# Sudan University of Science and Technology

## College of Postgraduate Studies

# Characterization of Constituents of*Corochorusolitorius*Seeds Fixed Oil by GC-MS and Antimicrobial Activity of the Oil

# توصيف مكونات الزيت الثابت لبذور الملوخية وفعالية الزيت المضاد للميكروبات

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


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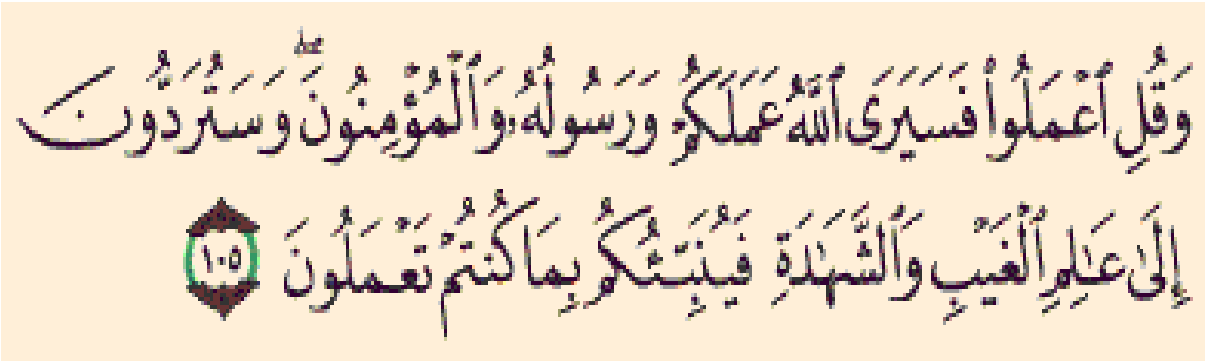

**(التوبة-105)**

# *Dedication*

*To*

*my parents*

*brothers and sisters*

## Acknowledgement

First of all I would like to thank **Almighty Allah** for giving me strength and patience to complete this research.

I would like to thank my supervisor Professor Mohammed abdelkarim for his suggestions, assistance patience and understanding throughout this research

Finally I wish to thank my parents and friends for their support and encouragement throughout my study.

## Abstract

The oil of the medicinally important species*corochorusolitorius* was extracted by hexane and analyzed by GC-MS. The analysis revealed the presence of 22 components. The major components are: 9, 12-
, hexadecanoic acid methyl .octadecadienoic acid (z,z)-,methyl ester
9, 12, 15-octadecatrien acid,2,3-dihydroxy و.,methyl stearate .ester
proply esterand eicosanoic acid, methyl ester.

In cup plate ager diffusion bioassay *corochorusolitorius* oil showed excellent activity against *Bacillus subtilis*. It also exhibited significant activity against *Candidaalbicans*.

## مستخلص البحث :iv

استخلصت بزور نبات الملوخيه بالهكسان حيث تم استخلاص الزيت الثابت . ثم حلل الزيت بتقنيه الكروموتوغرافيا الغازيه-طيف الكتله والتي اوضحت وجود 22مكونا اهمها:

9,12-octadecadienoic acid (Z,Z)-, methyl ester.

Hexadecanoic acid, methyl ester.

Methyl stearate.

9,12-Eicosanoic acid, methyl ester.

15- Octadecatrienoic acid , 2,3-dihydroxy propyl ester

وفي اختبار مضاد البكتريا ابدي الزيت فعاليه عاليه ضد (*Bacillussubtilis*) واعطى فعاليه ممتازه ضد*Candida albicans* .

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# Chapter 1

# Introduction

## 1.1-History and sources of essential oil

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin. The respective compounds are mainly derived from there biosynthetic pathway only , the mevalonate pathway leading to sesquiterpenes, the methyl erithrytol pathway leading to mono and diterpenes , shikimic acid pathway lead to phenylpropenes .nevertless ,There an almost uncountable number of single substance and tremendous variation in the composite of essential oils . Many of these volatile substance have diverse ecological functions .They can act as internal messengers ,as defensive substance against herbivores or as volatiles directing not only natural enemies to these herbivores but also attracting pollinating insect to their host[1].

All plants possess principally the ability to produce volatile compounds, ‘essential oil plant’ ’in particular are those plant

species delivering an essential oil of commercial interest. Two principal circumstances determine a plant to be used as an essential oil plant:

**(i)A**: Unique blend of volatiles like flower sent in rose (rosaspp), jasmine (jasminumsambac). Such flower produces and immediately emits the volatiles by the epidermal layers of

their petals[2]. Therefore the yield is even in intensive smellingflower is very low, and besides distillation special techniques, enfleurage had been applied to recover the volatile fragrance compounds.

**(ii)b:** Secretion and accumulation of volatiles in specializedanatomical structures. Leads to higher concentration of essential oil in plant such anatomical storage structures for essential oils can be secretory idioblasts (secretory cells),cavities/ducts, or glandular trichromes[3].

An essential 0il is concentrated hydrophobic liquid containing volatile hydrophobic liquid containing volatile (defined as ‘’the tendency of a substance to vaporize’’) 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. Oil is essential in the sense that it contains the ‘’essence of ‘’ the plant fragrance-the characteristic fragrance of the plant from which it is derived[4]. The term essential 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[5].

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[6].

**1.2- production**

Most common essential oils such as lavender, peppermint, tea tree oil, patchouli, and eucalyptus are distilled. Raw plant material, consisting of the flower, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As 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 us then collected in the receiving vessel.

Most oils are distilled in a single process. One exception is canangaodorata which is purified through a fractional distillation. Therecondensed water is referred to as a hydrosol, hydrolyte, herbal distillate, or plant water essence which may be sold as another fragrant product. Hydrosols include rose water, lavender water, lemon balm, clary sage, and orange blossom water. The use of herbal distillates in cosmetics is increasing [7].

### 1.3- Expression

To extract citrus oil, Peels are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relative large quantities of oil in truspeel 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[8].

### 1.4-Essential oil uses and benefits

Essential oils find many applications uses range from aromatherapy, household cleaning product, and natural medicine treatments. Essential oils come from distilling of different parts of plants, including the flowers, leaves, bark, roots, resin and peels .Essential oil benefits come from their antioxidant, antimicrobial and antiinflammatory properties. These healing oils are rapidly growing in populate because they are natural medicine without any side effects[9]

### 1.4.1-Essential oil remedies

The diverse uses of essential oils in talk medicine are discussed briefly below:

**i-Burns:**lavender essential oil is mixed with aloe Vera to treat burns.

**ii-Bug bites:** lavender oil is applied for bug bites and stings.

**iii-Bruises:** essential oil like lavender oil as a hot compress to treatbruises or other wounds

**iv-Motion sickness:** peppermint; lavender and ginger oil are usuallyapplied to reduce motion sickness.

**v-Colds:**Some drops of oil of oregano and frankincense are extremelyuseful for colds.

**vi- Neck pain:** peppermint, cypress and ginger oils with cayenne paperand coconut oil are the bases is for a homemade pain relieving muscle rub.

**vii- Headache relief:** A combination lavender oil and peppermint oil isapply to temples to help with headaches and migraines.

**viii-Cough or sinusitis:** Eucalyptus essential oil is known for itspowerful ability to fight coughs and open airways.

**ix- Broken bones:** To support healing of broken bones, helichrysum, firand cypress essential oils are used.

**x- Indigestion:** Ginger, peppermint and fennel essential oils supportdigestion and healing leaky gut.

**xi. Bronchitis and asthma:** A homemade vapor rubs is prepared bycombining eucalyptus, peppermint and coconut oil.

**xii.Bruises:**Levender and frankincense oils are treated with hot water and applied to affected area.

**xiv.Memary:** Bergamot, peppermint or grapefruit seed essential oilsmay increase concentration during the day.

**xv.Sore Feet:** Peppermint oil with Epsom salt is added to a warm waterfoot bath.

**xvi. Teeth grinding:** lavender is placed on the bottom of the feetand behind ears before bed.

**xvii. Eczema and psoriasis:** To treat eczema, psoriasis or red dry skin, a mixture of lavender essential oil with Shea butter is applied externally.

**xviii**. **Improving circulation**: Grapefruit essential oils added to warm abath water to improve circulation.

**xix. Balance of blood sugar:**peppermint and cinnamon essential oilsare in haled to reduce appetite and balance blood sugar.

**xx.fatigue:**Peppermint oil is inhaled before a workout to reduce fatigue 6.

**xxx.Fever:** Some drops of eucalyptus, peppermint and lavenderessential oils are added to a cool cloth to sponge the body.

**xL: Hangover symptoms:** Some drops each of juniper berry cedarwood, grapefruit, lavender, rosemary and lemon oil are added a warm bath.

**L: Arthritis relief:** Some drops of wintergreen, cypress and lemongrassoils are mass aged of into affected areas.

**Lx.Ringworm:**Somedrops of tea tree oil are combined with coconutoil and massage over the affected area twice a day.

**Lxx. Head lice treatment:** Some three drops of thyme, lavender and eucalyptus oils are applied to scalp. The head is covered with a shower cap and left on for 30 minutes.

**Lxxx. Blistered skin:** Some drops of tea tree oil are mixed with drops ofunscented oil and applied to the blistered area up to five times per day.

**xc. Sunburn:** Acombination of lavender or chamomile oil withcoconut oil is applied to the skin with a cotton ball to reduce swelling and pain.

**xcix. Immune system:** Some drops oregano oil is mixed with carrier oiland rubbed on the bottom of feet.

**c. Weigh loss:** Grapefruit, ginger and cinnamon oil and take as asupplement three times daily to support metabolism[10].

**1-5: Analysis of essential oils**

As widely acknowledged the composition of essential oils is mainly represented by mono and sesquiterpene hydrocarbons and oxygenated (hydroxyl and carbonyl) derivatives, along aliphatic aldehydes, alcohols and esters[11].

## 1-5-1: Modern analytical techniques

Most of the methods applied in the analysis of essential oils rely on chromatographic procedures .Which enable component separation and identification. Among such techniques (gas chromatography-mass spectrometry) is widely used for oil analysis[12].

## 1-5-1-1: Gas chromatography- mass spectrometry

Gc-Mass is a sophisticated instrumental technique that produces, separates, and detects ion in the gas phase. Gc-mass is a hyphenated analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances in a test sample[13] Application of Gc-Ms include drug detection , fire investigation , environmental analysis , explosives investigation , and identification of un known samples , including that of material samples obtained from planet Mars during probe missions as early as the 1970s. Gc-ms can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification .Like liquid chromatography-this hyphenated technique allows analysis and detection even of tiny amounts of a substance. Gc-Ms is very useful for forensic substance identification. It is used to perform a 100% specific test, which positively identifies the presence of a particular substance. A nonspecific test merely indicates is that any of several in a category of substances of present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification[1] Mass spectroscopy is used to determine the molecular formula of the unknown compound. Mass spectroscopy data that provides structural information tends to be unreliable and thus will only be used to verify a possible structure or in the event that the other spectral techniques are unsuccessful.

Mass spectrometry (MS) can be defined as the study of the systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge rations (m/z) and relative abundances[14.]

The analyte may be ionized thermally, by an electric filed or by impacting energetic electrons, ions. The potential of combined gas chromatography –mass spectrometers (Gc-Ms) for determining volatile compounds, contained in very complex flavor and fragment sample, is well known. The most frequent and simple identification method in Gc-Ms consists of comparison of the acquired unknown mass spectra with those chromatographic sample inlets[15]

GC-MS is a sophisticated instrument technique that produce, separated, and detects ion in the gas phase .Mass spectroscopy is used to determine the molecular formula of the unknown compound.

Mass spectroscopy data that provides structural information tend to be unreliable and thus will only be used to verify a possible structure or in the event that the other spectral techniques are unsuccessful[16]

Gas chromatography (GC) is a widely applied technique in many branches of science and Technology. For over half a century, GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture.

However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system.

The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum.

Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule. In a gas chromatographic system, the sample to beanalyzed may be a liquid solution or a collection of molecules adsorbed on a surface, e.g., the solid-phase micro extraction (SPME) system. During the transfer into the GC, the sample is volatilized by rapid exposure to a zone kept at relatively high temperature (200-300°C) and mixed with a stream of carrier gas (Ar , He, N2, or H2). The resulting gaseous mixture enters the separation section, a chromatographic column, which in its current version is a fused-silica tubular capillary coated internally with a thin polymer film.

Upon their displacement through the column, analyte molecules are partitioned between the gas carrier stream (mobile phase) and the polymer coating (stationary phase), to an extent which depends mainly on their chemical structure.

At the end of the separation section, the molecules reach a detection system in which a specific physical property (thermal conductivity) or aphysico-chemical process (ionization in a flame, electron capture) gives rise to an electric signal which is proportional to the amount of molecules of the same identity.

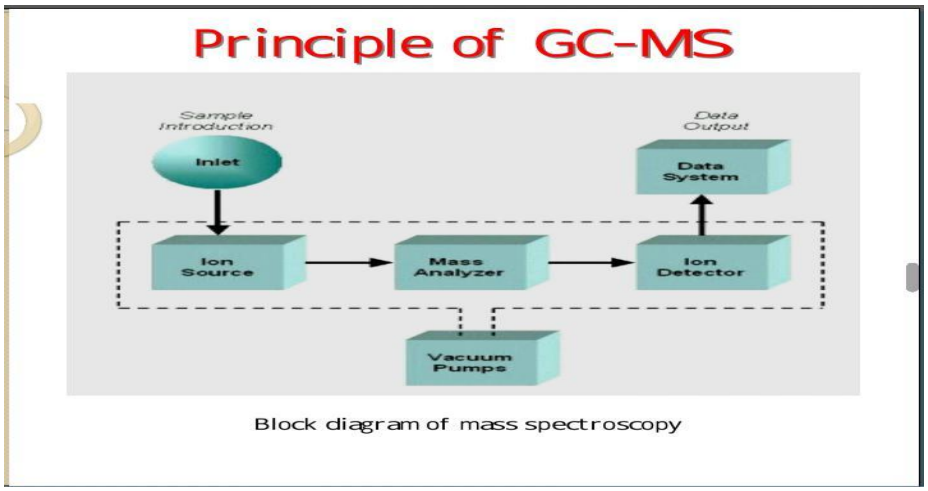
A data system permits to process these data to produce a graph of the variation of this detector signal with time (chromatogram). Thus, four principal sections are distinguishable in the chromatograph: introduction (injector), separation (chromatographic column) detection, and data handling units. Each section has its own function and its responsibility for the quality of the analysis. The injection system, for example, should ideally transfer the sample to the column quantitatively, without discrimination on molecular weights or volatility, and without chemical alteration (decomposition or isomerization).

It is a critical step, especially for quantitative analysis. For correct GC operation, among other conditions, this gateway to the column should remain unpolluted, clean, inert, and leak-free.

The main requirement for an analyte in GC is that it should be volatile enough to be present in detectable amounts in the mobile phase. Substances with low vapor pressure will not enter the chromatographic column, will accumulate at the injection system, and may eventually clog its conduits. Very polar, thermo labile, ionic and high-molecular weight compounds are not compatible with regular GC analysis. Depending on the molecular structure of the analyte and the functional groups available, it is possible in some cases to obtain a chemical

derivative which has a higher vapor pressure and is therefore more amenable to GC analysis[17]

### 1.5.1.2-Princible of GC-MS




The inlet transfers the sample into the vacuum of the mass spectrometer .In the source region, neutral sample molecules are ionized and then accelerated into the mass analyzer .The mass analyzer is the heart of the mass spectrometer .This section separates ions, either in space or in time, according to their mass to charge ratio. After the ions are separated, they are detected and the signal is transferred to a data system for analysis. All mass spectrometers also have a vacuum detected system and the signal is low pressure, which is called high vacuum, required for operation. High vacuum minimize ion molecule reactions, scattering, and neutralization of the ion[17, 18].

## 1.6- Antioxidant

### 1.6.1. Definition

Antioxidant are a group of compounds that facilitate survival in plants and many promote the health of humans that consume a variety of plant foods[19,20,21].In plant the term antioxidant often refers to a wide range of phenolic compounds that vary from simple phenolic acids to highly polymerized compounds such tannins. Phenolic compounds or poly phenols are categorized into 15 main classes with over 8000 identified compounds .The largest category is the flavonoid group[22, 23].

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radical in turn, these radical can start chain reaction that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediate, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agent such as thiols, ascorbic acid or polyphones[24]

Antioxidant is mainly used for two different groups of substance industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to

have beneficial health effects. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalyses and superoxide dismutase) produced internally or the dietary antioxidants: vitamin A, vitamin C, and vitamin E (24).Industrial antioxidants have diverse uses, including preservative in food and cosmetics, and oxidation inhibitors in fuels[25]. There are many ways to describe what antioxidants do inside the body, one definition of antioxidants is any substance that inhibits oxidation, especially one used to counteract the deterioration of stored food products or removes potentially damaging oxidizing agent in a living organism. Antioxidants include dozens of food based substances you many have heard of before, such as carotene , lycopene and vitamin C

.These are several examples of antioxidants that inhibit oxidation , or reaction promoted by oxygen , peroxides and\or free radicals[26].Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules free radicals.

Antioxidants help prevent oxidation, which can cause damage to cells and may contribute to aging. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. Antioxidants exist as vitamins, minerals and other compounds in foods. A diet containing plenty of fruits and vegetables, whole grains and nuts

can supply all the antioxidants your body needs. Diets rich in antioxidants can be very beneficial.

A few of the better known antioxidants include carotenoids (a form of vitamin A) — the substance that gives fruits and vegetables their deep rich colors. Apricots, broccoli, pumpkin, cantaloupes, spinach and sweet potatoes are good choices. Foods containing vitamins C and E are also good sources of antioxidants, as well as selenium and zinc.

## 1.7.2-Most commonly known antioxidants and their food sources

**Carotenoids:** (a form of vitamin A) the substance that gives fruitsand vegetables their deep rich colors. May be effective alliesAgainst prostate cancer sources include: Apricots, peaches, broccoli, pumpkin, canto loupes, carrots, spinach and sweet potatoes

(ii) **Vitamin C:** enhances the immune response and protects againstinfection. This vitamin is found in Citrus fruits like oranges and lime etc, green peppers, broccoli, green leafy vegetables, strawberries and tomatoes

(iii) **Vitamin E:** May help prevent the oxidation of LDL or “bad” cholesterol which contributes to plaque buildup in the arteries sources include: nuts and seeds, whole grains, green leafy vegetables, vegetable oil and liver oil.

(iv) **Selenium:** Sources of selenium include: Fish and shellfish, redmeat, grains, eggs, chicken and garlic

The flavonoids are common antioxidants and their food sources are shown below

i-Red wine

ii-purple grapes or Concord grapes

iii-pomegranate

iv-cranberries

v-tea

vi-Lycopene

vii-Tomato and tomato products

viii-pink grapefruit

ix-watermelon

x-Lute in

xi-dark green vegetables such as kale, broccoli, kiwi, brussels sprout and spinach

xii-Lignin

xiv-flax seed

xv-oatmeal

xvi-barley

xvii-rye

Some antioxidant enzyme made by the body is outlined below

i-superoxide dismutase (SOD)

ii-catalyses

iii-glutathione peroxides

**Benefits of antioxidants include**

(a)Protect Against Heart Disease

The American Heart Association recommends a diet high in fruits, vegetables and other foods that contain antioxidants to help fight cardiovascular disease. They do not recommend antioxidant supplements, however, because there is no scientific evidence to support the idea that they have any beneficial effect on heart disease. (b)Protect Against Cancer

Lycopene is concentrated in tomato soups, sauces, tomato paste and other tomato products, and is also available in smaller amount in fresh tomatoes, watermelon and pink grapefruit .Cancers of mouth, pharynx esophagus, stomach, colon and rectum can be prevented by lycopene and lutein may help decrease your risk of macular degeneration.

**(C)Boost Immunity**

Vitamin C ability to reduce the severity of the common cold is indicative of its effect on the immune system. Most fruits and vegetables provide some Vitamin C like Citrus fruits; kiwi, tomatoes and sweet peppers there are good sources.

**(d)Fight Aging**

While it has not been shown that antioxidants actually increase anyone's lifespan, they do protect against some of the degenerative effects on the body of age-related diseases that can lead to early death. Studies on laboratory animals suggest that a diet high in antioxidants, especially those found in blueberries, strawberries and spinach may also help fight the loss of brain function associated with aging. Eating a diet that includes a variety of fresh, deeply colored fruits and vegetables, such as broccoli, spinach, tomatoes, sweet peppers ,carrots, mangoes, kiwi, berries and cantaloupe and other plant foods, such as grains, legumes (beans, lentils, and split peas) and nuts, is the safest and most effective

way to boost your antioxidant supply and reap the health benefits these substances may convey[27].

## 1.7-Antibacterial activity of essential oils

Bacterial pathogens and their control are a serious problem in agriculture practice. spraying with antibiotics and copper compounds, usually

suggested to control bacterial diseases, have never been satisfactory. Furthermore, antibiotics are forbidden in many countries and copper compounds, because of their general toxicity, exert a negative impact on both yield and the environment. As an alternative strategy to prevent the spread of diseases, natural compounds of plant are being tested for their antimicrobial activity. Naturally occurring biologically active plant products can be a source of new pesticides or serve as templates for new, more effective compounds[28].

Investigations of aromatic and medicinal plants enable finding plants producing effective essential oils that have already found a considerable range of applications .Various essential oils are biocides against a broad range of organisms such as bacteria, fungi I, viruses, protozoa, insects and plants[29] .In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial Properties against bacteria and fungi. There is vast diversity among aromatic and medicinal plants[30].

Antimicrobials can be grouped according to the micro organism they act primarily agent.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 treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis[31].The science dealing with the study of the

prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. Its sub disciplines are virology (study of viruses), bacteriology (study of Bacteria), mycology (study of fungi), physiology (study of algae) and protozoology (study of protozoa).

For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders.

The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses),

bactericides (killing bacteria), algicides (killing algae) or fungicides (killing fungi) [32].

The antibacterial agents are classified in three categories:

**(i)** Antibiotics and chemically synthesized
Chemotherapeutic agents.

**(ii)** Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives)

**(iii)** Immunological products[33].

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach). which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics are applied to living tissue and help reduce infection during surgery and antibiotics tend to destroy microorganisms within the body. Antibacterial are used to treat bacterial infections. The drug toxicity to human and other animals from antibacterial is generally considered low. Prolonged use of certain antibacterial can decrease the number of gut flora, which may have a negative impact on health.

Antibacterial are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infection[34].

## 1.8-*Corchorus olitorius*

Leafy vegetables are known to add taste and flavour, as well as substantial amounts of protein, fiber, minerals, and vitamins to the diet[35,36]. Among the vegetables, genus *Corchorus* L. is one of the most important groups[36,37]. Nowadays, it belongs to the family of Sparmaniaceae[38]. It contains about forty species including *Corchorusolitorius*which is one of the most cultivated*Corchorus*species in theworld

The leaf juice, fried leaf, and some time whole green leaf, are used, among other reasons, as laxatives, in creams for skin care, and as a treatment for a wide range of diseases, respectively. The heterogeneous nature of jute leaf products may contribute to the diverse biological and therapeutic activities that have been observed. Variations in the composition of jute leaf can result in products with different chemical and physical properties, making the comparison of products difficult. This green, leafy vegetable is rich in beta-carotene for good eyesight, iron for healthy red blood cells, calcium for strong bones and teeth, and vitamin C for smooth, clear skin, strong immune cells, and fast wound-healing.

Vitamins A, C and E present in leaf.The plant can “spongeup” free radicals, scooping them up before they can commit cellular sabotage. The leaf as vegetable contains an abundance of antioxidants that have been associated with protection from chronic diseases such as heart

disease, cancer, diabetes, and hypertension as well as other medical conditions. Fresh leaf has higher demand.

Ayurvedics use the leaves for ascites, pain, piles (laxative) and tumors. Elsewhere the leaves are used for cystitis, dysuria, and fever. The cold infusion is said to restore the appetite and strength[38].

*Corchorusolitorius*

**Aim of this study**

This study was carried out to :

-Extract *Corchorusolitorius* seed oil.

-Investigate oil constituents by GC-MS.

-Evaluate the oil for its antimicrobial potential.

# Chapter tow

# 2-Materials and Methods

## 2.1-Materials

### 2.1.1- Instruments

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

### 2.1.2-Test organisms

*corochorusolitorius*oil was screened for antibacterial and antifungalactivities using the standard microorganisms shown in table(2.1).

**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 |

### 2.1.3-Plant material

Seeds of *corochorusolitorius* were purchased from the local market – Khartoum ,Sudan and authenticated by direct comparison with a herbarium sample.

## 2.2- Methods

### 2.2.1-Extraction of oil

Powdered seeds of *corochorusolitorius* (500g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at $4^{o}$C for further manipulation.

The target 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 sulphuricacid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes .

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

### 2.2.2- GC-MS analysis

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

Table 2: Oven temperature program

| Rate | Temperature($^{o}$C) | Hold Time (min.$^{-1}$) |
|---|---|---|
| - | 150.0 | 1.00 |
| 4.00 | 300.0 | 0.00 |

Table 3: Chromatographic conditions

| | |
|---|---|
| Column oven temperature | 150.0$^{o}$C |
| Injection temperature | 300.0$^{o}$C |

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

### 2.2.3-Antimicrobial assay

### (i)Bacterial suspensions

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

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about $10^8$-$10^9$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 saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

### ii)-Fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed

with sterile normal saline, and the suspension was stored in the refrigerator until used.

**iii)-Antibacterial test**

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed

into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

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

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After

incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

# Chapter 3

## 3-Results and Discussion

*Corochorusolitorius*fixed oil was successfully extracted from seeds bymaceration using hexane as solvent. The oil was analyzed by GC-MS which revealed the different constituents of the oil. In cup plate agar diffusion bioassay, the oil was assessed for antimicrobial activity against 5 standard human pathogens.

### 3.1- GC- MS analysis of *corochorusolitorius* oil

The GC-MS analysis of *corochorusolitorius* oil showed 22 components dominated by:

-9,12-octadecadienoic acid (Z,Z)-, methyl ester.

-Hexadecanoic acid, methyl ester.

-Methyl stearate.

-9,12 15- Octadecatrienoic acid , 2,3-dihydroxy propyl ester

-Eicosanoicacid , methyl ester

The total ions chromatograms is displayed in Fig. 1 , while Table 3.1 shows different constituents of the oil.

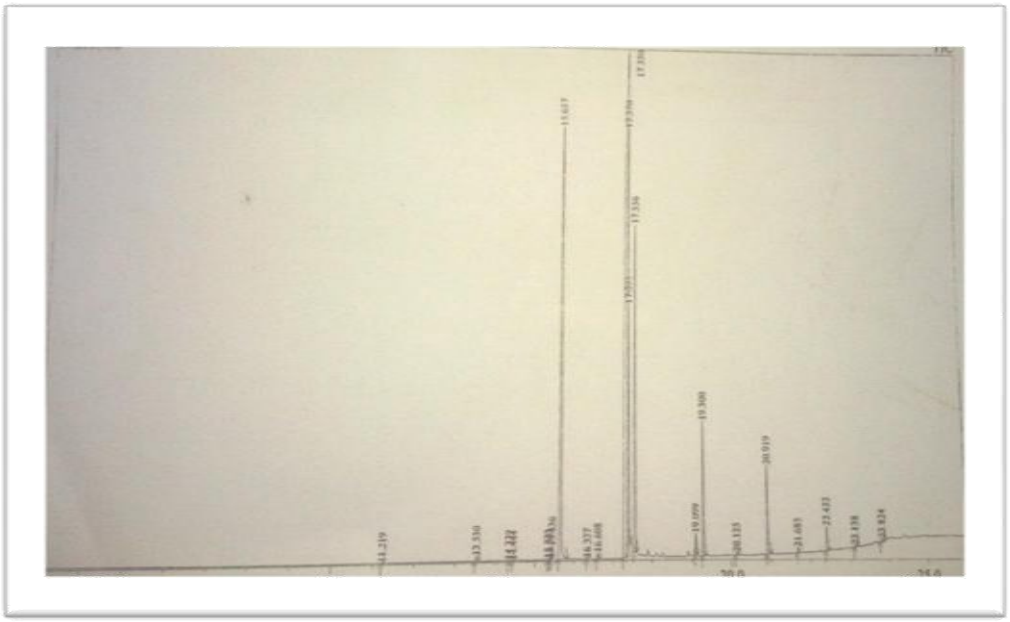


Fig.1: total ion chromatogram *corochorusolitorius* oil

Table 3.1: constituent of *corochorusolitorius* oil

| R.Time | Area | Area% | Name |
|---|---|---|---|
| 11.219 | 45027 | 0.02 | Dodecanoic acid, methyl ester |
| 13.530 | 339711 | 0.17 | Methyl tetradecanoate |
| 14.339 | 49057 | 0.03 | 6-Octadecenoic acid, methyl ester |
| 14.444 | 40047 | 0.02 | 5-Octadecenoic acid, methyl ester |
| 15.333 | 79677 | 0.04 | 7,10-Hexadecadienoic acid, methyl |
| 15.393 | 107387 | 0.05 | 7-Hexadecenoic acid, methyl ester, |
| 15.436 | 553411 | 0.28 | 9-Hexadecenoic acid, methyl ester, |
| 15.657 | 45658033 | 23.29 | Hexadecanoic acid, methyl ester |
| 16.337 | 56120 | 0.03 | Hexadecanoic acid, 14-methyl-, met |
| 16.608 | 370876 | 0.19 | Heptadecanoic acid, methyl ester |
| 17.350 | 92051404 | 46.95 | 9,12-Octadecadienoic acid (Z,Z)-, m |
| 17.370 | 5867125 | 2.99 | 9-Octadecenoic acid (Z)-, methyl est |
| 17.395 | 9432023 | 4.81 | 9,12,15-Octadecatrienoic acid, 2,3-di |
| 17.556 | 22760525 | 11.61 | Methyl stearate |
| 19.099 | 1288829 | 0.66 | cis-11-Eicosenoic acid, methyl ester |
| 19.300 | 8510936 | 4.34 | Eicosanoic acid, methyl ester |
| 20.125 | 215640 | 0.11 | Heneicosanoic acid, methyl ester |
| 20.919 | 5829961 | 2.97 | Docosanoic acid, methyl ester |
| 21.685 | 372457 | 0.19 | Tricosanoic acid, methyl ester |
| 22.422 | 1788213 | 0.91 | Tetracosanoic acid, methyl ester |
| 23.138 | 195536 | 0.10 | Pentacosanoic acid, methyl ester |
| 23.824 | 461335 | 0.24 | Hexacosanoic acid, methyl ester |
| | 196073330 | 100.00 | |

Major components of *corochorusolitorius* oil are discussed below:

**9, 12-octadecadienoic acid (Z,Z)-, methyl ester(46.95%).**

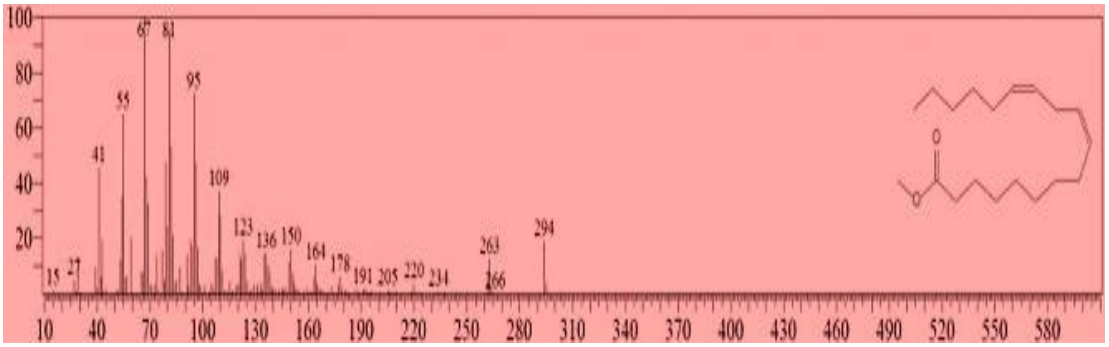



Fig 1: Mass spectrum of 9,12-octadcadienoic acid (z,z)-,methyl ester

The peak at m/z 294 corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ , while the signal at M/Z 263 corresponds loss of methoxyl function

**Hexadecanoic acid, methyl ester (23.29%)**

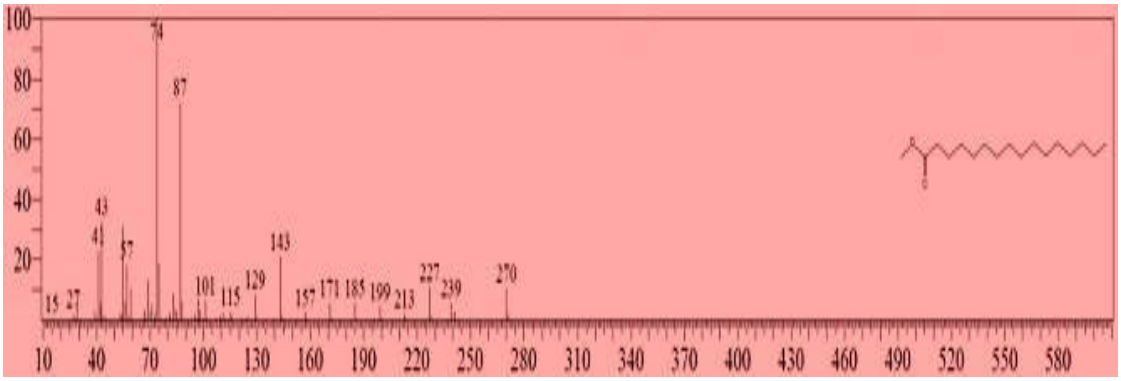



Fig. 2 : Mass spectrum of hexadecanoic acid ,methyl ester

The peak at m/z 270 corresponds to $M^{+}[C_{17}H_{34}O_2]^{+}$ .The signal at M/Z 239 corresponds loss of methoxyl function .

**Methyl stearate (11.61%).**

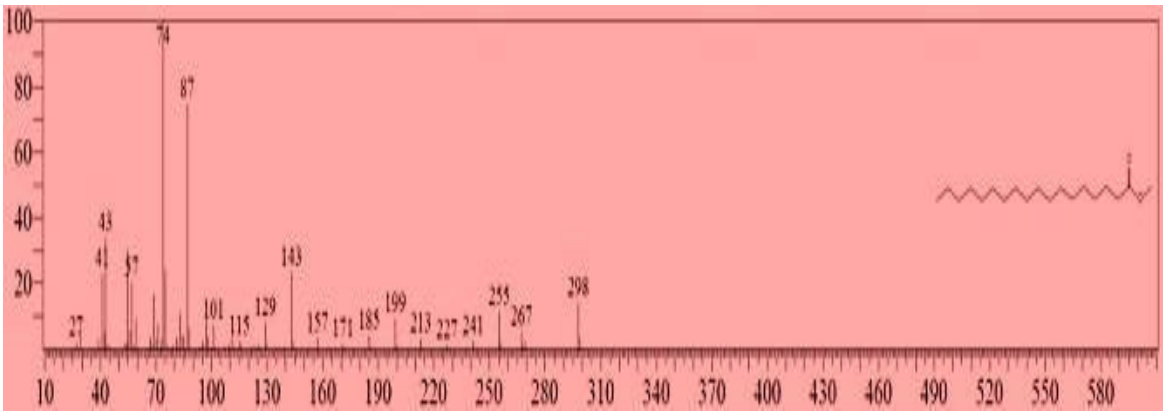



Fig. 3 :Mass spectrum of methyl stearate

The signal at m/z 298corresponds to $M^{+}[C_{19}H_{38}O_2]^{+}$ , while the signal at M/Z 267 corresponds loss of methoxyl function

**9,12, 15- Octadecatrienoic acid , 2,3-dihydroxy propyl ester(4.81)**

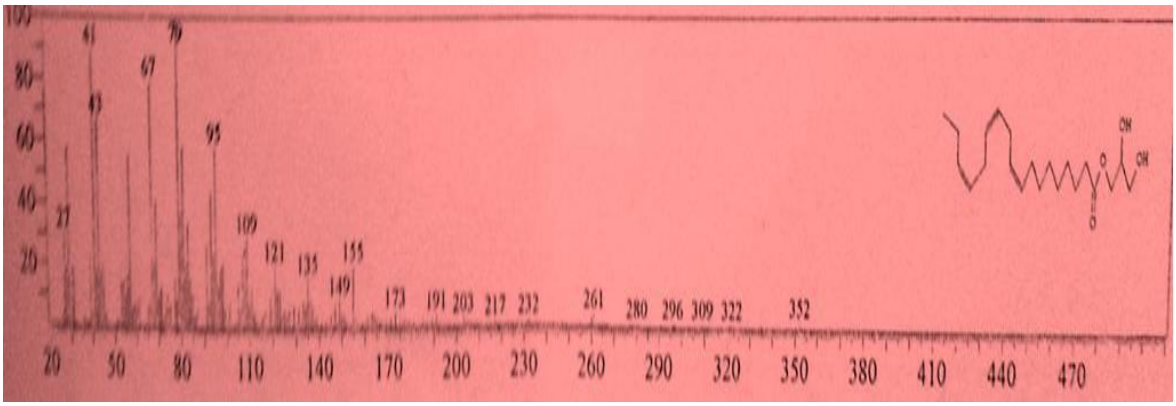



Fig. 4 : Mass spectrum 9,12,15,-octadecatrieonic acid-2,3-dihydroxy ester

As shown in the above figure , the peak at m/z 352corresponds to

$M^{+}[C_{21}H_{36}O_4]^{+}$ . The signal at M/Z 321 corresponds loss of methoxyl function.

**Eicosanoic acid, methyl ester (4.34%)**

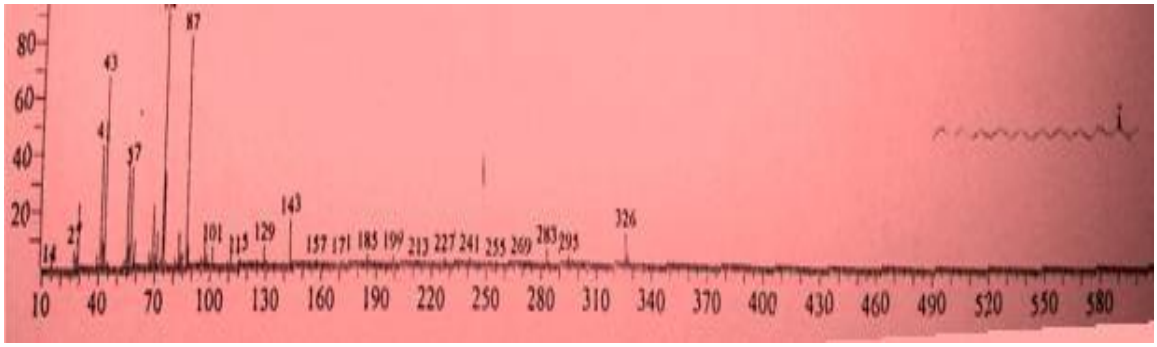



Fig.5 : Mass spectrum of eicosanoic acid, methyl ester

In figure 5 ,the peak at m/z 326corresponds to $M^{+}[C_{21}H_{42}O_2]^{+}$ the signal at M/Z 295 corresponds loss of methoxyl function

## 3.2- Antimicrobial test

*corochorusolitorius*oil was assessed for antimicrobial activity via thecup plate agar diffusion bioassay using five standard human pathogens.. The average of the diameters of the growth inhibition zones are shown in Table (3.2) .The results were interpreted in terms of the commonly used terms (>9mm: inative;9-12mm:partially active;13-18mm:

active;<18mm:very active) .Tables (3.3) and (3.4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 3.2 : Antibacterial activity of *corochorusolitorius* seed oil :M.D.I.Z (mm)

| Drug | Conc.(mg/ml) | Ec | Ps | Sa | Bs | Ca |
|---|---|---|---|---|---|---|
| *Corochorus Olitorius*oil | 100 | 14 | 14 | 13 | 17 | 20 |

Table 3.3 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

| Drug | Conc. mg/ml | Bs. | Sa. | Ec. | Ps. |
|---|---|---|---|---|---|
| Ampicillin | 40<br>20<br>10 | 15<br>14<br>11 | 30<br>25<br>15 | -<br>-<br>- | -<br>-<br>- |
| Gentamycin | 40<br>20<br>10 | 25<br>22<br>17 | 19<br>18<br>14 | 22<br>18<br>15 | 21<br>15<br>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*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*

The oil showed excellent activity against *Bacillus subtilis*. It also exhibited significant anticandidal potency. It showed moderate activity against other test microorganism.

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