



Sudan University of Science and Technology
College of post Graduate Studies



Chemical Charactrization of *Cumcumis melo var flexuosus* Seeds Oil and its BiologicalActivity
التوصيف الكيمياءى لزيى بذور العجور ونشاطه البيولوجى

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

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الإستهلال

بسم الله الرحمن الرحيم

قال تعالى:

{يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ

بِمَا تَعْمَلُونَ خَبِيرٌ} صدق الله العظيم

المجادلة (11)

DEDICATION

*To the souls of my father and mother,,,
My sisters and brothers, and my friends.....*

ACKNOWLEDGEMENT

First of all I would like to thank Almighty Allah, Most Gracious and Most Merciful, who gave me the serenity, means of strength and patience to finish this work.

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Abstract

The oil of *cucumismelo* var. flexuous seeds was extracted and analyzed by GC-MS technique. Twenty two components were detected major constituents are: 9, 12-z, z-octadecadienoic acid methyl ester (48.08%). 9, z-octadecenoic acid methyl ester (18.05%). hexadecanoic acid methyl ester (15.69%). And methyl ester (11.18%).

In the cup plate agar diffusion bioassay, the oil was evaluated for its antimicrobial efficiency against the pathogenic bacteria: *Staphylococcus Aureus*, *Escherichia Coli*, *Pseudomonas Aeruginosa*, *Aspergillus Niger*, *Escherichia Coli*, *Candida Albicans* and *Bacillus Stubilis* and promising results were obtained.

المستخلص

أستخلص زيت بذور العجور وأجري له تحليل عن طريق الكروماتوغرافية الغازية - مطافية

الكتلة ووجد أنه يتكون من 22 مكون، والمكونات الأساسية هي:

9, 12-z, z-octadecadienoic acid methyl ester (48.08%). 9, z-octadecenoic acid methyl ester (18.05%).hexadecanoic acid methyl ester(15.69%). And methyl ester(11.18%).

وأجري اختبار مضادات البكتريا ضد بعض الميكروبات القياسية:

Staphylococcus Aureus, Escherichia Coli, Pseudomonas Aeruginosa, Aspergillus Niger, Escherichia Coli, Candida Albicansand Bacillus Stubilis.

وأعطت هذه الإختبارات نتائج واعدة.

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Chapter one

Introduction

1- Introduction

1.1- The target species : *Cucumis Melo Var Flexuosus*

Comon name	: melon
Type	: Annual
Family	: Cucurbitaceae
Native Range	: India , Pakistan
Zone	: 2 to 11
Height	: 6.00 to 9.00 feet
Spread	: 1.00 to 3.00 feet
Bloom Description	; Yellow
Sun	: Full
Water	; Medium
Suggested Use	: Annual
Flower	: showy
Fruit	: Showy , Edible

i- Garden Locations

Melon is easily grown in loose , fertile , medium textured , organically rich , slightly acidic , well drained soils in full sun . Plant perform best in full sun .

This plant is typically grown as annuals in cages , on trellises or , space permitting , along the ground . Vertical growth on a trellis may be best for var .flexuosus because the fruits will grow straighter with less curvature . The plants thrives in hot summer day time weather with warm nights .

Cucumis melo , commonly called muskmelon or sweet melon , is a scarbid or hispid , scrambling or climbing , annual or perennial plant of

the ground family . Common name is in reference to the musky smell which emanates from many of fruits in the species vary considerably in terms of shape , size , rind , texture , flavor and flesh color . This is a polymorphic taxon that produces not only muskmelons (straight species) but also six different edible varieties or groups . Some experts prefer the term "group" because variety only applies to plants in the wild as follows ;

1/ Var. cantalupensis (cantaloupe developed at cantalupinear Room) ,

2/ Var.inodorous (winter melons including casaba and honeydew) ,

3/ Var. reticulates (netted or muskmelon with netted rind and musky sweet orange flesh) ,

4/ Var. conomon (orient al pickling melon) ,

5/ Var. chito group (magngomelons) ,

6/ Var.dudaim (stink melon or Queen Annes pocket melon)

7/ Var. flexuosus (Armenian cucumber, snake like or serpent melon with cucumber shape and appearance) . var. flexuosus (aka flexuosus group) most commonly called Armenian cucumber , is a frost-tender , tendril bearing annual vine that is grown for harvest of its edible , long and slender , cucumber-fruits consumed as a vegetable . Vines will typically grow to 6-9 long . This is an heirloom plant first cultivated in the 1400s in western Asia from Armenia and Turkey south along the eastern Mediterranean to Egypt . The cucumber that is most commonly grown for culinary consumption is the closely related *Cucumis sativus* .

In frost-free climates , yellow flowers with 5- parted corollas bloom throughout much of the year . Flowers give way to slender fruits with greenish-white flesh and thin corrugated pale green rinds . Fruits are best harvest when young (e.g.about 12 long and 1 diameter) , but will mature to as much as 36 (hence the sometimes used common name of yard-long cucumber) . Fruit flesh becomes drier and tougher as fruits mature . Stems are clad with rounded , wavy margined , rough pubescent (to 6 across) .Genus name from Latin means cucumber as derived from the Greek word kykyon also meaning cucumber^{1,2} .

ii-Health benefits of Cucumbers intake

Cucumbers contain an anti-inflammatory flavonol called fisetin that appears to play an important role in brain health . In addition to improving your memory and protecting your nerve cells from age related decline³, fisetin has been found to prevent progressive memory and learning impairments in mice with Alzheimers disease⁴ .

Cucumbers may help to (cool) the inflammatory response in your body , and animal studies suggest that cucumber extract helps reduce unwanted inflammation , in part by inhibiting the activity of pro-inflammatory enzymes (including cyclo-oxygenase -2 , or COX-2)⁵ .

Cucumbers contain polyphenols called lignans (pinoresinol , lariciresinol , and secoisolariciresinol) , which may help to lower your risk of breast , uterine , ovarian , and prostate cancer⁶ . They also contain

phytonutrients called cucurbitacins, which also have anti-cancer properties⁷.

Cucumbers contain numerous antioxidants, including the well-known vitamin C and beta-carotene. They also contain antioxidant flavonoids, such as quercetin, apigenin, luteolin, and kaempferol⁸, which provide additional benefits.

For instance, quercetin is an antioxidant believed to prevent histamine release-making quercetin-rich foods "natural antihistamines". Kaempferol, meanwhile, may help fight cancer and lower your risk of chronic diseases including heart disease.

Placing a cucumber slice on the roof of your mouth may help to rid your mouth of odor-causing bacteria. According to the principles of Ayurveda, eating cucumbers may also help to release excess heat in your stomach, which is said to be a primary cause of bad health⁹.

Cucumbers contain multiple B vitamins, including vitamin B₁, vitamin B₅, and vitamin B₇ (biotin). B vitamins are known to help ease feelings of anxiety and buffer some of the damaging effects of stress.

Cucumbers are rich in two of the most basic elements needed for healthy digestion; water and fiber. Adding cucumbers to your juice or salad can help you meet the ideal amount of fiber for your body needs. If you struggle with acid reflux, then drinking water can help suppress acute symptoms of acid reflux by temporarily raising stomach P^H; it is possible that water-rich cucumbers may have a similar effect.

Cucumber skins contain insoluble fiber , which helps add bulk to stool . This helps food to move through your digestive tract more quickly for healthy elimination .

Cucumbers are very low in calories , yet they make a filling snack (one cup off sliced cucumber contains just 16 calories)¹⁰ . The soluble fiber in cucumbers dissolves into a gel-like texture in the gut , helping to slow down your digestion . This helps to feel full longer and longer and is one reason why fiber-rich foods may help with weight control .

Cucumbers contain potassium , which is associated with lower blood pressure levels . A proper balance of potassium both inside and outside cells is crucial for the body to function properly¹¹ .

As an electrolyte , potassium is a positive charged ion that must maintain a certain concentration (about 30 times higher inside than outside your cells) in order to carry out its functions , which includes interacting with sodium to help control nerve impulse transmission , muscle contraction , and heart function .

Cucumbers are fermented in vinegar-based salads . Cucumbers make an ideal base for vegetable juice due to their mild flavor and high water content.

Conventionally grown cucumber varieties are recommended since organic cucumbers were ranked the 12th most contaminated food and the second in cancer risk due to their pesticide content according to the Environmental Working Group (EWG) .

Flavonoids and tannins in cucumbers have been found to have both free-radical scavenging and pain-relieving effects , while it has a number of traditional folk uses as well¹².

1-2-Oils , butter , fats , and waxes

Amongst the alchemist the term (oil) had a somewhat wider range of application than is usual at the present day , including various inorganic substances , such as oil of vitriol . Similarly butter of tin were metallic derivatives entirely dissimilar from cows butter in constitution , although resembling it in physical consistency . Even when such wholly inorganic compounds are excluded , the term oil has an extremely elastic meaning , being employed to designate a very large variety of liquid substances , natural and artificial , which have but few features in common beyond the fact that , being all organic in character , they are capable of burning with more or less facility under suitable conditions ; whilst- with but very few exceptions- they are practically insoluble in water , so as a rule lighter than water . When agitated an emulsion forms , from which the water and oil gradually separate on standing , the latter usually floating as a separate layer on the former¹³ .

The term (fatty matter) or more shortly (fat) is applied to substances which are more or less of a soft solid character at the ordinary temperature , but on gently heating pass to liquids closely resembling fluid oils in general characters ; (butters) being specially soft varieties of such fats possessing the peculiar physical texture of cows butter at the

atmospheric temperature of temperate climates . (Waxes) on other hand , possess a somewhat different and much firmer texture at the ordinary temperature , but when heated melt to fluids which closely resemble ordinary liquid oils and melted fats in their general physical characters¹⁴ . Oils are derived from animals , vegetables , and mineral sources, being mostly pre-contained in the tissues , seeds , or strata from which they are obtained by simple mechanical process such as pressure or pumping , or by means of solvents , or by volatilization ; certain products of destructive distillation , however , are also ranked amongst oils e.g., the light oils -creosote oils - obtained during the reflection of coal tar and similar substances formed by the breaking up of more complex organic matters under the influence of heat . Somewhat similar substances (fusel oils) are produced by analogous decomposition occurring during fermentative changes .

Oils capable of being converted into vapor by the application of heat without suffering material decomposition (volatile oils) are for the most part either artificial products of destructive distillation - like petroleum or essential oils i.e. volatile odorous matters extracted from numerous vegetable source , usually by distillation along with water¹⁵ .

Fixed oils , on the other hand , are substances not available without decomposition , and are essentially of animal and vegetable origin ; as also are butters , fats , and waxes practically become fixed oils on

slightly raising the temperature , with the exception of the so-called waxes of mineral origin , paraffin wax , ozokerite , cerasin .

From the point of view of general chemical composition , oils , fats , butters , and waxes may be divided into two leading classes-viz , those consisting of carbon and hydrogen , and those containing oxygen . Oils of the former class are practically all volatile without decomposition ; those of the second class are in some cases volatile without change (e.g., oxidized essential oils) , but , as a rule , are (fixed) undergoing destructive distillation when heated , so that vapors emitted are produced in consequence of decomposition¹⁶⁻¹⁸ .

1-3-Antimicrobials

An antimicrobial is an agent that kills microorganisms or inhibits their growth¹⁹ . Antimicrobial medicines can be grouped according to the microorganisms they act primarily against . For example , antibiotics are used against bacteria and antifungals are used against fungi . They can also be classified according to their function . Agents that kill microbes are called microbicidal , while those that merely inhibit their growth are called biostatic . The use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis .

The main classes of antimicrobial agents are disinfections (non selective antimicrobials such as bleach) , which kill a wide range of microbes on nonliving surfaces to prevent the spread of illness ; antiseptics (which are applied to living tissue and help reduce infection during surgery) ,

and antibiotics (which destroy microorganisms within the body) . The term (antibiotic) originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials , such as the sulphonamides , or fluoroquinones . The term is also used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature) , but its context has broadened to include all antimicrobials . Antibacterial agents can be further subdivided into bacterial agents which kill bacteria , and bacteriostatic agents , which slow down or stall bacterial growth .

Use of substances with antimicrobial properties is known to have been common practice for at least 200 years . Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infections²⁰ . More recently , microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine²¹ .

In 1928 Alexander Fleming became the first to discover a natural antimicrobial fungus he named penicillin Rubens. The substance extracted from the fungus he named penicillin and in 1942 it was successfully used to treat Streptococcus infection . Penicillin also proved successful in treatment of many other infections such as gonorrhoea²² , strep throat and pneumonia , which were potentially fatal to patients until then. Many antimicrobial agents exist , for use against a wide range of infections .

Antibacterials are used to treat bacterial infections . The toxicity to humans and other animals from antibacterials generally considered low . However , prolonged use of certain antibacterials can decrease the number of gut flora , which may have a negative impact on health . After prolonged antibacterial use consumption of probiotics and reasonable eating can help to replace destroyed gut flora . Stool transplants may be considered for patients who are having difficulty in recovering from prolonged antibiotic treatment , as for infections²³ .

The discovery , development and clinical use of antibacterials during the 20th century has substantially reduced mortality from bacterial infections . The antibiotic era began with pneumatic application of nitroglycerine drugs , followed by a (golden) period of discovery from about 1945 to 1970 , when a number of structurally diverse and highly effective agents were developed . However , since 1980 the introduction of new antimicrobial agents for clinical use has declined , in part because of the enormous expense of developing and testing new drugs . Paralleled to this there has been an alarming increase in resistance of bacteria, fungi , viruses and parasites to multiple existing agents^{24,25} .

Antibacterials are among the most used drugs ; however antibiotics are also among the drugs commonly misused by physicians , such as usage of antibiotic agents in viral respiratory tract infections²⁶ . As a consequence of wide spread and injudicious use of antibacterials , there has been an accelerated emergence of antibiotic-resistant pathogens ,

resulting in a serious threat to global public health . The resistance problem demands that renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibacterials possible strategies towards this objective include increased sampling from diverse environments and application of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms as well as the development of small molecules libraries customized for bacterial targets²⁷ .

Antifungals are used to kill or prevent further growth of fungi . In medicine , they are used as a treatment for infections such as athletes foot .

Antifungals work by exploiting differences between mammalian and fungal cells . They kill off the fungal organism without dangerous effects on the host . Unlike bacteria , both fungi and humans are eukaryotes . Thus , fungal and human cells are similar at the molecular level , making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism . Consequently , there are often side effects to some of these drugs . Some of these side effects can be life threatening if the drug is not used properly .

As well as their use in medicine , antifungals are frequently sought after to control mold growth in damp or wet home materials . Sodium bicarbonate blasted onto surfaces acts as an antifungal . Another antifungal serum applied after or without blasting by soda is a mix of

hydrogen peroxide and a thin surface coating that neutralizes mold and encapsulates the surface to prevent spore release . Some paints are also manufactured with an added antifungal agent for use in high humidity areas such as bathrooms or kitchens . Other antifungal surface treatments typically contain variants of metals known to suppress mold growth e.g. pigments or solutions containing copper , silver or zinc . These solutions are not usually available to the general public because of their toxicity²⁸ . Antiviral drugs are a class of medication used specifically for treating viral infections . Like antibiotics , specific antivirals are used for specific viruses . They are relatively harmless to the host and therefore can be used to treat infections . They should be distinguished from viricides , which actively deactivate virus particles outside the body .

Traditional herbalists used plants to treat infectious disease . Many of these plants have been investigated scientifically for antimicrobial activity , and some plant products have been shown to inhibit the growth of pathogenic microorganisms . A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use , suggesting that cross resistance with agents already in use may be minimal²⁹⁻³⁰ .

1-4-Essential Oils

Many essential oils included in herbal pharmacopoeias are claimed to possess antimicrobial activity , with the oils of bay , cinnamon , clove and thyme reported to be the most potent in studies with foodborne

bacterial pathogens^{31,32}. Active constituents include terpenoid chemicals and other secondary metabolites . Despite their prevalent use in alternative medicine , essential oils have limited use in mainstream medicine . While 25 to 50 % of pharmaceutical compounds are plant-derived , none are used as antimicrobials , though there has been increased research in this direction . Barriers to increased usage in mainstream medicine include poor regulatory oversight and quality control , mislabeled or misidentified products , and limited modes of delivery .

1-5- Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a hybrid analytical technique that couples the separation capabilities of GC with the detection properties of MS to provide a higher efficiency of sample analysis while GC can separate volatile components in a sample , MS helps fragment the components and identify them on the basis of their mass³³ .

GC-MS provides enhanced sample identification , higher sensitivity, an increased range of analyzable samples , and faster results , which enable a whole new range of applications for GC-MS in several areas .

GC-MS is used in screening tests for the detection of several congenital metabolic diseases . It detects trace levels of compounds present in the urine of patients with genetic metabolic disorders .

Monitoring environmental pollutants is a major application of GC-MS . It is widely used in the detection of dibenzofurans , dioxins , herbicides , sulfur , pesticides , phenols , and chlorophenols in air , soil , and water . Aromatic compounds such as fatty acids , esters , aldehydes , alcohols , and terpenes present in food and beverages can be easily analyzed using GC-MS . The technique can also be used to detect spoilage or contamination of food . The analysis of a wide range of oils such as lavender oil , olive oil , spearmint oil , and essential oils , perfumes , fragrances , allergens , menthol , and syrups is also possible using GC-MS³⁴ .

In the pharmaceutical industry GC-MS is used in research and development , production , and quality control . It is used in identification of impurities in active pharmaceutical ingredients . In medicinal chemistry , GC-MS is used in the synthesis and characterization of compounds and in pharmaceutical biotechnology .

Using GC-MS fire debris analysis can be performed as per the American Society for Testing Materials (ASTM) standards . GC-MS is widely used in forensic toxicology to identify poisons and steroids in biological specimens and in anti-doping labs to detect performance enhancing drugs such as anabolic steroids³⁵ .

Life science instruments manufacturer , Agilent life offers single and triple quadrupole GC-MS systems that are very sensitive with low

detection limits and are suitable for forensics applications , food testing and toxicology analysis .

GC-MS can be used for the bioanalysis of body fluids to detect narcotics , barbiturates , alcohols , and drugs such as anticonvulsants , anesthetics , antihistamines , sedative-hypnotics , and anti-epileptic drugs . It is also useful in detecting pollutants and metabolites in serum and in fatty acid profiling in microbes³⁶ .

GC-MS system from scientific solution provider , Thermo Fischer Scientific are coupled with software that helps streamline GC-MS workflows and data and can be seamlessly integrated with food , environmental , forensic and clinical applications .

Explosive detection system in public places uses GC-MS technique for the analysis and detection of chemical warfare agents³⁷ .

Due to its structurally significant mass spectral peaks , extended range of analyzable low volatility samples , enhanced molecular ions , and valuable isotope ratio information , GC-MS is a powerful tool for geochemical applications . GC-MS has been used to analyze the atmosphere of Venus and has also been used by the Viking program on Mars. Additionally , a chiral GC-MS system has been used by the Rosetta mission to analyze the materials in the comet 67p/churyumov-Gerasimenko³⁸ .

GC-MS is ideal for the analysis of organic gases and aromatic solvents , detection of impurities and allergens in cosmetics . It is also used in the

synthesis of cellulose acetate , polyethylene , polyvinyl , and synthetic fibers . Therefore it may concluded that automated GC-MS systems offer rapid and reproducible results in several applications³⁹.

1-6- Solvent Extraction

One of the most basic needs of mankind is an abundant and reliable food supply . In the modern world , one major source of protein and vegetable oil is from oil seeds , particularly the soybean –an abundant resource which is largely processed using solvent extraction , an efficient reliable means to separate the high-protein meal solids from the high-energy edible oil . The second most prevalent solvent –extracted oilseed is rapeseed and/or the varieties called canola . Sunflower is also quite high in volume⁴⁰.

A much lower volume or secondary use for soybean oil and rapeseed oil , gaining popularity in recent years , is as a feedstock for biodiesel fuels for diesel engines . There are many other products such as oleochemicals made from oilseeds- and often these are provided with a solvent extraction system as a part of the total supply process .

One early means of separation of oils was physical pressure to (squeeze the oil out) . The most energy efficient , practical embodiment of that method is the modern screw press . This is a conveyor screw with a slotted cage surrounding it and a screw with diminishing space for the solid material as the material proceeds from pitch to pitch of the screw . Eventually , as the free space is progressively restricted , the oil is

squeezed out of the solids and through the slots . More than half of the oil is easily removed in this way , but perhaps 7% or 8% residual oil is left in the solids , the process uses considerable horsepower , there is considerable wear and maintenance , and it takes many machines for high capacity . In comparison , solvent extraction with hexane (the primary solvent used worldwide) will remove all but ½% of residual oil , uses less horse power , and requires less maintenance . It is relatively efficient and reliable , and this is one reason why solvent extraction is the primary means of separating large tonnages of oil from protein meal⁴¹ .

Hexane has about the best characteristics of the many solvents tried over the years . With a boiling point of 156° F (69° C) it is a liquid in all but the most extreme climates of the world . With a fairly high volatility and a low sensible heat of a 144 BTU/lb (335KJ/kg) it is relatively easy to remove from the solids and oil with low energy use . It has an azeotrope , a slightly reduced 143° F (61.6° C) boiling temperature when in the presence of water or steam and resulting in a vapor coming off at about 95% by weigh hexane and 5% by weigh water . The azeotrope is convenient for efficient removal of the solvent from solids (or meal) using direct steam contact . Hexane has a good and aggressive capability to dissolve and mix with vegetable oils so that it can wash the desired oils out of a fibrous or solid material . It is selective and leaves the proteins , sugars and some undesired gums largely undistributed in the

meal . Also hexane has a relatively (tolerable) odor and a low tendency to cause discomfort when one is subjected to a brief exposure.

Table 1 :Physical properties of typical commercial hexane and isohexane

Property	Hexane	Isohexane mixed isomers
Flammable limits percent by vol	1.2-7.7	1.0-7.0
Ignition temperature (C)	225	264
Flash point (C) vlosed cup	-26	-18
Molecular weight	86.2	86.2
Melting point (C)	-94	-154
Boiling rang at 1atm. (1.0 bar)(C)	97-69	56-60
Specific gravity at 60 F(15.6 C)	0.68	0.66
Pounds per gal at 60 F (15.6 C)	5.63	5.52
Pounds per gal at 60 F (15.6 C)	3	3
Vapor density (air = 1)	79.6	N.A
Latent head of vaporization at 1 atm (1.0 bar) (kcal/kg)	38.1	39.4
Vapor pressure at 37.8 C (kpa)	0.531	N.A
Specific heat liquid (kcal per kg- C at) (15.6 C)	0.339	
Solubility in water (moles per L at 60 F 15.6 C)	Negligible	Negligible

Hexane has maintained the dominant position as a solvent for the major plants which extract oil from oil-seeds .. Wdespreed use and familiarity ensures that many people and resources will continue to improve on stubborn problems and optimize the hexane process . There is also the advantage of well-understood industrial expectations for performance , the availability of trained personal , and established standards for safe

use . Any proposed replacement must exceed hexanes basic advantages and still might be expected to require a lot of time , development and capital investment to work as well .

It should be noted that other solvents may be required to produce different and specific products . For example , an alcohol-water mixture is used in an additional extraction step (after hexane extraction) to produce soy protein concentrate (or SPC) by removing the sugars from standard soybean meal . Various alcohols , isohexane , heptanes , butane-many other solvents have found applications in markets . For the standard oil removal plant, only isohexane-an isomer with properties very close to hexane-has replaced hexane in a significant number of extractions .

Hexane does have one weakness which is universally mentioned; when hexane vapor is mixed with air in roughly range from 1.2% to 7.7% by volume , the mixture is flammable . Over the last century , many processing plants have had fires or even explosions which caused serious damage . The NFPA-36 standard is one result ; a set of rules for construction of these plants which ,if followed and well managed , should provide a very safe working environment and a reliable means of production . It should be noted that gasoline is also inherently and potentially dangerous , yet millions of grandmothers top off their gas tanks weekly . One result of this flammability , however , is the necessity to avoid significant amounts of hexane from escaping from the

plant process in the process air , water , meal or oil , or simply in losses due to poor equipment maintenance , frequent shutdowns , or poor housekeeping . The typical percolating extractor is designed to operate at a very slight vacuum of perhaps 0.4 water (-10mm water) in an effort to enhance safety .

Aim of This Study

- Extraction of fixed oil from *Cucumis Melo Var Flexuosus*.
- GC-MS analysis of the extracted oil.
- Assessment of antimicrobial activity.

Chapter Two

2- Materials and Methods

2.1- Materials

2.1.1-Plant material

Seeds of *Cucumis melo var. flecuous* were collected Khartoum state (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; 0.25um, thickness) was used.

2.1.3- Test organisms

Cucumis melo var. flecuous Seed oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table (2).

Table 2: Test organisms

Ser. No	Micro organism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
6	<i>Candida abilcans</i>	fungi

2.2- Methods

2.2.1- Preparations of reagents for phytochemical screening

a)-Falconoid test reagents

- Aluminium chloride solution

(1 g) of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

(1 g) of Potassium hydroxide was dissolved in 100 ml water.

- Ferric chloride solution

(1 g) of Ferric chloride was dissolved in 100 ml methanol.

b)- Alkaloid test reagents

Maeyer reagent

- Mercuric chloride solution: 1.36 g in 60 ml. water.

- Potassium iodide solution : 5 g in 10ml. water.

The two solution were combined and then diluted with water up to 100ml.

-Wagner reagent

(1.27 g) iodine and (2 g) of potassium iodide in (100 ml) water.

2.2.1.3- Preparation of plant extract for phytochemical screening

(100 g) of powdered air-dried seeds of *Cucumis Melo Var Flexuosus* were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract (PE) was used for phytochemical screening.

2.2.2- Phytochemical screening

i) Test for unsaturated sterols and for triterpenes

(10 ml) of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphite . (5 ml) portion of the solution was mixed with (0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

ii)- Test for flavonoids

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

iii)- Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on a water bath and 5 ml of 0.2 N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into portions:

To one portion a few drops of Maeyer reagent were added., to the other portion few drops of Wagner reagent were added.

iv)- Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium

chloride and freshly prepared distilled water) were added. The mixture cooled, filtrated and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

V)- Test for saponins

(1g) of dried powdered plant material was placed in a clean test tube. (10 ml) of distilled water were added and the tube was stopped and vigorously shaken for about 30 second, and allowed to stand.

2.2.3-Extraction of oil from seeds of *Cucumis Melo Var Flexuosus*

Powdered seeds of *Cucumis Melo Var Flexuosus* (300g) were exhaustively extracted with n-hexane (soxhlet). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°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. 92ml) 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. (5ml) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate (1u1) was injected in the GC-MS vial.

2.2.4- GC-MS analysis

The oil of *Cucumis Melo Var Flexuosus* was analysed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25 um, thickness) was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is given in Table 3, while other chromatographic condition are depicted in Table 4.

Table 3 : Oven temperature Program

Rate	Temperature	Hold Time (min)
-	150.0	1.00
4.00	300.0	0.00

Table 3 : Oven temperature Program

Column	150.0°C
Injection temperature	300. 0°C
Injection mode	Split
Flow control mode	Linear
Pressure	139.3 KPa
Total flow	50.0ml/min
Column flow	1.54ml/sec
Linear velocity	47.2cm/sec
Purge flow	3.0ml/min
Spilt ratio	-1.0

2.2.5- Antimicrobial test

i) Preparation of bacterial

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distribution 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 – 10^9 colony 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 of were made in sterile normal saline in tubes and one drop volumes (0.02ml) 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 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.

ii)- Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modification, to assess the antibacterial activity of Schiff bases and their Mannish bases. (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. (20ml) 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 above 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

Table 1: Constituents of *Cucumis Melo Var Flexuosus* oil

pk#	R.Time	Area	Area%	Name
1	13.570	524824	0.20	Methyl tetradecanoate
2	14.382	32839	0.01	cis-5-Dodecenoic acid, methyl ester
3	14.486	43712	0.02	5-Octadecenoic acid, methyl ester
4	14.645	219576	0.08	Pentadecanoic acid, methyl ester
5	15.374	88446	0.03	7,10-Hexadecadienoic acid, methyl ester
6	15.433	148432	0.06	7-Hexadecenoic acid, methyl ester, (Z)-
7	15.479	876033	0.33	9-Hexadecenoic acid, methyl ester, (Z)-
8	15.690	42472437	15.96	Hexadecanoic acid, methyl ester
9	16.650	776841	0.29	Heptadecanoic acid, methyl ester
10	17.392	127957677	48.08	9,12-Octadecadienoic acid (Z,Z)-, methyl
11	17.428	48043578	18.05	9-Octadecenoic acid (Z)-, methyl ester
12	17.604	29744195	11.18	Methyl stearate
13	18.943	2457377	0.92	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),
14	19.092	2043770	0.77	9-Octadecenoic acid, 12-hydroxy-, methyl
15	19.143	1303379	0.49	cis-11-Eicosenoic acid, methyl ester
16	19.342	2761768	1.04	Eicosanoic acid, methyl ester
17	19.397	4298664	1.62	PGH1, methyl ester
18	20.262	172461	0.06	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
19	20.961	454404	0.17	Docosanoic acid, methyl ester
20	21.730	298642	0.11	Tricosanoic acid, methyl ester
21	22.465	748550	0.28	Tetracosanoic acid, methyl ester
22	23.205	655029	0.25	Squalene
		266122634	100.00	

Main constituents of the oil are discussed below:

9,12-Z,Z-Octadecadienoic acid methyl ester (48.08%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.2. The peak at m/z 294(R.T. 17.392 -in total ion chromatogram) corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 corresponds methoxyl function.

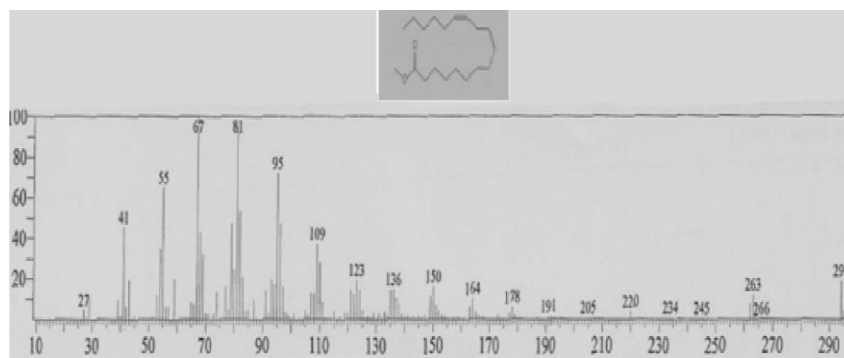


Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

9-Z-Octadecenoic acid methyl ester(18.05%%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.42 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z 266 accounts for loss of a methoxyl.

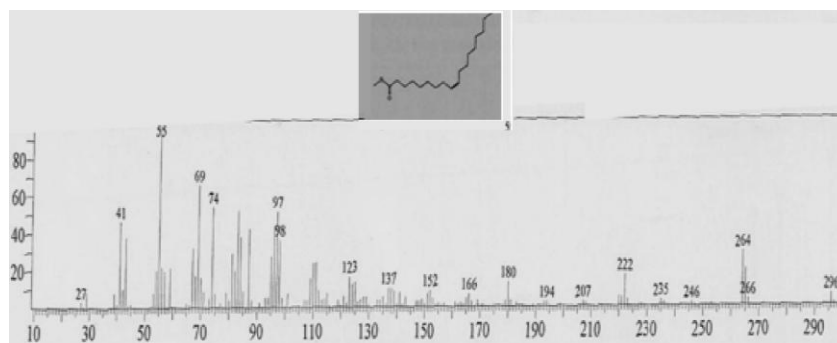


Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester(15.69%)

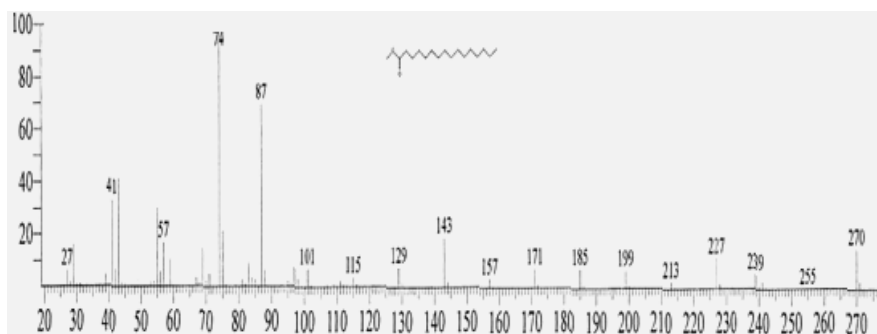


Fig. 4: Mass spectrum of hexadecanoic acid methyl ester

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4. The peak at m/z 270 (R.T.15.853) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 corresponds to loss of a methoxyl.

Methyl stearate(11.18%)

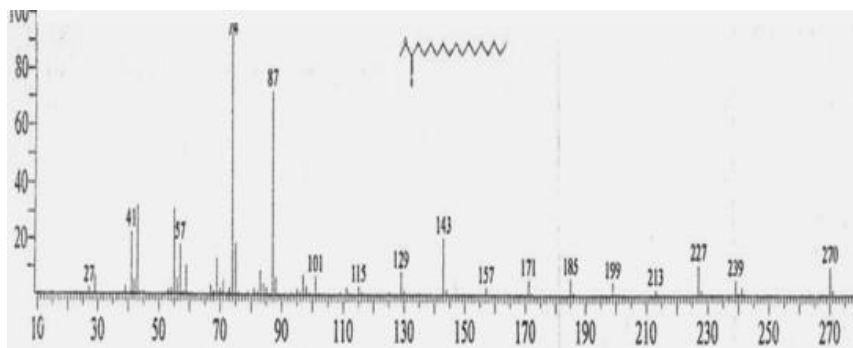


Fig. 5: Mass spectrum of methyl stearate

Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T.17.604) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 corresponds to loss of a methoxyl group.

Antibacterial activity

Cucumis Melo Var Flexuosus oil was assessed for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (2). Results were interpreted as follows : (<9mm: inactive;9-12mm:partially active;13-18mm: active;>18mm:very active). Tables (3) and (4) show the antibacterial and antifungal activities of standard drugs respectively.

Table 2 : Antibacterial activity of *Cucumis Melo Var Flexuosus*oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	10	14	16	12	10

Table 3.3 : Antibacterial activity of standard chemotherapeutic agents

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

Table 3.4 : Antifungal activity of standard chemotherapeutic agent

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

Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

An.: *Aspergillus niger Escherichia coli*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

The oil showed excellent activity against *Escherichia coli* at test concentration. It also showed good activity against *Bacillus subtilis*. However, It exhibited partial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* .The oil also displayed weak anticandidal potency.

Conclusion

The oil of *Cucumis Melo Var Flexuosus* was analyzed by GC-MS and furthermore evaluated for its antimicrobial activity.

GC-MS analysis of *Cucumis Melo Var Flexuosus* fixed oil was carried out. Identification of the constituents was based on the MS library (NIST) . Also the observed fragmentation pattern was discussed. 22 constituents were detected by GC-MS analysis. The oil showed excellent activity against *Escherichia coli* at test concentration. It also showed good activity against *Bacillus subtilis*. However, It exhibited partial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* .The oil also displayed weak anticandidal potency.

Recommendations

1-Other phytochemicals(alkaloids , steroids ..etc) present in *Cucumis Melo Var Flexuosus* may be isolated and identified.

2-The oil may be screened for antiinflammatory, antimalarial and other biological activities.

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