**Sudan University of Science and**

**Technology**

**College of Post Graduate Studies**

# *Charactization of Constituents of Catullus Colocynths Fixed Oil and its Biological Activity*

# دراسة مكونات الزيت الثابت لنبات الحنظل وآثار البيولوجي

**A Thesis Submitted in Partial Fulfillment for the Requirement of the M.Sc. Degree in Chemistry**

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بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ: ﴿ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍۚ وَٱللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صدق الله العظيم

المجادلة: ١١

# Dedication

*Dedicated to ,*

*my parents ,*

*brothers and sisters .*

# Acknowledgment

First Iwould like to praise**Almighty Allah** who give me the strength to do this work .

I wish to express my gratitude and thanks to Prof . Mohammed Abdel KarimMohammed for his suggestions , guidance , encouragement and support throughout the period of study .

Thank are due to the staff of Chemistry Department – Sudan University of Science and Technology for all facilities . I would like to extend my thanks to my colleagues for their valuable assistance .

# Abstract

In the present study , the fixed oil of Citrullus Colocynthis was extracted from seeds . The oil was analyzed by gas chromatography – mass spectrometry (GC-MS) and 41 components were identified and quantified. Major constituents were :9,12 –octadecanoic acid methyl ester (39.30%),hexadecanoic acid methyl ester (15.82%),methyl stearate (13,39%)and 9-octadecanoic acid methyl ester (12.51%).

Theoil was screened for antibacterial and antifungal activities and different antimicrobial responses were detected.

# المستخلص

استخلص الزيت الثابت لبذور نبات الحنظل ودرس بواسطة تقنية الكروموتوغرافيا الغازية – طيف الكتلة حيث اتضح ان الزيت يحتوى على41 مكونا ، المكونات الرئيسة هى :

octadecanoic acid methyl ester (39 . 30%), hexadecanoic acid methyl ester (15.82%),methyl stearate (13,39%) ,9-octadecanoic acid methyl ester (12.51%).

ثم اخضع زيت الحنظل لاختبارات بيولوجية كمضاد للميكروبات وكانت النتائج جيدة.

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# *Introduction*

# 1- Introduction

## 1.1- Gas Chromatography- Mass Spectrometry

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample **(Figure 1).** GC is used to separate the volatile and thermally stable substitutes in a sample whereas **GC-MS** fragments the analyte to be identified on the basis of its mass. The further addition of mass spectrometer in it leads to GC-MS/MS. Superior performance is achieved by single and triple quadrupole modes [1-3].

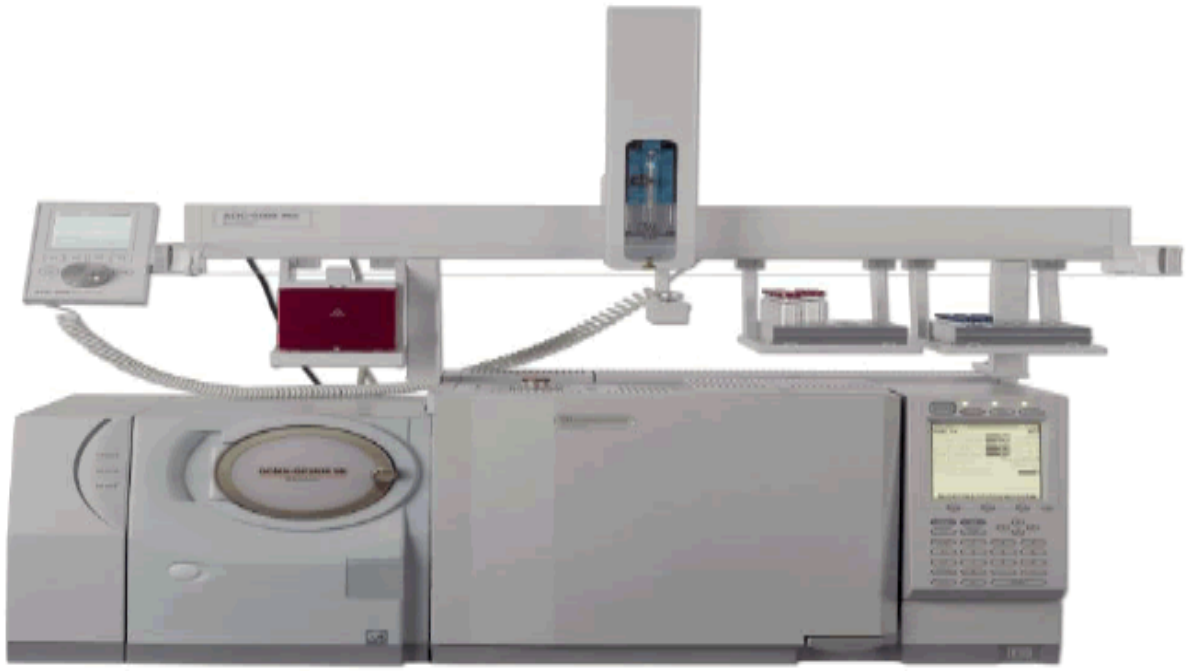


Figure 1: A typical GC-MS with head space of Shimadzu Company

### 1.1.2-Advantages of GC-MS

GC requires the analyte to have significant vapor pressure between 30 and 300°C. GC presents an insufficient proof of the nature of the detected compounds. The identification is based on retention time matching that may be inaccurate or misleading. **GC-MS** represents the mass of a given particle (Da) to the number (z) of electrostatic charges (e) that the particle carries. The term m/z is measured in DA/e. GCMS commonly uses electron impact (EI) and chemical ionization (CI) techniques. The main features of enhanced molecular ion, improved confidence in sample identification, significantly increased range of thermally labile and low volatility samples amenable for analysis, much faster analysis, improved sensitivity particularly for compounds that are hard to analyze and the many other features and options provide compelling reasons to use the GC-MS in broad range of areas [4,5].

### 1.1.3 Applications of GC-MS

### 1.1.3- Environmental monitoring

GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. The cost of GCMS equipment has decreased whereas the reliability has markedly increased. The determination of chloro-phenols in water and soil, polycyclic aromatic hydrocarbons (PAH), unleaded gasoline, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, sulphur in air is very convenient to

be screened by this technique. It can be used to screen the degradation products of lignin in bio-mass research, pesticides in spinach. Analysis of decacyclene, ovalene and even $C_{60}$ degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization[6,7]

### 1.13.2- Food, beverage, flavor and fragrance analysis

Foods and beverages have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. **GC-MS** is also used to detect and measure contaminants, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should be controlled and checked as regulated by governmental agencies. It is used in the analysis of piperine, spearmint oil, lavender oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrances, menthol, allergens, olive oil, lemon oil, peppermint oil, yiang oil, straw berry syrup, butter triglycerides, residual pesticides in food[8,9].

### 1.1.3.3- Forensic and criminal cases

GC-MS can analyze the particles from suspect to correlate his involvement in case. The analysis of fire debris using GC-MS can be established by American Society for Testing Materials (ASTM) standard for fire debris analysis. It is the key tool used in sports anti-doping laboratories to test athlete's urine samples for prohibited

performance enhancing drugs like anabolic steroids. It is also commonly used in forensic toxicology to find poisons, steroids in biological specimens of suspects, victims, or the deceased [10,11].

### 1.1.3.4- Biological and pesticides detections

GC-MS is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs like anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, sedative hypnotics, narcotics and food items. This technique could be used for detecting adulterations, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, organo-chlorinated pesticides in river water, drinking water, soft drinks by head space, pesticides in sunflower oil etc. [12].

### 1.1.3.5- Security and chemical warfare agent detection

For enhancing capability in homeland security and public health preparedness, traditional **GC-MS** units with the transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, soman, and VX [13,14].

### 1.1.3.6- Astro chemistry and Geo chemical Research

Scientist analyzed the atmosphere of Venus with GC-MS. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan. The material in the comet 67P/Churyumov-Gerasimenko has been analyzed with a chiral GC-MS in 2014.

Significantly enhanced molecular ions, major isomer and structurally significant mass spectral peaks, extended range of low volatility hydrocarbons that are amenable for analysis and unique isotope ratio information make **GC-MS** valuable for organic geochemical applications[15,16].

### 1.1.3.7- Medicine and Pharmaceutical Applications

Dozens of congenital metabolic diseases called as inborn error of metabolism are now detectable in newborn by screening tests using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering from metabolic disorders. This is easy, effective and efficient way to diagnose the problem like in case of genetic metabolic disorders by a urine test at birth. In combination with isotopic labeling of metabolite, the GCMS is used for determining metabolic activity. Most applications are based on the use of $^{13}C$ labeling and the measurement of 13C-12C ratios with an isotope ratio mass

spectrometer (IRMS); an MS with a detector designed to measure a few selected ions and return values as ratios. It is useful to detect oils in creams, ointments, lotion etc.

**GC-MS** is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. It is used for process and method development, identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc. [17,18].

### 1.1.3.8- Petrochemical and hydrocarbons analysis

Significantly enhanced molecular ions that are always observed, isomer and structurally significant mass spectral peaks and extended range of low volatilite hydrocarbons that are amenable for analysis including waxes up to $C_{74}H_{150}$ makes the GC-MS a most valuable technique. Broad range of petrochemicals, fuels and hydrocarbon mixtures, including gasoline, kerosene, naphthenic acids, diesel fuel, various oil types, transformer oil, biodiesel, wax and broad range of geochemical samples can be analyzed by **GC-MS**[19].

### 1.1.3.9- Clinical toxicology

Enhanced molecular ions, extended range of compounds amenable for analysis, superior sensitivity for compounds and faster analysis are the main attractive features of the clinical toxicology. The toxin and venoms are identified by GC-MS. It is extensively used in clinical toxicology [20].

### 1.1.3.10- Academic research

As a unique and powerful technology the **GC-MS** provides a rare opportunity to perform the analysis of new compounds for characterization and identification of synthesized or derivatized compound. It is widely used in pure and applied sciences like Chemistry, Polymers, Nanotechnology and Biotechnology etc. It yields useful information that can be used in research publication internationally [21,22].

### 1.1.3.11- Industrial applications

**GC-MS** is used in industries for the analysis of aromatic solvents, inorganic gases, amino alcohol in water, impurities in styrene, glycol, diols, xylene, allergens in cosmetics etc. GC-MS is used for the characterization of formic acid in acetic acid for industrial use. In Industries acetic acid is important intermediate in coal chemical synthesis. It is used in the production of poly ethylene, cellulose acetate and poly vinyl as well as synthesic fiber and fabrics [23].

### 1.1.3.12- Energy and fuel applications

**GC-MS** is used for the analysis of aromatic solvents, sulphur, impurities in polypropylene, sulphur in menthane, natural gases, 1,3 butadiene, ethylene, gas oil, unleaded gasoline, polyethene, diesel.oil, unleaded gasoline, polyethylene, diesel, modified biomass, grafted polymers etc. [24].

GC-MS has triggered a new arena of research and taken to new heights of impactful presentation and characterization of compounds by its wide range of applications [25-27].

## 1.2 Essential oil

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it contains the "essence of" the plant's fragrance—the characteristic fragrance of the plant from which it is derived.[28] The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism.[29].

Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. They are

used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products.

### 1.2.1-History

Essential oils have been used medicinally throughout history. The earliest recorded mention of the techniques and methods used to produce essential oils is believed to be that of Ibn al-Baitar (1188–1248), an Al-Andalusian (Muslim-controlled Spain) physician, pharmacist and chemist.[30]

Rather than refer to essential oils themselves, modern works typically discuss specific chemical compounds of which the essential oils are composed. For example: methyl salicylate rather than "oil of wintergreen".[31,32]

Interest in essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that uses essential oils and other aromatic compounds. Oils are volatilized, diluted in a carrier oil and used in massage, diffused in the air by a nebulizer, heated over a candle flame, or burned as incense.

Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments, and

treatment of cancers in particular, are now subject to regulation in most countries.

### 1.2.2 –Production of oils

#### 1.2.2.1- Distillation

Most common essential oils such as lavender, peppermint, tea tree oil, patchouli, and eucalyptus are distilled. Raw plant material, consisting of the flowers, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapors flow through a coil, where they condense back to liquid, which is then collected in the receiving vessel.

Most oils are distilled in a single process. One exception is ylang-ylang (Canangaodorata) which is purifed through a fractional distillation.

The recondensed water is referred to as a hydrosol, hydrolat, herbal distillate, or plant water essence, which may be sold as another fragrant product. Popular hydrosols include rose water, lavender water, lemon balm, clary sage, and orange blossom water. The use of herbal distillates in cosmetics is increasing. Some plant hydrosols have unpleasant smells and are therefore not sold.

### 1.2.2.2- Expression

Most citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus-fruit oils are cheaper than most other essential oils. Lemon or sweet orange oils are obtained as byproducts of the citrus industry.

Before the discovery of distillation, all essential oils were extracted by pressing.[33]

### 1.2.2.3- Solvent extraction

Most flowers contain too little volatile oil to undergo expression, but their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils [34]. Extracts from hexane and other hydrophobic solvents are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material.

Although highly fragrant, concretes contain large quantities of non-fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, which is more polar in nature, is used to extract the fragrant oil from the concrete. The alcohol solution is chilled to −18 °C (0 °F) for more than 48 hours which causes the waxes and lipids to precipitate out. The precipitates are then filtered out and the ethanol

is removed from the remaining solution by evaporation, vacuum purge, or both, leaving behind the absolute.

Supercritical carbon dioxide is used as a solvent in supercritical fluid extraction. This method has many benefits, including avoiding petrochemical residues in the product and the loss of some "top notes" when steam distillation is used. It does not yield an absolute directly. The supercritical carbon dioxide will extract both the waxes and the essential oils that make up the concrete. Subsequent processing with liquid carbon dioxide, achieved in the same extractor by merely lowering the extraction temperature, will separate the waxes from the essential oils. This lower temperature process prevents the decomposition and denaturing of compounds. When the extraction is complete, the pressure is reduced to ambient and the carbon dioxide reverts to a gas, leaving no residue.

### 1.2.2.4- Florasols extraction

Florasol is another solvent used to obtain essential oils. It was originally developed as a refrigerant to replace Freon. Although Florasol is an "ozone-friendly" product, it has a high global warming potential (GWP; 100-yr GWP = 1430).[8] The European Union has banned its use, with a phase-out process that began in 2011, to be completed in 2017.[34] One advantage of Florasol is that the extraction of essential oils occurs at or below room temperature so degradation through high temperature extremes does not occur. The essential oils are mostly pure and contain little to no foreign substances.

### 1.2.3-Pharmacology and medical uses

Carvacrol, a terpene found in oregano oil, inhibits the growth of several bacteria strains including Escherichia coli and Bacillus cereus. In Pseudomonas aeruginosa, it causes damages to the cell membrane of these bacteria and, unlike other terpenes, inhibits their proliferation. The cause of the antimicrobial properties is believed to be disruption of the bacteria membrane. Carvacrol is a potent activator of the human ion channels transient receptor potential V3 (TRPV3) and A1 (TRPA1).

Another example of the medicinal value of essential oils is thymol, isomeric with carvacrol and found in oil of the common spice thyme. Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol.[35-37]In addition, naturally occurring biocidal agents such as thymol can reduce bacterial resistance to common drugs such as penicillin. Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties. Research demonstrates that thymol and carvacrol reduce bacterial resistance to antibiotics through a synergistic effect, and thymol has been shown to be an effective fungicide, particularly against fluconazole-resistant strains. Carvacrol and thymol have been demonstrated to have a strong antimutagenic effect. In addition, there is evidence that thymol has

antitumor properties. Though the exact mechanism is unknown, some evidence suggests thymol effects at least some of its biocidal properties by membrane disruption. Thymol has been shown to act as a positive allosteric modulator of GABAA in vitro.

Studies have shown that certain essential oils may have the ability to prevent the transmission of some drug-resistant strains of pathogen, specifically Staphylococcus, Streptococcus and Candida.[38]

Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation. In the stomach, the effect is carminative, relaxing the gastric sphincter and encouraging eructation (belching). Further down the gut, the effect typically is antispasmodic.[39,40] Typical ingredients for such applications include eucalyptus oils, menthol, capsaicin, anise and camphor.

Different essential oils may have drastically different pharmacology. Those that do work well for upper respiratory tract and bronchial problems act variously as mild expectorants and decongestants. Some act as locally anesthetic counterirritants and, thereby, exert an antitussive effect.[41,42]

Some essential oils, such as those of juniper and agathosma, are valued for their diuretic effects.[43] With relatively recent concerns about the overuse of antibacterial agents,[44] many essential oils have seen a resurgence in off-label use for such properties and are being examined for this use clinically.[45]

Many essential oils affect the skin and mucous membranes in ways that are valuable or harmful. Many essential oils, particularly tea tree oil, may cause contact dermatitis.[46-49]They are used in antiseptics and liniments in particular. Typically, they produce rubefacient irritation at first and then counterirritant numbness. Turpentine oil and camphor are two typical examples of oils that cause such effects. Menthol and some others produce a feeling of cold followed by a sense of burning. This is caused by its effect on heat-sensing nerve endings. Some essential oils, such as clove oil or eugenol, were popular for many hundred years in dentistry as antiseptics and local anesthetics.

### 1.2.4-Use in aromatherapy

Essential oils are used in aromatherapy as part of, for example, essential oil diffusers.Aromatherapy is a form of alternative medicine in which healing effects are ascribed to the aromatic compounds in essential oils and other plant extracts. Aromatherapy appears to be useful to induce relaxation, especially when administered with massage.[50] Use of essential oils may cause harm including allergic reactions and skin irritation; there has been at least one case of death.[51]

## 1.3-Antibacterials

Antibacterials are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterials is

generally considered low.(depends) Prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, as for recurrent *Clostridium difficile* infections.[52,53]

The discovery, development and use of antibacterials during the 20th century has reduced mortality from bacterial infections. The antibiotic era began with the 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 discovered and developed. 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.[54] In parallel there has been an alarming increase in antimicrobial resistance of bacteria, fungi, parasites and some viruses to multiple existing agents.[55]

Antibacterials are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infections. As a consequence of widespread 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 a 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-molecule libraries customized for bacterial targets.[56]

## 1.4- The target species-*CitrullusColocynthis*

| **Scientific classification** | |
|---|---|
| Kingdom: | Plantae |
| (unranked): | Angiosperms |
| (unranked): | Eudicots |
| (unranked): | Rosids |
| Order: | Cucurbitales |
| Family: | Cucurbitaceae |
| Genus: | Citrullus |
| Species: | **C. colocynthis** |
| **Binomial name** | |
| **Citrulluscolocynthis** (L.) Schrad. | |

*Citrulluscolocynthis*, with many common names including colocynth, bitter apple, bitter cucumber,[57]desert gourd,egusi,[58]vine of Sodom[57], or wild gourd,[57] is a desert viny plant native to the Mediterranean Basin and Asia, especially Turkey (especially in regions such as İzmir), Nubia, and Trieste.

It resembles a common watermelon vine, but bears small, hard fruits with a bitter pulp. It originally bore the scientific name *Colocynthiscitrullus.*

### 1.4.1 Origin, distribution, and ecology

*C. colocynthis* is a desert viny plant that grows in sandy, arid soils. It is native to the Mediterranean Basin and Asia, and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India, and reaches also the north coast of the Mediterranean and the Caspian Seas. It grows also in southern European countries as in Spain and on the islands of the Grecian archipelago. On the island of Cyprus, it is cultivated on a small scale; it has been an income source since the 14th century and is still exported today. It is an annual or a perennial plant (in wild) in Indian arid zones and has a great survival rate under extreme xeric conditions.[59] In fact, it can tolerate annual precipitation of 250 to 1500 mm and an annual temperature of 14.8 to 27.8 °C. It grows from sea level up to 1500 meters above sea level on sandy loam, subdesert soils, and sandy sea coasts with a pH range between 5.0 and 7.8.[60]

### 1.4.2-Characteristics and morphology

#### 1.4.2.1- Roots and stems

The roots are large, fleshy, and perennial, leading to a high survival rate due to the long tap root. The vine-like stems spread in all

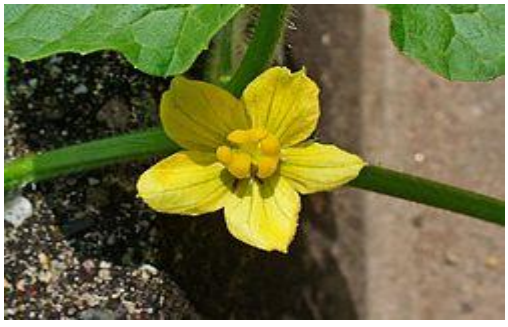
directions for a few meters looking for something over which to climb. If present, shrubs and herbs are preferred and climbed by means of axiliary branching tendrils.[59]

### 1.4.2.2- Leaves

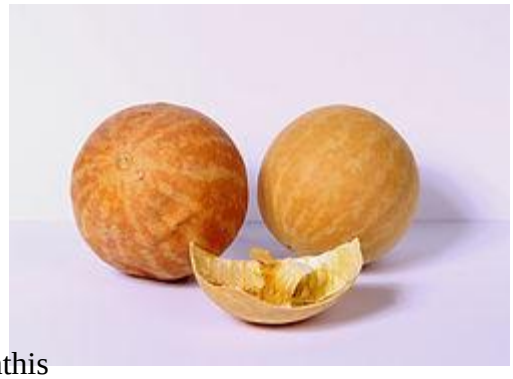
Very similar to watermelon, the leaves are palmate and angular with three to seven divided lobes.

### 1.4.2.3- Flowers

The flowers are yellow and solitary in the axes of leaves and are borne by yellow-greenish peduncles. Each has a subcampanulated five-lobed corolla and a five-parted calyx. They are monoecious, so the male (stamens) and the female reproductive parts (pistils and ovary) are borne in different flowers on the same plant. The male flowers' calyx is shorter than the corolla. They have five stamens, four of which are coupled and one is single with monadelphousanther. The female flowers have three staminoids and a three-carpelovary. The two sexes are distinguishable by observing the globular and hairy inferior ovary of the female flowers.[59]



*C. colocynthis* female flower



C. colocynthis

Ripe fruit of *C. colocynthis*

Seeds of*C.colocynthis*

### 1.4.2.4- Fruits

The fruit is smooth, spheric with a 5– to 10-cm-diameter and extremely bitter taste. The calyxenglobe the yellow-green fruit which becomes marble (yellow stripes) at maturity. The mesocarp is filled with a soft, dry, and spongy white pulp, in which the seeds are embedded. Each of the three carpels bears six seeds. Each plant produces 15 to 30 fruits.[60]

### 1.4. 2.5- Seeds

The seeds are grey and 5 mm long by 3 mm wide. They are edible but similarly bitter, nutty-flavored, and rich in fat and protein. They are eaten whole or used as an oilseed. The oil content of the seeds is 17–19% (w/w), consisting of 67–73% linoleic acid, 10–16% oleic acid, 5–8% stearic acid, and 9–12% palmitic acid. The oil yield is about 400 l/hectare.[61] In addition, the seeds contain a high amount of arginine, tryptophan, and the sulfur-containing amino acids.[62]

### 1.4.3 Cultivation

C. colocynthis, a perennial plant, can propagate both by generative and vegetative means. However, seed germination is poor due to the extreme xeric conditions, so vegetative propagation is more common and successful in nature. In the Indian arid zone, growth takes place between January and October, but the most favorable period for the vegetative growth is during summer, which coincides with the rainy season. Growth declines as soon as the rains and the temperature decrease and almost stops during the cold and dry months of December and January. Colocynth prefers sandy soils and is a good example of good water management which may be useful also on research to better understand how desert plants react to water stress.[63,64] To enhance production, an organic fertilizer can be applied.[65] Colocynth is also commonly cultivated together with cassava (intercropping) in Nigeria.

### 1.4.4-Uses

*C. colocynthis* can be eaten or elaborated for further uses in medicine and as energy source, e.g. oilseed and biofuel. The characteristic small seed of the colocynth have been found in several early archeological sites in northern Africa and the Near East, specifically at Neolithic Armant, Nagada in Egypt; at sites dating from 3800 BC to Roman times in Libya; and the prepottery Neolithic levels of the NahalHemar caves in Israel.[66]Zohary and

Hopf speculate, "these finds indicate that the wild colocynth was very probably used by humans prior to its domestication.[66]

### 1.4.5-Medical studies on *C.colocynthis*

Clinical studies have shown medicinal benefits of colocynth in patients with diabetes, diabetic neuropathy, and hyperlipidemia. In a randomized clinical trial (RCT), HbA1c and fasting blood glucose levels were decreased in patients using 300 mg of C. colocynthis dry fruit powder daily for 2 months.[67] In another trial, intake of 300 mg of powdered seed can lower the triglyceride and cholesterol concentration significantly in nondiabetichyperlipidemic patients.[68]

Another property of colocynth is hair growth stimulation: an experiment on rats demonstrated that hair growth initiation time was significantly decreased after treatment with colocynth petroleum ether extracts.[69]

## 1.5- Aim of this study

This study was designed to:

-Extract the fixed oil of Citrulluscolocynthis.

-To conduct a GC-MS analysis for the oil.

-To evaluate the oil for its antimicrobial potency.

# *Chapter Two*

# *Materials and Methods*

# 2- Materials and Methods

## 2.1 Materials

### 2.1.1 Plant Material

Seeds of citrulluscolocythis were purchased from the local market – Khartoum (sudan) and authenticated by the department of phytochemistry and taxonomy, national research center, at Khartoum – Sudan.

### 2.1.2 Instruments

GC-MS analysis was conducted on Shimadzo GC-MS QP2010 Ultra instrument with a RTX-5MS column (30ml, length; 0.24$\mu m$, thickness).

### 2.1.3 Test Organisms

Citrulluscoloythis oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table (1.2).

**Table (1.2): Test Organisms**

| **Ser. No.** | ***Microorganism*** | ***Type*** |
|---|---|---|
| 1 | *Bacillus Subtilis* | *G+ve* |
| 2 | *StaphococcusAureus* | *G+ve* |
| 3 | *Pseudomonas Aeroginosa* | *G-ve* |
| 4 | *Escherichia Coli* | *G-ve* |
| 5 | *Candida Albicans* | *Fungi* |

## 2.2 Methods

### 2.2.1 Presentation of Reagents for Phytochemical Screening

a) Flavonoid Test Reagents.

- Aluminum Chloride Solution:

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

- Potassium Hydroxide Solution:

(1g) of potassium chloride was dissolved in (100ml) water.

- Ferric Chloride Solution:

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

b) Alkaloid Test Reagents

Maeyer reagent

- Mercuric chloride solution: 1.36g in 60ml of water.
- Potassium iodide solution: 5g in 10ml of water.

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

- Wagner reagent

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

### 2.2.2 Presentation of Plant Extract for Phytochemical Screening

(100ml) of powderd air –dride seeds of citrulluscolocythis were extracted with 95% ehanol (soxhaustion. This prepared extract (PE) was used for phytochemical screening.

### 2.2.3 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 chlorform 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 aluminum chloride solution were added.

- To 3 mi. 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 mi of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into Two 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 was 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 stoppered and vigorously shaken for about 30 seconds, and allowed to stand.

### 2.2.4 Extraction of Oil

Powdered seeds of Citrulluscolocythis (500g) 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 (2rn1) was placed in a test tube and 7m1 of alcoholic sodium hydroxide were added followed by 7rn1 of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2m1) of supersaturated sodium chloride were added, then (2m1) of normal hexane were added and the tube was vigorously shaken for five minutes .The hexane layer was then separated.(5jil) of the hexane extract were mixed with 5m1 diethyl ether . The solution was filtered and the filtrate(1 ml) was injected in the GC-MS vial.

### 2.2.5- GC-MS Analysis

The oil of Citrulluscolocythis was analyzed by gaschromatograph-mass spectrornetry. A Shimadzo GC-MSQP2O 10 Ultra instrument with a RTX-5IVIS column (3.0 mm,length; 0.25mm diameter; 0.25 pm, thickness) was used.Helium(purity; 99.99 %) was used as carrier gas.Oven temperatureprogram is given in Table 2, while other chromatographicconditions are depicted in Table (2.3).

**Table (2.2): Oven Temperature Program**

| Rate | Temperature(°C) | Time ($Min.^{-1}$) |
|---|---|---|
| - | 150.0 | 1.00 |
| 4.00 | 300.0 | 0.00 |

**Table (2.3): Chromatographic Conditions**

| | |
|---|---|
| Column oven temperature | 150.0°C |
| Injection temperature | 300.0°C |
| Injection mode | Split |
| Flow control mode | Linear velocity |
| Pressure | 139.3 KPa |
| Total flow | 50.0 ml/ min |
| Column flow | l.54 ml/sec. |
| Linear velocity | 47.2cm/sec. |
| Purge flow | 3.0 ml/min. |
| Spilt ratio | - 1.0 |

### 2.2.6-Antimicrobial Test

#### i. Preparation of Bacterial Suspensions

One ml aliquots of 24 hours broth culture of the test organismswere aseptically distributed onto nutrient agar slopes andincubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterilenormal saline, and finally suspended in loo ml of normal salineto produce a suspension containing about $10^8$-$10^9$colonyforming units per ml.The suspension was stored in therefrigerator at 4°C until used. The average number of viableorganism per ml of the stock suspension was determined bymeans of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterilenormal saline in tubes and one drop volumes (0.02 ml) of theappropriate dilutions were transferred by adjustable volumemicropipette onto the surface of dried nutrient agar plates. Theplates were allowed to stand for two hours at room temperaturefor 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 agarincubated at 25°C for four days. The fungal growth washarvested and washed with sterile normal saline, and thesuspension was stored in the refrigerator until used.

### iii. Testing for Antibacterial Activity

The cup-plate agar diffusion method was adopted with someminor modifications, to assess the antibacterial activity of theoil. (2m1) of the standardized bacterial stock suspension weremixed with 200 ml of sterile molten nutrient agar which wasmaintained at 45°C in a water bath. (20 ml) Aliquots of theincubated nutrient agar were

distributed into sterile Petri dishes,the agar was left to settle and in each of these plates which weredivided into two halves, two cups in each half (10 mm indiameter) were cut using sterile cork borer (No 4), each one ofthe halves was designed for one of the compounds. SeparatePetri dishes were designed for standard antibacterialchemotherapeutic, (ampicillin and gentamycin).

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

The above procedure was repealed for different concentrationsof the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of theresultant growth inhibition zones were measured in triplicatesand averaged.

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