Sudan University of Science and Technology

College of Graduate Studies

Constituents of Celery (*Apium graveolens*) Seed Oil and its Biological Activity

مكونات زيت بذرة الكرافس وفعاليته البيولوجية

A Thesis Submitted in Partial Fulfillment for the Requirements of the Master Degree in Chemistry Science

by

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قال تعالى:

(وَقُلِ اعْمَلُوا فَسَيَرَى اللهُ عَمَلَكُمْ وَرَسُولُهُ وَالمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الغَيْبِ وَالشَّهَادَةِ فَيُنَبِئُكُمْ بَمَا كُنتُمْ تَعْمَلُونَ)

صَدَقَ اللهُ العَظِيمْ

سُورَةْ التَّوبَهْ الآيَة (105)

Dedication

To

My beloved father and mother

My sisters with love.

Acknowledgment

First and fore most profound thanks to **Allah** for providing us with unfailing support and good health well-being throughout our study and through the process of researching and writing this thesis .

I would like to express my sincere gratitude to my supervisor Prof. Mohamed Abdelkarim for his guidance , patience and continuous support throughout the period of this study.

Thanks are extended to the technical staff-Sudan University of Science and Technology for all facilities.

Thanks are due to my family for their infinite support.

Abstract

This study was aimed to extract the fixed oil of *Apium graveolens* through the process of maceration with normal hexane. The oil was analyzed by gas chromatography-mass spectrometry. The antimicrobial activity was also estimated.

The GC-MS analysis of the studied oil showed the presence of 45 constituents. Major components are:

i) 9-Octadecenoic acid methyl ester (56.04%)

ii) 9, 12-Octadecadienoic acid methyl ester (13.47%)

iii) Hexadecanoic acid methyl ester(12.05%)

iv) Methyl stearate(4.69%)

The oil showed partial antimicrobial activity against some test organism.

المستخلص

هدفت هذه الدراسة لاستخلاص الزيت الثابت لبذور نبات الكرافس عن طريق عملية النقع بواسطة الهكسان العادي. تم تحليل الزيت بواسطة جهاز كروموتوتغرافيا الغاز- مطياف الكتلة كما وتم قياس نشاط مضادات البكتريا والفطريات. اوضح التحليل وجود 45 مكونا أهمها:

i) 9-Octadecenoic acid methyl ester (56.04%)

ii) 9, 12-Octadecadienoic acid methyl ester (13.47%)

iii) Hexadecanoic acid methyl ester(12.05%)

iv) Methyl stearate(4.69%)

وفى اختبار مضاد الميكروبات ابدى الزيت فعالية جزئية ضد بعض الميكروبات.

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Introduction

1.1-Natural products

Thousands of years ago natural products have been used for disease prevention and health care. Ancient civilizations of Chinese, North Africans and Indians provide 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(Newman,2007).

Similarly mandrake was used for relief of pain. Turmeric was used for blood clotting. Gall bladder infections were treated by the roots of endive plants. Raw garlic was used to treat the circulatory disorders(Newman,2003).

These old medicines are still used in many countries as the alternative medicines. Until nineteenth century active components were not isolated from medicinal plants(Chin et.al.,2006).

In 1806 Friedrich Sertürner was a scientist who isolated morphine from the *Papaver somniferum*. Then natural products were extensively screened to obtain medicines. Atropine was obtained from the *Atropa belladonna* (Haefner,2003).

According to modern search 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(Koehn and Carter,2005).

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(Kong et. al.,2003, Phillipson,2001).

Plants play a vital 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 (Graham et. al.,2000). 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 (Sofowora,1982). 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(Jeychandran and Mahesh,2007).

1.1.1-Tannins

The tannin compounds 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) (Sofowora,1982).

1.1.2-Saponins

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative(Sofowora,1982)..

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(Sofowora,1982)..

1.1.3-Steroids

Steroids comprise a group of cyclic organic compounds whose most common characteristic is an arrangement of seventeen carbon atoms in a four-ring structure, where the rings are three 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(Sofowora,1982)..

1.1.4-Glycoside

In chemistry, a glycoside is a molecule 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(Sofowora,1982).

1.1.5-Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. 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(Sofowora,1982).

1.1.6-Flavonoids

Flavonoids are the low molecular weight polyphenolic secondary metabolic compounds, universally distributed in green plant kingdom, located in cell vacuoles. Flavonoids play a variety of 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(Sofowora,1982)..

Flavonoids form a family of well known natural products present in most of the plant families. More than 8000 different flavonoids have been isolated from their natural source to date. 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(Sofowora,1982)..

1.2-Essential oils

Essential oils (EOs) are obtained from aromatic and medicinal plants as a volatile mixture of chemical compounds with strong odor. EOs are extracted from the aromatic and medicinal plants using steam or hydrodistillation or Soxhlet extraction (solvent extraction or continuous extraction) methods developed in the middle ages by Arabs (Buchbauer ,2010)

EOs are considered as one of the most predominant plant products , as they exhibit antifungal, antibacterial, antioxidant, anticancer, antidiabetic, antiviral, insect- repellent, and anti-inflammatory properties (Buchbauer 2010; Swamy et al. 2016).

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 (Swamy and Sinniah, 2015, 2016).

Presently, 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 (Swamy and Sinniah 2015, 2016).

EOs, in perfumes, creams, soaps, in flavor and fragrance for foods, sanitary products and industrial solvents phytocompounds, 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 (Swamy and Sinniah 2015, 2016; Arumugam et al. 2016). 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).

EOs are widely used as fragrances. However, 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 (Carson and Riley 2003) .

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(Arumugam et al. 2016). 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.

1.2.1-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(Masotti et al. 2003; Swamy and Sinniah 2015; Swamy et al. 2016).

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 (Smith et al. 2005) 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 (Bruneton, 1995). 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:

i-Hydrodistillation

ii-Hydrodiffusion

iii- Effleurage

iv-Cold pressing

v-Microwave Assisted Process (MAP)

vi-Steam distillation

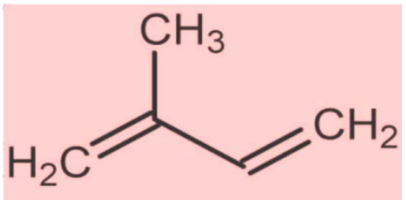
vii-Solvent extraction

viii-Carbon dioxide extraction

1.2.2-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 are obtained biosynthetically via mevalonate pathway (Swansom and Hohl 2006).



Isoprene

1.3-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 (Vigan ,2010). EOs are rich in terpenes, while phenylpropanoids more frequently occur in Apiaceae, Alliaceae, Lamiaceae, Myrtaceae, and Rutaceae plant families. 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 (Swamy and Sinniah 2015; Swamy et al. 2016).

1.3.1-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 (Swamy et al. 2016). 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 (Fujita et al. 2015). *O. basilicum* essential oil exhibits good antibacterial properties against Gram-positive bacteria (Al-Abbasy et al. 2015). 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* (Takarada et al. 2004). Tea tree

(*Melaleuca alternifolia*) oil is demonstrated to be sensitive to 15 genera of oral bacteria, indicating its potential applications in oral hygiene. *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 (Swamy et al. 2016).

1.3.2-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 phytocompounds, such as, phenolic compounds and terpenoids . 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(Tepe et al. 2004).

Thymol and carvacrol containing EOs in particular show strong antioxidant properties (Tepe et al. 2004). Likewise, EOs of

Cuminum cyminum, *Petroselinum sativum*, *S. cumini*, and *Coriandrum sativum* also exhibit efficient antioxidant. In addition, clove oil shows a much stronger antioxidant and radical scavenging activity compared to cinnamon, basil, oregano, nutmeg, and thyme EOs .

1.4-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.

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.

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 Oregon State University, 2012) by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog et al., 2007). 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.

1.4.1-Instrumentation and working of GC-MS

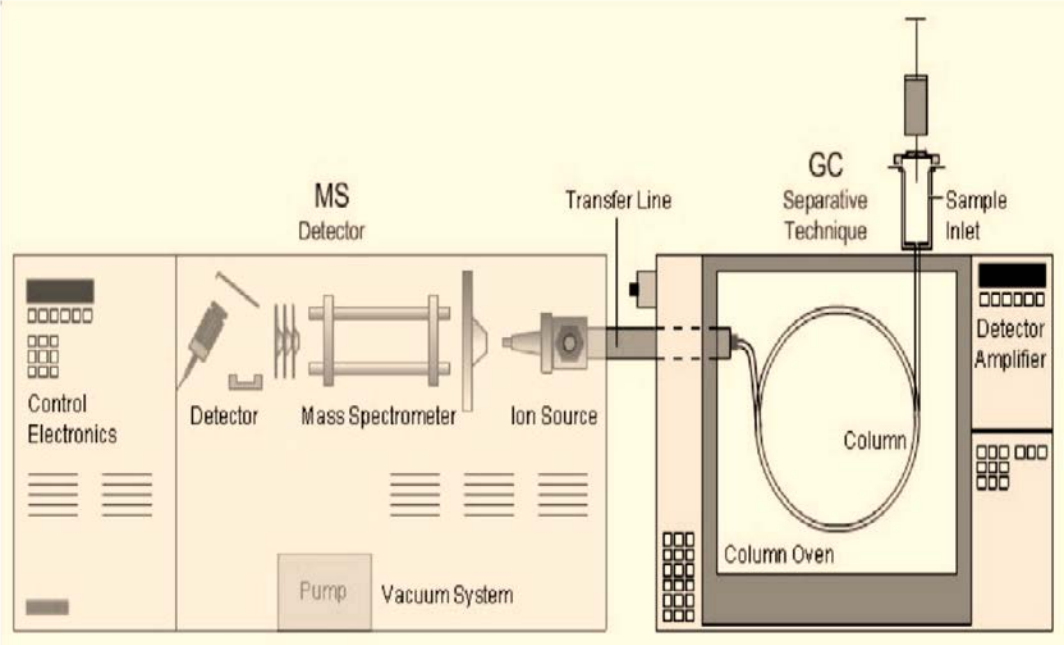


Fig. 1. Schematic diagram of GC-MS

Fig. 1 is the 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).

-Injector:

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

***-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).

-Ovens : *:*

Gas chromatography have ovens that are temperature programmable,

1.4.2-Mass spectrometry

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

differentiate ions. Ion source: In the ion source, the products are ionized prior to analysis in the mass spectrometer.

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.

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).

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.

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.

1.5-*Apium graveolens*

Apium grveolens L. is an annual or perennial plant in the family Apiaceae.It grows along tropical and subtropical Africa and Asia and throughout Europe(Gauri et.al.,2015).Phytochemical studies indicated the presence of many phytochemicals including alkaloids, steroids and flavonoids(Khare ,2008).The plant also contains carbohydrates beside vitamins A and C(Kooti ,2014).The seeds, leaves and essential oils are all used in ethnomedicine. Studies showed that *Apium grveolens* can reduce the risk of cardiovascular and liver diseases. It can also treat jaundice and gout and can protect against urinary tract obstruction(Bhattacharjee ,2004, Sowbhagya ,et.al.,2010, Nadkarni et.al.,2010 , Karnick ,1994).

Apium graveolens

Leaves can increase spermatogenesis and imrove fertility(Grzanna et.al.,2005, Zare et.al.2016, Kooti et.al.,2014(a)). *Apium grveolens* hypoglycemic, hypotensive and heart tonic(Kooti et.al.,2014(b) , Gelodar et.al.,1997, Lans ,2006).In vivo studies indicated that *Apium grveolens* possesses antifungal and antiinflammatory properties(Kooti et.al.,2014(a), Mencherini et.al.,2007).The antibacterial effect of *Apium grveolens* essential oil has been documented(Atta ,1998). The plant is used traditionally against asthma, skin infections, asthenopia, bronchitis, vomiting, fever and tumors(Khare,2007, Kritikar,2008) .

Aim of this study

This study was designed to:

-Extract oil from the medicinally important Apium graveolens.

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

-Evaluate the oil for its antimicrobial potential.

2-Materials and Methods

2.1-Materials

2.1.1--Plant material

Apium graveolens subsp. *Dulce* seeds were purchased from the local market Rhyadh-Saudi Arabia. The plant was authenticated by direct comparison with a herbarium sample. The seeds were shade – dried at room temperature and powdered

2.1.2-GC-MS analysis

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

2.1.3-Test organisms

The oil from *Apium graveolens* seeds was screened for antimicrobial activity 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
5	*Candida albicans*	fungi

2.2-Methods

2.2.1-Extraction of oil

Powdered shade-dried seeds of *Apium graveolens* (300g) were exhaustively extracted with n-hexane at room temperature.The solvent was removed under reduced pressure to give the oil.The oil was esterified as follows :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 n

ormal 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-Constitents of the oil

The studied oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument.Helium was used as carrier gas. Chromatographic conditions are presented below:

- *Oven temperature program*

Rate : --- ; Tempt. , 150.0^{0}C ; Hold time($min.^{-1}$) ,1.00

Rate : 4.00 ; Tempt. , 300.0^{0}C ; Hold time(min.$^{-1}$) ,0.00

Column oven temperature	150.0°C
Injection temperature	300.0°C
Rate	4/min
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

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to manufacture instructions.
Broth cultures($5.0x10^7$cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs(Oxid,6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria the plates were incubated at 37ºCfor 24h., while for fingi the plates were incubated at 25ºC for 3days.The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamycin and clotrimazole were used as positive control and DMSO as negative control.

3-Results and Discussion

3.1-*Apium graveolens* Subsp. *Dulce* oil

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

3.1.1-Constituents of oil

The GC-MS spectrum of the studied oil showed the presence of 45 constituents (Table 3.1)).The typical total ion chromatograms (TIC) is shown in Fig (3.1).

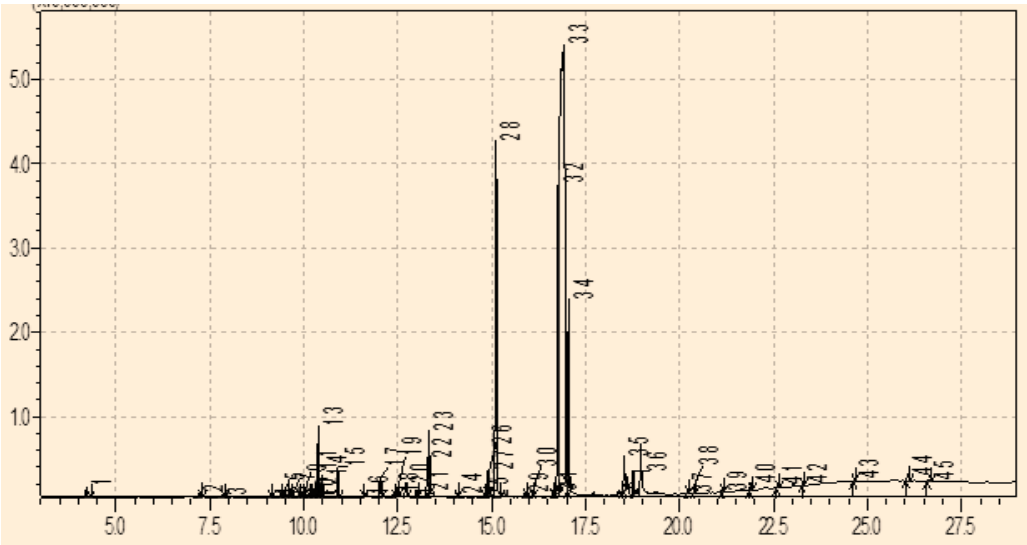


Fig (3.1): Total ion chromatograms

The major constituents of the oil are:

i) 9-Octadecenoic acid methyl ester (56.04%)

ii) 9, 12-Octadecadienoic acid methyl ester (13.47%)

iii) Hexadecanoic acid methyl ester(12.05%)

iv) Methyl stearate(4.69%)

The constituents of the oil are briefly discussed below:

i) 9-Octadecenoic acid methyl ester (56.04%)

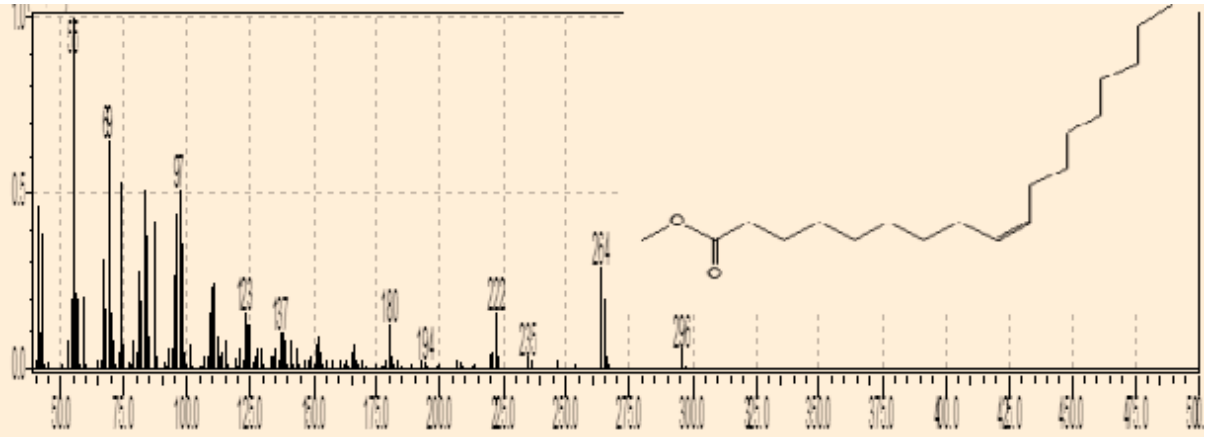


Fig (3.2): Mass spectrum of 9-octadecenoic acid methyl ester

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig(3.2).The peak at m/z 296, which appeared at R.T. 16.933 in total ion chromatogram, corresponds the molecular ion: $M^+[C_{19}H_{36}O_2]^+$ .The signal at m/z266 is due to loss of a methoxyl .

ii) 9,12-Octadecadienoic acid methyl ester(13.47%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Fig(3.3).The signal which was observed at m/z294(R.T. 16.774) is due to $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z263 corresponds to loss of a methoxyl.

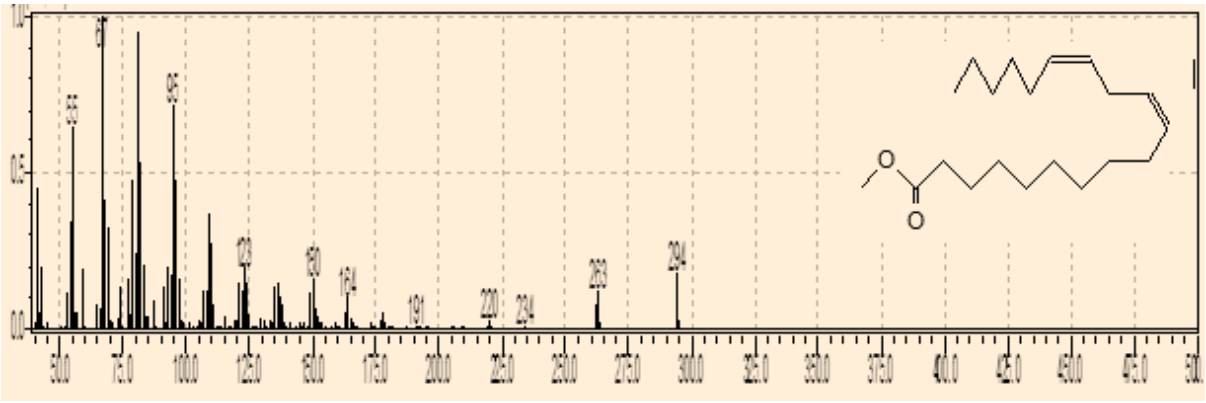


Fig (3.3): Mass spectrum of 9,12-octadecadienoic acid methyl ester

iii) Hexadecanoic acid methyl ester (12.05%)

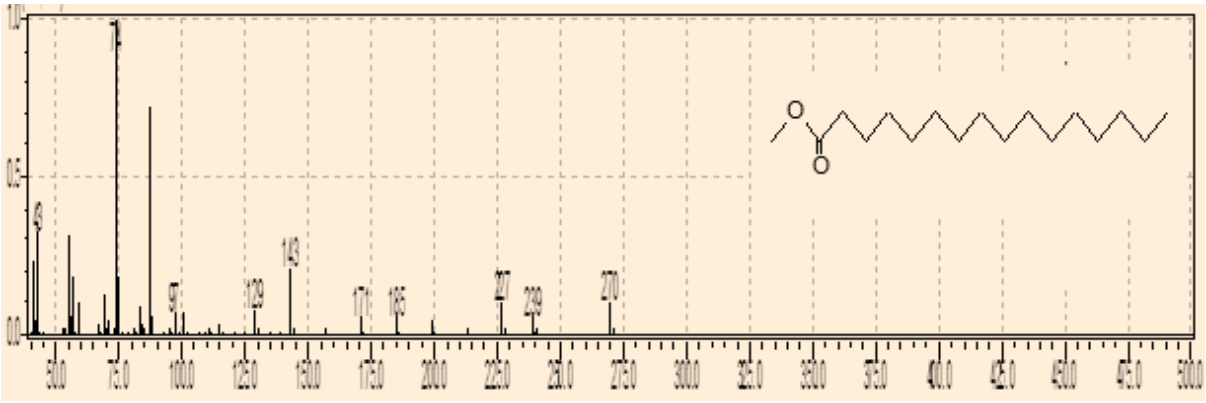


Fig (3.4): Mass spectrum of hexadecanoic acid methyl ester

Fig (3.4) shows the mass spectrum of hexadecanoic acid methyl ester.The peak m/z 270(R.T. 15.119) 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 .

ii) Methyl stearate(4.69%)

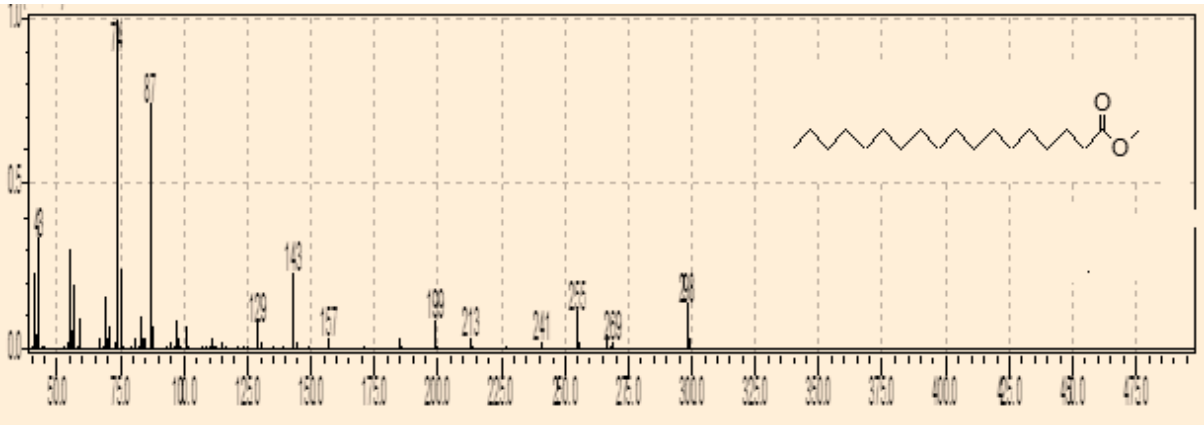


Fig (3.5): Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is displayed in Fig(3.3).The peak at m/z 298 (R.T. 17.042) is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl group.

3.1.2-Antimicrobial activity

Apium graveolens seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table(3.1).The results were interpreted in the following manner: (<9mm: inactive;9-12mm:partially active; 13-18mm: active;> 18mm:very active) .Tables(3.2) and (3.3) display the antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed partial activity against *Escherichia coli*, *Staphylococcus aureus* and the fungal species *Candida albicans.*

Table (3.1): Antimicrobial Activity of the *Azadirachta Indica* seed oil

Oil	Antibacterial activity				
	Gram positive		Gram negative		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	--	10	10	--	12

Table 3.2 : Antibacterial activity of standard chemotherapeutic agents

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

Table 3.3 : Antifungal activity of standard chemotherapeutic agent

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

Conclusion

Apium graveolens oil was investigated by GC-MS analysis.The analysis showed the presence of 45 constituents. Major components are:

i) 9-Octadecenoic acid methyl ester (56.04%)
ii) 9, 12-Octadecadienoic acid methyl ester (13.47%)
iii) Hexadecanoic acid methyl ester(12.05%)
iv) Methyl stearate(4.69%)

The oil was evaluated for antimicrobial activity. It showed partial activity 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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