1-Introduction

1.1 Natural products

Since the dawn of civilization natural products have been used for fighting diseases and health care. Ancient civilizations of many communities provide sufficient evidence for use of the natural sources in curing various types of diseases. The oldest known document is four thousand years old called Sumerian clay tablet used for various diseases[1]

For thousands of years turmeric was used for blood clotting. Gall bladder infections were treated by the roots of endive plants. Mandrake was used for relief of pain. Raw garlic was used to treat the circulatory disorders[2]. Such natural medicines are still used worldwide as an alternative medicines. Until nineteenth century active components were not isolated from medicinal plants [3]

When 1806 Friedrich Sertürner isolated morphine from the *Papaver somniferum.* Natural products were since that date extensively screened to obtain medicines[4] . It was revealed by World Health Organization that almost 80% of world's population depends on the traditional medicines. Almost 121 drugs used in USA in these days come from the natural sources. From these 90 drugs come from plant sources indirectly or directly[4].

Almost 47 % of anticancer drugs come from the natural products. Between years from 1981 to 2006 about 100 anticancer agents were developed. From these 25 were the derivatives of natural products, 18 were mimics of natural products and 11 were derived from the natural product called pharmacophore. There were also 9 anticancer agents which were purely natural products. Thus the natural sources are significant source of caring in the health system[1-3].

It is well established that plants play a central role in the management of various diseases and have been heavily utilized in the sustainable development of drugs that provide a major focus in global health care delivery · Plants have been used for the treatment diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry[1].

1.1.1 Tannins

Tannins are natural products which are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or

tea. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruits.Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins)[1].

1.1.2 Saponins

Saponins constitute a class of natural products which are found in particular abundance in various plant species. More specifically, saponins glycosides charcterized by the soap-like foaming which they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative[1]. The aglycone (glycoside-free) portions of the saponins are termed sapogenins. The number of saccharide chains attached to the sapogenin/aglycone core can vary – giving rise to another dimension of nomenclature (monodesmosidic, bidesmosidic, etc.) – as can the length of each chain. A somewhat dated compilation has the range of saccharide chain lengths being 1–11, with the numbers 2-5 being the most frequent, and with both linear and branched chain saccharides being represented. Dietary monosaccharides such as D-glucose and D-galactose are among the most common components of the attached chains[1].

1.1.3 Steroids

The most common characteristic of steroids is an skeleton consisting of an arrangement of seventeen carbon atoms in a four-ring structure, where three rings are composed of 6-carbons (rings A, B, and C) followed by one with 5-carbons (ring D). Further common features are an 8-carbon side chain attached to a carbon on ring D, and two or more methyl groups at the points where adjacent rings are "fused". Hundreds of distinct steroids are found in animals, fungi, plants, and elsewhere, and specific steroids underlie proper structure and function in many biological processes. Their core tetracyclic ring structure is synthesized in each organism by biochemical pathways that involve cyclization of a thirty-carbon chain, squalene, into an intermediate, either lanosterol or cycloartenol. From such intermediates, organisms then derive critical steroids such as cholesterol, the sex hormones estradiol and testosterone and bile acids. Based on such structures, synthetic and medicinal chemists synthesize novel steroids for use as drugs such as the anti-inflammatory agent dexamethasone[1].

1.1.4 Glycoside

Glycosides are a molecules in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as

medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body [1,2].

1.1.5 Alkaloids

Alkaloids are defined as basic nitrogen-containing compounds with pronounce physiological activity. Unlike the steroids they do not possess a common skeleton and their structures are quite diverse.a group of naturally occurring chemical compounds that. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus[1].

1.1.6 Flavonoids

Flavonoids are secondary metabolic which are widely distributed in the plant kingdom bwing located in cell vacuoles.

The flavonoids do possess many biological activities in plants, animals, and bacteria. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for color and aroma of flowers, fruit to attract pollinators consequently fruit dispersion; help in seed germination, growth and development of seedling. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, Function as signal molecules,

allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimation and freezing tolerance [1].

Up to date more than 8000 different flavonoids have been isolated from their natural matrices. The structural variations of these flavonoids are associated with many different biological and pharmacological activities, including anticancer activity, protection against cancer formation (chemo-protection), antioxidant activity, cardiovascular and hepatic protection, antibacterial, antifungal and antiviral activity. Flavonoids have also been reported to play an important role in hormone-related female diseases, such as breast cancer and menopausal syndrome. Natural flavonoids have therefore been subjected to many chemical modifications in order to improve their activity [2].

1.2 Essential oils

Essential oils which are endowed with characteristic odor are obtained from aromatic and medicinal plants as a volatile mixture of chemical compounds .

Essential oil are extracted from plant material mainly by using steam or hydrodistillation or Soxhlet extraction (solvent extraction or continuous extraction)[1].

Essential oils are one of the most predominant plant products which exhibit a wide spectrum of physiological activities including: antifungal, antibacterial, antioxidant, anticancer, antidiabetic, antiviral, insect- repellent, and anti-inflammatory properties [2,3].

Research on artificial pharmaceutical substances reveals the significance of EOs extracted from medicinal and aromatic plants, as their therapeutic properties have numerous applications. Consequently, researchers and farmers have been motivated to expand the cultivation and market these substances[3,4].

Approximaltely about 100 herbs are known for their EOs, while more than 2000 herbs scattered across 60 families, such as Umbelliferae, Lamiaceae, Lauraceae, Myrtaceae, etc., could produce medicinally valued EOs. In global markets, only 300 among 3000 known types of EOs are deemed to be of commercial importance. EOs have found application in agricultural sectors and can be potentially used in other industries, such as pharmaceuticals, drugs, food, perfumes, makeup products, sanitary products, dentistry, food preservatives, additives, cosmetics, and natural remedies[3,4] .

In many industries like perfumes, creams, soaps foods and sanitary products,essential oils such as limonene, patchoulol, geranyl acetate, etc… have been widely used.

Moreover, essential oil blends are used in bath products and in aromatherapy. Furthermore, many EOs are particularly valued for their

medicinal properties[3,4]. For example, menthol is used as natural bug repellent, as well as for treating joint pain, respiratory allergies, muscle pain, headache, hair growth, and fever relief, as well as in cancer treatment (menthol protects against cell death and DNA damage). Essential oils are extensively used as fragrances,but their application in human health, agricultural industry, and environmental protection requires better understanding of their biological properties. Some of the EOs and their chemical constituents are viable as alternatives to the synthetic compounds, presently widely used in the chemical industry. This is because EOs are not associated with harmful side effect .

In nature, EOs play an important role in providing plant protection against pathogenic bacteria, viruses, and fungi and preventing the attack by insect pests. In addition, EOs can attract or repel insects when present in pollen and seeds. The use of EOs in pharmaceutical, food, bactericidal, and fungicidal is becoming more prevalent in recent times. EOs - yielding medicinal and aromatic plants are normally native to warm countries, where they represent an important traditional pharmacopeia. EOs are less dense than water. They are volatile and mostly colorless, as well as soluble in organic solvents. All plant parts, such as buds, leaves, fruits, bark, root, stems, twigs, and flowers, can contain Eos[4].

1.3 Extraction of essential oils

Different methods can be applied for essential oil extraction, such as hydrodistillation, steam distillation, and solvent extraction (including liquid carbon dioxide or microwave extraction). For example, hydrodistillation or steam distillation is typically used for Citrus and Lamiaceae family members. Various factors, such as the extraction method, geographical conditions, type of soil, plant material, and harvesting stage, are being reported to influence on the occurrence of number of chemical constituents in EOs and variations in EO quality and yield[3,4].

In order to ensure a constant chemical composition, quality, and quantity, EOs should be extracted under the same conditions, such as using same plant organs, extraction method, harvesting period or season, and growing plants in the same soil types. Many of the EOs are commercialized and chemotyped by gas chromatography - mass spectrometry (GC-MS), and the results have been published in international organizations like the ISO, WHO, EP (European pharmacopoeia), and Council of Europe to protect good grade and amount of EOs.

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process[5,6]. The oil droplets being stored in the oil glands or sacs can be removed by

either accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are[5,6]:

i-Hydrodistillation

ii-Hydrodiffusion

iii- Effleurage

iv-Cold pressing

v-Microwave Assisted Process (MAP)

vi-Steam distillation

vii-Solvent extraction

viii-Carbon dioxide extraction

1.4 Chemical composition of essential oils

Most constituents of oil belong to the large group of terpenes. Terpenes usually refer to hydrocarbon molecules consisting of isoprene (2-methylbuya-1, 3-diene). The isoprene unit, which can build upon it in various ways, is a five-carbon molecule. Two of the molecules of isoprene give terpene, sesquiterpenes contain three molecule of isoprene, four isoprene gives diterpene, six isoprene gives triterpene. Isoprene units (Fig. 1) are obtained biosynthetically via mevalonate pathway[7].

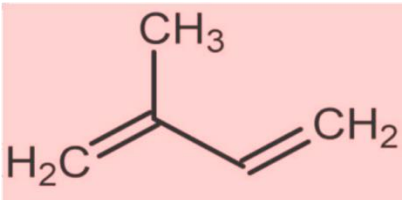

Fig. 1. Isoprene

1.5 Biological effects of essential oils

At present, around 60 plant families are known to produce EOs, which are valued in medicinal, pharmaceutical, flavor and fragrance, and agricultural industries. Several plant species belonging to the Apiaceae, Alliaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae, and Rutaceae family produce EOs with medicinal and industrial values[8]. EOs are rich in terpenes, while phenylpropanoids more frequently occur in Apiaceae, Alliaceae, Lamiaceae, Myrtaceae, and Rutaceae plant families[9]. These family plants are used for the commercial level manufacture of EOs. For example, patchoulol, coriander, anise, dill, and fennel EOs are extracted from *P. cablin*, *C. sativum*, *P. anisum*, *A. graveolens* and *F. vulgare*, respectively. These EOs are well known for their antimicrobial and anticancer activities. The plants belonging to the Lamiaceae and Apiaceae family are popular for antimicrobial, anticancer, antibacterial, antimutagenic, antiinflammatory, and antioxidant activities [3,4].

1.5.1 Essential oils as antibacterial agents

Many essential oils have been investigated for their antibacterial and antifungal activities, as well as their potential against Gram-positive and Gram-negative bacteria[4]. EOs show good antibacterial properties against *Salmonella*, *Staphylococcus,* and other bacterial pathogens. Thus, it is essential to study their effects as very good alternatives to antibiotics. *O. basilicum* essential oil exhibits good antibacterial properties against Gram-positive bacteria[10] . In the investigations of antibacterial effects, manuka oil has been shown to exhibit good antibacterial activity. Similarly, eucalyptus, rosmarinus, *Lavandula* oil, and tree oil were found effective against *Streptococcus mutans, S. sobrinus, Fusobacterium nucleatum*, and *Porphyromonas gingivalis*[11]. Tea tree (*Melaleuca alternifolia*) oil is demonstrated to be sensitive to 15 genera of oral bacteria, indicating its potential applications in oral hygiene[12]. *Pittosporum undulatum* and *Hedychium gardnerianum* EOs show the highest antibacterial activities against *Staphylococcus epidermis* and *S. aureus*.

Despite the discovery of new antibiotics, bacterial infectious/diseases still pose a serious threat to human health, predominantly due to the appearance of antibiotic- resistant strains. In addition, as the global people continues to expand, this will result in a greater prevalence of bacterial diseases, low immunity, and increased drug resistance. Therefore, bacterial infections will be more likely to be fatal[4].

1.5.2 Essential oils as antioxidant agents

Modern era has brought about different health problems, such as noncommunicable diseases (e.g., cancer, diabetes, and Alzheimer's, Parkinson's, and heart diseases) which are attributed to oxidative stresses. EOs exhibit a significant antioxidant activity due to their phtocompounds, such as flavonoids, terpenoids, and phenolic compounds[13]. Among many EOs, *O. majorana*, *T. filifolia*, *B. monnieri*, *C. longa*, *S. cryptantha*, *millefolium*, *S. multicaulis*, *M. officinalis*, *M. alternifolia*, *Ocimum*, and *Mentha* sp. have been reported to possess significant antioxidant activity

Thymol and carvacrol containing EOs in particular show strong antioxidant properties[13]. Likewise, EOs of *Cuminum cyminum*, *Petroselinum sativum*, *S. cumini*, and *Coriandrum sativum* also exhibit efficient antioxidant[13]. In addition, clove oil shows a much stronger antioxidant and radical scavenging activity compared to cinnamon, basil, oregano, nutmeg, and thyme Eos[13].

1.6 Gas Chromatography-mass spectrometry

Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a

column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase[14].

The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass Spectrometry (MS), the detector for the GC is the Mass Spectrometer (MS). As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint. Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gasliquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semivolatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them[14] .

GC/MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to

analyze complex organic and biochemical mixtures (Skoog et al., 2007). The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed.

1.6.1 Instrumentation and working of GC-MS

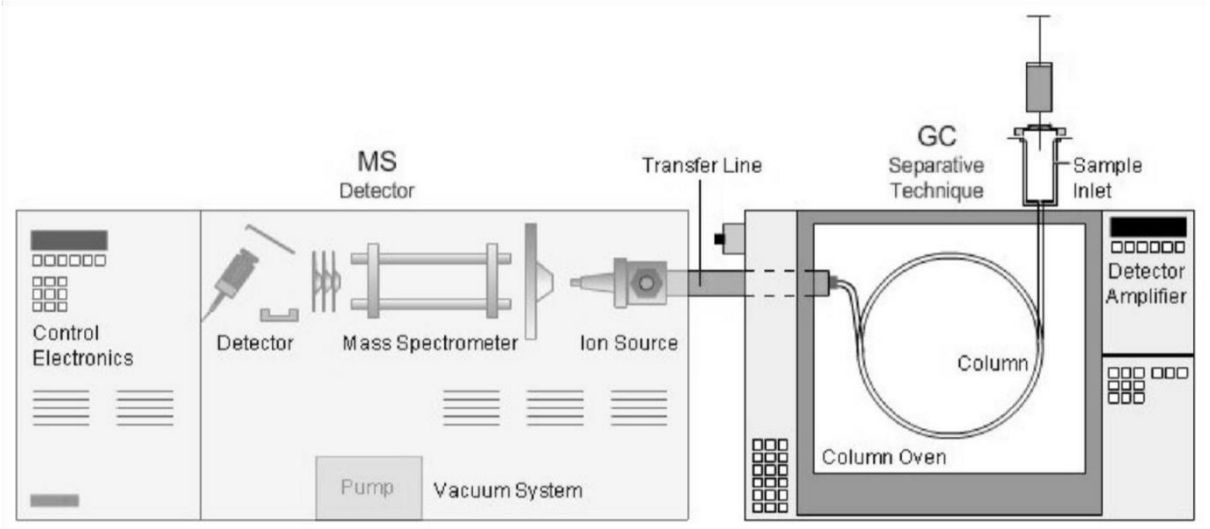

Fig. 2. Schematic diagram of GC-MS

Fig.2 illustrates a schematic diagram of GC-MS. Its different parts and their functions are discussed below.

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure (Gas Supply and Pressure Control from theory and Instrumentation of GC-GC Channel)[14].

-Injector:

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column[14].

***-Column*:**

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter (GC columns from Theory and Instrumentation of GC)[24].

-Ovens : *:*

Gas chromatography have ovens that are temperature programmable,

1.6.2 Mass Spectrometer

The separation of the phase ions is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions.

i)Ion source

In the ion source, the products are ionized prior to analysis in the mass spectrometer.[14]

ii) Mass analyzer

There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis**.** Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way[14].

The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges)[14].

The MS parameters can be selected and controlled from this panel. Modern instruments will also allow to control MS parameters from a computer by using specially designed software. The mobile-phase called as carrier gas, must be chemically inert. The helium gas is most commonly used, however, argon, nitrogen, and hydrogen are also used. These gases are held in pressurized tanks and use pressure regulators, gauges, and flow meters to control the flow rate of the gas. Flow rates usually range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular capillary columns, and are assumed to be constant if inlet pressure is constant. This is often accompanied by a molecular sieve to purify the gas before it is used[14].

Samples are introduced as a plug of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port which should be about 50°C above the boiling point of the least volatile constituent of the sample. After the sample is introduced, it is carried to the column by the mobile phase. The temperature of the column is an important variable, so the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As the mobile phase carrying the sample is passed through the stationary phase in the column, the

different components of the sample are separated. After being separated, the sample is run through a detector 19which ionizes the sample and then separates the ions based on their massto-charge ratio. This data is then sent to a computer to be displayed and analyzed. The computer linked to the GCMS has a library of samples to help in analyzing this data .Data for the GC-MS is displayed in several ways. One is a total-ion chromatogram, which sums the total ion abundances in each spectrum and plots them as a function of time. Another is the mass spectrum at a particular time in the chromatogram to identify the particular component that was eluted at that time. A mass spectra of selected ions with a specific mass to charge ratio, called a mass chromatogram, can also be used[14].

1.7 The targeted plant species

1.7.1 *Annona senegalensis* Persoon

Annona senegalensis **Persoon** is a small tree or herb in the family Annonaceae.Usually the plant is 4-6m tall, but under favorable conditions it may reach 11 m in height.

Fig. 3 :*Annona senegalensi*s

In the African continent *Annona senegalensi*s occurs in forests,swamps, river banks and savannah woodland[15].All parts of the plant find some applications in ethnomedicine.Leaves are used by traditional healers against tuberculosis, small pox and yellow fever[16,17].Root is used against snake bite, gastritis, sexual impotency, infectious diseases and erectile dysfunction[18-20].Plant juice is a natural remedy for chicken pox[21].The plant is also used as antidiabetic, antimalarial and as antidote for venomous bites[22-24].Bark is used against open sores[25]. Phytochemical screening of *Annona senegalensi*s revealed the presence of many bioactive components including flavonoids, tannins, Alkaloids, saponins and steroids beside volatile oil [12-17]. *Annona senegalensi*s is considered as a rich source of many nutrients.This plant contains minerals including Zn,Fe,Mg,K,Cu, Mn and Cr.It also contains ascorbic acid and some amino acids[32,33].It has been reported that all parts of the

plant contains volatile oil dominated by p-cymene(36.0%) and phellandrene(25%)[34].Several pharmacological studies have been conducted on different parts of *Annona senegalensi*s. The antimicrobial activity of bark extract has been reported[35] and the anticonvulsant effect of *Annona senegalensi*s has been demonstrated[36].The in vitro cytotoxicity has been documented[37,38].Various fractions of leave extracts exhibited cytotoxic activity[39-41].Root extract showed antiplasmodial potential[42] and root bark detoxified snake venom[43].The antiinflammatory, analgesic,hypnotic, antioxidant, anthelmintic[33], antitrypanosomal properties have been reported[44-53].

1.7.2 *Cucurbita maxima*

Cucurbita maxima is a creeper in the family Cucurbitaceae.For centuries the plant has been used in ethnomedicine against a wide range of ailments. Cucurbita maxima is diuretic and has a deworming effect.The plant is also valued as a nervine tonic. Seeds exhibited significant free radical scavenging capacity and are recognized for several health promoting properties including: reduction of prostate size reduction of urethral pressure hypoglycemic effect and lowering risk of breast lung and colorectal cancer[54].

Fig.4 : Cucurbita maxima

Aim of this study

This research was designed to:

-Extract the oils from two medicinal plants: *Annona senegalensis* and ta *Cucurbita maxima.*

-Identification of the constituents of the oils by GC/MS.

-Evaluation of the oils for their antimicrobial activity.

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Annona senegalensis* and *Cucurbita maxima* were collected from a forest reserve around Damazin-Sudan. The plants were authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2- Instruments

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

2.1.3-Test organisms

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

Table 1: Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungi

2.2- Methods

2.2.1-Extraction of oil

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

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes .The

hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

2.2.2- GC-MS analysis

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

Table 2: Oven temperature program

Rate	Temperature(ºC)	Hold Time (min.$^{-1}$)

- 4.00	150.0 300.0	1.00 0.00

Table 3: Chromatographic conditions

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

2.2.3-

Antimicrobial assay

i)-Preparation of bacterial suspensions

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

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

suspension was determined by means of the surface viable counting technique.

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

ii)-Preparation of fungal suspensions

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

iii)-Testing for antibacterial activity

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

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

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

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

3-Results and Discussion

3.1 *Cucurbita maxima*

Oil of *Cucurbita maxima* was extracted with hexane. The oil was investigated by GC-MS and the constituents of the oil have been characterized. The oil was also evaluated for its antimicrobial potential using the cup plate agar diffusion bioassay.

3.1.1 GC- MS analysis of *Cucurbita maxima* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of constituents *Cucurbita maxima* oil. The analysis revealed the presence of 40 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

Fig. 3.1: Total ion chromatograms

Table 3.1: Constituents of *Cucurbita maxima* oil

No.	Name	R.Time	Area %
1	5-Hexenoic acid methyl ester	3.219	0.01
2	6-Heptenoic acid methyl ester	4.455	0.02
3	L-alpha -Terpeneol	6.983	0.06
4	Methyltetradecanoate	13.565	0.02
5	Pentadecanoic acid methyl ester	14.639	0.11
6	7,10-Hexadecadianoic acid methyl ester	15.368	0.02
7	7-Hexadecenoic acid methyl ester	15.428	0.03
8	9-Hexadecenoic acid methyl ester	15.473	0.27
9	Hexadecanoic acid methyl ester	15.702	17.50
10	9,12-Octadecdienoic acid methyl ester	16.434	0.17
11	Heptadecanoic acid methyl ester	16.646	0.43
12	9,12-Octadecdienoic acid methyl ester	17.430	43.39
13	9-Octadecenoic acid methyl ester	17.460	9.73
14	Methyl stearate	17.625	15.60
15	Methyl 9-cis-11-trans-,13-trans octadecanoate	18.586	0.43
16	Cyclopentaneoctanoic acid	18.941	4.03
17	9-Octadecenoic acid ,12-hydroxy,methyl ester	19.088	0.83
18	Cis-11-Eicosenoic acid methyl ester	19.136	0.64
19	Eicosanoic acid methyl ester	19.337	2.67
20	PGHi, methyl ester	19.394	0.39
21	1 H-Naphthol[2,1-b]pyran-8(4aH)-one	19.746	0.60
22	Methyl 2-octylcyclopropene-1-octanoate	20.385	0.45
23	Docasanoic acid methyl ester	20.953	0.60
24	Tricosanoic acid methyl ester	21.719	0.11
25	Tetracosanoic acid methyl ester	22,457	0.51
26	2,6,10,14,18-Pentamethyl2,6,10,14,18-	23.195	0.74

	eicosanoate		
27	Hexacosanoic acid methyl ester	2.3860	0.16
28	Stigmasta-7,16-dien-3-ol	24.351	0.22
29	Gamma-Tocopherol	24.832	0.10

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

ii) 9 12-Octadecenoic acid methyl ester (43.19%)
ii) Hexdecanoic acid (17.50 %)
iii) Methyl stearate(15.60%).

Fig. 3.2 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.430)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester

is presented in Fig. 3.3.The peak at m/z 270 which appeared at (RT.15.702) is due to M^+ $[C_{17}H_{32}O_2]^+$.

Fig. 3.4 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.625) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 accounts for loss of a methoxyl.

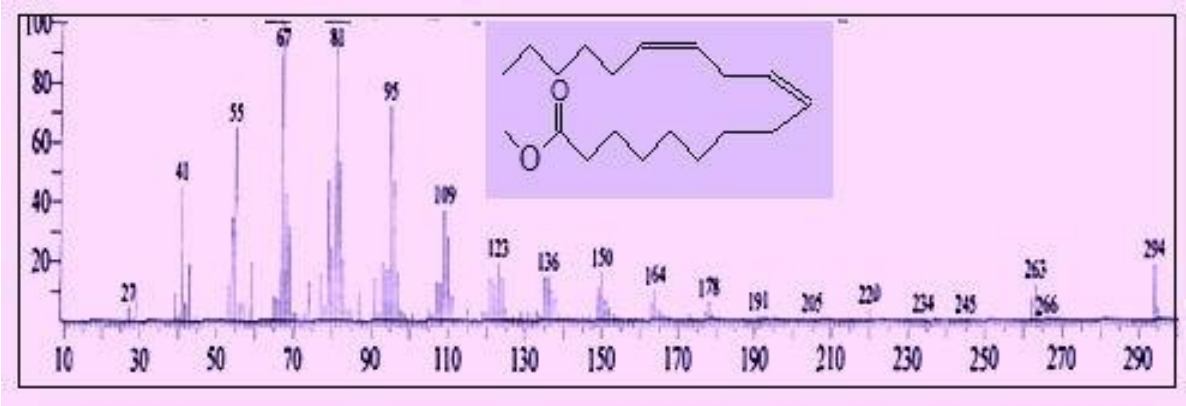

Fig.3.2: Mass spectrum of 9,12-octadecadienoic acid

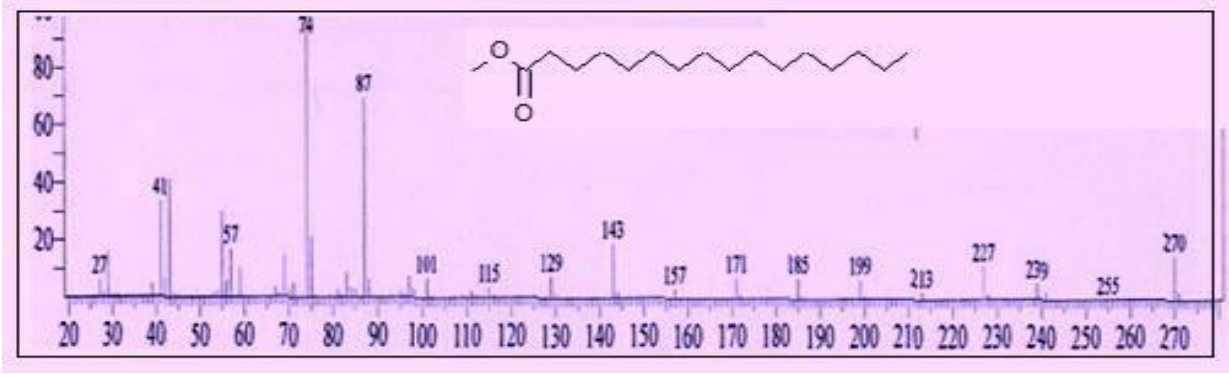

Fig. 3.3: Mass spectrum of hexadecanoic acid methyl ester

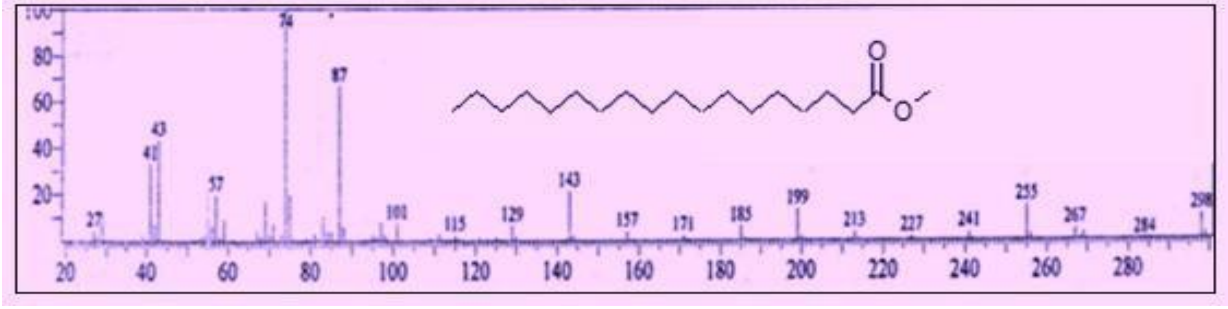

Fig.3. 4: Mass spectrum of methyl stearate

3.1.2 Antimicrobial activity of *Cucurbita maxima* oil

Cucurbita maxima oil was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.2).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed moderate activity against *Pseudomonas aeruginosa Escherichia coli* and *Candida albicans*.

Table : 3.2 : Inhibition zones(mm/mg sample)

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

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

3.2 *Annona senegalensis*

3.2.1-Gas chromatography – mass spectrometry of *Annona senegalensis* oil

Annona senegalensis oil was studied by GC-MS. The GC-MS analysis showed 22 constituents which were confirmed by the retention times and mass spectra fragmentation pattern. Major components are:

i-9,12-Octadecadienoic acid methyl ester(38.36%)
ii-9-Octadecenoic acid methyl ester(21.57%)
iii- Hexadecanoic acid methyl ester(18.12%)
iv- Methyl stearate(14.47%)

Table 3.3: Constituents of *Annona senegalensis* oil

No.	Name	R.Time	Area %
1	L –Alpha Terineol	6.980	0.10
2	Methyl tetradecanoate	13.565	0.33
3	5-Octadecenoic acid methyl ester	14.480	0.01
4	Pentadecanoic acid methyl ester	14.640	0.05
5	7,10-Hexadecadienoic acid methyl ester	15.370	0.01
6	7-Hexdecenoic acid methyl ester	15.429	0.03
7	9-Hexadecenoic acid methyl ester	15.474	0.29
8	Hexadecanoic acid methyl ester	15.688	18.12
9	6-Octadecenoic acid methyl ester	16.436	0.17
10	Heptadecanoic acid methyl ester	16.645	0.29
11	9,12-Octadecadienoic acid methyl ester	17.384	38.36
12	9-Octadecenoic acid methyl ester	17.432	21.57
13	Methyl stearate	17.604	14.47
14	Cyclopentaneoctanoic acid methyl ester	18.938	1.48

15	9-Octadecenoic acid, 12-Hydroxy methyl ester	19.093	0.43
16	Cis-11-Eicosecoic acid methyl ester	19.136	0.35
17	Eicosanoic acid methyl ester	19.337	2.04
18	Phenol, 2,2-methylbis[6-1,1-dimethylene]	20.258	0.12
19	Docosanoic acid methyl ester	20.954	0.55
20	Tricosanoic acid methyl ester	21.718	0.11
21	Tetracosanoic acid methyl ester	22.456	0.36
22	Squalene	23.194	0.77
			100.00

The mass spectra of the major constituents are discussed below:

i-9,12-Octadecadienoic acid methyl ester(38.36%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 3.5.The peak at m/z 294, which appeared at R.T. 17.384 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

ii-9-Octadecenoic acid methyl ester(21.57%)

Fig.3.6 shows the mass spectrum of oleic acid. The peak at m/z 282(RT., 17.432) accounts for : $M^+[C_{18}H_{34}O_2]^+$.

iii- Hexadecanoic acid methyl ester(18.12%)

Fig.3.7 shows the mass spectrum of hexadecanoic acid methyl.The peak m/z 270(R.T. 15.688) was detected in the spectrum . It

corresponds $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 is due to loss of a methoxyl.

iv- Methyl stearate(14.47%)

The EI mass spectrum of methyl stearate is displayed in Fig 3.8.The peak at m/z 298 with R.T. 17.604 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl group.

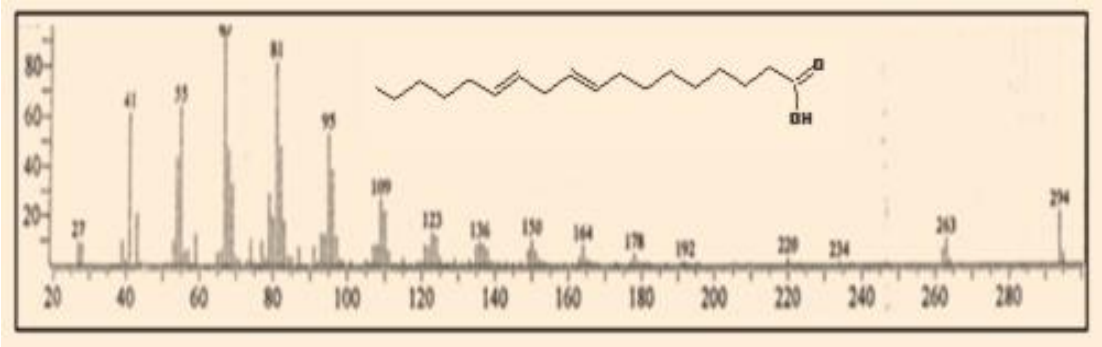

Fig. 3.5: Mass spectrum of 9,12-octadecanoic acid methyl ester

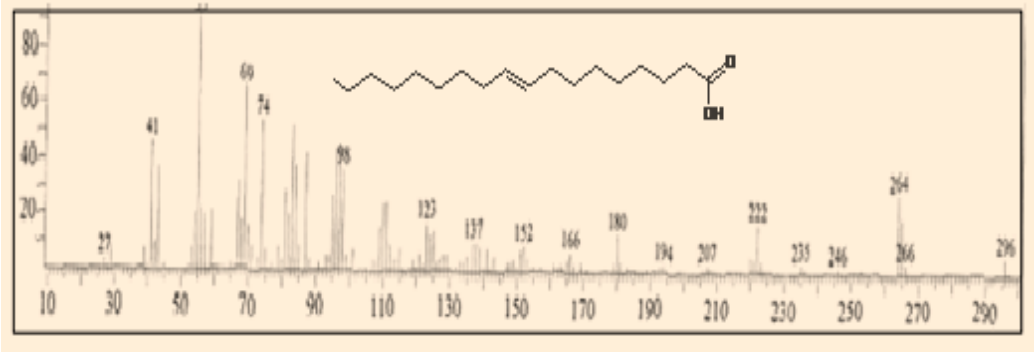

Fig.. 3.6 : Mass spectrum of 9-octadecenoic acid methyl ester

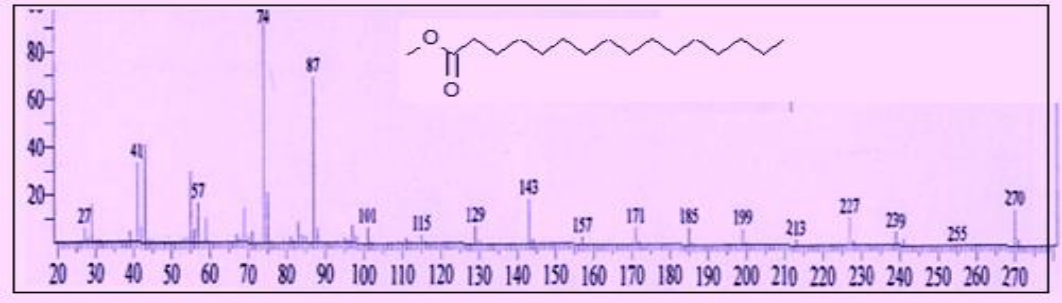

Fig. 3.7: Mass spectrum of hexadecanoic acid methyl ester

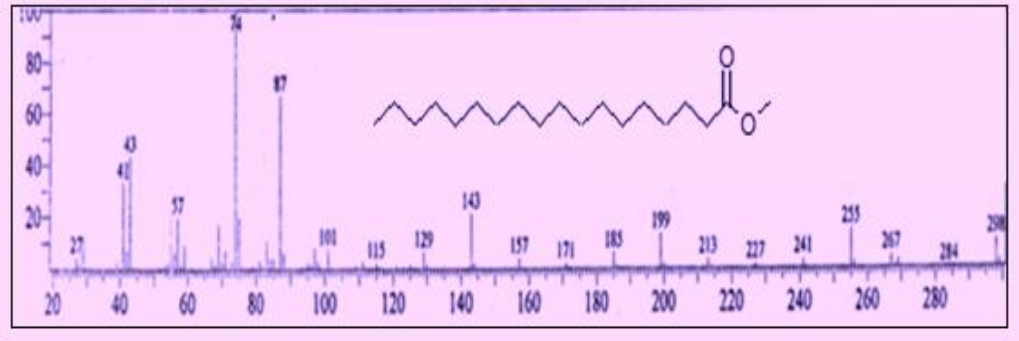

Fig. 3.8: Mass spectrum of methyl stereate

3.2.2 Antimicrobial activity of *Annona senegalensis* oil

Annona senegalensis oil was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.4).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Escherichia coli* and *Candida albicans*.

Table 3.4 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	13	--	14	16	14
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

Conclsusion

The oils from two potential medicinal plants have been extracted and the constituents of the oils have been identified by GC/MS. *Cucurbita maxima* oil revealed 29 constituents while the oil from *Annona senegalensis* showed 22 components.

The oils have been assessed for their antimicrobial activity and both oils gave moderate activity against most test organisms.

Recommendations

1- The extracted oils may be evaluated for other biological activities like antimalarial , antiviral …etc.

2- Other phytochemicals of the targeted plant species may be isolated and identified. Furthemore they be evaluated for their biological activity.

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