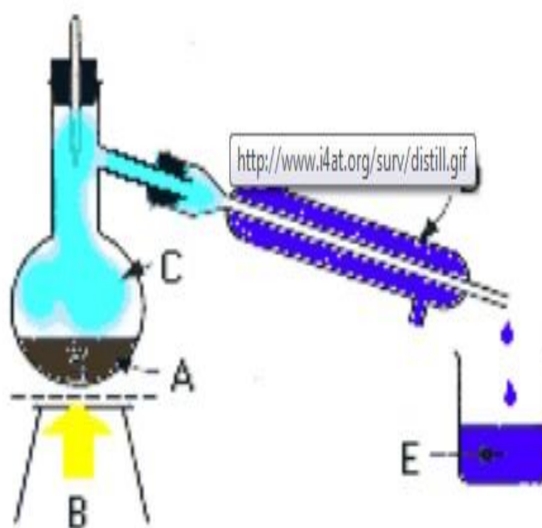
Supercritical CO_2 extraction includes carbon dioxide warmed to

30.6 °C and pumped through the plant material at around 551.58 bars, under these conditions the carbon dioxide is contrasted with a "thick haze" or vapor. With the arrival of the weight in either prepare, the carbon dioxide escapes in its vaporous structure, deserting the Essential Oil. The typical strategy for extraction is through steam refining. After extraction, the properties of decent quality crucial oil ought to be as close as could allowed to the pith of the first plant. The way to decent fundamental oil is through low weight and low temperature

handling. High temperatures, quick handling and the utilization of solvents change the sub-atomic structure, will annihilate the helpful esteem and modify the scent[72].

1.9.6- Water Distillation

In this strategy, the material is totally submerged in water, which is boiled by applying heat by direct fire, steam coat, shut steam coat, shut steam loop or open steam curl as shown in figure 2.1 below.



The procedure is that, there is immediate contact between boiling water and plant material. When the still is warmed by direct fire, sufficient safety measures are important to keep the charge from overheating. When a steam coat or shut steam loop is utilized, there is less peril of overheating. In any case, with open steam, care must be taken to counteract gathering of dense

water inside of the still. In this way, the still ought to be all around protected. The plant material in the still should be upset as the water boils, generally collections of thick material will settle on the base and turn out to be thermally debased. Certain plant materials like cinnamon bark, which are rich in adhesive, must be powdered so that the charge can promptly scatter in the water; as the temperature of the water increases, the separation occurs and the rest settles at the bottom of the still. This enormously builds the consistency of the water charge blend, permitting it to boil. Before any field refining is done, small- scale water refining in a dish is performed to find out whether any advances happen amid the refining process. From this trial, the yield of oil from a known weight of the plant material can be resolved. Amid water refining, all parts of the plant charge must be kept in movement by boiling water; this is feasible when the refining material is charged freely and stays free in the boiling water. Thus, just water refining has one particular point of interest, i.e. it permits processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate.

1.9.7- Turbo distillation extraction

Turbo refining is suitable for difficult to separate or coarse plant material, for example, bark, roots, and seeds of plants. In this

procedure, the plants absorb water and steam is coursed through this plant and water blend. All through the whole process, the same water is consistently reused through the plant material. This technique permits quicker extraction of fundamental oils from difficult to concentrate plant materials[72].

1.9.8- Steam distillations method

As the name implies, direct steam refining is the procedure of refining plant material with steam produced outside the still in a satellite steam generator. With the direct steam refining technique, the plant material is bolstered on a punctured framework over the steam gulf. A genuine reason for preference of satellite steam generator is that the quantity of steam can be promptly controlled. Since steam is produced in a satellite heater, the plant material is warmed to around 100°C and, thus, it ought not to experience warm corruption. The steam which then contains the fundamental oil is passed through a cooling system to condense the steam, which form a fluid from which the essential oil and water is then separated. Direct steam refining (DSD) is the most broadly acknowledged procedure for the generation of crucial oils on substantial scale. In the flavor and fragrance supply business, the direct steam distillation method is a standard practice since this method does not change the composition of the oil. A conspicuous disadvantage to steam distillation method is the much higher capital cost expected to

construct such a facility. The cost of essential oils such as Rosemary, Chinese cedarwood, lemongrass, litsea cubeba, Spike Lavender, Eucalyptus, citronella, cornmint, across the world are sufficiently high to legitimize their generation by steam distillation method .

1.10- Uses of essential oils

Essential oils have been utilized for a large number of years in different societies for restorative and wellbeing purposes. Essential oil utilization ranges from fragrance based treatment, family unit cleaning items, individual excellence consideration and regular drug medicines. The particles in essential oils originate from refining or separating the diverse parts of plants, including the blooms, leaves, bark, roots, sap and peels. In old times, Jews and Egyptians made essential oils by absorbing the plants oil and afterward separating the oil through a material sack.

1.10.1 Importance of essential oil in pharmaceuticals

Essential Oils have flexible applications in pharmaceutics. The germicide properties of Essential Oil make them dynamic against extensive variety of microorganisms as anti-microbial safe strains. Notwithstanding this they are used likewise against parasites and yeasts. The most well-known wellsprings of essential oils utilized as cleaning agents seem to be: Cinnamon, thyme, clover, eucalyptus, culinsavory, and lavender. citral,

geraniol, linalool and thymol are considerably stronger than phenol. At the point when utilized remotely, essential oils (L"essence de terebenthine) expand microcirculation and give a slight neighborhood sedative activity. Till now, essential oils are utilized as part of various treatments. They are known not exceptionally to be viable in diminishing sprains and other articular agonies. Oral administration of essential oils like eucalyptus or pin oils, arouse ciliated epithelial cells to emit bodily fluid. On the renal system, these are known to increase vasodilation and in consequence bring about diuretic effect[72].

Fundamental oils from the Umbellifereae family, Mentha species and verbena are alleged to diminish or dispense with gastrointestinal fits. These essential oils expand discharge of gastric juices. In different cases, they are known to be powerful against sleep deprivation[72].

1.11 Biological activities of essential oils

1.11.1 Anticancer properties

A very promising feld of treatment with EOs is their application against tumors. Especially since the 1990s the anticancer properties of EOs and/or their main constituents and/or metabolites have gained more and more interest, inasmuch as such a “natural” therapy is accepted all over the world by the patients. One of the most prominent compounds in that sense is either *d*-limonene, the main constituent of the EO of sweet

orange peel oil (*Citrus sinensis*, Rutaceae) as well as of other citrus fruit peel oils, or perillyl alcohol, the most important metabolite of this monoterpene hydrocarbon. Perillyl alcohol has been developed as a clinical candidate at the National Cancer Institute because of its greater potency than limonene, which may enable potentially effective systemic concentrations of the active principles to be achieved at considerably lower doses[73]. Perillyl alcohol is effective as an inhibitor of farnesyl transferase. In the early developmental stages of mouse lung carcinogenesis the *ras*-protein undergoes a series of modifcations, and farnesylation at the cysteine is one of these, which leads to the anchoring of *ras*-p-21-gen to the plasma membrane in its biologically active state. Perillyl alcohol administered to test mice showed a 22% reduction in tumor incidence and a 58% reduction in tumor multiplicity[74]. Perillyl alcohol reduced the growth of hamster pancreatic tumors (>50% of the controls), or even led to a complete regression (16%). Thus, perillyl alcohol may be an effective chemotherapeutic agent for human pancreatic cancer[75,76] . Perillyl alcohol also inhibited signifcantly the incidence (percentage of animals with tumors) and multiplicity (tumor/animals) of invasive adenocarcinomas of the colon and exhibited increased apoptosis of the tumor cells. Scientists from the Purdue University report that the rate of apoptosis is over sixfold higher in perillyl alcohol-treated pancreatic adenocarcinoma cells than in

untreated cells, and that the effect of perillyl alcohol on pancreatic tumor cells is signifcantly greater than its effect on nonmalignant pancreatic ductal cells[77]. Moreover, this monoterpene alcohol-induced increase in apoptosis in all of the pancreatic tumor cells is associated with a 2–8-fold increase in the expression of a proapoptotic protein which preferentially stimulates the apoptosis in malignant cells. Perillyl alcohol is also effective in reducing liver tumor growth. Two weeks after diethyl nitrosamine exposure was discontinued, the animals were divided into perillyl alcohol-treated and untreated groups. The mean liver tumor weight for the perillyl alcohol-treated rats of perillyl alcohol treatment was 10-fold less than that for the untreated animals[78]. A newer study found that this monoterpene alcohol potentially attenuates ferric-nitrilo-acetate-induced oxidative damage and tumor promotional events[79]. Monoterpenes such as *d*-limonene and perillyl alcohol, as well as other terpene alcohols, such as geraniol, carveol, farnesol, nerolidol, b-citronellol, linalool, and menthol, showed inhibitory activities on induced neoplasia of the large bowl and duodenum. Nerolidol, especially, has an impact on the protein prenylation and is able to reduce the adenomas in rats fed with these compounds to an extent of about 82% compared to the controls[80]. Geraniol prevents the growth of cultured tumor cells, especially those of rat hepatomas and melanomas[81]. Dietary geraniol increased the 50% survival time of mice signifcantly

and even 20% of the animals remained free of tumors when fed a geraniolcontaining diet 14 days before an intraperitoneal transfer of the tumor cells[82]. Similar studies indicate that the colon tumors of animals fed with perillyl alcohol exhibited increased apoptosis as compared to those fed the control diet[83]. Therefore, consumption of diets containing fruits and vegetables rich in monoterpenes, such as *d*-limonene, reduces the risk of developing cancer of the colon, mammary gland, liver, pancreas, and lung[84].

1.11.2- The antimicrobial properties

The antimicrobial properties of essential oils and of their constituents have been considered[55,85]and the mechanism of action has been studied in detail[63]. An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable[23]. This can then cause leakage of ions and other cellular molecules[86,87,88,89] . Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death[90]. EOs and/or their constituents can have a single target or multiple targets of their activity. For instance, trans-cinnamaldehyde can inhibit the growth of *Escherichia coli* and *Salmonella typhimirium* without disintegrating the OM or depleting

intracellular ATP. Similar to thymol and carvacrol, trans-cinnamaldehyde likely gains access to the periplasm and deeper portions of the cell[91]. Carvone is also ineffective against the OM and does not affect the cellular ATP pool[92]. It has been reported that EOs containing mainly aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol were characterized by the highest antibacterial activity, followed by EOs containing terpene alcohols. Other EOs, containing ketones or esters, such as β-myrcene, α-thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive[93,94]. Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, have important antibacterial activities[63,93,95] by EOs containing terpene alcohols. Other EOs, containing ketones or esters, such as β-myrcene, α-thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive[93,94]. 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[18,23,96]. The chemical structure of essential oils affects their mode of action concerning their antibacterial activity[94]. The importance of the presence of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, was confirmed[22,94,97]. However, the relative position of the phenolic

hydroxyl group on the ring does not appear to influence the intensity of the antibacterial activity. The action of thymol against *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol, for example[63,89] . However, carvacrol and thymol act differently against Gram-positive and Gram-negative species[95]. 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*[98,99]. Other families of compounds also have valuable antibacterial properties: certain alcohols, aldehydes, and ketones, monoterpene (geraniol, linalol, menthol, terpineol, thujanol, myrcenol, citronelîaî, neral, thujone, camphor, carvone, etc.), phenylpropanes (cinnamaldehyde), and monoterpenes (γ-terpinene, p-cymene). Among these compounds, carvacrol is the most active. Known to be non-toxic, it is used as a preservative and food flavoring in drinks, sweets, and other preparations. It is important to mention that essential oils are more active against Gram-positive than Gram-negative bacteria[100,101,102,103] . The latter are less susceptible to the action of essential oils with the outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide film[102] . Furthermore, the antibacterial activity of essential oils related to their

chemical composition, the proportions of volatile molecules, and their interactions[95,99,103]. An additive effect is observed when the combination is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less important when they are tested together than when used individually[104]. A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects[105]. Some studies have shown that the use of the whole essential oil provides an effect which is greater than that of the major components used together[106]. This suggests that minor components are essential for activity and may have a synergistic effect. It has been reported additive and synergistic effects of the combinations of 1,8-cineole and aromadendrene against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) and *Enterococcus faecalis* by using checkerboard and time-kill assays, respectively[107].The combined effects of plant volatile oils and benzoic acid derivatives against *L. monocytogenes* and *S. enteritidis* are considered as synergistic since the combined components allowed ≥log10 higher inhibition than the sum of the inhibitory effects of the components used separately[108]. Increased antifungal effects were caused by combinations (1:5, 1:7, and 1:9) of essential oils of *S. aromaticum* (clove) and *Rosmarinus officinalis* against *C. albicans*[109]. Moreover[63], reported that, combined, carvacrol and

thymol showed additive effects against *S. aureus* and *P. aeruginosa* by using half-fold dilutions within the Bioscreen plat. Two hypotheses have been proposed to explain synergistic effects of cinnamaldehyde/thymol or cinnamaldehyde/carvacrol against *S. typhimurium*: proving, on one hand, that thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell, and, on the other hand, that thymol or carvacrol could increase the number, size, or duration of the existence of the pores created by the binding of cinnamaldehyde to proteins in the cell membrane[110]. These facts justify a synergistic effect achieved when these two components are used in combination. Mechanisms of interaction that produced antagonistic effects were less studied[111]. In addition, essential oils have also revealed to be effective on the inhibition of growth and reduction in numbers of the more serious foodborne pathogens, such as *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes*[108].

1.11.3-AntioxidantActivity

Numerous studies have demonstrated the antioxidant properties of essential oils. The antioxidant potential of an essential oil depends on its composition. It is well established that phenolics and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties[112]. Most of the essential oils are dominated by oxygenated monoterpenes such

as alcohols (*Achillea filipendulina*), aldehydes (*Galagania fragrantissima*), ketones (*Anethum graveolens*, *Artemisia rutifolia*, *Hyssopus seravschanicus*, *Mentha longifolia*, and *Ziziphora clinopodioides*), and esters (*Salvia sclarea*). *Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons, whereas phenolic terpenoids, such as thymol or carvacrol, characterize *Origanum tyttanthum* and *Mentha longifolia* EOs, which would explain why both plants exhibited generally the strongest antioxidant activity. Thymol and carvacrol, which are predominant in *Origanum tyttanthum*, are also responsible for the antioxidant activity of several other essential oils, such as *Mentha longifolia* and *Thymus serpyllus*[113]. The essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties[110]. 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[107]. The antioxidant activity of essential oils is also due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-cineoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: α-terpinene, β-terpinene and α-terpinolene110.

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[110,111]. EOs have shown their action as hepatoprotective agents in ageing polyunsaturated fatty acids mammals and it has been proved that they possess a beneficial impact upon the PUFAs, in particular the long chain C20 and C22 acids[114]. Moreover, essential oils being able to scavenge free radicals may also play an important role in some disease prevention, such as brain dysfunction disease, and immune system decline[115].

1.11.4 Anti-Inflammatory Activity

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self cells) and to remove dead or damaged host cells. The inflammatory response induces an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines, such as interleukins and tumor necrosis factor-α (TNF-α). It also stimulates the activity of several enzymes (oxygenases, nitric oxide synthases, peroxidases, etc.), as well as the arachidonic acid metabolism. Recently, essential oils have been used in clinical settings to treat inflammatory diseases, such as rheumatism, allergies, or arthritis[111]. *Melaleuca*

alternifolia EO was reported to have a considerable anti-inflammatory activity[112,113,114] .This activity is correlated with its major compound: α-terpineol115. The active compounds act by inhibiting the release of histamine or reducing the production of inflammation mediators. Geranium essential oil is another example[111]. Linalool and linalyl acetate showed anti-inflammatory activity on oedema of paw-induced mouse carrageenan[116,117]. The oil of *Torreya nucifera*[118] , mainly constituted by limonene, δ-3-carene, and α-pinene, have an inhibitory effect on COX-2, thus inducing a significant inhibitory effect on prostaglandin (PGE2) production. Furthermore, 1,8-cineole, present in many essential oils, was reported as an inhibitor of leukotrienes (LTB4) and PGE2, biogenerated both from pathways of arachidonic acid metabolism[118]. The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. Essential oils, therefore, represent a new option in the treatment of inflammatory diseases.

1.11.5 -Cancer chemoprotective activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researchers for their potential activity against cancer. Essential oils would act in the prevention

of cancer, as well as at its removal. It is well known that certain foods, such as garlic and turmeric, are good sources of anticancer agents[119]. Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer[120,121] . Diallylsulfide, diallyldisulfide, and diallyltrisulfide are examples. According to[122], these compounds activate, in rats, the enzymes involved in the detoxification process of hepatic phase 1 (disintegration of chemical bonds that link carcinogenic toxins to each other) and phase 2 (bonds to toxins released detoxifying enzymes, such as glutathionebS-transferase). Metabolism happens mainly in the liver, the body‟ s largest internal organ. The portal vein carries blood from the small intestine directly to the liver. Sixty percent of liver tissue is made up of hepatic cells. More chemical processes happen in these than in any other group of cells in the body. Phase 1 metabolism involves chemical reactions, such as oxidation (most common), reduction, and hydrolysis. There are three possible results of phase 1 metabolism. The drug becomes completely inactive. In other words, the metabolites are pharmacologically inactive. One or more of the metabolites are pharmacologically active, but less so than the original drug. The original substance is not pharmacologically active, but one of its metabolites is. The original substance is called a prodrug. Phase 2 metabolism involves reactions that chemically change the drug or phase 1

metabolites into compounds that are soluble enough to be excreted in urine. In these reactions, the molecule (drug or metabolite) is attached to an ionisable grouping. This is called conjugation and the product is called a conjugate. Metabolites formed in phase 2 are unlikely to be pharmacologically active. Some drugs undergo either phase 1 or phase 2 metabolism, but most undergo phase 1 metabolism followed by phase 2 metabolism. Another example is myristicin, an allylbenzene present on a certain essential oil, especially that of nutmeg (*Myristica fragrans*). This molecule is known to activate glutathione *S*-transferase in mice[123] and inhibit carcinogenesis induced by benzo(a)pyrene in the lungs of mice [58]. Recently, it has been discovered that myristicin induces apoptosis in neuroblastoma (SK-N-SH) in humans[124]. There are other volatile compounds that showed a cytotoxic activity against various cancer cell lines[109]. Geraniol decreases the resistance of colon cancer cells (TC118) to 5-fluorouracil, an anticancer agent. Therefore, geraniol enhances this inhibitory effect of tumour growth 5-fluorouracil[125,126] . The essential oil of balsam fir and α-humulene, showed significant anticancer activity in several cell lines and low toxicity to healthy cells[127]. In addition, anticancer activity of D-limonene, the main component of Citrus essential oil has been proven, especially at the level of stomach cancer and liver[128]. The α-bisabolol, an abundant sesquiterpene alcohol in chamomile essential oil (*Matricaria*), has an

antigliomale activity[129]. Many essential oils have a cytotoxic activity namely *Melissa officinalis*[130], *Melaleuca alternifolia*[131], *Artemisia annua*[132] , and *Comptonia peregrina*[133] .

1.11.6 Cytotoxicity

Due to their complex chemical composition, essential oils have no specific cellular ligands[134]. As lipophilic mixtures, they are able to cross the cell membrane and degrade the layers of polysaccharides, phospholipids and fatty acids, and permeabilize. This cytotoxicity appears to include such membrane damage. In bacteria, the membrane permeabilization is associated with the loss of ions and the reduction of the membrane potential, the collapse of the proton pump and the depletion of the ATP pool[89,135,136]. Essential oils may coagulate the cytoplasm[63] and damage lipids and proteins[89,106] . Damage to the wall and the cell membrane can lead to the leakage of macromolecules
and lysis[63,87,137]. In addition, essential oils change membrane fluidity, which becomes abnormally permeable, resulting in a leakage of radicals, cytochrome C, the Ca^{2+} ions, and proteins, like in the case of oxidative stress. This permeabilization of the outer and inner membranes causes cell death by apoptosis and necrosis[138,139]. Ultrastructural alteration of the cell can be observed at a plurality of compartments[118,140,141]. The interruption of the viral envelope herpes simplex virus HSV by

essential oils can also be observed by electron microscopy[142]. The induction of membrane damage was also confirmed by an analysis showing that microtubule *Saccharomyces cerevisiae* genes involved in the biosynthesis of ergosterol, the absorption of sterols, lipid metabolism, the structure and function of cell wall cellular detoxification, and transport are affected by treatment with α-terpinene[143]. Recent work on the yeast *Saccharomyces cerevisiae*, has shown that the cytotoxicity of some essential oils based on the ability to form colonies differs significantly in relation to their chemical composition. Generally, essential oil cytotoxicity mainly correlates to the presence of phenols, alcohols, and monoterpene aldehydes[144,145]. The cytotoxic properties of essential oils are of great importance because they assume their use not only against certain human pathogens and animal parasites, but also in the preservation of agricultural and marine products against microbial attack. Indeed, some components of essential oils are effective against a variety of microorganisms as bacteria[146], viruses147, fungi143,148,149,150 , protozoa151, parasites152,153 , mites,

and others. In addition, α-humulene shows cytotoxicity against breast cancer cells in vitro. α-humulene was reported to be responsible for cytotoxicity (CI50 55 mM)[154] . It induced a dose- and time-dependent decrease in cellular glutathione (GSH) content and an increase in reactive oxygen species (ROS) production. Furthermore[155], focusing on the effects of

carvacrol, one of the main compounds in the EO of oregano, on the DNA synthesis of *N*-ras transformed mouse myoblast CO25 cells, finding that this monoterpenic phenol was able to inhibit the DNA synthesis in the growth medium and ras-activating medium, which contained dexamethasone. They proposed that it may be valuable in cancer therapy because of its growth inhibition of myoblast cells, even after activation of mutated *N*-ras-oncogene. The EO of the Anonaceae *Xylopia aethiopica* (Ethiopian pepper), a plant grown in Nigeria, showed, at a concentration of 5 mg/mL, a cytotoxic effect in the carcinoma cell line (Hep-2)[156] . Moreover[157], tested the essential oil of the rhizome of the *Aristolochiaceae Aristolochia mollissima* for its cytotoxicity on four human cancer cell lines (ACHN, Bel-7402, Hep G2, HeLa). The rhizome oil possessed a significantly greater cytotoxic effect on these cell lines than the oil extracted from the aerial plant. Linalool inhibited only moderate cell proliferation; however, in subtoxic concentrations potentiates doxorubicin-induced cytotoxicity and proapoptotic effects in both cell lines, MCF7 WT and MCF7 AdrR. This monoterpene improves the therapeutic index in the management of breast cancer, especially multidrug resistance (MDR) tumors[158]. An in vitro cytotoxicity assay indicated that the EO of *Cyperus rotundus* (Cyperaceae) characterized by the predominance of cyperene, α-cyperone, isolongifolen-5-one, rotundene, and cyperorotundene, was very effective against

L1210 leukemia cells, which correlates with significantly increased apoptotic DNA fragmentation[159].

1.12- The studied plant species

1.12.1-*Indigofera arrecta*

Indigofera arrecta is a perennial growing to 2 m (6ft) at a medium rate. It can fix nitrogen.Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid and neutral soils and can grow in very acidic soils. It cannot grow in the shade. It prefers moist soil.

Indigofera arrecta

The leaves are used in traditional medicine for treating epilepsy and nervous disorders. An aqueous extract of the leaves from immature shoots is administered orally to patients with diabetes mellitus[160]. A medicine for the management of peptic ulcer and methods of its preparation and use have been patented[160]. The leaves are applied externally to heal sores and ulcers[161]. An infusion or decoction of the leaves and roots is abortifacient,

antispasmodic, diuretic, febrifuge, purgative, sedative, stomachic and vermifuge. It is used to treat conditions such as gum infections, snakebites, gonorrhoea, epilepsy and jaundice[1]. The leaves and roots are used externally to treat itching[1]. The fruits and seeds are used to treat ophthalmia[160].

The plant is a major source of the blue dye 'indigo'[161]. The leaves and twigs do not actually contain indigo but colourless precursors that must be extracted and then processed in order to produce the indigo dye[160].

1.12.2-*Indigofera hirsuta*

Indigofera hirsuta (Fabaceae) is commonly known as hairy indigo has several medicinal uses.It is used as chest medicine and in Tanganyika whole plant is prepared as an external application for backache[162] . Whole plant extract is used for injury to the eye ball and inflammation of eye lids; root decoction is used to counteract various poisons[163]. Leaf is used against infant immunity[164] and urinary tract disorders[165].

Indigofera hirsuta

Decoction of leaf is used in case of stomach problems[166] and against diarrhea[167]. *I. hirsuta* leaf methanol and ethanol extracts showed effective activity at 100 µg/ml on *E.coli* and *B.subtilis* than *P.aeruginosa* and *S.aureus*, which also supports the presence of flavonoids like rutin, quercetin and kaempferol, and phenols like protocatecheuic acid, chlorogenic acid, trans-p-coumaric acid, caffeic acid, cis-p-coumaric acid, P-hydroxybenzoic acid, coumarin and cinnamic acid[168]. Fruits of *I. hirsuta* consist of nearly 14 phytoconstituents mainly in aqueous, methanol, alcohol, ethyl acetate and chloroform extracts. Fixed oils are totally absent. Antibacterial activity against selected four bacterial strains was very effective than the control drug ampicillin and the MIC values ranges from 0.019 mg to 0.312 mg. Hence *I. hirsuta* proved its herbal usage against diarrohea, chest and body pains, infant immunity and skin diseases[169] and this is due to the presence of various bioactive constituents like alkaloids, tannins, lignins, phenols, flavonoids, terpenoids and glycosides. *I.hirsuta* leaf, fruit alcohol and methanol extracts showed effective anthelmintic activity than the control drug Albendazole[170]

Indigofera is a large genus of about 700 species of flowering plants that belongs to the Fabaceae family. It is commonly known as hairy Indigo. It originated from Africa and Asia but now widespread and naturalized in Australia and Southern Asia[171,172].. It is used as chest medicine; leaves are used to boost

immunity in infant, for urinary tract infections and for the treatment of impotency and weak erection. The leaves are used against diarrhoea and stomach problems. Whole plant paste is applied as an external application for backache, for eye ball injury and inflammation of eye lids[173,174]. It was reported that I. *hirsuta* leaves extracts has antibacterial activity against *E. coli, B. subtilis, Ps. aeruginosa, S. aureus*[174].

1.12.3-*Lagenaria siceraria*

Lagenaria siceraria is a vigorous, annual, running or climbing vine with large leaves and a lush appearance. It grows fast and *may begin to flower only 2 months after seeding. The thick stem* is furrowed longitudinally. The vine is branched and climbs by means of tendrils along the stem. *Lagenaria siceraria* commonly known as bottle gourd is official in Ayurvedic Pharmacopoeia. It is one of the excellent fruit for human having composition of all the essential constituents that are required for normal and good human health. *Lagenaria siceraria* fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion strings, alternative purgative, cooling effects. It cures pain, ulcers and fever and used for pectoral cough, asthma and other bronchial disorders-especially syrup prepared from the tender fruits[175,176,177]. The pulp of the fruit is considered cool,

diuretic, and useful in coughs and as antidote to certain poisions[176,177].

The tribal communities in some African countries use the dry hard shells of bottle gourd fruits for various purposes. The plant is used traditionally as a cure for headache (external application) by mixing the seed oil with castor oil. The pulp of the fruit is considered cool and diuretic[176,178]. Leaves of *Lagenaria siceraria* are emetic.

Lagenaria siceraria

Crushed leaves are used for baldness and applied on the head for the headache. Leaves are also used as alternative purgative[179]. Fruit juice is traditionally claimed to cure various diseases including flatulence, diabetes mellitus, hypertension, liver diseases, and as a diuretic. The seeds of this crop are rich in essential amino acids and oil.

1.12.4-*Fallopia convolvulus*

Fallopia convolvulus is a troublesome summer annual that twines around and drags down both cereals and root crops[180].

There is evidence that this plant was a weed of crops in the Bronze Age[181]. It was frequently associated with cereals[182]

Fallopia convolvulus

1.12.5- *Dicoma tomentosa*

Dicoma is a genus of approximately 35 species in the family Compositeae[183].Most of these species are small shrubs or even trees and they may grow in diverse habitats including deserts[184]. *Dicoma tomentosa* is distributed in tropical Africa and Asia.In African system of medicine the plant is used against malaria[185]. *Dicoma tomentosa* contains sesquiterpenes[186,187,188], sterols and triterpenes[190,191] . It also contains some flavonoids[192,193]

Dicoma tomentosa

Dicoma tomentosa is used by some communities as tooth cleaner [194]. It is used traditionally against wounds and as febrifuge[195].The antiplasmodial activity of *Dicoma tomentosa* has been reported[196,197,198].

1.12.6-*Carica papya*

Carica papya is a large perennial plant in the family Caricaceae[199].This plant is cultivated in tropical and subtropical regions for its economic and medicinal importance[200]. All parts of the plant(leaves,roots,peel,flowers and seeds) are used as natural remedy against a wide array of ailments[201].Fruit has a nutritional value. It contains vitamins, mineals,enzymes, proteins, polysaccharides beside sterols, flavonoids, alkaloids and saponins [202,203].

Carica papya

Carica papya has been used traditionally against many diseases including heart diseases, low sperm count, kidney failure and uterus fibroid[204].Leaves are rich in flavonoids and alkaloids.They are traditionally used as hypoglycaemic, antiinflammatory, hepatoprotective, antihypertensive, antiviral, antimalarial and anticancer[205,206,207].In vitro and in vivo studies demonstrated that the leaves possess antiinflammatory, antioxidant, antiplasmodial, antibacterial , antidenge and anti cancer effect[208].Flowers are emmengogue, febrifuge and are used in ethnomedicine against jaundice,hypertension, intestinal helminthiasis,malaria, diabetes and cancer[209,210,211].Flowers are used traditionally as dressing for wounds.

Seeds have some pharmacological activities including antiinflammatory, antimicrobial, anthelmintic, contraceotive and analgesic effects[212].Seeds are fermifuge, pain alleviator and thirst quencher.They are used by local healers for hypertension,

hypercholesterolemia, diabetes and intestinal worms[209,213].Peel extracts exhibited a range of biological activities including antibacterial, antioxidant and anticancer activites[214,215,216].Root is a remedy for typhoid fever, wounds, urethritis, gastroenteritis, abdominal pain and pneumonia[217,218].

Aim of this study

This study was carried out to:

-Extract oils from six medicinal plants used in Sudanese ethnomedicine.

-Identify and quantify the constituents of the oils by GC-MS analysis.

-Evaluate the studied oils for antimicrobial activity.

2 Materials and Methods

2.1 Materials

2.1.1 Plant materials

Seeds of (*Indigofera arrect, Indigoferra hirsutal, Lagenaria siceraria, Fallopia conuolulus, Dicoma tonentoza and Carica papaya*) were collected from Damazin – Sudan. The plants were identified and authenticated by The Medicinal and Aromatic Plants Research Institute – Khartoum – Sudan.

2.1.2 Instruments

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

2.1.3 Test organisms

The following test organisms used in this study: *Bacillus subtillis,* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

2.2 Methods

2.2.1 Extraction of oil

Powdered plant material (300 g) were macerated with n- hexane for 48h. The solvent was removed under reduced pressure giving the oil.

2.2.2 Gas chromatography-Mass spectroscopy analysis

(2 mL) of the oil was mixed thoroughly with 7 mL of alcoholic sodium hydroxide that was prepared by dissolving 2g in 100 mL methanol. (7 mL) alcoholic sulfuric acid (1 mL H_2SO_4 in 100 mL methanol) was added. The mixture was then shacked for 5 minutes. The content of the test tube was left to stand overnight. Then (1 mL) of supersaturated sodium chloride was added and the tube was shaken for 5 min. (2 mL) of normal hexane were added and the contents were shaken thoroughly for 5 minutes. (5µL) of the n-hexane were diluted with (5 mL) of diethyl ether and dried over anhydrous sodium sulphite. (1 µL) of the diluted sample was injected in the GC-MS vial.

The qualitative and quantitative analysis of the sample was carried out by using a Shimadzu machine- model (GC/MS-QP2010-Ultra). The sample was injected under the following chromatographic conditions: column oven temperature: 150.0ºC ; injection temperature: 300ºC ; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow 50.0 mL/min; column flow 1.54 mL/sec.; linear velocity: 47.2 cm/sec. ; purge flow: 3 mL/min. ; split ratio: -1.0. Oven temperature program is presented in table 1.

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

Table 2.1 : Oven temperature program

2.2.3 Antimicrobial assay

The paper disc diffusion method was used to screen the antibacterial activity of the oil and performed by using Mueller Henton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999) with some minor modifications. Bacterial suspension was diluted with sterile physiological solution to 10^8 mL (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper disc (Whitman No. 1, 6 mm in diameter) were placed on the surface of MHA and soaked with 20 µL of a solution of test sample. The inoculated plates were incubated at 37ºC for 24h in the inverted position. The diameters (mm) of the inhibition zone were measured and recorded as average of two replicates.

3-Results and Discussion

In this study the oils from six plants of medicinal medicinal attributes (*Indigofera arrect*, *Indigofera hirsutal*, *Lagenaria siceraria*, *Fallopia conuolulus* , *Dicoma tomentoza and Carica papya*) have been investigated by GC.MS and the antimicrobial activity has been screened.

3.1-*Indigofera arrect*

3.1.1-GC/MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Indigofera arrect* oil. The analysis revealed the presence of 16 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

Fig. 3.1: Total ion chromatograms

Table 3.1: Constituent of the oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.201	0.37
2.	Pentadecanoic acid, methyl ester	15.329	0.09
3.	Hexadecanoic acid, methyl ester	16.410	34.14
4.	Hexadecanoic acid, ethyl ester	17.105	1.49
5.	Heptadecanoic acid, methyl ester	17.441	0.18
6.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.165	31.76
7.	9-Octadecenoic acid (Z)-, methyl ester	18.206	0.86
8.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.244	15.56
9.	Methyl stearate	18.431	7.10
10	Linoleic acid ethyl ester	18.800	1.25
11	Ethyl 9,12,15-octadecatrienoate	18.880	0.60
12	Octadecanoic acid, ethyl ester	19.058	0.45
13	Eicosanoic acid, methyl ester	20.286	1.76
14	Docosanoic acid, methyl ester	21.990	2.18
15	Tricosanoic acid, methyl ester	22.794	0.72
16	Tetracosanoic acid, methyl ester	23.567	1.49

The following compounds were detected in the chromatograms as major constituents:

i) Hexadecanoic acid methyl ester (34.14 %)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (31.76 %)
iii) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (15.56 %)

The GC-MS analysis gave a spectrum characteristic of hexadecanoic acid methyl ester (Fig.3.2).The peak at m/z 270(RT. 16.410) corresponds M^+ $[C_{17}H_{32}O_2]^+$.The analysis also revealed a mass spectrum (Fig. 3.3) identical of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z 294(RT. 18.165)corresponds M^+ $[C_{19}H_{34}O_2]^+$. A Mass spectrum (Fig.3.4) characteristic of 9,12,15-octadecatrienoic acid methyl ester also appeared.The signal at m/z292 is due to : $M^+[C_{19}H_{32}O_2]^+$.

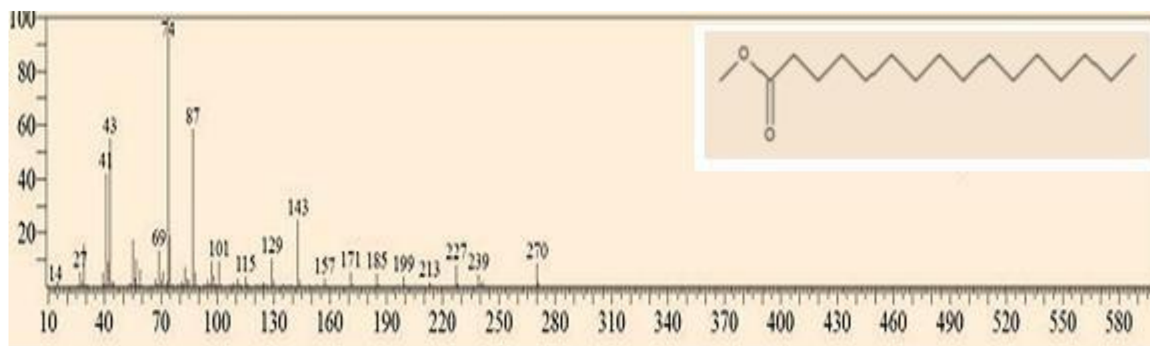


Fig. 3.2: Mass spectrum of hexadecanoic acid, methyl ester

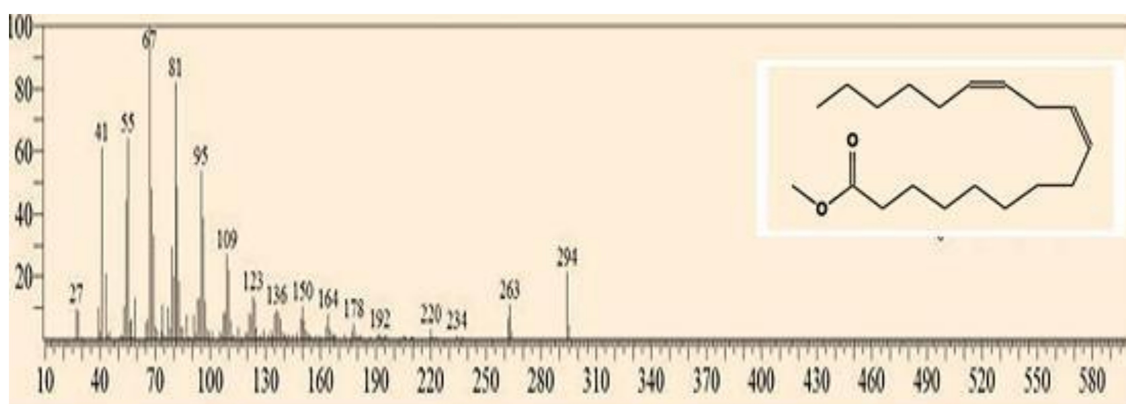


Fig. 3.3 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

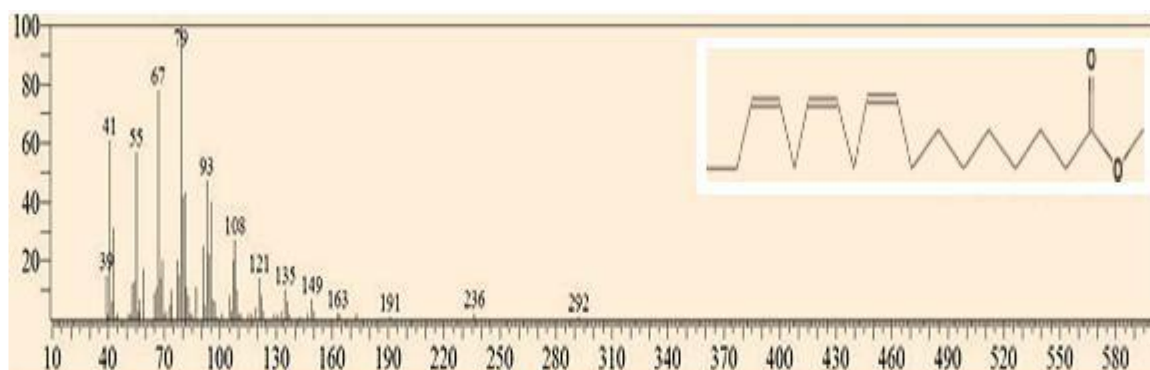


Fig.3.4: Mass spectrum 9,12,15-octadecatrienoic acid methyl ester

3.1.2-Antimicrobial activity

Indigofera arrect oil was screened for antimicrobial activity against five standard organisms. The inhibition zones are presented in Table 3.2. The oil showed significant activity against *Escherichia coli* and moderate activity against *Staphylococcus aureus.* and *Candida albicans*.Tables 3.3 and 3.4 illustrate the antimicrobial activity of standard drugs.

Table 3.2: Inhibtion zones(mm) of the oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil 100mg/ml	14	---	20	13	15

Sa.: Staphylococcus aureus.
Bs.: Bacillus subtilis.
Ec.: Escherichia coli.
Pa.: Pseudomonas aeroginosa.

Ca.: Candida albicans.

Table 3.3 : Inhibition zones of standard antibacterial agents

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

Table 3.4 : Inhibition zone (mm)s of standard antifungal agent

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

3.2-*Indigofera hirsutal*

3.2.1-GC-MS analysis

Gas chromatography - mass spectrometry analysis showed the presence of 19 components - Table (3.5).The total ion chromatogram is presented in Fig.3.5.

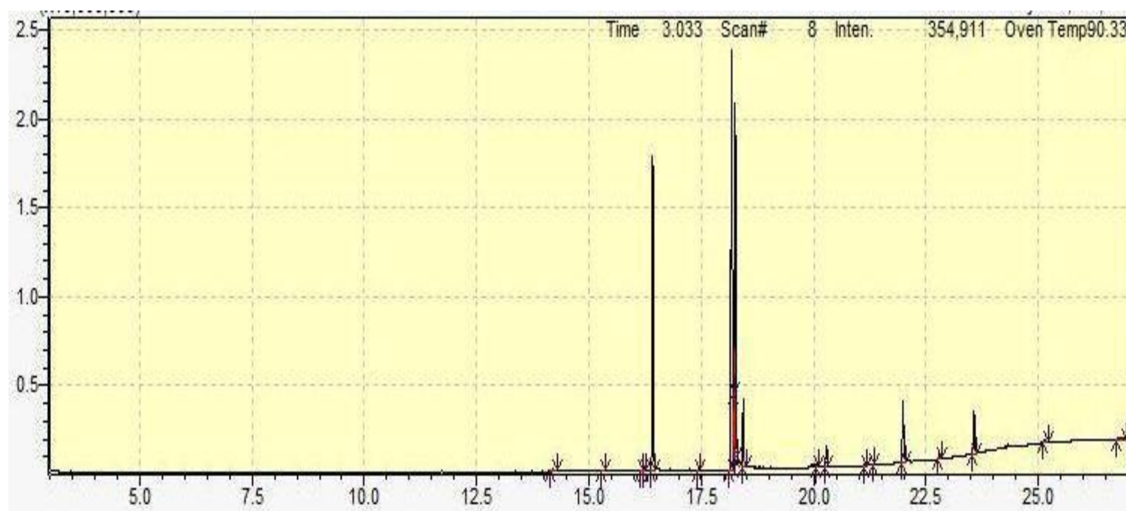


Fig. 3.5 :Total ions chromatograms

Table 3.5 : Constituents of the oil

No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	14.196	0.35
2	Pentadecanoic acid, methyl ester	15.328	0.10
3	7-Hexadecenoic acid, methyl ester, (Z)-	16.185	0.03
4	9-Hexadecenoic acid, methyl ester, (Z)-	16.212	0.10
5	Hexadecanoic acid, methyl ester	16.416	22.49
6	Heptadecanoic acid, methyl ester	17.441	0.26
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.173	32.40
8	9-Octadecenoic acid (Z)-, methyl ester	18.225	1.83
9	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.253	25.63
10	Methyl stearate	18.430	4.58
11	cis-11-Eicosenoic acid, methyl ester	20.078	0.29
12	Eicosanoic acid, methyl ester	20.280	1.46
13	Heneicosanoic acid, methyl ester	21.149	0.20
14	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.328	0.07
15	Docosanoic acid, methyl ester	21.985	5.03

16	Tricosanoic acid, methyl ester	22.788	0.83
17	Tetracosanoic acid, methyl ester	23.563	3.45
18	Hexacosanoic acid, methyl ester	25.134	0.43
19	.beta.-Tocopherol	26.868	0.47

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (32.40 %)

ii) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (25.63 %)

iii)9-Octadecnoic acid methyl ester(22.49%)

The GC-MS analysis showed a mass spectrum(Fig.3.6) characteristic of of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.173)corresponds M^+ $[C_{19}H_{34}O_2]^+$.It also showed a mass spectrum (Fig.3.7) corresponding to 9,12,15-octadecatrienoic acid, methyl ester.The signal at m/z292(RT.18.253) is due to : $M^+[C_{19}H_{32}O_2]^+$. The GC-MS analysis gave a spectrum characteristic of hexadecanoic acid methyl ester (Fig. 3.8).The peak at m/z 270(RT. 16.416) accounts for: M^+ $[C_{17}H_{34}O_2]^+$.

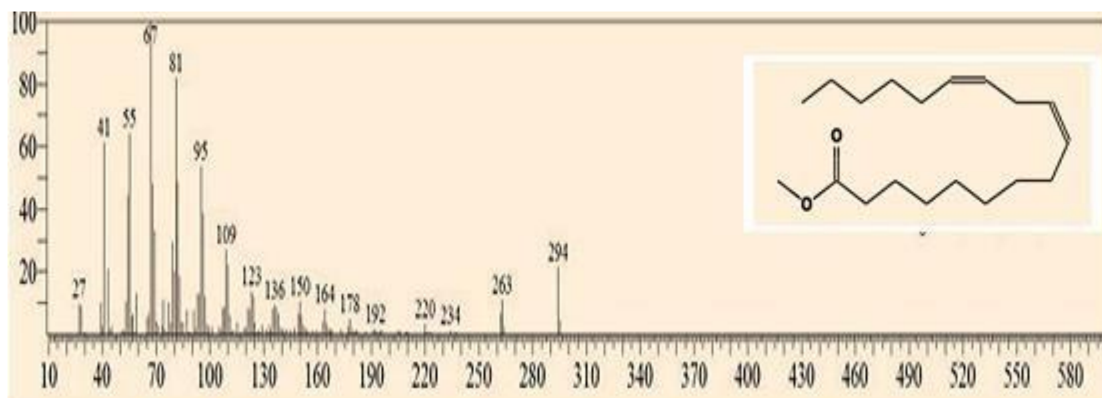


Fig. 3.6 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

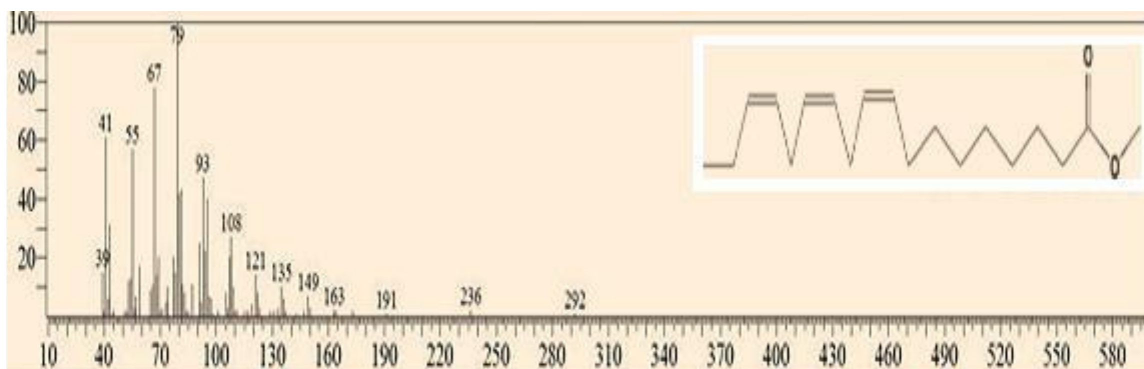


Fig.3.7: Mass spectrum 9,12,15-octadecatrienoic acid methyl ester

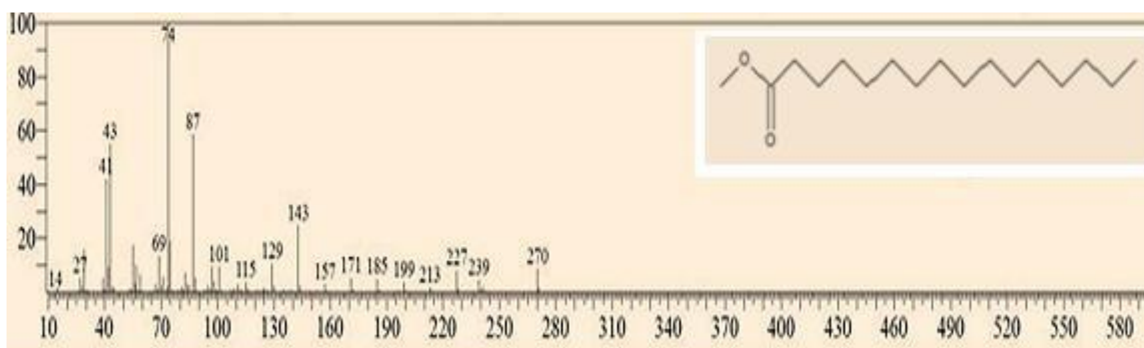


Fig 3.8 : Mass spectrum of hexadecanoic acid, methyl ester

3.2.2-Antimicrobial activity of *Indigofera hirsutal* oil

Indigofera hirsutal oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are presented in Table 3.6. The oil showed significant activity against *Staphylococcus aureus* and *Bacillus subtilis*. .

Table 3.6: Inhibtion zones(mm) of ***Indigofera hirsutal*** oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil 100mg/ml	18	19	--	10	--

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

3.3-*Lagenaria siceraria*

3.3.1-GC-MS analysis

GC/MS was conducted for *Lagenaria siceraria* oil. The analysis revealed the presence of 9 components - Table (3.7).The total ion chromatogram is presented in Fig.3.9.

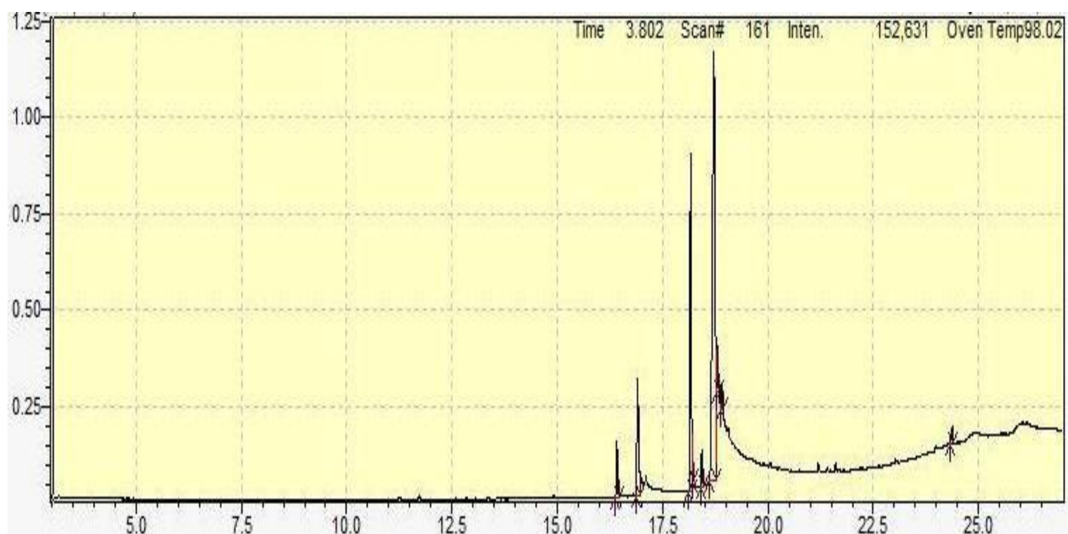


Fig. 3.9 :Total ions chromatograms

Table 3.7 : Constituents of the oil

No.	Name	Ret.Time	Area%
1	Hexadecanoic acid, methyl ester	16.411	3.39
2	n-Hexadecanoic acid	16.908	10.15
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.164	19.56
4	9-Octadecenoic acid (Z)-, methyl ester	18.205	1.43
5	Methyl stearate	18.428	2.02
6	Linoleic acid ethyl ester	18.716	57.96
7	9,11-Octadecadienoic acid, methyl ester, (E,E)-	18.797	3.15
8	Octadecanoic acid	18.891	1.41
9	Squalene	24.354	0.93

The following compounds were detected in the chromatogram as major constituents:

i) Linoleic acid ethyl ester (57.96 %)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (19.56 %)

iii) Hexadecanoic acid (10.15%)

The GC-MS analysis showed a mass spectrum(Fig.3.10) characteristic of linoleic acid ethyl ester.The signal at m/z308(RT. 18.716) is due to $M^+[C_{20}H_{36}O_2]^+$. It also showed a mass spectrum(Fig.3.11) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.164) corresponds M^+ $[C_{19}H_{34}O_2]^+$. The GC-MS analysis gave a spectrum(Fig.3.12) characteristic of hexadecanoic acid .The peak at m/z 256(RT. 16.908) accounts for: M^+ $[C_{16}H_{32}O_2]^+$.

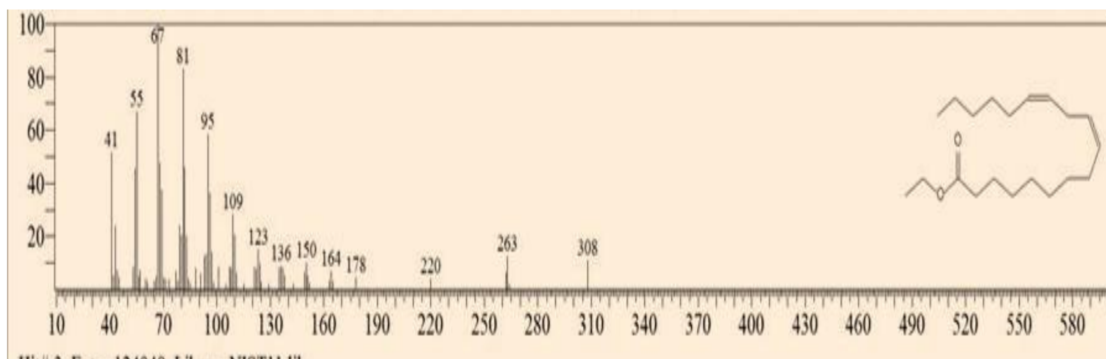


Fig.3.10 : Mass spectrum of linoleic acid ethyl ester

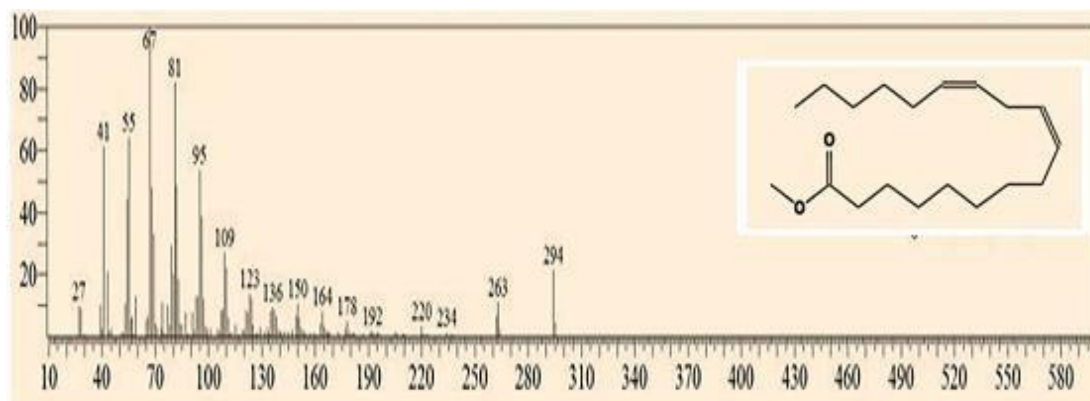


Fig.3.11: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

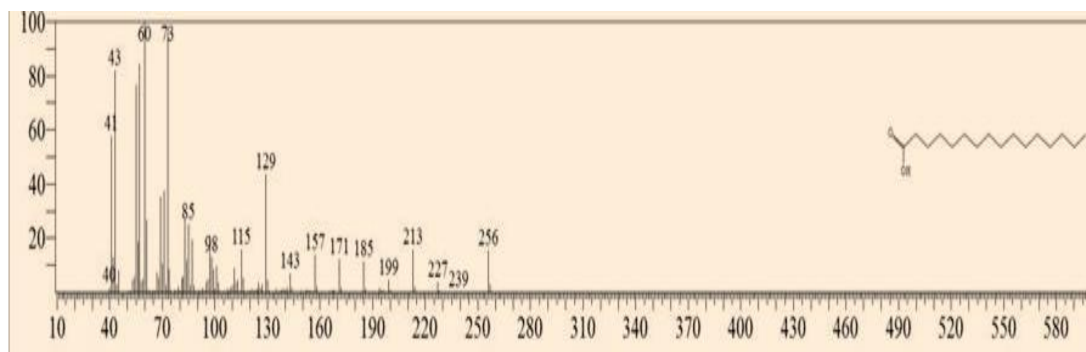


Fig. 3.12 : Mass spectrum of hexadecanoic acid

3.3.2-Antimicrobial activity of *Lagenaria siceraria* oil

Lagenaria siceraria oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are presented in Table 3.8. The oil showed moderate activity against *Pseudomonas aeroginosa.*

Table 3.8: Inhibtion zones(mm) of *Lagenaria siceraria* oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil 100mg/ml	12	11	--	16	--

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

3.4-*Fallopia conuolulus*

3.4.1-GC/MS analysis

GC/MS was conducted for the studied oil. The analysis revealed the presence of 23 components - Table (3.9).The total ion chromatogram is presented in Fig.3.13.

Fig. 3.13 :Total ions chromatograms

Table 3.9 : Constituents of the oil

No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	14.196	0.23
2	5-Octadecenoic acid, methyl ester	15.053	0.01
3	Pentadecanoic acid, methyl ester	15.329	0.03
4	7-Hexadecenoic acid, methyl ester, (Z)-	16.170	0.03
5	9-Hexadecenoic acid, methyl ester, (Z)-	16.212	0.38
6	Hexadecanoic acid, methyl ester	16.435	22.67
7	cis-10-Heptadecenoic acid, methyl ester	17.229	0.06
8	Heptadecanoic acid, methyl ester	17.442	0.18
9	6,9,12-Octadecatrienoic acid, methyl ester	18.026	0.81
10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.203	32.34
11	9-Octadecenoic acid (Z)-, methyl ester	18.257	25.77
12	Methyl stearate	18.445	11.17
13	8,11,14-Docosatrienoic acid, methyl ester	19.927	0.04
14	9-octadecenoic acid, 2,2,2-trifluoroethyl ester	20.005	0.32
15	cis-11-Eicosenoic acid, methyl ester	20.076	0.78
16	Eicosanoic acid, methyl ester	20.281	3.02
17.	Heneicosanoic acid, methyl ester	21.148	0.05
18.	13-Octadecenal, (Z)-	21.768	0.64
19.	Docosanoic acid, methyl ester	21.985	0.77
20.	Tricosanoic acid, methyl ester	22.788	0.09
21.	Hexatriacontane	23.279	0.07
22.	8-(2-Octylcyclopropyl)-8-oxooctanoic acid, methyl ester	23.392	0.16
23.	Tetracosanoic acid, methyl ester	23.563	0.38

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (32.34 %)
ii) 9-Octadecenoic acid methyl ester(25.77%)
iii) Hexdecanoic acid methyl ester(22.67 %)
iv) Methyl stearate(11.17%)

The GC-MS analysis showed a mass spectrum(Fig.3.14) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.203)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The analysis showed a mass spectrum(Fig.3.15) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 18.257) corresponds : $M^+[C_{19}H_{36}O_2]^+$.

The GC-MS analysis also gave a spectrum(Fig.3.16) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT. 16.435) accounts for: M^+ $[C_{17}H_{34}O_2]^+$. The analysis also exhibited a mass spectrum (Fig. 3.17) identical with that of methyl stearate. The peak at m/z 298 (RT. 18.445) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$.

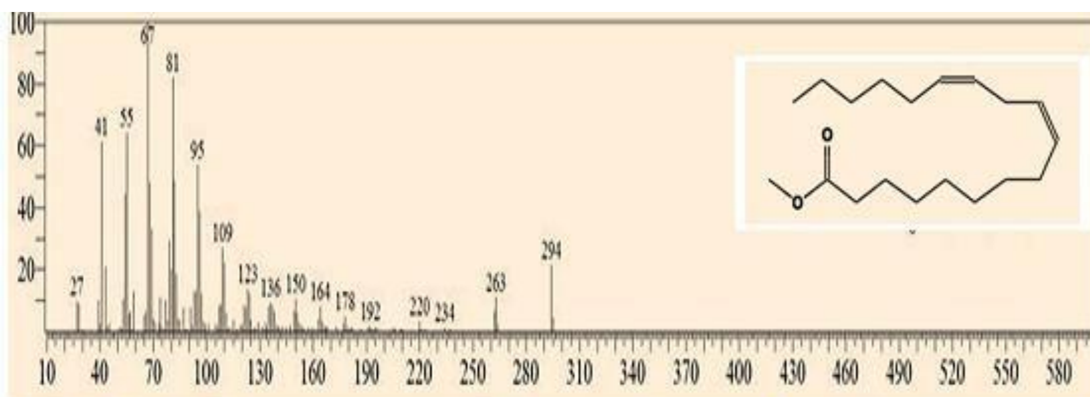


Fig.3.14: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

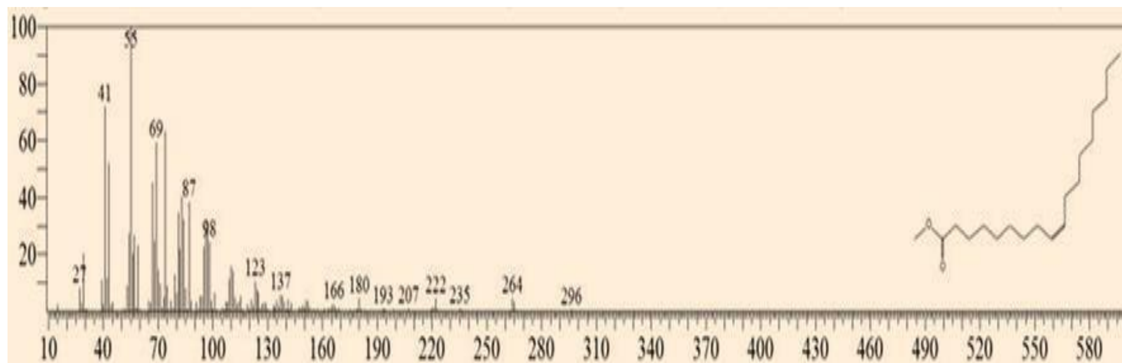


Fig.3.15: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

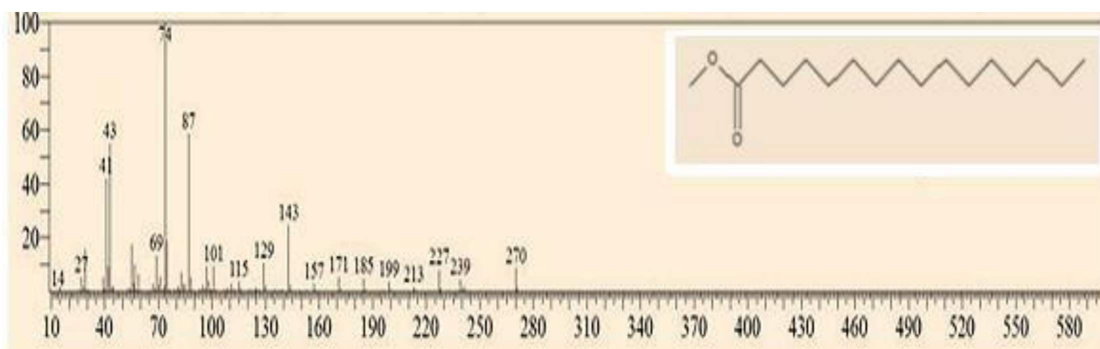


Fig3.16: Mass spectrum of hexadecanoic acid, methyl ester

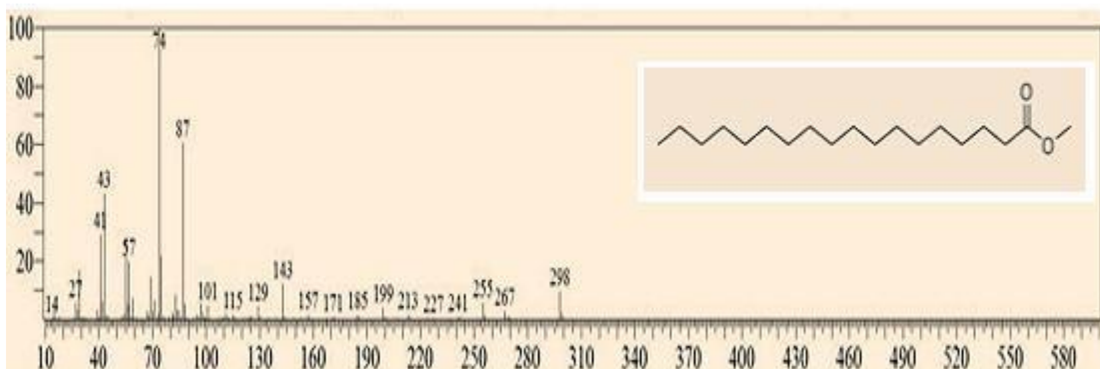


Fig.3.17 : Mass spectrum of methyl stearate

3.4.2-Antimicrobial activity of *Fallopia conuolulus* oil

Fallopia conuolulus oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are presented in Table 3.10. The oil showed moderate activity against *Staphylococcus aureus* beside moderate anticandidal activity.

Table 3.10: Inhibtion zones(mm) of *Fallopia conuotulus* oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil 100mg/ml	16	--	--	13	14

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

3.5-*Carica papaya*

3.5.1-GC-MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Carica papaya* oil. The analysis revealed the presence of 20 components - Table (3.11).The total ion chromatogram is presented in Fig.3.18.

Fig. 3.18: Total ion chromatograms

Table 3.11: Constituent of *Carica papaya* oil

No.	Name	Ret.Time	Area%
1.	Hexadecanoic acid, methyl ester	16.396	7.78
2.	n-Hexadecanoic acid	16.834	1.98
3.	Hexadecanoic acid, ethyl ester	17.090	3.22
4.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.143	4.29
5.	9-Octadecenoic acid (Z)-, methyl ester	18.188	28.06
6.	Methyl stearate	18.409	2.53
7.	Oleic Acid	18.630	12.86
8.	Ethyl Oleate	18.817	11.52
9.	Octadecanoic acid, ethyl ester	19.036	0.84
10	Hexadecanoic acid, tetradecyl ester	19.886	0.28
11	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.951	0.29
12	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	20.030	1.67
13	Oleoyl chloride	21.194	1.36
14	6-Octadecenoic acid, (Z)-	21.412	0.58
15	Decyl oleate	21.476	0.46
16	Octadecanoic acid, 2-propenyl ester	21.536	0.42
17	cis-9-Hexadecenal	21.601	3.04
18	Stigmasterol	22.847	7.57
19	2-Dodecen-1-yl(-)succinic anhydride	23.051	5.85
20	.gamma.-Sitosterol	23.979	5.40

The following compounds were detected in the chromatogram as major constituents:

i) 9-Octadecenoic acid methyl ester (18.182 %)

ii)Oleic acid (12.961 %)
iii) Ethyl oleate(11.52 %)

iv)Stigmasterol (7.57%)

v)2-Dodecen-1-yl(-)succenic acid anhydride (5.85%)

vi)gamma-Sitosterol(5.40%)

The GC-MS analysis of the studied oil showed a mass spectrum (Fig. 3.19) identical with that of 9-octadecenoic acid methyl ester where the peak at m/z 296 (RT.18.188) accounts for : M^+ [$C_{19}H_{36}O_2$].The analysis also showed a mass spectrum(Fig.3.20) characteristic of oleic acid.The molecular ion : $M^+[C_{18}H_{34}O_2]^+$ appeared at m/z282(RT.18.630). A Mass spectrum(Fig.3.21) characteristic of ethyl oleate was also shown: the peak at m/z310(RT.18.817) is attributed to the molecular ion :$M^+[C_{20}H_{38}O_2]^+$.Stigmasterol was also detected by its retention time(22.847) and mass spectrum(Fig.3.22) which revealed m/z412 for : $M^+[C_{29}H_{48}O]$. 2-Dodecen-1-yl(-)-succenic anhydride was also detected . The peak(Fig.23) at m/z266(RT.23.051) is due to the molecular ion: $M^+[C_{16}H_{26}O_3]^+$. A mass spectrum characteristic of gamma – sitosterol (Fig.3.24)was observed. The signal at m/z414(RT.23.979) is due to : $M^+[C_{29}H_{50}O]$.

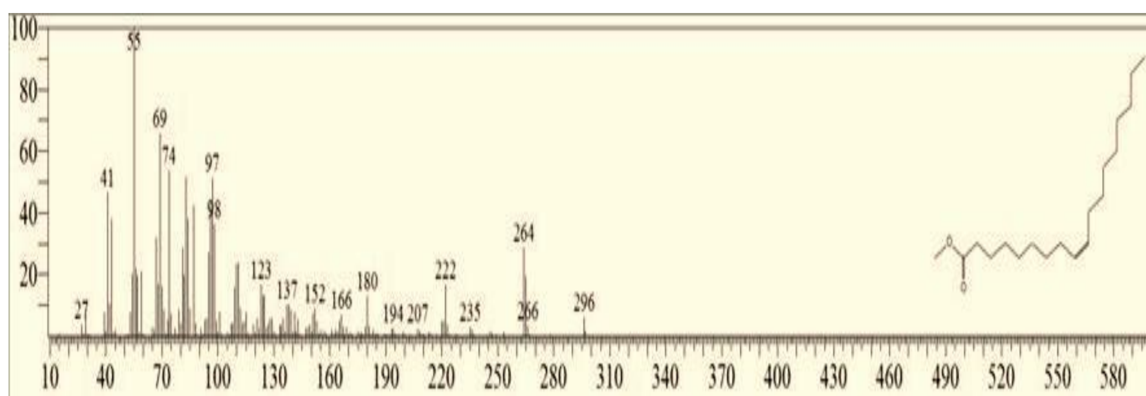


Fig.3.19 : Mass spectrum of 9-octadecenoic acid methyl ester

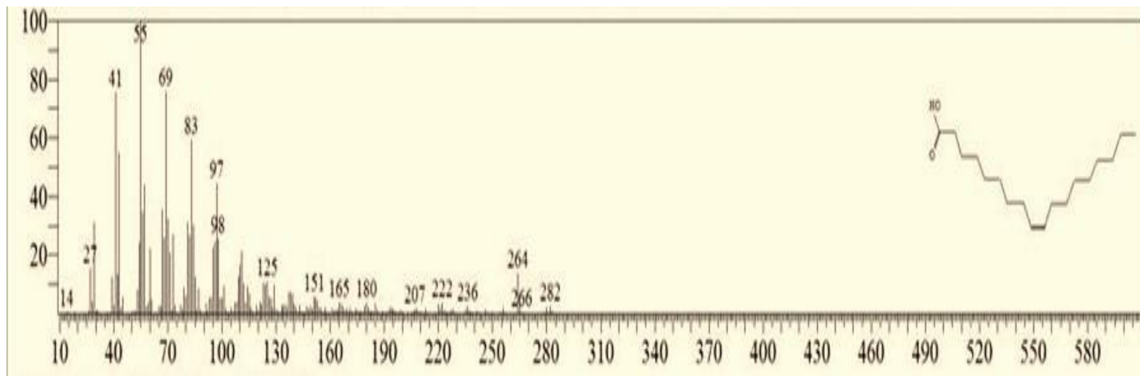


Fig.3.20 : Mass spectrum of oleic acid

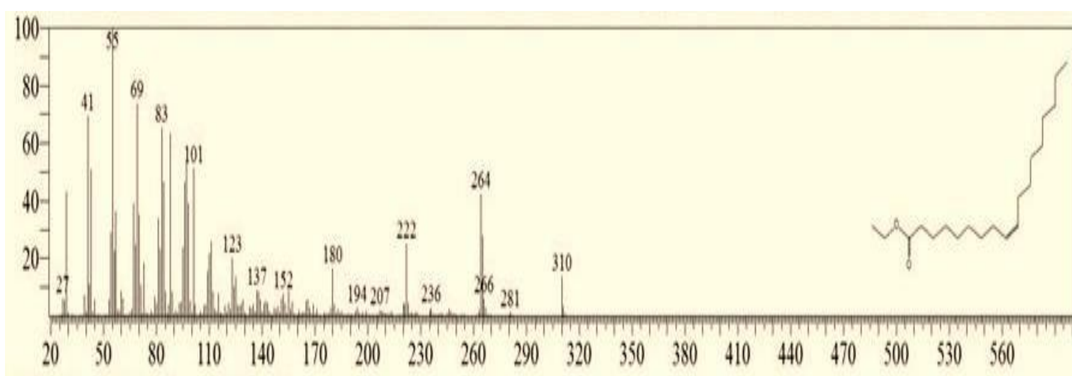


Fig. 3.21: Mass spectrum of ethyl oleate

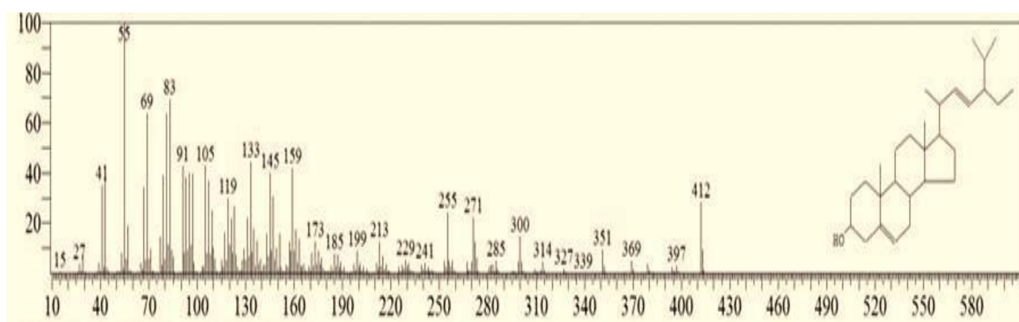


Fig.3.22 : Mass spectrum of stigmasterol

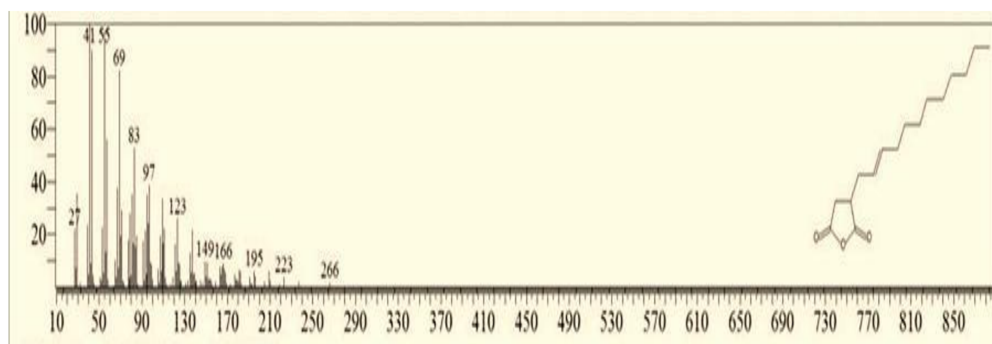


Fig. 3.23: Mass spectrum of 2-dodecen-1-yl(-)-succenic anhydride

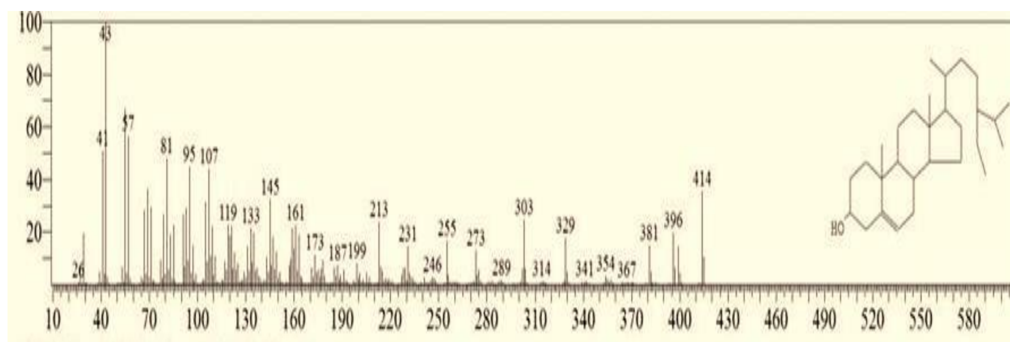


Fig.3.24 : Mass spectrum of gamma-sitosterol

3.5.2-Antimicrobial activity of *Carica papaya* oil

Carica papaya oil was screened for antimicrobial activity against five standard organisms. The inhibition zones are presented in Table 3.12. The oil showed significant activity against *Escherichia coli* and *Pseudomonas aeroginosa* .It also exhibited moderate activity against *Bacillus subtilis* and *Staphylococcus aureus* beside weak anticandidal activity.

Table 312: Inhibtion zones(mm) of the oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil 100mg/ml	15	16	19	19	11

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

6- *Dicoma tomentoza*

6.1.1-GC/MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Dicoma tomentoza* oil. The

analysis revealed the presence of 18 components - Table (3.13).The total ion chromatogram is presented in Fig.3.25.

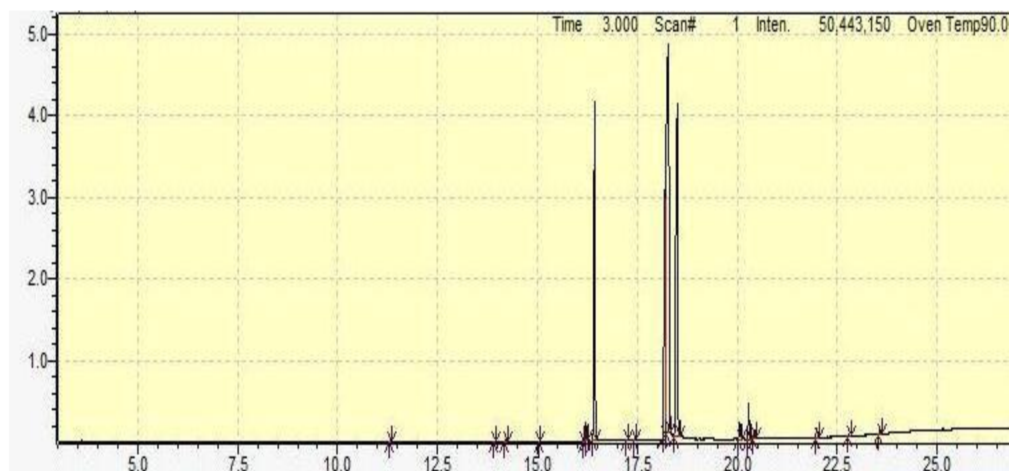


Fig. 3.25: Total ion chromatograms

Table 3.13: Constituents of the oil

No.	Name	Ret.Time	Area%
1	.gamma.-Muurolene	11.305	0.03
2	Methyl myristoleate	13.914	0.01
3	Methyl tetradecanoate	14.195	0.11
4	cis-5-Dodecenoic acid, methyl ester	15.055	0.01
5	7-Hexadecenoic acid, methyl ester, (Z)-	16.163	0.15
6	9-Hexadecenoic acid, methyl ester, (Z)-	16.211	0.69
7	Hexadecanoic acid, methyl ester	16.430	16.57
8	cis-10-Heptadecenoic acid, methyl ester	17.229	0.05
9	Heptadecanoic acid, methyl ester	17.441	0.08
10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.186	14.50
11	9-Octadecenoic acid (Z)-, methyl ester	18.274	39.78
12	Methyl 5,6-octadecadienoate	18.496	24.57
13	cis-11-Eicosenoic acid, methyl ester	20.055	0.95
14	Eicosanoic acid, methyl ester	20.280	1.47
15	6,9-Octadecadienoic acid, methyl ester	20.430	0.27
16	Docosanoic acid, methyl ester	21.983	0.48
17	Tricosanoic acid, methyl ester	22.789	0.08
18	Tetracosanoic acid, methyl ester	23.563	0.20

The following compounds were detected in the chromatogram as major constituents:

i) 9-Octadecenoic acid methyl ester (39.78 %)

ii) Methyl 5;6-octadecadienoate(24.57%)

iii) Hexdecanoic acid methyl ester(16.57 %)

iv) 9;12-Octadecadienoic acid methyl ester(14.50%)

The GC/MS analysis revealed a mass spectrum(Fig.3.26) characteristic of 9-octadecenoic acid methyl ester where the peak at m/z 296 (RT.18.274) is due to $M^+ [C_{19}H_{36}O_2]^+$.It also showed a spectrum(Fig.3.27) matching that of methyl 5;6-octadecadienoate :the signal at m/z294(RT.18.496) is due to the molecular ion : $M^+[C_{19}H_{34}O_2]^+$.

The GC/MS analysis also revealed the presence of hexdecanoic acid methyl ester.The peak at m/z 270 (Fig.3.28)which appeared at (RT.16.430) is due to $M^+ [C_{17}H_{34}O_2]^+$. The analysis gave a mass spectrum(Fig.3.29) characteristic of 9;12-octadecadienoic acid methyl ester . The molecular ion: $M^+ [C_{19}H_{34}O_2]^+$ appeared at m/z 294(RT. 18.186).

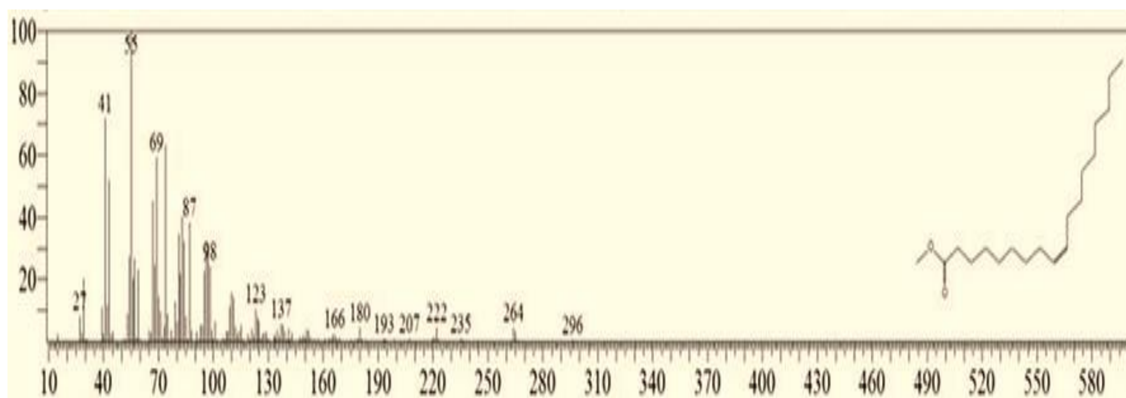


Fig.3.26: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

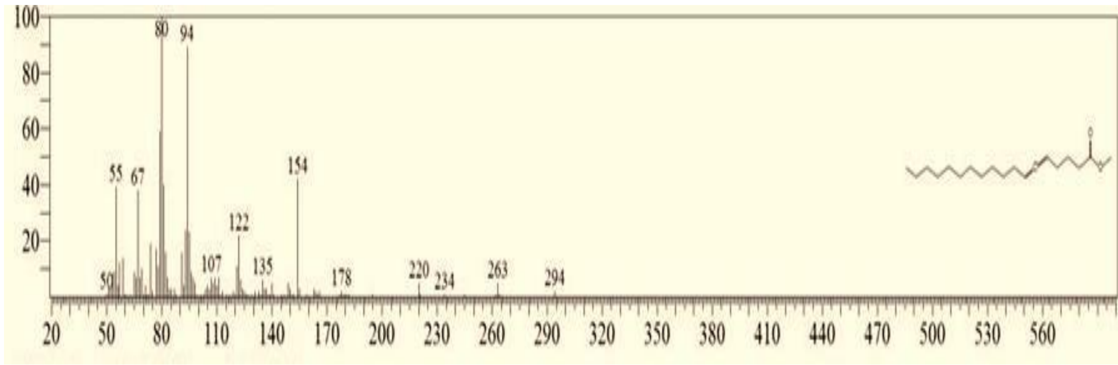


Fig.3.27: Mass spectrum of methyl 5;6-octadecanoate

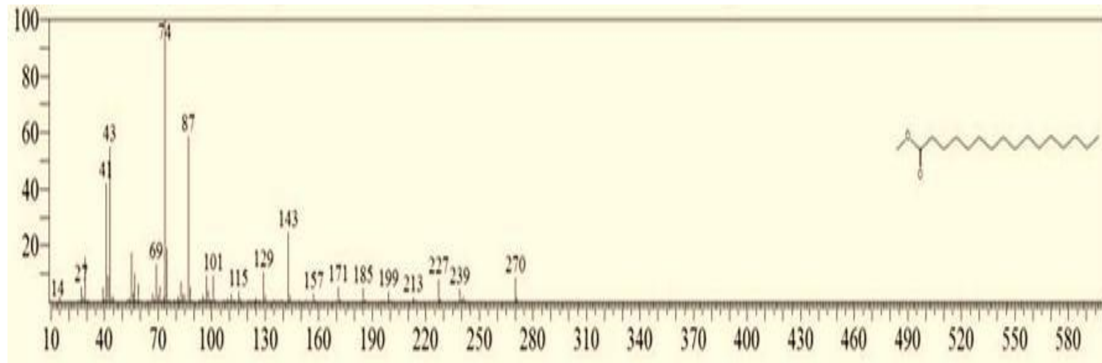


Fig. 3.28: Mass spectrum of hexadecanoic acid, methyl ester

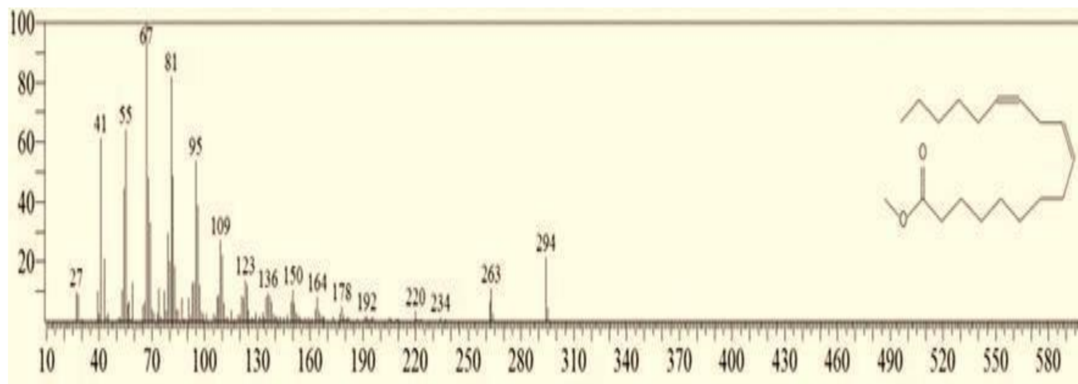


Fig. 3.29 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

3.6.2-Antimicrobial activity of *Dicoma tomentoza* oil

Dicoma tomentoza oil was assessed for antimicrobial activity against five standard organisms. The inhibition zones are presented in Table 3.14. The oil showed significant activity against *Escherichia coli*. It also showed moderate activity against

Staphylococcus aureus , *Pseudomonas aeroginosa* and *Candida albicans*.

Table 3.14 : Inhibtion zones(mm) of *Dicoma tomentoza* oil

Sample	Sa	Bs	Ec	Pa	**Ca**
Oil(100mg/ml)	14	---	20	13	15

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

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