بسم الله الرحمن الرحيم

SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

College of science

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EXTERACTION AND ANALYSIS OF FIXED OIL OF CICER ARIETINUM

Dissertation Submitted In Partial Fulfillment Of The Requirements Of The Degree of B.Sc. Chemistry

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الآيـــــــــة

قال تعالى:

بسم الله الرحمن الرحيم

ورُ السَّمَـوَ اتِ (وَاللَّهُ نُورُ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ
كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لاَ شَرْقِيَّةٍ وَلاَ غَرْبِيَّةٍ يَكَادُ
زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ
الأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

صدق الله العظيم

سورة النور الآية رقم (35)

Dedication

To parents

Brothers and sisters

Acknowledgment

All praise is rise to my creator; the merciful and the biggest helper who gave us the strength and determination in this work. I wish am able to give hem appreciation he deserves ,he never stopped giving me effort and time , and he offered me advice , support and never stopped giving them to me , really I am unable to thank hem : prof.Mohammed Abdel Kareem Mohammed . I am indebted to those who supported and guided me in this research. To all those who contributed I am extremely great full.

Abstract

The fixed oil of Cicer arietinum seeds was extracted by using maceration method and n-hexane using it as solvent.
The oil was analyzed by anti-microbial assay and results were recorded. In addition, it has a lot of medicinal uses as well as the use in the food products.

الخلاصة

تم استخلاص الزيت الثابت لبذور نبات الحمص (الكبكبي) باستخدام طريقة النقع بواسطة استخدام الهكسان كمذيب وتم اجراء تحليل للزيت وسجلت نتائج التحليل .

بالاضافة الى وجود عدد من الاستخدامات الطبية بجانب استخدامه في المنتجات الغذائية .

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Chapter one

1-Introduction

1.1-Essential oils.

Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants, for example, leaves, peels, barks, flowers, buds, seeds. They can be extracted from plant materials by several methods, steam distillation, expression. Among all methods, for example, steam distillation method has been widely used, especially for commercial scale production. Essential oils have been widely used as food flavors. Essential oils found in many different plants, especially the aromatic plants, vary in odor and flavor, which are governed by the types and amount of constituents present in oils. Additionally, the amount of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods. Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with

hydrophobicity in nature of essential oils, can be improved. Almost any part of a plant may be the source of the oil, which could be extracted and fully exploited for food applications or others. Modern technologies have been continuously developed to conquer the limitation of conventional methods, and to enhance the extraction efficacy. Due to the increasing attention in natural additives, essential oils from several plants have been used more widely, especially in conjunction with other preservations under concept of “hurdle technology.” Thus, essential oils can serve as the alternative additives or processing aid as green technology.

1.2- Chemical Composition of essential oils

Several plants contain essential oils, however, parts of plants, which serve as the major source of essential oil can be different .Those include roots, peels, leaves, seeds, fruits, barks. Plant essential oils are usually the complex mixture of natural compounds, both polar and non-polar compounds. In general, the constituents in essential oils are terpenes (monoterpenes and sesquet trpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) .Compounds and aroma of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds.

-Terpene hydrocarbons

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C5), which are the combinations of 2 isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C10) and sesquiterpenes (C15), which are hydrocarbons with the general formula $(C_5H_8)_n$. The diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentration. Terpenoids (a terpene containing oxygen) is also found in essential oils. Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$(MW 136 amu) and $C_{15}H_{24}$ (MW 204 amu), respectively. Although sesquiterpenes are larger in molecules, structure and functional properties of sesquiterpenes are similar to the monoterpenes. For diterpenes, triterpenes, and tetraterpenes, they have the larger molecule than monoterpenes and sesquiterpenes, but they are present at very low concentration in essential oils.

- Oxygenated compounds

These compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils.

Oxygenated compounds can be derived from the terpenes, in which they are termed “terpenoids.” Some oxygenated compounds prevalent in plant essential oils are shown as follows: - Phenols: thymol, eugenol, carvacrol, chavicol, thymol, and so on. - Alcohols: Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, and so on. Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, and so on. - Aldehydes: citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, and so on. - Ketones: carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, and so on. - Esters: bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, and so on. - Oxides: 1,8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, and so on. - Lactones: bergaptene, nepetalactone, psoralen, aesculatine, citroptene, and so on. - Ethers: 1, 8-cineole, anethole, elemicin, myristicin, and so on. Different constituents in essential oils exhibit varying smell or flavor. (Also, the perception of individual volatile compounds depends on their threshold.

1.3-Extraction of Essential Oils

Essentia**l** oils can be extracted from several plants with different parts by various extraction methods. The manufacturing of essential oils, and the method used for essential oil extraction are normally dependent on botanical material used. State and form of material is another factor used for consideration. Extraction

method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss in bioactivity and natural characteristics. For severe case, discoloration, off-odor/flavor as well as physical change such as the increased viscosity can occur. Those changes in extracted essential oil must be avoided. Extraction of essential oils can be carried out by various means.

1.3.1- **Distillation**

i) Steam distillation:

Steam distillation is the most widely used method for plant essential oil extraction. The proportion of essential oils extracted by steam distillation is 93% and the remaining 7% can be further extracted by other methods. Basically, the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and break down of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released. The temperature of heating must be enough to break down the plant material and release aromatic compound or essential oil. A new process design and operation for steam distillation of essential oils to increase oil yield and reduce the loss of polar compounds in wastewater was developed by Masango (2005). The system consists of a packed bed of the plant

materials, which sits above the steam source. Only steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the distillate is reduced. Also, water-soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent (Masango 2005). Yildirim and others (2004) reported that the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activities of essential oils from steam distillation process were markedly higher than those of oils extracted using hydro distillation (HD).

ii) Hydro distillation:

HD has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate non water-soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling. This method protects the oils extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The steam and essential oil vapor are condensed to an aqueous fraction.

The advantage of this technique is that the required material can be distilled at a temperature below 100 °C. Okoh and others (2010) studied the different extraction processes on yield and properties of essential oil from rosemary (Rosmarinus of- ficinalis L.) by HD

and solvent-free microwave extraction (SFME). The total yields of the volatile fractions obtained through HD and SFME were 0.31% and 0.39%, respectively. HD oil contained more monoterpene hydrocarbons (32.95%) than SFME-extracted oil (25.77%), while higher amounts of oxygenated monoterpenes (28.6%) were present in the oil extracted by SFME in comparison with HD (26.98%). Golmakani and Rezaei (2008) studied the microwave-assisted HD (MAHD), which is an advanced HD technique utilizing a microwave oven in the extraction process. MAHD was superior in terms of saving energy and extraction time (75 min, compared to 4 h in HD). Ohmic-assisted HD (OAHD) is another advanced HD technique.

OAHD method had the extraction time of 24.75 min, while HD took 1 h for extraction of essential oil from thyme. No changes in the compounds of the essential oils obtained by OAHD were found in comparison with HD.

iii) Hydro diffusion:

Hydro diffusion extraction is a type of steam distillation, which is only different in the inlet way of steam into the container of still. This method is used when the plant material has been dried and is not damaged at boiling temperature. For hydro diffusion, steam is applied from the top of plant material, whereas steam is entered from the bottom for steam distillation method. The process can

also be operated under low pressure or vacuum and reduces the steam temperature to below 100 °C.

Hydro diffusion method is superior to steam distillation because of a shorter processing time and a higher oil yield with less steam used. Bousbia and others (2009) compared the HD and innovative microwave hydro diffusion and gravity (MHG) methods for their effectiveness in the isolation of essential oil from rosemary leave (R. officinalis). The MHG method exhibits the excellent advantages over traditional alternatives including shorter isolation times (15 min against 3 h for HD), environmental impact (energy cost is fairly higher to perform HD than that required for rapid MHG isolation), cleaner features (no residue generation and no water or solvent used), increased antimicrobial and antioxidant activities. Farhat and others (2011) studied the microwave steam diffusion (MSDf), which is an advanced steam diffusion (SDf) technique utilizing microwave heating process for extraction of essential oils from by-products of orange peel. The essential oils extracted by MSDf for 12 min had similar yield and aromatic profile to those obtained by SDf for 40 min.

1.3.2-Solvent extraction

Conventional solvent extraction has been implemented for fragile or delicate flower materials, which are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane,

petroleum ether, methanol, or ethanol can be used for extraction. For general practice, the solvent is mixed with the plant material and then heated to extract the essential oil, followed by filtration. Subsequently, the filtrate is concentrated by solvent evaporation. The concentrate is resin (resinoid), or concrete (a combination of wax, fragrance, and essential oil). From the concentrate, it is then mixed with pure alcohol to extract the oil and distilled at low temperatures. The alcohol absorbs the fragrance and when the alcohol is evaporated, the aromatic absolute oil is remained. However, this method is a relatively time-consuming process, thus making the oils more expensive than other methods. Essential oil with antioxidant activity from Ptychotisverticillata was extracted using solvent extraction method. The oil was dominated by phenolic compounds (48.0%) with carvacrol (44.6%) and thymol (3.4%) as the main compounds. Ozen and others (2011) studied the chemical composition and anti-oxidant activity of separated essential oils from Thymus praecox subsp. skorpilii var. skorpilii (TPS) extracted using different solvents. TPS essential oil was found to contain thymol (40.31%) and o-cymene (13.66%) as the major components. The ethanol, methanol, and water extracts exerted significant free-radical scavenging activity. The water extract has the highest total phenolics (6.211) mg gallic acid/g dry weight) and flavonoids (0.809 mg quercetin/g dry weight).

Moreover, Sarikurkcu and others (2009) reported that the water extract exhibited higher antioxidant activity than other extracts (hexane, dichloromethane, ethyl acetate, and methanol). However, solvent residue could be retained in the final product due to incomplete removal. This may cause allergies, toxicity, and affect the immune system.

1.3.3-Supercritical carbon dioxide

Conventional methods including solvent extraction and steam distillation have some shortcomings such as long preparation time and large amount of organic solvents. Moreover, the losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds, and toxic solvent residue in the extract may be encountered.

Therefore, supercritical fluids have been considered as an alternative medium for essential oil extraction. Carbon dioxide (CO_2) is the most commonly used supercritical fluid because of its modest critical conditions. Under high-pressure condition, CO_2 turns into liquid, which can be used as a very inert and safe medium to extract the aromatic molecules from raw material. No solvent residue remains in the final finished product since the liquid CO_2 simply reverts to a gas and evaporates under normal atmospheric pressure and temperature. Despite high solubilities of essential oil components in supercritical CO_2, the extraction rates

were relatively slow with pure CO2 (ca. 80% recovery after 90 min). However, the combination methods by a 15-min static extraction with methylene chloride as a modifier followed by a 15-min dynamic extraction with pure CO2 yielded high recoveries. The extraction efficacy was equivalent to HD, which was performed for 4 h. The volatile compounds such as monoterpenes can be collected from the supercritical fluid extraction (SFE) effluent by >90%. SFE was able to recover some organic compounds that were not extracted by HD. Pereira and Meireles (2007) showed that the supercritical fluid extraction is economically viable than steam distillation. This is mainly caused by the lower yield and the higher energy consumption of the latter.

1.3.4-Subcritical water

The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure high enough to maintain water under liquid state and temperature in the range of 100 to 374 °C). Jimenez-Carmona and others (1999) reported that the efficiency (in terms of volume of essential oil/1 g of plant) of continuous subcritical water extraction was 5.1 times higher than HD method. This method is quicker (15 min compared with 3 h), provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes), and allows substantial savings of costs, in terms of both energy and

plant material. Kubatova and others (2001) studied the subcritical water extraction of lactones from a kava (Piper methysticum) root, compared to a Soxhlet extraction with water. The extraction of ground samples with subcritical water at 100 °C took 2 h, but the shorter time (20 min) was required when extraction was carried out at 175 °C. Boiling for 2 h and extraction with Soxhlet apparatus for 6 h showed the lower yields by 40% to 60%, compared with that obtained using subcritical water.

1.3.5-Solvent-free microwave

The disadvantages of conventional methods such as solvent or hydro diffusion extraction are the losses of some volatile compounds, low extraction efficiency, long extraction time, degradation of unsaturated or ester compounds through thermal or hydrolytic effects, and toxic solvent residue in the extract. These disadvantages have led to the consideration of the use of SFME. It is a rapid extraction of essential oils from aromatic herbs, spices, and dry seeds. SFME has several advantages, involving higher yield and selectivity, shorter time, and environmental friendly. SFME is a combination of microwave heating and dry distillation, performed at atmospheric pressure without any solvent or water. Isolation and concentration of volatile compounds are performed by a single stage. Using oregano as a raw material, SFME offered significantly higher essential oil yields (0.054 mL/g), compared to

HD (0.048 mL/g). When microwave power at 662 W was used in SFME, process time was reduced by 80%, compared with conventional process. Ferhat and others (2007b) reported that microwave method offers the important advantages over traditional alternatives, such as shorter extraction times (30 min compared with 3 h for HD and 1 h for cold pressing [CP]); better yields (0.24% compared with 0.21% for HD and 0.05% for CP); environmental impact (energy cost is appreciably higher for performing HD and for mechanical motors (CP) than that required for rapid microwave extraction); cleaner features (as no residue generation and no water or solvent used); and high antimicrobial activities. Farhat and others (2010) reported that essential oils of caraway seeds isolated by microwave dry-diffusion and gravity (MDG) exhibited the similar yield and aromatic profile to those obtained by HD, but MDG was better than HD in terms of shorter process time (45 min compared with 300 min), energy saving, and cleanliness. The present apparatus permits fast and efficient extraction, reduces waste, avoids water and solvent consumption, and allows substantial energy savings.

1.4-The target species *Cicer arietinum* (chickpea)

chickpea (English); Bengal gram (India); garbanzo (Spanish); hummus, hamaz (Arabic); nohut (Turkish); shimbra (Ethiopia);

pois chiche (French); grão de bico, gravanço, ervanço (Portuguese); mdengu (Swahili).

-Key Uses:

Food and drink, fodder, traditional medicine.

-Known hazards:

In India, chickpea is sometimes adulterated with cheaper, but potentially toxic, grass pea (*Lathyrus sativus*).

-Taxonomy

Class:
Equisetopsida

Subclass:
Magnoliidae

Superorder:
Rosanae

Order:
Fabales

Family:
Leguminosae/Fabaceae - Papilionoideae

Genus ;Cicer.

A member of the pea and bean family (Leguminosae/Fabaceae), *Cicer arietinum* is one of 43 species in the genus *Cicer*. *Cicer* is Latin for chickpea and is thought to be the origin of the surname Cicero (as in the Roman philosopher Marcus Tullius Cicero, 106–43 BC).

Chickpea is the third most important pulse in the world (after beans and peas). Its seeds have been eaten by humans since around 7,000 BC. It is widely cultivated for its nutritious seeds, which are harvested when immature and eaten raw, roasted, or boiled or when mature and dry processed into flour. Chickpea is a major protein source for poor communities in many parts of the semi-arid tropical areas of Africa and Asia.

Synonym:

Cicer album hort., *C. nigrum* hort., *C. grossum* Salisb., *C. sativum* Schkuhr, *C. physodes* Rchb., *C. rotundum* Jord. ex Alef. (full list available on The Plant List)

Cicer arietinum is not known as a wild plant but is believed to have originated in the central part of the Fertile Crescent (in modern Turkey, Syria and Iran).

Evidence suggests that *C. reticulatum* (sometimes treated as *C. arietinum* subspecies *reticulatum*) from southeastern Turkey might be the wild progenitor of the domesticated plant.

Chickpea is cultivated in tropical, subtropical and warm temperate zones, including the Mediterranean, the Canary Islands, western and central Asia and northeastern tropical Africa, including Madagascar. It is grown up to 2,500 m above sea level.

It is not suited to the humid and hot lowland tropics where it fails to flower.

-Description

Overview: A slender, erect annual growing up to 100 cm tall, with simple or branched stems.

Roots: Extensive root system. Roots bearing nodules containing nitrogen-fixing bacteria (including *Mesorhizobium ciceri* and *M. mediterraneum*).

Leaves: Divided into 5–7 pairs of leaflets. Leaflets up to 16 mm long and 14 mm wide with toothed margins and weak, spreading,

glandular hairs. Triangular stipules (leaf-like appendages) are borne at the leaf base.

Flowers: Typical pea flowers, up to 12 mm long, borne singly, with white or lilac to violet petals.

Fruits: A small, inflated and rounded pod, up to 3 cm long and 1.5 cm wide, with glandular hairs.

Seeds: Roughly spherical, with smooth or rough surface, up to 14 mm in diameter. Variable in colour, usually creamy-whitish when dried. One or two seeds per pod.

Many cultivars of chickpea have been described. There are two main groups in cultivation:

- Desi (microsperma) cultivars – producing small, angular seeds with rough, yellow-brown coats. The desi forms predominate in the Indian subcontinent, Ethiopia, Mexico and Iran. They are often used for split peas (dahl) or flour after the hulls are removed.
- Kabuli (macrosperma) cultivars - producing relatively large, plump seeds with a smooth, cream-coloured coat. The kabuli forms predominate in Afghanistan through western Asia to North Africa and in southern Europe and America (excluding Mexico). They are usually sold whole.

-Threats and conservation

Seeds from *Cicer* species have been stored in the ICRISAT seed bank in Patancheru, India (about 17,000 chickpea accessions), the ICARDA seed bank in Aleppo, Syria (about 10,000 accessions) and the Australia Temperate Field Crops Collection, Victoria, Australia (about 7,700 accessions).

Many *Cicer* species from central Asia (most of which are perennial) are not yet represented in seed collections.

-Uses

i)Food

Chickpea is a major pulse crop with world production of well over 9 million tonnes. India is the world's main producer and consumer of chickpea. Other major producers include Turkey, Pakistan and Iran. It is also a significant export crop in Australia, New Zealand and Canada.

The earliest remains of chickpea seed have been found in Syria & Turkey and date back to around 7,000 BC. Chickpea was gradually introduced to the western Mediterranean region and Asia and had reached the Indian subcontinent by 2,000 BC.

Chickpea seeds are an excellent source of protein and contain a wide range of amino acids. They are high in fibre, low in fat and contain phosphorus, calcium and iron.

Immature seeds are consumed fresh, boiled or roasted and salted as snacks. Canned chickpea seeds are popular in the United States and Europe. In the Indian subcontinent, most chickpeas are processed into flour (Bengal gram, besan flour) for cooking bhajis, pakoras and breads. Chickpea flour can also be used to make gluten-free cakes.

Dhal is a dish made from split chickpeas with the seed coats removed. The seeds are often dried and then cooked to make a thick soup or ground into flour for snacks and sweetmeats.

Hummus is a dip or spread made using cooked and mashed chickpea seeds (mixed with tahini (sesame seed paste), olive oil, lemon juice, garlic and salt) and is a traditional dish in the Middle East, Turkey and North Africa.

Sprouted chickpea seeds are eaten as a vegetable or added to salads. Young plants and green pods are eaten like spinach. Chickpea seeds are ground to make flour, which is used to make soup, dhal and bread. Chickpea seeds are prepared with pepper, salt and lemon and served as a side dish.

Roasted chickpea roots have been used as a coffee substitute.

ii)Uses - animal feed, medicine, others

a)Animal feed

Chickpea plants are used as fodder in many developing countries. Seed husks and green or dried stems and leaves are used for stock feed, but they contain appreciable quantities of oxalic acid and are not good as forage. Whole seeds are sometimes milled for animal feed. *Cicer* hay has been reported as being toxic to horses.

b)Traditional medicine

Glandular secretions of the leaves, stems and pods of chickpea include malic and oxalic acids. These sour-tasting acid exudates can be applied medicinally or used as vinegar. In India these acids used to be harvested by spreading thin muslin over the crop during the night. In the morning the soaked cloth was wrung out and the liquid collected in bottles.

Chickpea acid exudates have been used to treat bronchitis, catarrh, cholera, constipation, diarrhoea, dyspepsia, flatulence, snakebite, sunstroke and warts. They have also been used as an aphrodisiac and to lower blood cholesterol levels. Germinated chickpea has been reported to be effective in controlling cholesterol level in rats.

In Chile, a cooked chickpea-milk mixture has been fed to infants, effectively controlling diarrhoea.
Chickpea seeds are considered to be anti-bilious (to combat nausea, abdominal discomfort, headache, constipation and gas caused by an excessive secretion of bile).

c)Other uses

Chickpeas can be used to make an adhesive that is suitable for plywood, although it is not water-resistant.

Chickpea yields starch suitable for textile sizing and gives a light finish to silk, wool and cotton cloth. Chickpea leaves are said to yield an indigo-like dye.

Chickpeas are usually rapidly boiled for 10 minutes and then simmered for a longer period. Dried chickpeas need a long cooking time (1–2 hours) but will easily fall apart when cooked longer. If soaked for 12–24 hours before use, cooking time can be shortened by around 30 minutes. Chickpeas can also be pressure cooked or *sous vide* cooked at 90 °C (194 °F).

Chickpeas (*Cicer arietinum*) do not cause lathyrism. Similarly named "chickling peas" (*Lathyrus sativus*) and other plants of the genus *Lathyrus* contain the toxins associated with lathyrism.

Mature chickpeas can be cooked and eaten cold in salads, cooked in stews, ground into gram flour (also known as chickpea flour and *besan* and used frequently in Indian cuisine), ground and shaped in balls and fried as *falafel*, or stirred into a batter and baked to make *farinata* or *panelle*.

Chickpeas are popular in the Iberian Peninsula. In Portugal, they are one of the main ingredients in *rancho*, eaten with pasta and meat, including Portuguese sausages, or with rice. They are used in other hot dishes with *bacalhau* and in soup. In Spain, they are used cold in *tapas* and salads, as well as in *cocido madrileño*. In Italy, chickpeas are eaten with pasta or in soup. In the southern regions such as Sicily, grounded chickpeas flour is used to produce a famous local street food called *panelle*. In Egypt, they are used as a topping for *kushari*.

Hummus is the Arabic word for chickpeas, which are often cooked and ground into a paste and mixed with *tahini* (sesame seed paste), the blend called *hummus bi tahini*, or chickpeas are roasted, spiced, and eaten as a snack, such as *leblebi*. By the end of the 20th century, hummus had become commonplace in American cuisine.[14] By 2010, 5% of Americans consumed hummus on a regular basis, and it was present in 17% of American households.

Some varieties of chickpeas can be popped and eaten like popcorn.

Chickpeas and Bengal grams are used to make curries and are one of the most popular vegetarian foods in the Indian subcontinent and in diaspora communities of many other countries. Popular dishes in Indian cuisine are made with chickpea flour, such as *mirchi bajji* and *mirapakaya bajji Telugu*. In India, as well as in the Levant, unripe chickpeas are often picked out of the pod and eaten as a raw snack and the leaves are eaten as a leaf vegetable in salads.Chickpeas serve as an energy and protein source as animal feed.

Raw chickpeas have a lower trypsin and chymotrypsin inhibitor content than peas, common beans, and soybeans. This leads to higher nutrition values and fewer digestive problems in nonruminants. Nonruminant diets can be completed with 200 g/kg of raw chickpeas to promote egg production and growth of birds and pigs. Higher amounts can be used when chickpeas are treated with heat.

Experiments have shown that ruminants grow equally well and produce an equal amount and quality of milk when soybean or cereal meals are replaced with chickpeas. Pigs show the same performance, but growing pigs experience a negative effect of raw

chickpea feed; extruded chickpeas can increase performance even in growing pigs. In poultry diet experiments with untreated chickpeas, only young broilers (starting period) showed worse performance. Fish performed equally well when their soybean or cereal diet was replaced by extruded chickpeas.

Secondary components of legumes — such as lecithin, polyphenols, oligosaccharides, and amylase, protease, trypsin and chymotrypsin inhibitors — can lead to lower nutrient availability, thus to negative effects in growth and health of animals (especially in nonruminants). Ruminants have generally less problems to digest legumes with secondary components, since they can inactivate them in the rumen liquor. Their diets can be supplemented by 300 g/kg or more raw chickpea seeds.[17] However, protein digestibility and energy availability can be improved through treatments, such as germination, dehulling, and heat. Extrusion is a very good heat technique to destroy secondary components in legumes, since the proteins are irreversibly denatured. Overprocessing may decrease the nutritional value; extrusion leads to losses in minerals and vitamins, while dry heating does not change the chemical composition.

-Nutrition

Chickpeas, mature seeds, cooked no salt

Nutritional value per 100 g (3.5 oz)

Energy	686 kJ (164 kcal)
Carbohydrates	27.42 g
Sugars	4.8 g
Dietary fibre	7.6 g
Fat	2.59 g
Saturated	0.269 g
Monounsaturated	0.583 g
Polyunsaturated	1.156 g
Protein	8.86 g
Vitamin	
Vitamin A equiv.	(0%) 1 μg
Thiamine (B1)	(10%) 0.116 mg
Riboflavin (B2)	(5%)

	0.063 mg
Niacin (B3)	(4%)
	0.526 mg
Pantothenic acid (B5)	(6%)
	0.286 mg
Vitamin B6	(11%)
	0.139 mg
Folate (B9)	(43%)
	172 µg
Vitamin B12	(0%)
	0 µg
Vitamin C	(2%)
	1.3 mg
Vitamin E	(2%)
	0.35 mg
Vitamin K	(4%)
	4 µg

Minerals

Calcium	(5%)
	49 mg
Iron	(22%)
	2.89 mg

Magnesium	(14%) 48 mg
Phosphorus	(24%) 168 mg
Potassium	(6%) 291 mg
Sodium	(0%) 7 mg
Zinc	(16%) 1.53 mg

Other constituents

Water	60.21 g

-Effects of cooking

Cooking treatments do not lead to variance in total protein and carbohydrate content. Soaking and cooking of dry seeds possibly induces chemical modification of protein-fibre complexes, which leads to an increase in crude fibre content. Thus, cooking can increase protein quality by inactivating or destroying heat-labile antinutritional factors. Cooking also increases protein digestibility, essential amino acid index, and protein efficiency ratio. Although cooking lowers concentrations of amino acids such as tryptophan,

lysine, total aromatic, and sulphur-containing amino acids, their contents are still higher than proposed by the FAO/WHO reference. Diffusion of reducing sugars, raffinose, sucrose and others into cooking water reduces or completely removes these components. Cooking also significantly reduces fat and mineral contents. The B vitamins riboflavin, thiamin, niacin, and pyridoxine dissolve into cooking water at differing rates.

-Germination

Germination of chickpeas improves protein digestibility, although at a lower level than cooking. Germination degrades proteins to simple peptides, so improves crude protein, nonprotein nitrogen, and crude fiber content. Germination decreases lysine, tryptophan, sulphur and total aromatic amino acids, but most contents are still higher than proposed by the FAO/WHO reference pattern.

Oligosaccharides, such as stachyose and raffinose, are reduced in higher amounts during germination than during cooking. Minerals and B vitamins are retained more effectively during germination than with cooking. Phytic acids are reduced significantly, but trypsin inhibitor, tannin, and saponin reduction is less effective than cooking.

Protein digestibility is improved by all treatments of cooking. Essential amino acids are slightly increased by boiling and microwave cooking when compared to autoclaving and germination. Overall, microwave cooking leads to a significantly lower loss of nutrients compared to autoclaving and boiling.

Finally, all treatments lead to an improved protein digestibility, protein efficiency ratio, and essential amino acid index. Microwave cooking seems to be an effective method to prepare chickpeas because of its improvement of nutritional values and its lower cooking time.

-Leaves

Malnutrition and insufficient micronutrient supply have been reported in many regions where chickpeas are a major part of the diet. However, this nutritional lack is not due to the consumption of chickpeas but due to the overall inadequate food supply for people. In some parts of the world, young chickpea leaves are consumed as cooked green vegetables. Especially in malnourished populations, it can supplement important dietary nutrients [23] Chickpea leaves have a significantly higher mineral content than cabbage and spinach.[*citation needed*] In natural settings, environmental factors and nutrient availability could influence mineral concentrations.[*citation needed*] Nevertheless, consumption of chickpea

leaves is recommended for areas where chickpeas are produced as food for humans. Preliminary research shows that chickpea consumption may lower blood cholesterol.

-Kew's research into chickpea disease-resistance

Chickpea is a major protein source for poor communities in many parts of the semi-arid tropical areas of Africa and Asia. Chickpea crops can be totally destroyed by insects and diseases. Their wild relatives, however, are often resistant to these pests and pathogens.

Kew scientists have identified compounds in these wild species that confer this resistance and therefore traditional breeding methods could use these as markers to help introduce resistance into commercial varieties.

-Cultivation

Chickpea thrives in a sunny site in a cool, dry climate on well-drained soils. It is generally grown on heavy black or red soils with a pH of 5.5–8.6. Frost, hailstones and excessive rain can damage the crop. Some cultivars can tolerate temperatures as low as -9.5°C in the early stages or under snow cover.

The most important chickpea disease worldwide is ascochyta blight caused by the seed-borne fungus *Ascochyta rabiei*. Chickpea

roots can be affected by the nematode worm *Meloidogyne javanica* (root-knot).

Pods can be damaged by moth larvae such as *Helicoverpa armigera* and the cutworm *Agrotis ipsilon*. Integrated pest management practices, including the selection of tolerant cultivars, pest population monitoring and the use of bio-pesticides and natural enemies, have been developed to reduce reliance on chemical insecticides.

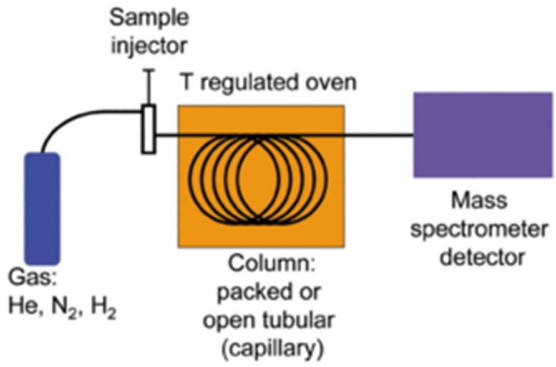
1.5-**Gas chromatography–mass spectrometry**

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. 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.

GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a

specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A *non-specific test* merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.



GC-MS schematic

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic

retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample.

1.5.1-Applications

i)Environmental monitoring and cleanup

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies. There are some compounds for which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective.

ii)Criminal forensics

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

iii)Law enforcement

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

iv)Sports anti-doping analysis

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.

Aim of this study

This study was designed to:

-Extract the fixed oil from Cicer arietinum.

-Evaluate the oil for antimicrobial activity.

Chapter Two

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of Cicer arietinum were purchased from local market-Khartoum. The plant was identified by direct comparison with reference herbarium sample

2.1.2-Test organisms

Cicer arietinum oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table(1).

Table (1): Test organisms

Ser.No	Micro-organism	Type
1	Bacillus subtilis	G+ve
2	Staphylococcus aureus	G+ve
3	Bacillus subtilis	G-ve
4	Escherichia coli	G-ve
5	Candida albicans	fungi

2.2Methods

2.2.1-Extraction of oil from seeds

Powdered shade-dried seeds of Cicer arietinum (500g) were macerated with n-hexane for 48hr..The solvent was removed under

reduced pressure and the oil was kept in the fridge at 4º C for further manipulation.

2.2.2-Antimicrobial assay

2.2.2.1-Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (**Miles and Misra, 1938**). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count

of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.2.2.2-Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.2.2.3-Testing of antibacterial susceptibility

The cup plate agar method was used to screen the antibacterial activity of the oil and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6

mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts.The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Chapter Three

3-Result and discussion

Antibacterial and antifungal activity:-

The oil was evaluated for their antimicrobial activity using the cup plate agar diffusion method. The average of the diameters of the growth inhibition zones are shown in Table (2) .The results were interpreted in terms of the commonly used terms : 13-18mm growth inhibition zones is considered to be active; more than 18mm: very active. Values less than 9 mm indicate inactivity. Values ranging from 9-12 indicate partial activity. Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

The oil was partially active against *Staphylococcus aureus*. It showed significant activity against *Escherichia coli* and Pseudomonas aeruginos

Table 2: Antibacterial activity of synthesized Compounds: M.D.I.Z (mm)

Compd.	Conc.(mg/ml)	Ec	Pa	Sa	Bs	Ca
Oil	100	15	15	13-11	-	16-15

Table 3: Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Pa
Ampicillin	40	15	30	-	-

	20 10	14 11	25 15	- -	- -
Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table 4: Antifungal activity of standard chemotherapeutic agents against standard fungi

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

- S.a*: Staphylococcus aureus*
- E.c*: Escherichia coli*
- P.a: *Pseudomonas aeruginosa*
- A.n: *Aspergillus niger*
- C.a: *Candida albicans*
- S.t: *Salmonella typhi*
- *B.a: Bacillus subtilis*

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