Sudan University of Science and Technology

College of Graduate Studies

Constituents of Argel (*Solenostemma argel*) Oil and Its Biological Activity

مكونات زيت الحرجل وفعاليته البيولوجية

A Thesis Submitted in Partial Fulfillment for the Requirement of Master Degree in Chemical Science

by

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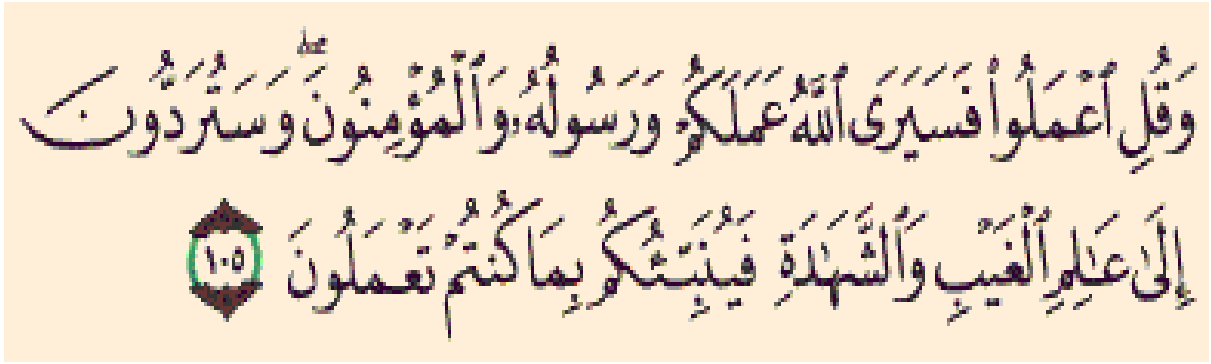

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Dedication

To

My parents

Brothers and sisters

Acknowledgement

First of all, I would like to thank **Allah Almighty** for giving me the ability and strength to accomplish this work.

I would like to express my thanks , gratitude and respect to my supervisor Prof. Mohamed Abdel Kareem ,for his interest ,close supervision and continuous advice.

Thanks for the technical staff, Dept. of chemistry, Sudan University of Science and Technology for all facilities.

Deep thanks to my family for their support.

Abstract

Solenostemma argel oil was extracted by maceration and the oil has been analyzed by GC-MS. The analysis revealed the presence of 31 components being dominated by:

(i) 7-Hexadecenal, (z) (18.23%)

(ii) 9.12 Octadecadienoic acid (z,z)methyl ester (15.17%)

(iii) Hexadecanoic acid methyl ester (12.27%)

(iv) Methyl stearate (11.82%)

(v) 9-Octadecenoic acid (Z)-, methyl ester (9.84%).

In the cup plate agar diffusion bioassay, the oil was screened for antimicrobial activity against five standard human pathogens. *Solenostemma argel* oil showed excellent activity against *Escherichia coli* and *Pseudomonas aeruginosa* .

الملخص

استخلص زيت نبات الحرجل بواسطة التقطير ثم اجرى تحليل الكروموتوغرافيا الغازية –طيف الكتلة حيث اتضح ان الزيت يحوى 31 مكونا أهمها:

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اجرى اختبار مضاد الميكروبات ضد خمسة ميكروبات قياسية . وقد اعطى الزيت فعالية عالية ضد كل من :

Escherichia coli and *Pseudomonas aeruginosa* .

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Chapter One

Introduction

Introduction

1.1-Essential oils

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenou layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant [1] . Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelica -archangelica), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated factors, climatic, seasonal and geographical conditions, harvest period and extraction techniques [1] · The yield of oils from the plants can also be affected by the stages of the plant growth [1] .

Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators. The oxygenated molecules of essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers [2,3,4]. These properties were established after the oils have been extracted from the plant materials [3].

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are odorous and volatile compounds found only in 10% of the plant kingdom and are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts [5-12] .

Since ancient times, essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. They continue to be of paramount importance until the present day. Essential oils have been used

as perfumes, flavors for foods and beverages, or to heal both body and mind for thousands of years [13-16] .

Plants are capable of synthesizing two kinds of oils: fixed oils and essential oils (volatile oils). Fixed oils consist of esters of glycerol and fatty acids (triglycerides or triacylglycerols), while essential oils (EOs) are complex mixtures of volatile and semivolatile organic compounds originating from a single botanical source that determines the specific aroma of plants and the flavor and fragrance of the plants [17] .essential oils of plant origin are an important product of agriculture-based industries. Plant essential oils have various applications, mainly in the health, agriculture, cosmetics, and food industries. In particular, the essential oils of aromatic plants and spices have been used in food preservation and as flavoring agents in food products, drinks, perfumeries, and cosmetics. Extensive phytochemical analysis has lead to the characterization and identification of major components of essential oils, which are of wide interest, especially to the cosmetics and pharmaceutical industries. At present, there is a growing interest in essential oils and their components, particularly for their broad-spectrum antimicrobial activity, which can provide, for instance, alternative functional ingredients to extend the shelf life of food products and ensure microbial safety for consumers [18].

Essential oils may constitute 20–100 different plant secondary metabolites belonging to a variety of chemical classes [19]. Around 3000 essential oils have been produced from at least 2000 plant species, out of which 300 are important from a commercial point of view [20]. They are usually stored in the oil ducts, resin ducts, glands, or trichomes (glandular hairs) of plants [21].

1.2-Sources of natural essential oils

Essential oils are generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavander), leaves (e.g. mint, Ocimum spp., lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), bark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g. ginger, calamus, curcuma, orris) and gums or oleoresin exudations[21].

1.3-Chemical composition of essential oils

Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma[21].

There are two main groups of metabolites that can be found In nature: primary and secondary metabolites , Primary metabolite are universal compounds, present in all living organisms, and include proteins, carbohydrates, lipids, and nucleic acids. Secondary metabolites are found only in some species and are classified as terpenoids,shikimates, polyketides, and alkaloids. Essential oils are composed of different chemical compounds. The constituents of plant essential oils fall mainly into two distinct chemical classes: terpenes and phenylpropanoids. Although terpenes and their oxygenated derivatives (terpenoids) are more frequent and abundant in essential oils, certain species contain high quantities of shikimates; namely, phenylpropanoids, and when these compounds are present, they provide a specific odor and flavor to the plant [22] .

1.3.1-Terpenes

Terpenes and terpenoids result from the condensation of isoprene (2-methyl-1,3-butadiene), a pentacarbonate unit with two unsaturated bonds, and therefore are many times called isoprenoides . They have many isomeric cyclic or linear structures, and various degrees of unsaturations, substitutions, and oxygenated derivatives, generally called terpenoids [21،23] . Isoprene units are joined in one direction. The branched end of the chain is referred to as the head of the molecule and the other end as the tail. Therefore, the arrangement of the

structure is called head-to-tail joining. This pattern of coupling can be explained by the biosynthesis of terpenoids[24] . Furthermore, terpenes are the largest and most diverse class of volatile organic compounds (VOCs). Terpenes are classified into different structural and functional classes. Terpenes are classified according to the number of isoprene units in their structure, for example, hemiterpenes (one unit), monoterpenes (two units), sesquiterpenes (three units), diterpenes (four units), and so on. Most essential oils are highly complex mixtures of monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$), and include biogenetically related phenols (phenylpropanes and cinnamates), along with carbohydrates, alcohols, ethers, aldehydes, and ketones that are responsible for their characteristics. Furthermore, sometimes trace amounts of heavier terpenes, such as diterpenes, may also be present in essential oils with four isoprene units, but theseusually do not contribute to the odor of essential oils, as with the diterpenes found in ginger oil [22,25,26] .

i)- Monoterpenes

The main properties of monoterpenes are anti-bacterial, analge-sic, stimulant, and expectorant. Monoterpenes are naturally occurring constituents in essential oil plants with the majority being unsaturated hydrocarbons (C_{10}). Alcohols, ketones and carboxylic acids are present as substituents in the oxygenated derivatives of monoterpenes,which are

collectively known as monoterpenoids [27] . The branched-chains of C_{10} hydrocarbons, forming a linkage of two isomers, are widely distributed in herbal plants with more than 400 naturally occurring monoterpenes identified [28] .

ii)- Sesquiterpenes

Sesquiterpenes are biogenetically isolated from farensyl pyrophosphate, an intermediate in both the mevalonate and non-mevalonate pathways used by organisms in the biosynthesis of terpenoids, sterols, and terpenes. The structures of sesquiterpenes may be linear, monocyclic or bi- and tricyclic. Linear structures of sesquiterpenes, referred to as farnesenes, are branched hydrocarbons with four double bonds [29] . The farnesenes usually found in essential oils are E,E-farnesene and E-farnesene, while E,Z-farnesene and Z,Z-farnesene are not reported to occur in nature. Farnesols is a primary alcohol in oxidation products of farnesenes, as well as E- and Z-nerodiol as tertiary alcohol are occasional essential oil constituents. The aldehydes components of farnesenes consist of a-sinensal and B-sinensal, where they occur as minor components in essential oils from various Citrus species [30]. In monocyclic compounds of sesquiterpenes, there are bisobolenes groups with C_6 ring structures, and their isomers such as Z-bisabolene, ZZ-bisabolene, E-bisabolene, and Z-bisabolene which occur in several essential oils. Besides, Zingiberene, Curcumene, and arcurcumene also

found in sesquiterpenes group of essential oils. Zingiberene is the main component inside ginger essential oil (Zingiber officinale) and the pungent smell of this oil is due to the aromatic structure, gingerol (phenols). Curcumene is the main constituent found in Curcuma oils (Curcuma aromatic and Curcuma longa), while the aromatic curcumene usually found in Curcuma and Zingiber species [30] .

The bicyclicbergamotenes represent a structure of cyclobutane ring. In the lemon essential oil and lodgepole pine (Pinuscontorta), Trans-bergamotene occurs as a minor constituent. Caryophyllene (E-caryophyllene) derived from humulene presents a C_9 ring fused to a cyclobutane ring. It is broadly found as a major component in the essential oils from cannabis, oregano, and rosemary, and also found in the vegetative parts of many plants. Caryophyllene selectively binds to the CB(2) receptor so that it acts as a functional CB(2) agonist. Additionally, through in vivo study, this natural constituent exerts cannabimimetic effects with antiinflammatory action [31] .

iii)- Diterpenes

Diterpenes are made up from combining four units of isoprene. They are considered as too heavy components which do not evaporate easily during the extraction process using steam distillation; hence, it is impossible to be found in isolated aromatic oils. Diterpenes are found in all plant

families with C_{20} chemical structures. Almost 2500 known diterpenes were discovered which belong to 20 major structural types. Diterpenic derivatives can be found in plant hormones and phytol, where they occur as a side chain on chlorophyll [32].

1.3.2-Phenylpropanoids

Phenylpropanoids contain one or more C_6–C_3 units, with C_6 being a benzene ring. They usually have a methyl ether functional group attached to the ring, and a propenyl tail (three-carbon chain with one C = C bonded to the ring by one end). Many of the phenylpropanoids found in essential oils are phenols or phenol ethers, and in some cases the side chain is shortened (C_1). Their main representatives in essential oils include the oxygenated hydrocarbons anethole, eugenol, and safrole, which all possess a carbon–carbon double bond in the side chain (and are hence phenylpropanoid alkenes, or phenylpropanoids). α-Asarone, β-asarone, estragole, methyleugenol, and safrole are all phenylpropanoids that are rodent carcinogens [33,23].

1.3.3-Hydrocarbons

Hydrocarbons are the first main category of compounds and are composed entirely of carbon and hydrogen atoms, which vary greatly in size and complexity. They are very soluble in lipids (lipophilic), but are very poorly soluble in water. Simple hydrocarbons, such as alkanes, alkenes, and

benzenoids, are called nonterpenoid hydrocarbons .Another class of hydrocarbons is known as the aromatic class. These compounds usually contain a benzene ring (C_6H_6) and include phenyl, benzyl, phenylethyl, and phenylpropyl compounds, as well as polycyclic structures, such as naphthalene and benzo[α]pyrene. The name "aromatic" derives from the first benzene derivatives isolated from plants, which were found to be pleasant smelling. Subsequently, however, less pleasant derivatives were discovered [33] .

1.3.4-Alcohols

Alcohols in essential oils provide some excellent properties like anti-septic, anti-viral, anti-bacteria, and germicidal. Alcohols naturally occur as a single component or combined with a terpene or ester. The attachment of terpenes with oxygen and hydrogen atom can result in the formation of alcohols. The term Monoterpenol is used to describe a monoterpene that contains hydroxyl groups inside its hydrocarbon structure. Alcohols are considered safe to be used since their amounts are extremely low or totally absent of toxic reaction in the body or onto the skin [34] .

1.3.5-Esters

The formation of esters is due to the interaction between alcohols and acids. The ester component inside essential oils offers soothing and balancing effects. Due to the presence of alcoholic groups inside esters, they are able to provide anti-

inflammatory activities. In medical sciences, esters are considered to have antifungal and sedative properties, with balancing action on the nervous system. They are generally free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system [30] . The common esters found in essential oils are linyl acetate and geranyl formate which are present in bergamot and lavender, and geranium essential oils [30] .

1.3.6- Ketones

Ketones have anti-catarrhal, cell proliferant, expectorant, and vulnerary properties and are often found in plants that are used for upper respiratory tract complaints. Essential oils with ketone group are advantageous for wound healing and improve scar tissue. Thujone is a very toxic ketone component that exists in sage, mugwort, thuja, tansy, and wormwood essential oils. Besides, pulegone is another toxic ketone component found in essential oils such as pinocamphone and pennyroyal oils [30] . Other than that, jasmone, fenchone, carvone and menthone are the non-toxic ketone components found in jasmine oil,fennel oil,spearmint and dill oil,and peppermint oil [30].

1.4-Method of extraction of essential oils

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft

drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential oils are obtained from plant raw material by several extraction methods [35,36] .

1.4.1- Classical and Conventional Methods:

There are several by the numbers methods of extraction behavior of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused in copious parts of the globe. Hydrodistillation (HD), Steam distillation (SD), Solvent extraction, Enfleurage, Cohobation , and Maceration are the roughly traditional and generally used methods [36].

1.4.1.1- Hydrodistillation (HD):

Hydrodistillation is a traditional method for removal of essential oils. Water or hydrodistillation is one of the oldest and easiest methods. Being used for the extraction of essential oils [37] .

Hydrodistillation normally used to isolation essential oils from the aromatic and medicinal plant. The conventional method for the extraction of essential oils is hydrodistillation (HD), in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed

by the liquefaction of the vapors in a condenser.The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively . The principle of extraction is based on the isotropic distillation. In fact, at atmospheric pressure and during extraction process (heating), water or other solvent and oils molecules. Hydrodistillation (HD) is a variant of steam distillation, which is bespoke by the French Pharmacopoeia for the extraction of Essential oils from dried plants . There are three types of hydrodistillation: with water immersion, with direct vapor injection and with water immersion and vapor injection. It is a multilateral process that can be utilized for large or small industries. The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of essential oil, but does add unwanted high boiling point compounds and oxidation products [38] .

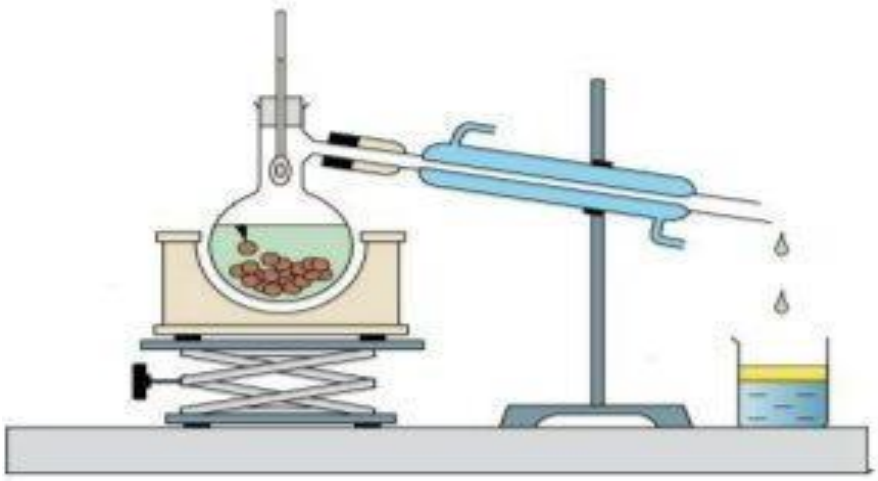

Fig.1: Apparatus for hydrodistillation.

1.4.1.2-Steam Distillation:

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation still important in certain industrial sectors [38] . Steam distillation is one of ancient and official approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material . This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected [39] . The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 ℃ so that the volatile components with the boiling points ranging from 150 to 300 ℃ can be evaporated at a temperature close to that of water. Furthermore, this technique

can be also carried out under pressure depending on the essential oils extraction difficulty [38] .

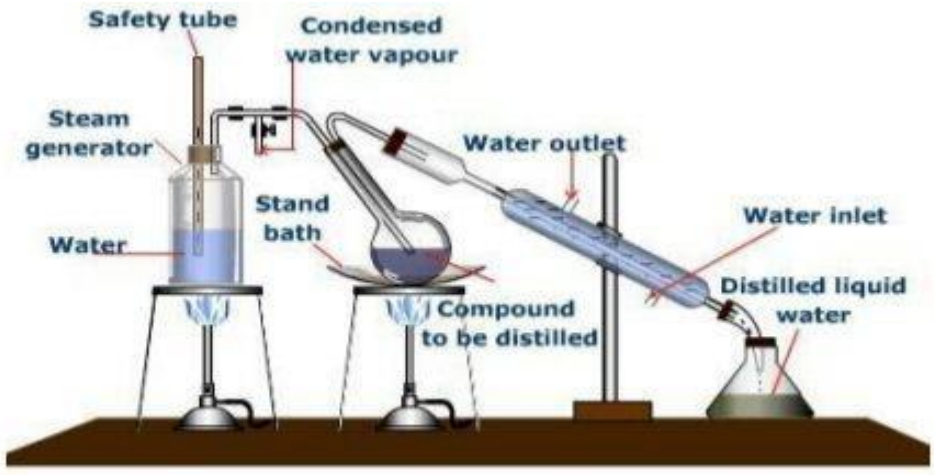

Fig.2: Apparatus for steam distillation

1.4.1.3-Solvent extraction:

Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example, water and an organic solvent . In the Solvent-Extraction method of Essential Oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost [40] . The most frequently applied sample preparation

procedure in plant material analysis. The quality and quantity of extracted mixture are determined by the type of extra heat applied because of the method is limited by the compound solubility in the specific solvent used. Although the method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility [41].

1.4.1.4- Soxhlet Extraction:

A Soxhlet extractor is a piece of laboratory apparatus [42] invented in 1879 by Franz von Soxhlet [43] .It was originally designed for the extraction of a lipid from a solid material . Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid [44]. This procedure is

repeated until virtually complete extraction is achieved. There are several advantages of Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed [17,41].

1.4.1.5-Cold Pressing method:

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure.Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant . It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method[17,42].

Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils [45] . In this process, the outer layer of the plants contains the oil are removed by scrubbing.Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation [17,42].

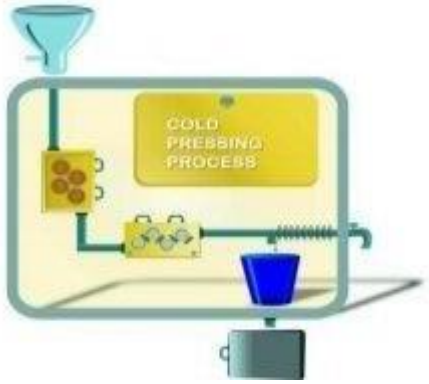

Fig.3: Apparatus for cold pressing method

1.4.2-Innovative Techniques Of Essential Oils Extraction

One of the disadvantages of conventional techniques is related with the thermolability of Essential oils components which undergo chemical alterations (hydrolyse, isomerization, oxidation) due to the high applied temperatures. The quality of extracted Essential oils is therefore extremely damaged

particularly if the extraction time is long. It is important that extraction methods could maintain Essential oils chemical composition and natural proportion at its original state. Since economy, competitiveness, eco-friendly, sustainability, high efficiency and good quality become keywords of the modern industrial production, the development of essential oils extraction techniques has never been interrupted. Strictly speaking, conventional techniques are not the only way for the extraction of essential oils. Novel techniques, for example, abide by green extraction concept and principles have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of official methods. New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO2 emissions [41,42].

Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on a commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as the production of costly essential oils in a natural state without any alteration of their thermosensitive components or the extraction of essential oils for micro-analysis [41,43].

1.4.2.1- Supercritical Fluid Extraction (SFE):

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO2) for several practice reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32Co), CO2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [46] . The main drawback of CO2 is its lack of polarity for the extraction of polar analytes [47] . These essential oils can include limonene and other straight solvents.Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods [48] . This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon

dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled.Supercritical extracts proved to be of superior quality with better functional and biological activities[49]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product [48].

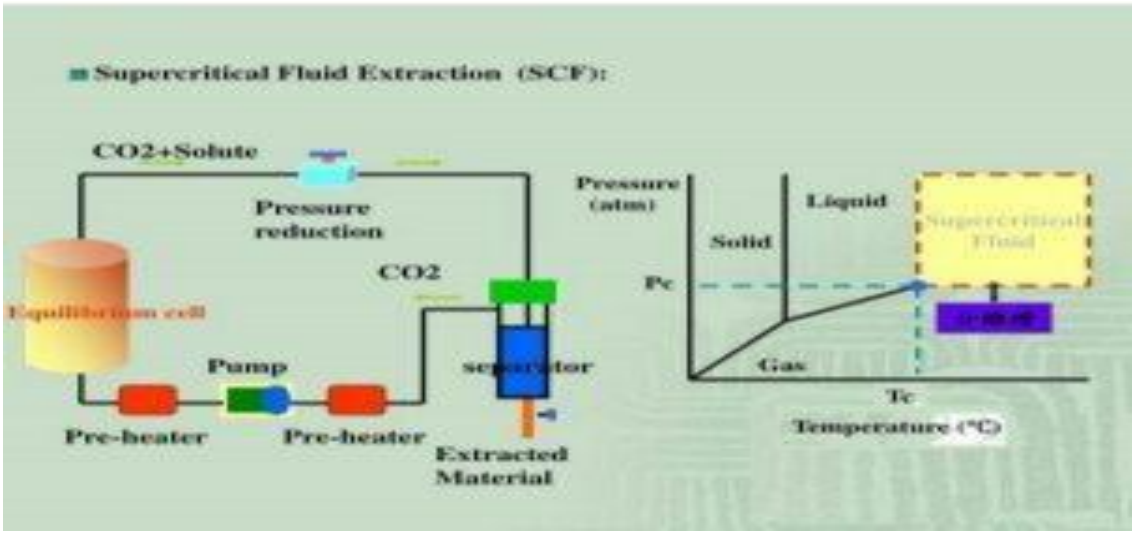

Fig.4: Scheme of supercritical fluid extraction (SFE)

1.4.2.2-Microwave-Assisted Hydrodistillation (MAHD):

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizinga microwave oven in the extraction process. Golmakani et al [50]. reported some recently published studies have successfully utilized a microwave oven for the extraction of active components from plants. The efficiency of Microwaveassisted hydrodistillation is strongly

dependent on the dielectric constant of water and the sample [51] .Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step [52] .High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique . Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO2 in atmosphere [53,54] and consuming only a fraction of the energy used in conventional extraction methods [55] . The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions [55] .

Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process.The principle of heating using Microwave-assisted hydrodistillation is based upon its direct impact with

polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously [56] .

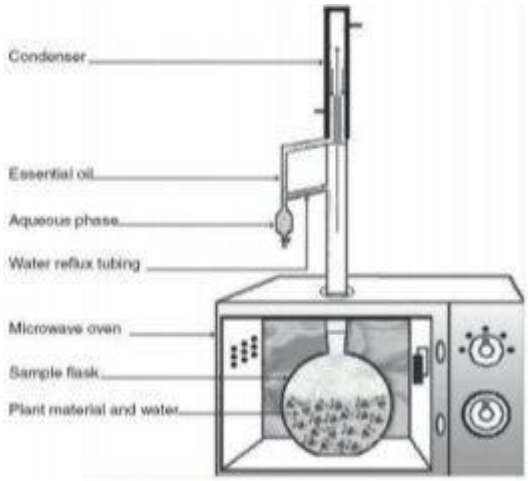

Fig.5: Apparatus of microwave-assisted hydrodistillation

1.4.2.3- Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could Involved to the increase in the estimate of some food by-products when used as sources of natural compounds or plant material [57] .The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. This technique was developed in 1950 at laboratory apparatus [58] . Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with

other techniques for example solvent extraction and hydrodistillation [58].

Ultrasound technology has been Featured as a valuable method in food engineering processes and plants [57], and become this field from the techniques active. In these applications the power ultrasound increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material affecting cluster transfer [59]. The plants raw material is immersed in water or another solvent(Methanol or ethanol or anyone from the solvents) and at the same time, it is subjected to the work of ultrasound [60]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds [61].

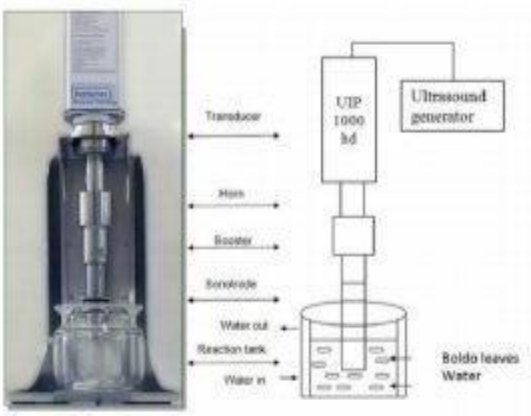

Fig.6: Apparatus of ultrasound-assisted extraction (UAE)

1.4.2.4- Solvent-free microwave extraction (SFME):

Solvent-free microwave extraction (SFME) is in the extraction procedure of essential oil which is cloaca by the in site water of the plant material without added any solvent [62] . Developed this method by Cheat and co-workers [53] .Based on the integration of dry distillation and microwave heating energy . It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent [63] . In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature,and extraction time were controlled by the panel in the instrument. The separated essential oil was dried over anhydrous sodium sulfate and stored at 4 0C in the dark. The extraction yield of essential oil was calculated as follows:

$$\text{Extraction yield (ml/kg)} = V/M$$

where V is the volume of essential oil in herb samples (ml), and M is the mass of the herb samples (kg) [63] .

Fig.7:Apparatus of solvent-free microwave extraction (SFME)

1.4.2.5-Microwave hydro diffusion and gravity (MHG):

Is a new green technique for the extraction of essential oils. This green extraction technique is an original microwave blend microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of materialplants [64] .

Microwave hydro diffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and as it does require less energy [65] .The performances and advantages of this technique are a reduction of extraction time (in the case of hydro-distillation it takes 90min or more but in this technique only 20min) and reducing environmental impact and power saving [66] .

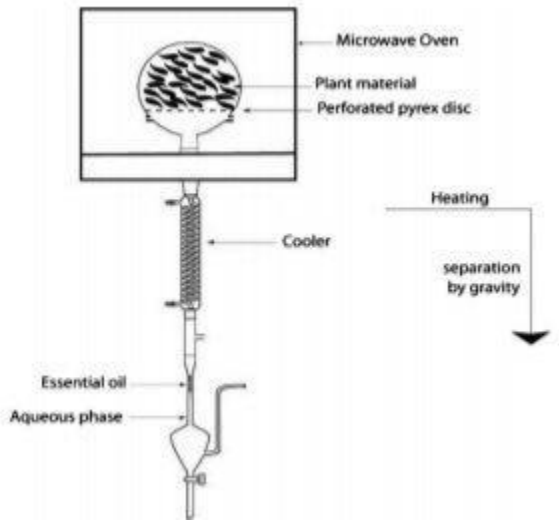

Fig.8:Apparatus of microwave hydro diffusion and gravity

1.5-Solenostemma argel

Solennostemma argel (Apocynaceae) is a desert plant widely distributed in Egypt with the name "hargel" [67] ,and in Sudan which is its richest source [68] . It the most important one from the many Egyptian plants which are known to be of potential medicinal value in herbal medicine [69] . An extract from the leaves of this plant showed fungitoxic activity [70] .The leaves are used in herbal medicine for the treatment of some diseases such of liver and kidney and allergies . it is an effective remedy for bronchitis and is used to treat neuraigia and sciatica . It is used in the treatment of measles , and some crushed and used as remedy for suppurating wounds [71].

Fig.9: Solenostemma argel

The leaves are infused to treat gastro-intestinal cramps, stomachache, colic, cold, and urinary tract infections and is effective as an anti-syphilitic where it is used for prolonged periods of 40-80 days [71,72] . Leaves possess purgative properties which may be due to the latex present in the stems . Several active compounds have been extracted from S.argel . The native Sudanese have commonly used solenostemma argel to suppress stomach pain, pains due to child birth, and loss of appetite .It has been proved that its crude aqueous extract possessed larvicidal activity against mosquito larval [69].

From the previous phytochemical studies , it was found that the leaves are characterized by high carbohydrates , low crude fiber, proteins, crude oil, ash, and high potassium,magnesium, sodium, and low copper, ferrous, manganese, lead, and contained phytic acid and tannins [73] . Also S . argel contains acylated phenolic,glycosides,pregnene glycoside(solenoside A) kaempferol-3-O-glucoside and 3-O-rutinoside(R) . Also it was found that its aerial parts contained two monoterpene glucosides, a pregnane glucoside, benzyl alcohol β-apiofuranosyl(1-6),β-glucopyranoside(1-6),β-glucopyranoside astragalin and kaempferol-3-O-neohesperidose [73] .

Aim of this study

This study was aimed to fulfill the following :

-Extraction of oil from the medicinally important Solenostemma argel.

-Conducting a GC-MS analysis to identify and quantify the oil constituents .

-Evaluating the oil for its antimicrobial potential

Chapter Two

Materials and Methods

Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Solenostemma argle* collected from Shendi (Sudan) and 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 oil was assessed 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 *Solenostemma argle* (300g) were exhaustively extracted with n-hexane by maceration.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C 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 oil was 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(^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions

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

2.2.3-Antimicrobial assay

i)-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.

Chapter Three

Result and Discussion

Results and Discussion

3.1-*Solenostemma argel*

The constituents of *Solenostemma argel* – an underutilized medicinal plant of potential applications – were investigated.

3.1.1-GC-MS analysis

The oil was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra . The GC-MS analysis revealed the presence of 31 components Table 1. The typical total ion chromatograms(TIC) is depicted in Fig. 10.

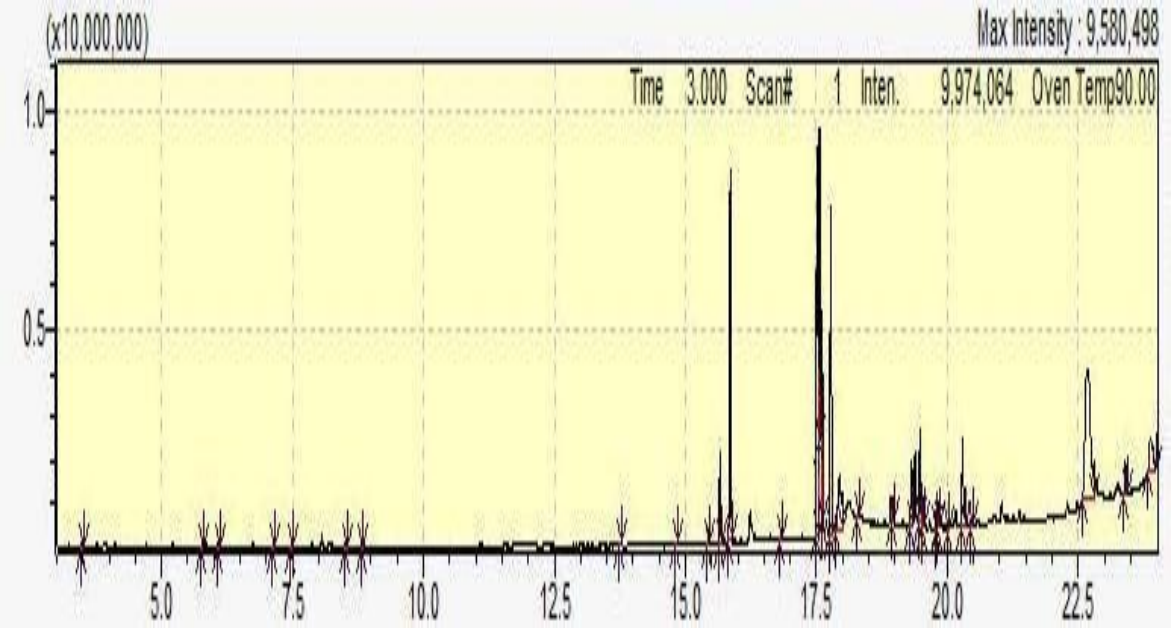

Fig.10: Typical total ion chromatogram

Table 4 : Contituents of *Solenostemma argel* oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.480	144785	0.12	Hexanoic acid, methyl ester
2	5.783	69690	0.06	1,6-Octadien-3-ol, 3,7-dimethyl-
3	6.085	103725	0.09	Octanoic acid, methyl ester
4	7.151	155514	0.13	L-.alpha.-Terpineol
5	7.486	270614	0.22	Nonanoic acid, methyl ester
6	8.512	210736	0.17	2,4-Decadienal
7	8.827	351695	0.29	2,4-Decadienal, (E,E)-
8	13.746	110588	0.09	Methyl tetradecanoate
9	14.823	122182	0.10	Pentadecanoic acid, methyl ester
10	15.440	598062	0.49	Tonalid
11	15.661	3181745	2.62	9-Hexadecenoic acid, methyl ester, (Z)-
12	15.857	15443492	12.72	Hexadecanoic acid, methyl ester
13	16.832	286107	0.24	Heptadecanoic acid, methyl ester
14	17.516	19072090	15.71	9,12-Octadecadienoic acid (Z,Z)-, methyl e
15	17.562	11943428	9.84	9-Octadecenoic acid (Z)-, methyl ester
16	17.608	7395521	6.09	9-Octadecenoic acid, methyl ester, (E)-
17	17.776	14348504	11.82	Methyl stearate
18	17.941	5359711	4.42	Oleic Acid
19	18.302	461279	0.38	Hexadecanoic acid, butyl ester
20	18.951	840076	0.69	4,8-Ethano-4H-1,3-benzodioxin, hexahydr
21	19.318	2492312	2.05	4-Oxo-.beta.-isodamascol
22	19.476	3368614	2.78	Octanoic acid, 2-propenyl ester
23	19.531	954705	0.79	Eicosanoic acid, methyl ester
24	19.791	620150	0.51	n-Propyl 9,12-octadecadienoate
25	19.818	846611	0.70	2,3-Dihydroxypropyl elaidate
26	20.015	324356	0.27	Octadecanoic acid, butyl ester
27	20.289	4440203	3.66	Octadecanoic acid, 9,10-dihydroxy-, methy
28	20.464	393729	0.32	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
29	22.686	22120646	18.23	7-Hexadecenal, (Z)-
30	23.400	1194043	0.98	Squalene
31	23.875	4143332	3.41	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
		121368245	100.00	

The GC-MS analysis revealed the following major constituents:

(i) 7-Hexadecenal, (z) (18.23%)

The EI mass spectrum of 7-hexadecenal, (z) is shown in Fig. 11, the peak at m/z 185 which appeared at R.T 22.686 corresponds $M^+[C_{16}H_{30}O]^+$.The signal at m/z 154 accounts for loss methoxyl function.

(ii) 9.12 Octadecadienoic acid (z,z)methyl ester (15.17%)

The mass spectrum of 9,12- octadecadienoic acid (z,z) methyl ester is depicted in Fig.12.The signal at m/z 294(R.T 17.516)

corresponds $M^+[C_{19}H_{34}O]^+$, while the signal at m/z 263 is attributed to loss methoxyl function.

(iii) Hexadecanoic acid methyl ester (12.27%)

Mass spectrum of Hexadecanoic acid methyl ester is depicted in Fig. 13. The peak at m\z 270, which appeared at R.T.15.857 accounts for $M^+\{C_{17}H_{34}O_2\}^+$, while the peak at m\z 239 is attributed to loss of methoxyl function.

(iv) Methyl stearate (11.82%)

Mass spectrum of methyl stearate is shown in Fig. 14. The peak at m/z 298which appeared at R.T. 17.775 corresponds to $M^+\{C_{19}H_{38}O_2\}$. The peak at m/z 267 is due to loss of methoxyl function.

(v) 9-Octadecenoic acid (Z)-, methyl ester (9.84%)

Fig. 15 displays the mass spectrum of 9-octadecenoic acid (z)-, methyl ester . The peak at m/z 296 which appeared at R.T. 17.562 corresponds to $M^+ [C_{19} H_{36} O_2]^+$ The signal at m/z 264 is attributed to loss of a methoxyl function**.**

(vi) 9-Octadecenoic acid, methyl ester, (E) (6.09%)

Fig.16 shows the mass spectrum of 9-octadecenoic acid, methyl ester. The peak at m/z 296 (R.T.17. 608) corresponds to M^+ $[C_{19}H_{36}O_2]$, whilst the peak at m/z 264 accounts to loss of methoxyl function**.**

(vii) Oleic Acid (4.42%)

Mass spectrum of Oleic acid) is shown in Fig.17. The peak at m/z 264 which appeared at R.T 17.941 corresponds to$C_{18}H_{34}O_{2.}$ The peak at m/z 233 corresponds to loss of methoxyl function**.**

(viii) Octadecanoic acid,9,10-dihydroxy-, methyl (3.66%)

The mass spectrum of Octadecanoic acid,9,10-dihydroxy-, methyl is shown in Fig. 18. The peak at m/z 187 which appeared at R.T 20.289 corresponds to $C_{19}H_{38}O_{4.}$ The peak at m/z 155 corresponds to loss of methoxyl function**.**

(ix) E,E,Z-1,3,12-Nonadecatriene-5,14-diol (3,41%)

Mass spectrum E,E,Z-1,3,12-nonadecatriene-5,14-diol is shown in Fig.19. The signal at m/z 292 (R.T 23.875) corresponds to M^+ $[C_{19}H_{38}O_4]^+$. The peak at m/z 261 is due to loss of methoxyl function**.**

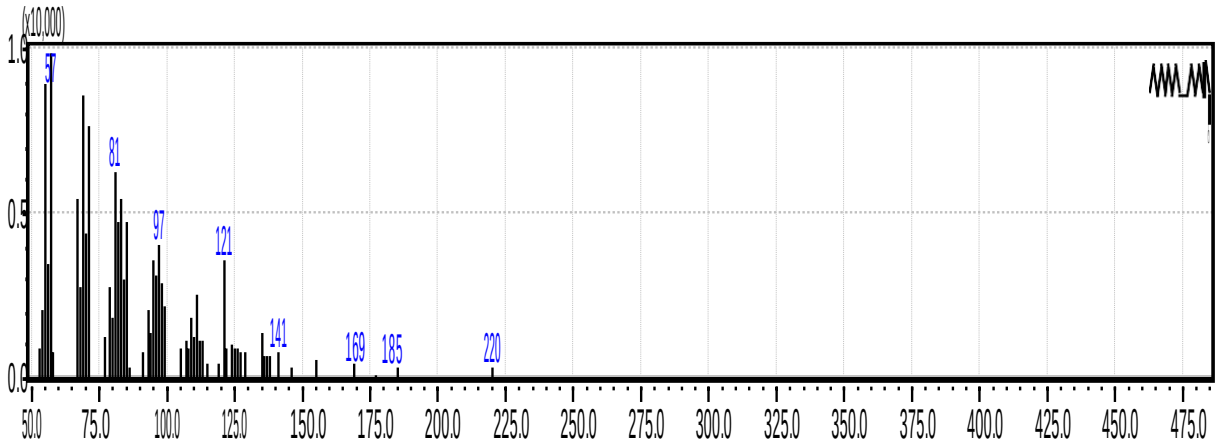

Fig. 11: Mass spectrum of 7-hexadecenal

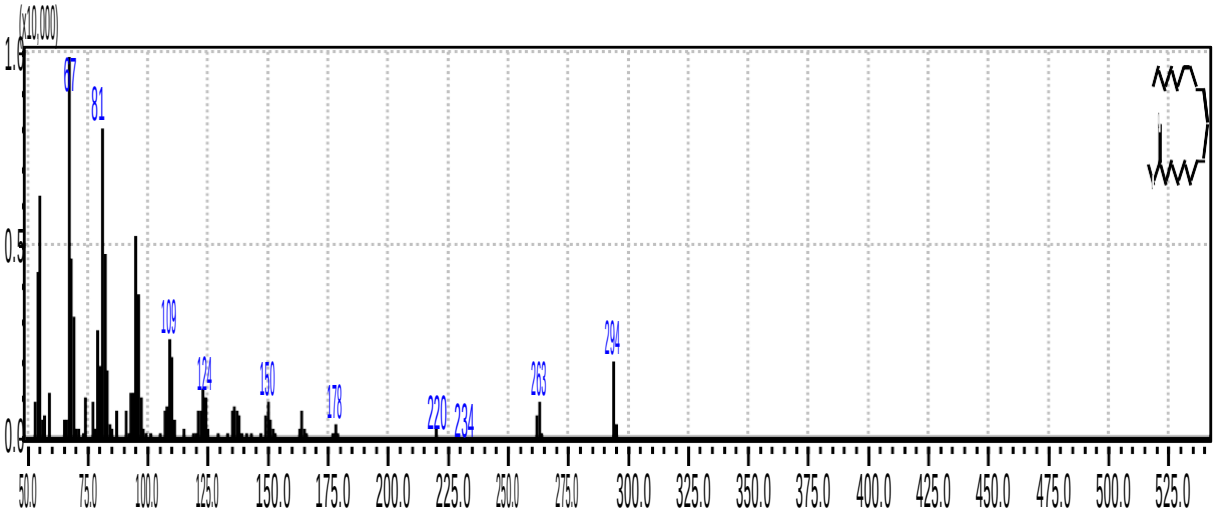

Fig:12 : Mass spectrum of 9-12-octadienoic acid(z,z)methyl ester

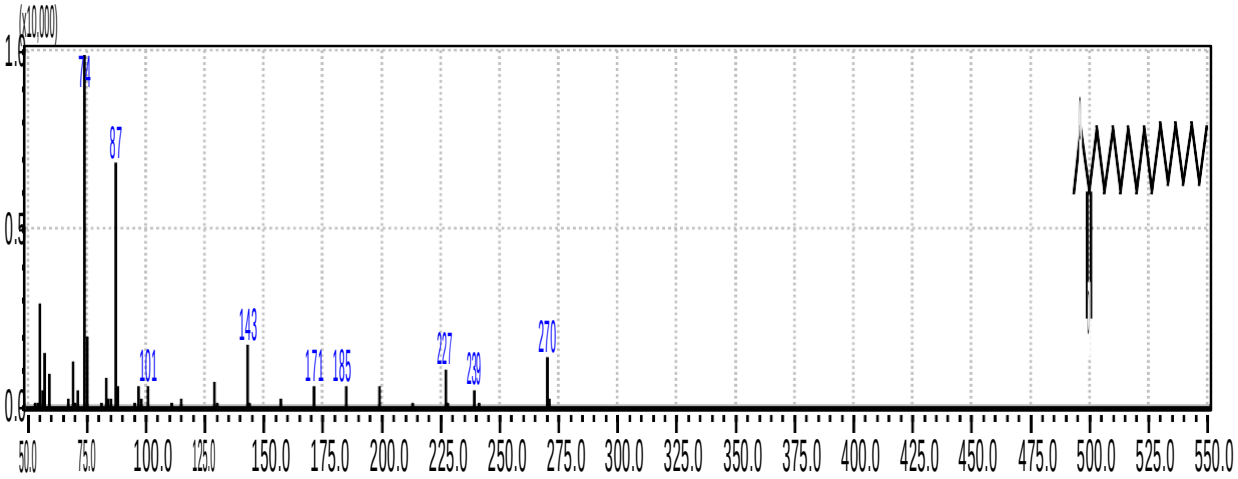

Fig.13:Mass spectrum of hexadecanoic acid methyl ester

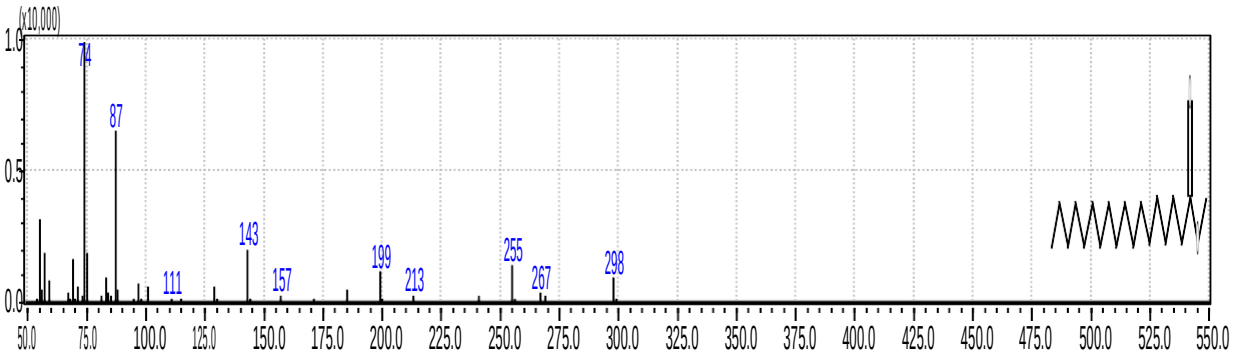

Fig. 14: Mass spectrum of methyl stearate

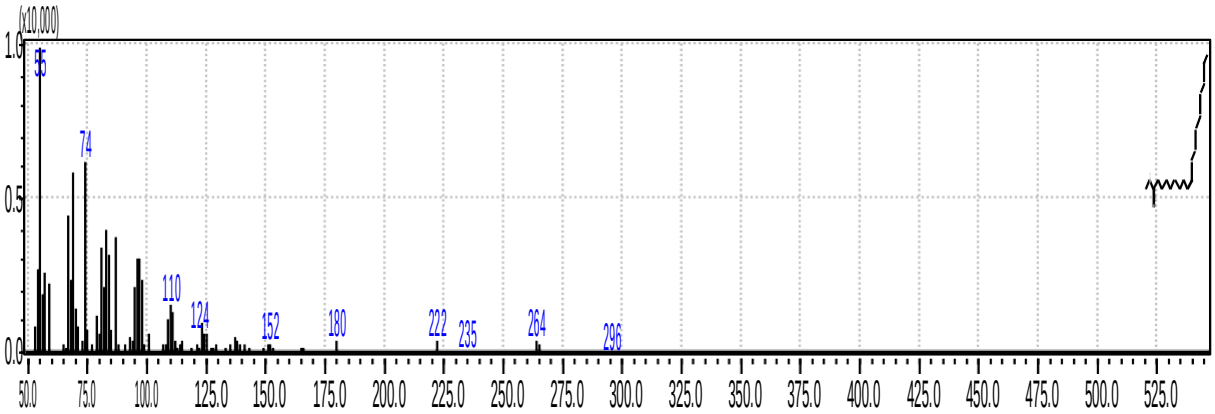

Fig.15 :Mass spectrum of 9-octadecenoic acid (z)-, methyl ester

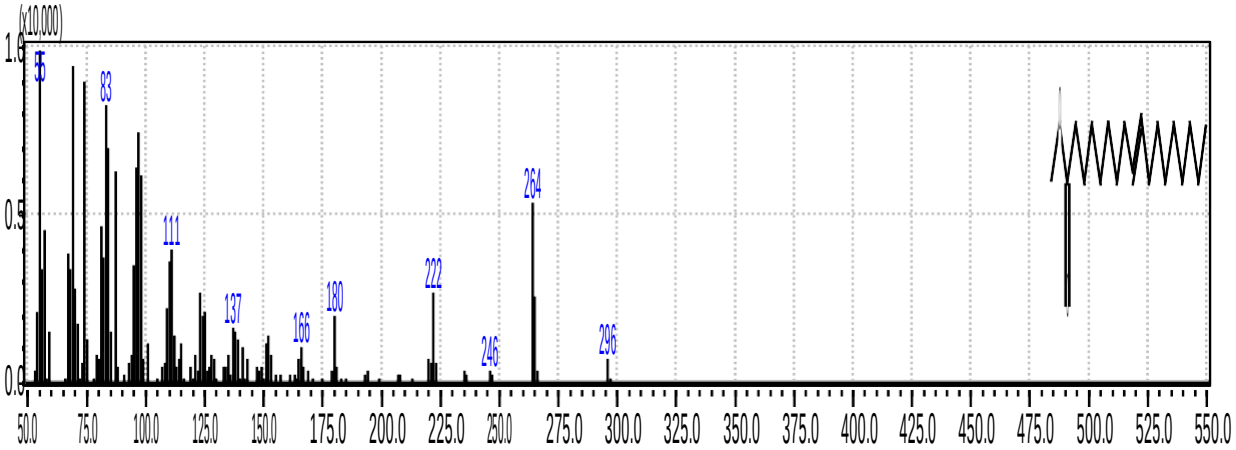

Fig.16 : Mass spectrum of 9-Octadecenoic acid, methyl ester(E)

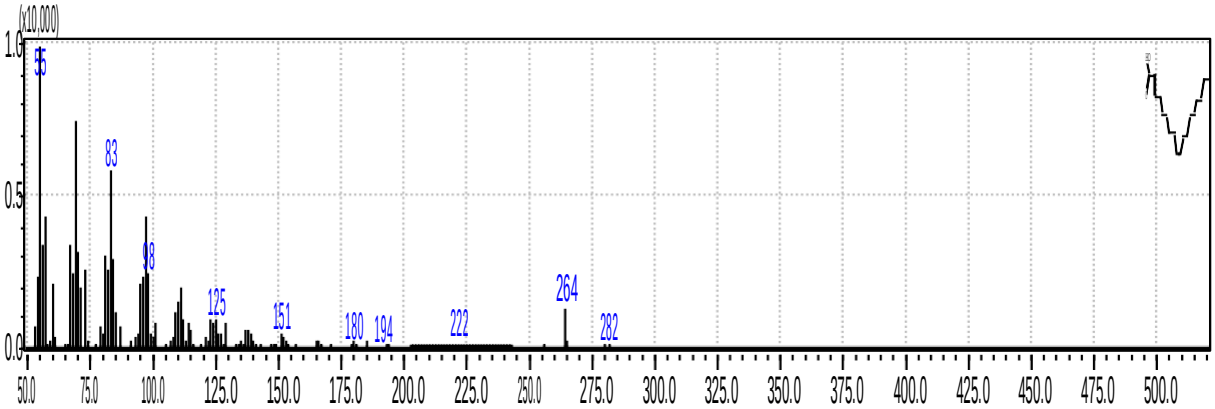

Fig. 17: Mass spectrum of oleic acid

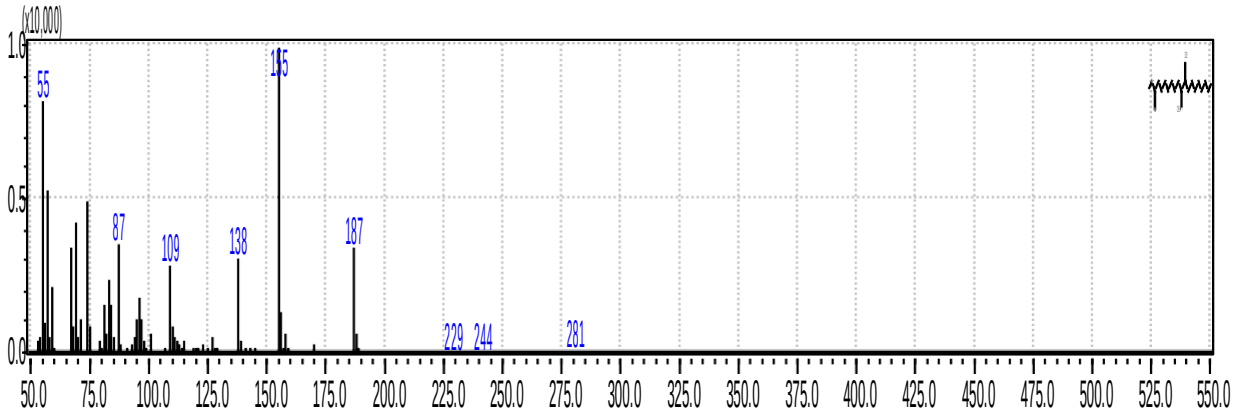

Fig. 18: Mass spectrum of octadecanoic acid,9,10-dihydroxy-, methyl ester

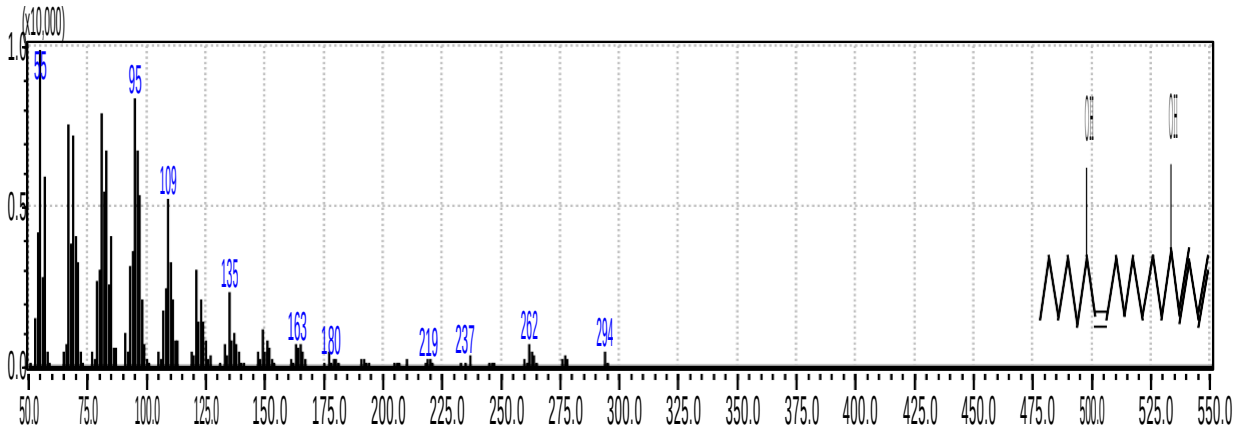

Fig.19:Mass spectrum E,E,Z-1,3,12-nonadecatriene-5,14-diol

3.1.2-Antimicrobial activity

In cup plates agar diffusion assay the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 5.

Table 5 : Antimicrobial activity of *Solenostemma argel* oil

Type	Ec.	Ps.	Sa.	Bs.	Ca.
Oil 100mg/ml	22	18	16	--	15

Sa: Staphylococcus aureus.

Ec: Escherichia coli.

Ps: Pseudomonas aeruginosa.

Bs: Bacillus subtilis.

Ca: Candida albicans.

Solenostemma argel oil showed excellent activity against *Escherichia coli* and *Pseudomonas aeruginosa* . The oil exhibited very good activity against *Staphylococcus aureus* . It also showed good anticandidal potency.

Conclusion

Solennostemma argel oil was investigated by GC-MS analysis. The analysis showed the presence of 31 constituents being dominated by:

(i) 7-Hexadecenal, (z) (18.23%)

(ii)9.12 Octadecadienoic acid (z,z)methyl ester (15.17%)

(iii)Hexadecanoic acid methyl ester (12.27%)

(iv)Methyl stearate (11.82%)

(v)9-Octadecenoic acid (Z)-, methyl ester (9.84%).

The oil was evaluated for antimicrobial activity. It showed excellent against some of the test organisms.

Recommendations

Recommendations include the following:

1-The extracted oil may be evaluated for other pharmacological properties such as antimalarial and antiviral activity.

2-Other biologically active constituents of the targeted plant may be isolated and their bioactivity could be assessed.

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