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Impact of *Coriandrum sativum* extract on the peroxide, acid and saponification value of storage and reused Sunflower oil

A thesis submitted in fulfillment of the requirements for the degree of M.Sc. in chemistry

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October **2017**

قال تعالى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَىٰ ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَٰلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ ﴿٩٩﴾)

صدق الله العظيم

سورة الانعام

DEDICATION

To soul of my mother who taught me that the success secret is patience.

To my father who always encourages and pushes me forward.

To my sister and my brothers whom did not leave anything to help me.

To my wife who provided me the comforts to completing this search.

Acknowledgement

Thank God for facilitated the completion of this research on the face which I wish and satisfy me. Then my great thanks to supervisor Mr. Dr. Faroug Bakheit Elsonni, who has credited after Allah almighty, since the topic title is an idea until became a thesis. My thanks to Dr. Ibrahim Alkabashi, head of statistics department for great helpful in statistical analysis of the study results. Also I would to thank Dr. Mohamed Abbas, Abu Bakr and Mohamed Damona from center of researches and industrial consulting. Also I'm not forgetting to thank Valinteena, technician of science college, Khartoum University.

Abstract

The coriander plant was used from ancient times in different fields such as food spices, medicine, pharmacy and perfumes, and it has not any toxic effect on human life. These advantages were drawing the attention of researcher to detect more benefits and knowledge about coriander. This study was designed to assess the effect of coriander oil on the chemical properties (peroxide, acid and saponification value) of storage and reused sunflower oil. Coriander oil was extracted by steam distillation process then GC-MS was used to determine the chemical profile of coriander oil which revealed that the coriander seeds oil contains 24 compounds were; monoterpens, Diterpenes, sesquiterpenes, aldehydes, alcohols, phenols, acids and esters. The chemical properties of sunflower oil were tested before and after the addition of coriander oil. The study showed that the coriander extract had a positive effect on chemical properties with clear significant difference ($P < 0.05$). Coriander oil also had a clear impact on the chemical properties values of reused sunflower oil which was used in several cooking processes where the study revealed that there is a significant difference between the values of the chemical properties of reused edible oil before and after the addition of coriander oil ($p < 0.05$). The study was attributed this influence on the chemical properties to the plenty of antioxidant in coriander oil such as monoterpens and sesquiterpenes.

The antibacterial activity of coriander extract was detected by investigation of six types of bacteria which classified on three gram- positive and three gram-negative. The examination was carried out by using four different concentrations (12.5%, 25%, 50% and 100%) of extract. The results showed that the coriander oil has positive impact on all types of bacteria mainly gram-positive type. Coriander oil contains a number of antioxidant organic compounds that serve as a good source of food and oil preservation during storage, also contains antimicrobial activity that can be used in the preparation of medications.

الملخص

أُستخدم نبات الكزبرة منذ قديم الزمان في عدة مجالات مثل توابل الغذاء ، الطب ، الصيدله وصناعة العطور ، لاسيما إنه ليس له سميه على حياة الإنسان. قد لفت هذه المميزات إنتباه الباحث لمعرفة المزيد عن فوائدها وإستخداماتها. صممت هذه الدراسة بغرض تحديد أثر مستخلص نبات بذور الكزبرة على الخواص الكيميائية (رقم البيروكسيد ، رقم الحمضية و رقم التصبن) لعينتى زيت زهرة الشمس مخزنه وأخرى مستخدمه فى عدة عمليات طهى (مكرر). تم إستخلاص زيت بذور الكزبرة بواسطة التقطير البخاري المائي و من ثم أُستخدم جهاز كروماتوغرافيا الغاز- مطيافي الكتلة لتحديد مكونات زيت الكزبرة. أوضحت الدراسة إن للزيت 24 مكونا تتبع فى مجملها إلى التربينات الأحاديه ، التربينات الأكسجينيه ، التربينات الثلاثيه ، الألدهيدات ، الكحولات ، الفينولات ، الأحماض والإسترات.

أُختبرت الخواص الكيميائية لزيت زهرة الشمس قبل وبعد إضافة زيت الكزبرة حيث أظهر زيت الكزبرة أثر إيجابي على الخواص الكيميائية (رقم البيروكسيد ، الحمضية والتصبن) وكان ذلك مصحوباً بفرق معنوي واضح ($P < 0.05$) في قيم هذه الخواص الكيميائية. كما كان لزيت الكزبرة كذلك الأثر الواضح على الخصائص الكيميائية للزيت المكرر حيث بينت الدراسة ان هنالك فرقا واضحا بين قيم الخواص الكيميائية لزيت الطعام المكرر قبل وبعد إضافة زيت الكزبرة ($P < 0.05$) . عزت الدراسة التأثير الواضح لمستخلص زيت الكزبرة على الخصائص الكيميائية في المقام الأول إلى مركبات مضادات الأكسدة الموجودة في الزيت المستخرج من الكزبرة مثل التربينات الأحادية.

تم تقييم النشاط البكتيري لزيت الكزبرة من خلال تجهيز ستة أنواع من البكتيريا (ثلاثة موجبة صبغ جرام و ثلاثة سالبة صبغ جرام) وأجري الفحص بواسطة طريقة الأجار باستخدام أربعة تراكيز مختلفة (12.5٪، 25٪، 50٪ و 100٪). أظهرت النتائج أن جميع تراكيز زيت الكزبرة لها نشاط مضاد للبكتيريا التي تم إختيارها كعينات للاختبار.

زيت الكزبرة يحتوي على عدد من المركبات العضوية المضادة للأكسدة والتي تعمل كمصدر جيد للمحافظة علي الأطعمة والزيوت طوال فترة التخزين كما يحتوي على مضادات بكتيريه متعدده يمكن إستخدامها فى أنتاج مختلف المضادات الحيويه. للأمراض البكتيريه.

List of Abbreviations

AOAC	Association of official analytical chemists
ASTM	American society for testing and materials
AV	Acid value
BPC	British pharmacopoeia commission
BS	British standards
EOA	Essential oil association
EP	European pharmacopoeia
FAO	Food and agricultural organization
FFA	Free fatty acid
GC-MS	Gas chromatography-mass spectrometry
IS	Indian standards
ISO	International standards organization
IV	Iodine value
MIZD	Mean Inhibition zones diameter
NTP	National toxicology program
PV	Peroxide value
NRA	National renderers association
SV	Saponification value

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CHAPTER ONE

CHAPTER ONE

1. Introduction and literature review

1.1. Introduction:

Coriandrum Sativum,Coriander is an annual apiaceae herb, which grows in mediterranean countries. The fun fact about coriander is that, ancient reasoning attributed anything with such a pronounced and unpleasant odor to possess powerful curative or preventative attributes. Coriander seeds have been found in Egyptian tombs dating to the 21st dynasty (Coskuner, and Karababa 2007).

Coriander is widely used in food and pharmaceutical industries. In traditional medicine, seeds are used in the treatment of gastrointestinal problems, rheumatism and pain joints (Wangesteen,n and Samuelsen,2004). Recent studies have also demonstrated a hypoglycemic action and effects on carbohydrate metabolism.It has also been reported the antimicrobial effect of coriander leaves and seeds against several microorganisms (Delaquis, and Stanich,2002).Furthermore in food industry, leaves and seeds are employed as condiment, being used to flavour various commercial foods, as liqueurs, teas, meat products and pickles (Dharmalingam, and NazniP, 2013).

The essential oils represent a small fraction of a plant's composition but confer the characteristics for which aromatic plants are used in the pharmaceutical, food and fragrance industries. Essential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetals, ethers and esters) .The essential oils and extracts of aromatic plants and spices have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies. Currently, it is necessary to investigate those plants scientifically, for the composition of essential oil and its biological activities, which have been used in traditional medicine to improve the quality of healthcare. The essential oil contents in different species are varied inherently, influenced greatly by culture conditions and environment, as well as by crop and post-crop processing, and hence evaluations of the oils from many medicinal plants are being conducted(Bhuiyan, and Begum, 2009).

One of the most useful essential oil bearing spices as well as medicinal plants is *Coriandrum sativum* containing essential oil in its leaves, stem, flowers and fruits/seeds), and thus updates on its usefulness, based upon the scientific studies, are required for its better maintenance and scientific use for the mankind.Essential oil of coriander is used in perfumery and cosmetic (Asuquo, and Anusiem, 2012).Coriander possess good antioxidant potential, which can be useful in treatment of many disorders caused by oxidative stress such as inflammations, diabetes, cancer, neurodegenerative and cardiovascular diseases and many others
(Anita,and, Sharad, 2014).

Oils and fats are produced from animal and vegetable sources, with the former group declining in market share though not in production tonnage. Tallow, lard and butter still occupy the fifth, sixth and seventh positions after the four dominant vegetable oils (palm, soybean, rapeseed and sunflower seed). Edible oil is oxidized during processing and storage via autoxidation and photosensitized oxidation, in which triplet oxygen ($3O_2$) and singlet oxygen ($1O_2$) react with the oil, respectively. Lipid hydroperoxides formed by three moles of oxygen are conjugated dienes, where one mole of oxygen produces both conjugated and non-conjugated dienes. Autoxidation of oil is accelerated by the presence of free fatty acids, mono- and diacylglycerols, metals such as iron, and thermally oxidized compounds(Choe, and Min, 2006).

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 (Harris, and Daniel ,1999).

Objectives of the study:

This study searches to achieve this aims:

- To extract the coriander oil and to determine its chemical profile of coriander.
- To determine the chemical properties of coriander oil.
- To determine the effect of coriander oil on the chemical properties of storage edible oil (sunflower oil).
- To determine the effect of coriander oil on the reused sunflower oil.
- To detect the antibacterial activity of coriander oil.

1.2. Literature Review

1.2.1. Coriander Sativum:

1.2.1.1. Common Names:

Arabic: kuzbara; English: coriander, cilantro; French: coriandre; German: wanzendill; Greek: koriannon; Chinese: yuan sui; Hindi: dhania; Italian: coriandolo; Japanese: koendoro; Portuguese: coriandro; Sanskrit: dhanayaka; Spanish: coriandro; Swedish: coriander (Nadeem, and, Anjum, 2013).

1.2.1.2. Taxonomic Classification:

Kingdom:Plantae
Subkingdom:Tracheobionta
Superdivision:Spermatophyta
Division:Magnoliophyta
Class:Magnoliopsida
Subclass:Rosidae
Order:Apiales
Family:Apiaceae
Genus:Coriandrum
Species:*Sativum*(Nurzynska, and Wierdak, 2013)

1.2.1.3. Distribution:

Coriandrum sativum (coriander) probably originated from Eastern Mediterranean and it was spread as a spice plant to India, China, Russia, Central Europe, and Morocco, and has been cultivated since human antiquity . However, now it was distributed in Europe (Denmark, Finland, Ireland, Norway, Sweden, UK, Belgium, Czechoslovakia, Germany, Hungary, the Netherlands, Poland, Switzerland, Belarus, Estonia, Latvia Lithuania, Moldova, Ukraine, Albania, Bulgaria, Greece, Italy, Romania, Yugoslavia, France, Portugal and Spain), Northern Africa (Algeria, Morocco, Tunisia and Ethiopia), Asia (Afghanistan, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, southern Russia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, China, India and Pakistan) and Austria (Pino, and. Rosado, 2006).

1.2.1.4.Growing of Coriander

Coriander seeds are often sown in autumn but may also be sown in the spring. When growing coriander from seeds, sown the coriander seeds in rows about 15 inches apart

spaced 1 inch asunder and one-half inch deep. Many herb gardeners grow coriander primarily for cilantro leaves and only leave a plant or two to produce seeds(Ravi, and Prakash,2007)..

1.2.1.5.Description:

Coriander is an upright and short-lived herbaceous plant usually growing 1-2m.

Stems: The branched stems are hairless, mostly hollow, and have fine lengthwise grooves.They are pale green in colour but covered in distinctive purplish or pinkish blotches. The alternately arranged leaves are borne on long hollow leaf stalks that tend to sheath the stem at their bases. They are deeply divided, with toothed segments, and are ferny in appearance(Yeung, and Bowra, 2011).

Leaves:The leaves of the coriander plant were once thought to be unsuitable for eating due to their strong odor and odd flavor. The leaves odor is such as to suggest the flavor of "buggy" raspberries we sometimes gather in the fence rows (Shahwar, and El-Ghorab , 2012)

***Flowers*:**Flowers are umbels of small whitish flowers that are followed by pairs of brownish-yellow, deeply furrowed "seeds". The white flowers are borne in large numbers in dense flat-topped clusters at the tips of the branches. Individual flowers are small (2-4 mm across), have five incurved petals and five stamens, and are borne on stalks up to 5 cm long. Many (about 15) of these stalks radiate from the same point and form a small cluster of flowers, with several of these smaller clusters being grouped together into a much larger cluster that is subtended by several small leafy bracts (about 5 mm long). Flowering occurs mostly during spring and summer (Burdock, and Carabin, 2009).

Fruits:The fruit turns from green to greyish-brown in colour as it matures and resembles a capsule. It actually consists of two one-seeded structures that readily split apart when the fruit is fully mature. Each of these (seeds), 2-4 mm long, hairless, but has five prominent yellowish-coloured ribs. There are many varieties of coriander which differ in the fruit size and oil yield (Pavia, and,Donald 2006).

Coriander seeds remain viable for 5-6 years and do not have the odd smell of the plant but have a rather agreeable smell and a moderately warm, pungent taste. Once the flowers have finished and the seed heads or fruit begin to form, can either cut or bundle the stems and store them in paper bags to dry or cover the flower heads on the plants securely with a paper bag and harvest at the end of the growing season. Generally, the yield of coriander seeds is fairly small. More than enough for next year's crop but perhaps not enough to harvest the spice(Ramezani, and Rasouli 2009).

1.2.1.6.Physicochemical Composition:

In general, the total physiochemical composition of coriander can classified in:

- Total ash: not more than 6 per cent,
- Acid insoluble ash: not more than 1.5 per cent,
- Water -soluble extractive: notmore than 19 per cent,
- Alcohol soluble extractive: not more than 10 per cent.
- Volatile oil: not less than 0.3%v/w (Shivanand, 2010).

1.2.1.7.The Chemistry of Coriander Composition:

The chemical constituents of coriander leaves and seeds are; protein 21.93 and 12.37 g, total lipid (fat) 4.78 and 17.77 g, carbohydrate 52.10 and 54.99 g, fiber 10.40 and 41.9 g, calcium 1246 and 709 mg, iron 42.46 and 16.32 mg, phosphorus 481 and 409 mg, magnesium 694 and 330 mg, potassium 4466 and 1267mg, sodium 211 and 35 mg, zinc 4.72 and 4.70 mg, vitamin C 566.7 and 21 mg, thiamin 1.252 and 0.239 mg, riboflavin 1.500 and 0.290 mg, niacin 10.707 and 2.130 mg, vitamin B-120.00 and 0.00 μg, vitamin A, RAE 293 and 0.00 μg, vitamin D (D2 + D3) 0.00 and 0.00 μg, respectively(Raal, and, Arak,2004).

The phytochemical screening of plant showed the presence of essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides . The most important constituents of coriander fruits were the essential oil and fatty oil. The essential oil content of dried coriander fruits varies between 0.03 and 2.6%, while the fatty oil content varies between 9.9 and 27.7%.The variations in the oil constituents of coriander leaves and seeds could be attributed to the variations in the cultivar and not due to geographic divergence and ecological conditions(Dharmalingam, and, NazniP , 2013).

However, the compounds isolated from coriander essential oil were included: Monoterpene hydrocarbons (p-cymene, camphene, Δ-3-carene, limonene (dipentene), myrcene, cis- and trans-ocimene, α-phellandrene, β-phellandrene, α- pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinolene, α-thujene); Monoterpene oxides and Carbonyls (Camphor, 1,8- cineole, linalol oxide, carvone, geranial); Monoterpene alcohols (Borneol, citronellol, geraniol, linalool, nerol, α-terpineol, 4-terpinenol); Monoterpene Esters (Bornyl acetate, geranyl acetate, linalyl acetate, α-terpinyl acetate); Sesquiterpenes (β-Caryophyllene, caryophellene oxide, elemol, nerolidol); Phenols (Anethole, myristicin, thymol); Aliphatic hydrocarbons (Heptadecane, octadecane); Aliphatic alcohols (Decanol, dodecanol); Aliphatic aldehydes (Octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, tetradecanal, 3 - octenal, 2-decenal, 5-

decenal, 8-methyl-2-nonenal,8- methyl-5-nonenal, 6-undecenal, 2-dodecenal, 7-dodecenal, 2-tridecenal, 8- tridecenal, 9-tetradecenal, 10-pentadecenal,3,6-undecadienal, 5,8-tridecadienal) and Miscellaneous compounds: Acetic acid, α-pdimethyl styrene) (Figueiredo, and, Marques, 2004).

A range of aldehyde compounds are detected and it largely responsible for the aroma of coriander leaves. The largest proportions ofthese are those aldehydes with 6-10 carbon atoms, particularly decyl (10) and nonyl (9) aldehydes (Bhuiyan,et al. 2009). Coriander leaves contain high levels of organic compounds called aldehydes.The same aldehydes, or similar, are often commonly found in soaps and lotions. Studies have also suggested that crushing coriander leaves may lead to faster breakdown of aldehydes, diminishing the soapy taste(Raal, and Arak,2004).

The analysis of the essential oil conducted by gas chromatography-mass spectroscopy, revealed 33 components, representing 99.99% of the total oil from the seeds of coriander. The major components were linalool (55.09%), α-pinene (7.49%), 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (5.70%), geraniol (4.83%), 3-Cyclohexene-1- methanol, α,α,4-trimethyl- (4.72%), hexadecanoic acid (2.65%), tetradecanoic acid (2.49%), 2-α-pinene (2.39%), citronellyl acetate (1.77%), and undecanal (1.29%) (Dauenhauer, Paul, 2015).Sudanese *coriandrum sativum* oils contained seventy eight compounds with sabinene (17.63%), camphor (15.5%), cis-beta-ocimene (10.11%), trans-beta-ocimene (5.64%), alpha pinene (4.69%) and norboreneolacetate (4.09%) as the main constituents (Dharmalingam, and Nazni, 2013).

Chromatographic analysis yielded 93.0% neutral lipids, 4.14% glycolipids, and 1.57% phospholipids. Six triacylglycerol molecularspecies were detected but one component (C54:3) corresponding to tripetroselinin, and/or dipetroselinoyloleoyl glycerol comprised more than 50% of the totaltriacylglycerols. Sterol content was estimated to be at ahigh level (5186 µg /g oil). Stigmasterol, β -sitosterol, Δ 5-avenasterol, and campesterol were found to be the sterol markers. The major phospholipid subclasses were phosphatidylcholine followed by phosphatidylethanolamine, phosphatidy-linositol and phosphatidylserine (Ramadan, and , Kroh ,2002).

The leaves and stems of Korean *coriandrum sativum* were extracted and the essential oil composition was studied. Thirty-nine components representing 99.62% of the total oil were identified from the leaves. The major components were cyclododecanol (23.11%), tetradecanal (17.86%), 2-dodecenal (9.93%), 1-decanol (7.24%),13-tetradecenal (6.85%), 1-dodecanol (6.54%), dodecanal (5.16%), 1-undecanol (2.28%), and decanal (2.33%). On the other hand, thirty-eight components representing 98.46%

of the total oil were identified from the stems of the coriander. The major components were phytol (61.86%), 15- ethyltricyclo[6.5.2(13,14),0(7,15)]-pentadeca-1,3,5,7,9,11,13-heptene (7.01%), dodecanal (3.18%), and 1-dodecanol (2.47%) (Neffati, and Marzouk ,2008).

The leaf oil of *coriandrum sativum* from Bangladesh contained 44 compounds mostly of aromatic acids, the major were 2-decenoic acid (30.82%), E-11-tetradecenoic acid (13.4%), capric acid (12.7%), undecyl alcohol (6.4%) and tridecanoic acid (5.5%). Other major constituents in the leaf oil were undecanoic acid (2.13%), 2-dodecanal (1.32%), 2-undecenal (3.87%), cyclododecane (2.45%), decamethylene glycol (1.15%), decanal (1.35%) and dodecanoic acid (2.63%). The seed oil contained 53 compounds, the major compounds were linalool (37.7%), geranyl acetate (17.6%) and γ-terpinene (14.4%) (Potter, and, Fagerson ,1990).

Rajeshwari and Andallu found that the ethanolic extract of coriander seeds contained many flavonoids including caffeic acid, chlorogenic, quercetin and rutin (Rajeshwari, 2012).However, the total polyphenolic content of the seeds was found to be 12.2 gallic acid equivalents (GAE)/g while total flavanoid content was found to be 12.6 quercetin equivalents/g (Deepa, 2011). The amount of flavonoids in 70% ethanol extract was found to be 44.5 μg and that of the total phenols was 133.74 μg gallic acid equivalents per mg of the hydro-alcohol extract of coriander leaves (Romeilah,and Fayed, 2010)

1.2.1.8.Useful of coriander:

The use of coriander dated back to around 1550 BC, and it was one of the oldest spice crops in the world. Medicinally, it was used as stimulant, aromatic and carminative. The powdered fruit, fluid extract and oil are chiefly used medicinally as flavouring to disguise the taste of active purgatives and correct their griping tendencies. However, seeds were applied locally to alleviate swelling and pains. Paste of green coriander was used for headache. Externally, powdered green coriander was used to alleviate burning sensation and pain in diseases like inflammation caused by erysipelas and lymphadenopathy. Decoction of green coriander was used in stomatitis. Nasal drops of green coriander act as a haemostat and thus stop bleeding in epistaxis. Juice or decoction of green coriander was used in conjunctivitis (Coskuner and Karababa, 2007). The whole plant is used in the treatment of ulcers, cough and insomnia (Duke and Ayensu, 1985), vomiting, dysentery and biliousness (Mahendra, and Bisht, 2011). The leaves are used as a tonic, for urinary infection ,as a carminative, stimulant and pectoral , for leucoderma ,and skin disease (Mahendra, and Bisht, 2011).The seeds were included in many prescriptions as carminative and for the treatment of fever, diarrhoea,

vomiting and indigestion. Coriander was used internally as tonics. It was also used for syncope and memory loss. Fresh juice of leaves was used as gargle in sore throat and stomatitis. Paste of leaves was applied for swelling and boils and was applied over forehead and temples for headache (Craker and Simon, 2002).

The fruit (seed) is used in menstrual disorders . as an aphrodisiac. The fruits are used as astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic,expectorant, anodyne, and anti-diabetic and dyspepsia . The fruits have been used as a traditional medicinein many cultures to treat various medical conditions, including drug for indigestion, against worms and as acomponent of embrocations for rheumatism and pains in the articulations (Shahwar, and , El-Ghorab 2012).

All parts of coriander plant are edible, but the fresh leaves and the dried seeds are commonly used in cooking. The coriander fruits are used in the preparation of fish and meat, which is a spiced, hot red-pepper powder used for numerous meat andvegetarian dishes (Msaada,and,Hosni,2007). Today, most coriander is consumed in the form of curry powder, of which it forms 25-40%. The fatty oil is obtained either by pressing or by extraction of the fruits (Shahwar, and El-Ghorab,2012).

1.2.2. Essential Oils:

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are odorous and volatile compounds found only in 10% of the plant kingdom and are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts. The total essential oil content of plants is generally very low and rarely exceeds 1% (Bowles, 2003), but in some cases, for example clove and nutmeg, it reaches more than 10% (Morone,*et al*., 2010).

1.2.2.1. Essential oils characteristics:

Essential oils are hydrophobic, are soluble in alcohol, non polar or weakly polar solvents, waxes and oils, but only slightly soluble in water and most are colourless or pale yellow, with exception of the blue essential oil of chamomile andmost are liquid and of lower density than water (sassafras, vetiver, cinnamon and clove essential oils being exceptions).Due to their molecular structures (presence of olefenic double bonds and functional groups such as hydroxyl, aldehyde, ester); essential oils are readily oxidizable by light, heat and air(Eisenmenger, *et al*., 2010).

Essential oils are highly complex mixtures of volatile compounds, and many contain about 20 to 60 individual compounds, albeit some may contain more than 100 different components, such as jasmine, lemon and cinnamon essential oils.The major volatile constituents are hydrocarbons (e.g. pinene, limonene, bisabolene), alcohols (e.g. linalol, santalol), acids (e.g. benzoic acid, geranic acid), aldehydes (e.g. citral), cyclic aldehydes (e.g. cuminal), ketones (e.g. camphor), lactones (e.g. bergaptene), phenols (e.g. eugenol), phenolic ethers (e.g. anethole), oxides (e.g. 1,8 cineole) and esters (e.g. geranyl acetate) . All these compounds may be classified into two main categories:terpenoids and phenylpropanoids. or also into hydrocarbons and oxygenated compounds .The fragrance and chemical composition of essential oils can vary according to the geo-climatic location and growing conditions (soil type, climate, altitude and amount of water available), season (for example before or after flowering), and time of day when harvesting is achieved, etc (Zawislak G., 2011).

In addition, there is another important factor that influences the chemical composition of essential oils, namely the genetic composition of the plant. Therefore, all these biotope factors (genetic and epigenetic) influence the biochemical synthesis of essential oils in a given plant. Thus, the same species of plant can produce a similar essential oil, however with different chemical composition, resulting in different therapeutic activities. These variations in chemical composition led to the notion of chemotypes.

The chemotype is generally defined as a distinct population within the same species (plant or microorganism) that produces different chemical profiles for a particular class of secondary metabolites(Seader, et al.,1998).

1.2.2.2. Classes of essential oil compounds and their biological activities:

1.2.2.2.1. Hydrocarbons:

The majority of essential oils fall into this category; these contain molecules of hydrogen and carbon only and are classified into terpenes (monoterpenes: C10, sesquiterpenes: C15, and diterpenes: C20). These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic or tricyclic) or aromatic. Limonene, myrcene, *p*-menthane, α-pinene, β-pinene, α-sabinene, pcymene, myrcene, α–phellandrene, thujane, fenchane, farnesene, azulene, cadinene and sabinene are some examples of this family of products. These compounds have been associated with various therapeutic activities (Zare-Shehneh, *et al*., 2014)

1.2.2.2.2. Esters:

Esters are sweet smelling and give a pleasant smell to the oils and are very commonly found in a large number of essential oils. They include for example, linalyl acetate, geraniol acetate, eugenol acetate and bornyl acetate. Esters are anti-inflammatory, spasmolytic, sedative, and antifungal (Delaquis, et al.,2002).

1.2.2.2.3. Oxides:

Oxides or cyclic ethers are the strongest odorants, and by far the most known oxide is 1,8- cineole, as it is the most omnipresent one in essential oils. Other examples of oxides are bisabolone oxide, linalool oxide, sclareol oxide and ascaridole .Their therapeutic benefits are expectorant and stimulant of nervous system (Mageed, *et al*., 2012).

1.2.2.2.4. Lactones:

Lactones are of relatively high molecular weight and are usually found in pressed oils. Some examples of lactones are nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone, epinepetalactone, aesculatine, citroptene, and psoralen. They may be used for antipyretic, sedative and hypotensive purposes, but their contraindication is allergy, especially such involving the skin (Sriti,*et al*., 2011)

1.2.2.2.5. Alcohols

In addition to their pleasant fragrance, alcohols are the most therapeutically beneficial of essential oil components with no reported contraindications. They are antimicrobial, antiseptic, tonifying, balancing and spasmolytic. Examples of essential oil alcohols are linalol, menthol, borneol, santalol, nerol, citronellol and geraniol (Shibamoto, et al., 2010).

1.2.2.2.6. Phenols:

These aromatic components are among the most reactive, potentially toxic and irritant, especially for the skin and the mucous membranes. Their properties are similar to alcohols but more pronounced. They possess antimicrobial, rubefacient properties, stimulate the immune and nervous systems and may reduce cholesterol. Phenols are often found as crystals at room temperature, and the most common ones are thymol, eugenol, carvacrol and chavicol (Teshale, et al., 2013).

1.2.2.2.7. Aldehydes:

Aldehydes are common essential oil components that are unstable and oxidize easily. Many aldehydes are mucous membrane irritants and are skin sensitizers. They have characteristically sweet, pleasant fruity odors and are found in some of our most well known culinary herbs such as cumin and cinnamon. Therapeutically, certain aldehydes have been described as: antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic and spasmolytic. Common examples of aldehydes in essential oils include citral (geranial and neral), myrtenal, cuminaldehyde, citronellal, cinnamaldehyde and benzaldehyde (Zawislak ,et al., 2011).

1.2.2.2.8. Ketones:

Ketones are not very common in the majority of essential oils; they are relatively stable molecules and are not particularly important as fragrances or flavor substances. In some cases, ketones are neurotoxic and abortifacients such as camphor and thujone but have some therapeutic effects. They may be mucolytic, cell regenerating; sedative, antiviral, analgesic and digestive. Due to their stability, ketones are not easily metabolized by the liver. Common examples of ketones found in essential oils include carvone, menthone, pulegone, fenchone, camphor, thujone and verbenone (Muhtassib,*et al*., 2000).

1.2.2.3.Bioavailability of essential oils:

The term bioavailability, one of the principal pharmacokinetic properties of drugs, is used to describe the fraction of an administered dose of unchanged drug that reaches the systemic circulation and can be used for a specific function and/or stored. By definition, when a drug is administered intravenously, its bioavailability is 100%. However, when a drug is administer via other routes (such as oral), it has to pass absorption and metabolic barriers, before it reaches the general circulation system, and its bioavailability is prone to decrease (due to gastro-intestinal metabolism, incomplete absorption or first-pass metabolism)(Zare-Shehneh, and, Askarfarashah 2014).

Bioavailability is measured by pharmacokinetic analysis of blood samples taken from the systemic circulation and reflects the fraction of the drug reaching the systemic circulation. If a compound is poorly absorbed or extensively metabolized beforehand, only a limited fraction of the dose administered will reach the systemic circulation. Thus, in order to achieve a high bioavailability, the compound must be of sufficiently high absorption and of low renal clearance (measurement of the renal or other organ excretion ability). Various factors can affect bioavailability such as biochemical, physiological, physicochemical interactions; habitual mix of the diet; individual characteristics (life-stage and life-style) as well as the genotype(Ates, and Erdogrul 2003).

In the case of essential oils, the comprehension of their bioavailability by studying their absorption, distribution, metabolism and excretion in the human body is necessary. Unfortunately, there exists only limited data on the bioavailability of essential oils, and most studies are based on animal models. All findings confirm that most essential oils are rapidly absorbed after dermal, oral, or pulmonary administration and cross the blood-brain barrier and interact with receptors in the central nervous system, and then affect relevant biological functions such as relaxation, sleep, digestion. Most essential oil components are metabolized and either eliminated by the kidneys in the form of polar compounds following limited phase I enzyme metabolism by conjugation with glucuronate or sulfate, or exhaled via the lungs as CO_2. For example, after oral administration of (-)-menthol, 35% of the original menthol content was excreted renally as menthol glucuronide .The same happens with thymol, carvacrol, limonene and eugenol. After their oral administration, sulphate and glucuronide forms have been detected in urine and in plasma respectively.The fast metabolism and short half-life of active compounds have led to the belief that there is aminimum risk of accumulation in body tissues (Ates, et al. 2003).

1.2.2.4. Mechanism of the biological activities of essential oils:

So far, there is no study that can give us a clear idea and be accurate on the mode of action of the essential oils. Given the complexity of their chemical composition, everything suggests that this mode of action is complex, and it is difficult to identify the molecular pathway of action. It is very likely that each of the constituents of the essential oils has its own mechanism of action (Derbesy, and Uzio . 1993)

1.2.2.5. Antimicrobial activity of essential oil:

1.2.2.5.1. Antibacterial action:

Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils.One of the possibilities for action is the generation of irreversible damage to the membrane of bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore toits death(Bakkali,*et al*., 2008). Another possibility of action is inhibition of production of amylase and protease which stop the toxin production, electron flow and result in coagulation of the cell content (Pasqua ,*et al*., 2007).

1.2.2.5.2. Antifungal action:

Antifungal actions are quite similar to those described for bacteria. However, two additional phenomena inhibiting the action of yeast are worth mentioning: the establishment of a pH gradient across the cytoplasmic membrane and the blocking of energy production of yeasts which involve the disruption of the bacterial membrane (Darughe ,*et al*., 2008).

1.2.2.5.3. Antiviral activity:

The complex mixture of essential oils usually shows a higher antiviral activity than individual compounds (due probably to synergism phenomena); with exception of β-caryophyllene which is the most famous antiviral compounds found in many different essential oils from different plant families. Different mechanisms of antiviral activity of different essential oils and their constituents seem to be present. The antiviral activity of the essential oil is principally due to direct virucidal effects (by denaturing viral structural proteins or glycoproteins). Proposed mechanisms suggest that essential oils interfere with the virus envelope by inhibiting specific processes in the viral replication cycle or bymasking viral components, which are necessary for adsorption or entry into host cells, thus, they prevent the cell-to-cell virus diffusion (Darughe ,*et al*., 2007).

1.2.2.6. Extraction of essential oils:

Oils contained within plant cells are liberated through heat and pressure from various parts of the plant matter; for example, the leaves, flowers, fruit, grass, roots, wood, bark, gums and blossom. The extraction of essential oils from plant material can be achieved by various methods, of which hydro-distillation, steam and steam/water distillation are the most common method of extraction (Surburg and Panten, 2006).Other methods include solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction and phytonic process. This later process has been newly developed; it uses refrigerant hydrofluorocarbons solvents at low temperatures (below room temperature), resulting in good quality of the extracted oils.(Porto, *et al*., 2009).

1.2.3. Distillation of oils:

Distillation is the process of separating the component or substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of the mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction (Kister,and Henry. 1992).

1.2.3.1. History of distillation:

Distillation was known in the ancient Indian subcontinent, evident from baked clay retorts and receivers found at Taxila and Charsadda in modern Pakistan, dating back to the early centuries of the Common Era. These "Gandhara stills" were only capable of producing very weak liquor, as there was no efficient means of collecting the vapors at low heat (Hassan, and, A. 2014).Evidence of distillation also comes from alchemists working in Alexandria, Roman Egypt, in the 1st century, when Alexander of Aphrodisias described the process work on distilling other liquids continued in early Byzantine Egypt, under Zosimus of Panopolis in the 3rd century. Distillation in China could have begun during the Eastern Han dynasty (1st–2nd centuries), but the distillation of beverages began in the Jin (12th–13th centuries) and Southern Song (10th–13th centuries) dynasties according to archaeological evidence. Clear evidence of the distillation of alcohol comes from the Arab chemist Al-Kindi, in 9th-century Iraq, The process later spread to Italy, where it was described by the school of Salerno in the 12th century .Fractional distillation was developed by Tadeo Alderotti in the 13th century (Malterud, 2004).

In the early 19th century the basics of modern techniques including pre-heating and reflux were developed in 1822, Anthony Perrier developed one of the first continuous stills. In 1826, Robert Stein improved that design to make his patent still. In 1830, Aeneas Coffey got a patent for improving that design. Coffey continuous still may be regarded as the archetype of modern petrochemical units. The French engineer Armand Savalle developed his steam regulator around 1846. In 1877, Ernest Solvay was granted a U.S. Patent for a tray column for ammonia distillation and the same and subsequent years saw developments of this theme for oil and spirits (James,1970). In the early 20th century provided the impetus for the development of accurate design methods such as the McCabe–Thiele method and the Fenske equation. The availability of powerful

computers has also allowed direct computer simulation of distillation columns (Forbes,and, R.1970).

1.2.3.2. Applications of distillation:

Early forms of distillation were batch processes using one vaporization and one condensation. Purity was improved by further distillation of the condensate. Greater volumes were processed by simply repeating the distillation. Chemists were reported to carry out as many as 500 to 600 distillations in order to obtain a pure compound.The application of distillation can roughly be divided in four groups: laboratory scale, industrial distillation, distillation of herbs for perfumery and medicinals (herbal distillate), and food processing. The latter two are distinctively different from the former two in that in the processing of beverages and herbs, the distillation is not used as a true purification method but more to transfer all volatiles from the source materials to the distillate (Stephen, 2012).The main difference between laboratory scale distillation and industrial distillation is that laboratory scale distillation is often performed batch-wise, whereas industrial distillation often occurs continuously (Hassan,2014).

1.2.3.2.1. Batch distillation:

Heating an ideal mixture of two volatile substances A and B (with A having the higher volatility, or lower boiling point) in a batch distillation setup (such as in an apparatus depicted in the opening figure) until the mixture is boiling results in a vapor above the liquid which contains a mixture of A and B. The ratio between A and B in the vapor will be different from the ratio in the liquid: the ratio in the liquid will be determined by how the original mixture was prepared, while the ratio in the vapor will be enriched in the more volatile compound (Kravchenko, 2014).

The vapor goes through the condenser and is removed from the system. This in turn means that the ratio of compounds in the remaining liquid is now different from the initial ratio (i.e., more enriched in B than the starting liquid).The result is that the ratio in the liquid mixture is changing, becoming richer in component B. This causes the boiling point of the mixture to rise, which in turn results in a rise in the temperature in the vapor, which results in a changing ratio of A : B in the gas phase (as distillation continues, there is an increasing proportion of B in the gas phase). This results in a slowly changing ratio A:B in the distillate.If the difference in vapor pressure between the two components A and B is large (generally expressed as the difference in boiling points), the mixture in the beginning of the distillation is highly enriched in component

A, and when component A has distilled off, the boiling liquid is enriched in component B (Hassan and Ahmad,2014)

1.2.3.2.2. Continuous distillation:

Continuous distillation is an ongoing distillation in which a liquid mixture is continuously (without interruption) fed into the process and separated fractions are removed continuously as output streams occur over time during the operation. Continuous distillation produces a minimum of two output fractions, including at least one volatile distillate fraction, which has boiled and been separately captured as a vapor, and then condensed to a liquid. There is always a bottoms (or residue) fraction, which is the least volatile residue that has not been separately captured as a condensed vapor (Holmyard,1990).

Continuous distillation can be run at a steady state for an arbitrary amount of time. For any source material of specific composition, the main variables that affect the purity of products in continuous distillation are the reflux ratio and the number of theoretical equilibrium stages, in practice determined by the number of trays or the height of packing. Reflux is a flow from the condenser back to the column, which generates a recycle that allows a better separation with a given number of trays. Equilibrium stages are ideal steps where compositions achieve vapor–liquid equilibrium, repeating the separation process and allowing better separation given a reflux ratio. A column with a high reflux ratio may have fewer stages, but it refluxes a large amount of liquid, giving a wide column with a large holdup. Conversely, a column with a low reflux ratio must have a large number of stages, thus requiring a taller column (Kravchenko,2011).

1.2.3.3: Types of distillation:

1.2.3.3.1. Industrial Distillation:

Large scale industrial distillation applications include both batch and continuous fractional, vacuum, azeotropic, extractive, and steam distillation. The most widely used industrial applications of continuous, steady-state fractional distillation are in petroleum refineries, petrochemical and chemical plants and natural gas processing plants (Kister and Henry,1992).

1.2.3.3.2. Laboratory scale distillation

Laboratory scale distillations are almost exclusively run as batch distillations. The device used in distillation, sometimes referred to as a still, consists at a minimum of a reboiler or pot in which the source material is heated, a condenser in which the heated vapor is cooled back to the liquid state, and a receiver in which the concentrated or

purified liquid, called the distillate, is collected. Several laboratory scale techniques for distillation exist:

1.2.3.3.2.1.*Simple Distillation:*

In simple distillation, the vapor is immediately channeled into a condenser. Consequently, the distillate is not pure but rather its composition is identical to the composition of the vapors at the given temperature and pressure. That concentration follows Raoult's law. As a result, simple distillation is effective only when the liquid boiling points differ greatly (rule of thumb is 25 °C) or when separating liquids from non-volatile solids or oils. For these cases, the vapor pressures of the components are usually different enough that the distillate may be sufficiently pure for its intended purpose (Harwood and Moody, 1989).

1.2.3.3.2.2.*Steam distillation:*

Steam distillation is a method for distilling compounds which are heat-sensitive. The temperature of the steam is easier to control than the surface of a heating element, and allows a high rate of heat transfer without heating at a very high temperature. This process involves bubbling steam through a heated mixture of the raw material. By Raoult's law, some of the target compound will vaporize (in accordance with its partial pressure). The vapor mixture is cooled and condensed, usually yielding a layer of oil and a layer of water. Steam distillation of various aromatic herbs and flowers can result in two products; an essential oil as well as a watery herbal distillate. The essential oils are often used in perfumery and aromatherapy while the watery distillates have many applications in aromatherapy, food processing and skin care (Figueira, G.M.,2014)

1.2.3.3.2.3. *Short Path Distillation:*

Distillation technique that involves the distillate travelling a short distance, often only a few centimeters, and is normally done at reduced pressure.A classic example would be a distillation involving the distillate travelling from one glass bulb to another, without the need for a condenser separating the two chambers. This technique is often used for compounds which are unstable at high temperatures or to purify small amounts of compound. The advantage is that the heating temperature can be considerably lower (at reduced pressure) than the boiling point of the liquid at standard pressure, and the distillate only has to travel a short distance before condensing.

1.2.3.3.2.4.*Zone distillation:*

Zone distillation is a distillation process in long container with partial melting of refined matter in moving liquid zone and condensation of vapor in the solid phase at condensate pulling in cold area. The process is worked in theory. When zone heater is moving from the top to the bottom of the container then solid condensate with irregular impurity distribution is forming. Then most pure part of the condensate may be extracted as product. The process may be iterated many times by moving (without turnover) the received condensate to the bottom part of the container on the place of refined matter. The irregular impurity distribution in the condensate (that is efficiency of purification) increases with number of repetitions of the process (Kravchenko, 2014).

1.2.3.3.2.5.*Azeotropic distillation:*

Interactions between the components of the solution create properties unique to the solution, as most processes entail non-ideal mixtures, where Raoult's law does not hold. Such interactions can result in a constant-boiling azeotrope which behaves as if it were a pure compound (i.e., boils at a single temperature instead of a range). At an azeotrope, the solution contains the given component in the same proportion as the vapor, so that evaporation does not change the purity, and distillation does not affect separation traditional (Kayisoglu, B., Ulger,2006). For example, ethyl alcohol and water form an azeotrope of 95.6% at 78.1 °C. If the azeotrope is not considered sufficiently pure for use, there exist some techniques to break the azeotrope to give a pure distillate. Some techniques achieve this by "jumping" over the azeotropic composition (by adding another component to create a new azeotrope, or by varying the pressure). Others work by chemically or physically removing or sequestering the impurity. For example, to purify ethanol beyond 95%, a drying agent (or desiccant, such as potassium carbonate) can be added to convert the soluble water into insoluble water of crystallization(Khanahmadi, M., 2006)

1.2.3.3.2.6.*Freeze Distillation:*

Is an analogous method of purification using freezing instead of evaporation. It is not truly distillation, but a recrystallization where the product is the mother liquor, and does not produce products equivalent to distillation. This process is used in the production of ice beer and ice wine to increase ethanol and sugar content, respectively. It is also used to produce applejack. Unlike distillation, freeze distillation concentrates poisonous congeners rather than removing them; As a result, many countries prohibit such applejack as a health measure. However, reducing methanol with the absorption of 4A molecular sieve is a practical method for production. Also, distillation by evaporation can separate these since they have different boiling points Energy (Choe, 2006)

1.2.4. Edible Oils:

Edible oil is a triglyceride extracted from a plant. The term “edible oil " can be narrowly defined as referring only to substances that are liquid at room temperature, or broadly defined without regard to a substance's state of matter at a given temperature. For this reason edible oil that are solid at room temperature are sometimes called edible fats.Edible oil are composed of triglycerides, as contrasted with waxes which lack glycerin in their structure. Although many plant parts may yield oil, in commercial practice, oil is extracted primarily from seeds (Applewhite, 1978).

Plant and animal or synthetic fat used in frying, baking, and other types of edible. It is also used in food preparation and flavoring not involving heat, such as salad dressings and bread dips, and in this sense might be more accurately termed edible oil. Edible oil is typically a liquid at room temperature, although some oils that contain saturated fat, such as coconut oil, palm oil and palm kernel oil are solid. There are a wide variety of cooking oils from plant sources such as olive oil, palm oil, soybean oil, canola oil, corn oil, peanut oil and other vegetable oils, as well as animal-based oils like butter and lard. Oil can be flavored with aromatic foodstuffs such as herbs, chillies or garlic (Oyedeji, and Oderinde, 2006).

1.2.4.1. Production of edible oils:

There are several steps to result in desirable refined edible oil which are concluded in:

1.2.4.1.1. Extraction of edible oil:

General Food fats and oils are derived from oilseed and animal sources. Vegetable oils are obtained by the extraction or the expression of the oil from the oilseed source. To produce edible oil, the oil first needs to be removed from the oil-bearing plant components, typically seeds(Asuquo, et al.2012). This can be done via:

1.2.4.1.1.1. *Mechanical extraction:*

Oils can be removed via mechanical extraction, termed "crushing" or pressing." This method is typically used to produce the more traditional oils (e.g., olive, coconut and sunflower etc.).There are several different types of mechanical extraction; expeller-pressing extraction is common. Oil seed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive (Porto, and Decorti,2009).

1.2.4.1.1.2. *Solvent extraction:*

The processing of edible oil in commercial applications is commonly done by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive. The most common solvent is petroleum-derived hexane. This technique is used for most of the newer industrial oils such as soybean and corn oils. Supercritical carbon dioxide can be used as a non-toxic alternative to other solvents(Patel, and Desai,2012).

Historically, cold or hot expression methods were used. These methods have largely been replaced with solvent extraction or pre-press/solvent extraction methods which give better oil yield. In this process the oil is extracted from the oilseed by hexane (a light petroleum fraction) and the hexane is then separated from the oil, recovered, and reused. Because of its high volatility, hexane does not remain in the finished oil after processing. (Asuquo, and Asuquo, 2012).

The fats and oils obtained directly from rendering or from the extraction of the oilseeds are termed "crude" fats and oils. Crude fats and oils contain varying but relatively small amounts of naturally occurring non-glyceride materials that are removed through a series of processing steps. For example, crude soybean oil may contain small amounts of protein, free fatty acids, and phosphatides which must be removed through subsequent processing to produce the desired shortening and oil products. Similarly, meat fats may contain some free fatty acids, water, and protein which must be removed. It should be pointed out, however, that not all of the nonglyceride materials are undesirable elements. Tocopherols, for example, perform the important function of protecting the oils from oxidation and provide vitamin E. Processing is carried out in such a way as to control retention of these substances. (Keskin, et al. 2011)

1.2.4.1.2. Degumming or refining:

Crude oils having relatively high levels of phosphatides (e.g., soybean oil) may be degummed prior to refining to remove the majority of those phosphatides, proteinaceous, and mucilaginous substances. The process generally involves treating the crude oil with a limited amount of water to hydrate the phosphatides and make them separable by centrifugation. The phospholipids are often recovered and further processed to yield a variety of lecithin products. A relatively new process in the United States is enzymatic degumming. An enzyme, phospholipase, converts phospholipids, present in crude oil, into lysophospholipids that can be removed by centrifugation(Kravchenko, et al.2011).

Crude oil, pre-treated with a combination of sodium hydroxide and citric acid, is mixed with water and enzymes (phospholipase) by a high shear mixer, creating a very stable emulsion. The emulsion allows the enzyme to react with the phospholipids, transforming them into water-soluble lysophospholipids. This emulsion is broken by centrifugation, separating the gums and phospholipids from the oil. This process generates a better oil yield than traditional degumming/refining. Enzymatic degumming is currently not widely commercialized. The process of refining generally is performed on vegetable oils to reduce the free fatty acid content. Oils low in phosphatide content (palm and coconut) may be physically refined (i.e., steam stripped) to remove free fatty acids. After alkali refining, the fat or oil is water-washed to remove residual soap (Frega, et al. 1999).

1.2.4.1.3. Bleaching:

The term "bleaching" refers to the process for removing color producing substances and for further purifying the fat or oil. Normally, bleaching is accomplished after the oil has been refined. The usual method of bleaching is by adsorption of the color producing substances on an adsorbent material. Acid-activated bleaching earth or clay, sometimes called bentonite, is the adsorbent material that has been used most extensively. This substance consists primarily of hydrated aluminum silicate. Anhydrous silica gel and activated carbon also are used as bleaching adsorbents to a limited extent (Seader, and, Henley.1998).

1.2.4.1.4. Deodorization:

Deodorization is a vacuum steam distillation process for the purpose of removing trace constituents that give rise to undesirable flavors, colors and odors in fats and oils. Normally this process is accomplished after refining and bleaching. The deodorization of fats and oils is simply a removal of the relatively volatile components from the fat or oil using steam. This is feasible because of the great differences in volatility between the substances that give flavors, colors and odors to fats and oils and the triglycerides. Deodorization is carried out under vacuum to facilitate the removal of the volatile substances, to avoid undue hydrolysis of the fat, and to make the most efficient use of the steam. Deodorization does not have any significant effect upon the fatty acid composition of most fats or oils. Depending upon the degree of unsaturation of the oil being deodorized, small amounts of trans fatty acids may be formed. In the case of vegetable oils, sufficient 9 tocopherols remain in the finished oils after deodorization to provide stability (Othmer ,et al 1982).

1.2.4.1.5. Fractionation (Including Winterization):

Fractionation is the removal of solids by controlled crystallization and separation techniques involving the use of solvents or dry processing. Dry fractionation encompasses both winterization and pressing techniques and is the most widely practiced form of fractionation. It relies upon the differences in melting points to separate the oil fractions. Winterization is a process whereby material is crystallized and removed from the oil by filtration to avoid clouding of the liquid fraction at cooler temperatures. The term winterization was originally applied decades ago when cottonseed oil was subjected to winter temperatures to accomplish this process. Winterization processes using temperature to control crystallization are continued today on several oils. A similar process called de-waxing is utilized to clarify oils containing trace amounts of clouding constituents. Pressing is a fractionation process sometimes used to separate liquid oils from solid fat (Seader, and, Henley,1998**)**

1.2.4.1.6. Partial Hydrogenation:

Hydrogenation is the process by which hydrogen is added to points of unsaturation in the fatty acids.Oils may be partially hydrogenated to produce various ingredient oils. Lightly hydrogenated oils have very similar physical characteristics to regular soya oil, but are more resistant to becoming rancid. Hardening edible oil is done by raising a blend of edible oil and a catalyst in near-vacuum to very high temperatures, and introducing hydrogen. This causes the carbon atoms of the oil to break double-bonds with other carbons, each carbon forming a new single-bond with a hydrogen atom(Hudlický,and, Milos1996).

Hydrogenation was developed as a result of the need toconvert liquid oils to the semi-solid form for greater utility in certain food uses, and increase the oxidative and thermal stability of the fat or oil. It is an important process to our food supply, because it provides the desired stability and functionality to many edible oil products. In the process of hydrogenation, hydrogen gas reacts with oil at elevated temperature and pressure in the presence of a catalyst. The catalyst most widely used is nickel which is removed from the fat after the hydrogenation processing is completed.The hydrogenation process is easily controlled and can be stopped at any desired point. As hydrogenation progresses, there is generally a gradual increase in the melting point of the fat or oil(Paul ,and, Rylander, 2005).

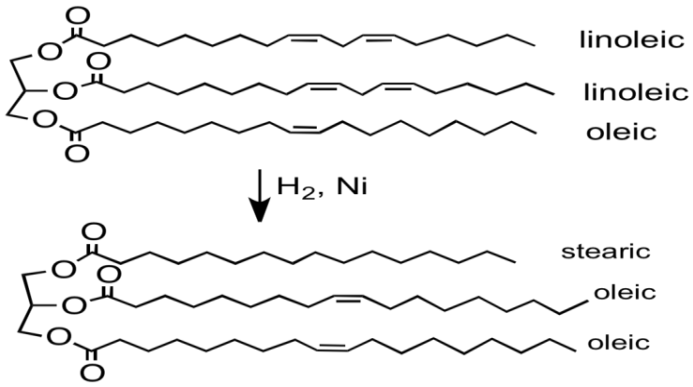


Figure (1-1) Hydrogenation reaction of edible oils (Dunn, 2005)

If the hydrogenation of cottonseed or soybean oil, for example, is stopped after only a small amount of hydrogenation has taken place, the oils remain liquid. These partially hydrogenated oils are typically used to produce institutional cooking oils, liquid shortenings and liquid margarines. Further hydrogenation can produce soft but solid appearing fats which still contain appreciable amounts of unsaturated fatty acids and are used in solid shortenings and margarines. Hydrogenated edible oils differ in two major ways from other oils which are equally saturated. During hydrogenation, it is easier for hydrogen to come into contact with the fatty acids on the end of the triglyceride, and less easy for them to come into contact with the center fatty acid. This makes the resulting fat more brittle than tropical oil(Alfred, and, Thomas 2002).

The other difference is that trans-fatty acids (often called trans-fat) are formed in the hydrogenation reactor, and may amount to as much as 40 percent by weight of partially hydrogenated oil. Hydrogenated oils especially partially hydrogenated oils with their higher amounts of trans fatty acids are increasingly thought to be unhealthy. Hydrogenated oils have been shown to cause what is commonly termed the "double deadly effect", raising the level of low density lipoproteins (LDLs) and decreasing the level of high density lipoproteins (HDLs) in the blood, increasing the risk of blood clotting inside blood vessels. Biological hydrogenation of polyunsaturated fatty acids occurs in some animal organisms, particularly in ruminants. This accounts for the presence of some trans isomers that occur in the tissues and milk of ruminants(Eisenmenger, and, Dunford,2006).

1.2.4.1.8. Interesterification:

Another process used by oil processors is interesterification which causes a redistribution of the fatty acids on the glycerol fragment of the molecule. This rearrangement process does not change the composition of the fatty acids from the starting materials. Interesterification may be accomplished by chemical or enzymatic

processes. Chemical interesterification is a process by which fatty acids are randomly distributed across the glycerol backbone of the triglyceride. This process is carried out by blending the desired oils, drying them, and adding a catalyst such as sodium methoxide. When the reaction is complete, the catalyst is neutralized and the rearranged product is washed, bleached, and deodorized to give a final oil product with different characteristics than the original oil blends. The second process is enzymatic interesterification. This process rearranges the fatty acids (can be position specific) on the glycerol backbone of the triglyceride through the use of an enzyme. Higher temperatures will result in inactivation of the enzyme. After interesterification, the oil is deodorized to make finished oil products. The predominant commercial application for interesterification in the US is the production of specialty fats. These processes permit further tailoring of triglyceride properties to achieve the required melting curves (Grob, Konrad, 1997)

1.2.4.1.9. Esterification:

Fatty acids are usually present in nature in the form of esters and are consumed as such. Triglycerides, the predominant constituents of fats and oils, are examples of esters. When consumed and digested, fats are hydrolyzed initially to diglycerides and monoglycerides which are also esters. Carried to completion, these esters are hydrolyzed to glycerol and fatty acids. In the reverse process, esterification, an alcohol such as glycerol is reacted with an acid such as a fatty acid to form an ester such as mono-, di-, and triglycerides. In an alternative esterification process, called alcoholysis, an alcohol such as glycerol is reacted with fat or oil to produce esters such as mono- and diglycerides. Using the foregoing esterification processes, edible acids, fats, and oils can be reacted with edible alcohols to produce useful food ingredients (Harwood,et al,1989).

1.2.4.1.10. Additives and Processing Aids:

Manufacturers may add low levels of approved food additives to fats and oils to protect their quality in processing, storage, handling, and shipping of finished products. This insures quality maintenance from time of production to time of consumption. When their addition provides a technical effect in the end-use product, the material added is considered a direct food additive. When additives are included to achieve a technical effect during processing, shipping, or storage and followed by removal or reduction to an insignificant level, the material added is considered to be a processing aid(Nanditha,et al,2008).

1.2.4.1.11. Emulsifiers:

Many foods are processed and/or consumed as emulsions, which are dispersions of immiscible liquids such as water and oil, e.g., milk, mayonnaise, ice cream, icings, and sauces. Emulsifiers, either present naturally in one or more of the ingredients or added separately, provide emulsion stability. Lack of stability is results in separation of the oil and water phases. Some emulsifiers also provide valuable functional attributes in addition to emulsification. These include aeration, starch and protein complexing, hydration, crystal modification, solubilization, and dispersion (Neffati, et al,2008)

1.2.4.2. Physiochemical Properties of edible oil:

1.2.4.2.1. Physical properties:

1.2.4.2.1.1.*Colour:*

Colour, flavour and texture are key factors in food acceptability. Additionally, colour may be used to evaluate composition and chemical changes in foodstuffs, being one of the indicators of product quality (Ravi, R.M., 2007). The method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond tintometer (Soares,et al, 2012).

1.2.4.2.1.2. *Specific Gravity (SG):*

The mass density or density of a material is defined as its mass per unit volume. The symbol most often used for density is ρ (the Greek letter rho). In some cases (for instance, in the United States oil and gas industry), density is also defined as its weight per unit volume although this quantity is more properly called specific weight. In some cases density is expressed as the dimensionless quantities specific gravity (SG) or relative density (RD), in which case it is expressed in multiples of the density of some other standard material, usually water or air/gas. For example, a specific gravity less than one mean that the substance floats in water(Halvorsen, 1993).This physical property is an important criterion for quality and purity of an essential oil. For determining the specific gravity accuracy to at least the third decimal place is necessary used(Sonestedt,et al,2008).

1.2.4.2.1.3.*Viscosity:*

Viscosity is a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress. In everyday terms (and for fluids only), viscosity is "thickness" or "internal friction". Thus, water is "thin", having a lower viscosity, while honey is "thick", having a higher viscosity. Put simply, the less viscous the fluid is, the greater its ease of movement (fluidity) (Pino,et al, 1996).One of the most common

instruments for measuring kinematic viscosity is the glass capillary viscometer (Zoubiri,et al,2010).Viscosity is independent of pressure (except at very high pressure) and decreasing temperature increases viscosity

1.2.4.2.1.4.*Refractive Index:*

Refractive index is defined as a ratio of the sin of the angle of incident to the sin of the angle or refraction when a ray of light of defined wavelength passes from air to the material kept at constant temperature. For measuring refractive index of oil, usually refractometer is used. The ratio of velocity of light in vacuum to the velocity of light in the oil or fat; more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of Sodium) passes from air into the oil or fat. Refractive index varies with temperature and wavelength (Attwood, and, David ,1999).

1.2.4.2.2. Chemical properties:

1.2.4.2.2.1. *Peroxide value:*

The peroxide value is defined as the amount of peroxide oxygen milliequivalents per 1 kilogram of fat or oil. The double bonds found in fats and oils play a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation (oxidative rancidity) is determination of the peroxide value. Peroxides are intermediates in the autoxidation reaction. Peroxide values of fresh oils are less than 10 milliequivalents /kg; when the peroxide value is between 30 and 40 milliequivalents/kg, a rancid taste is noticeable Chakrabarty,et al, 2011).

1.2.4.2.2.2.*Saponification Value*

The number of mg of potassium hydroxide required to saponify 1 gram of oil/fat. The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution.The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa.The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid. (J.I.S. K 0070-1992).

1.2.4.2.2.3.*Acid Value:*

The number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids

are normally formed during decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid. The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution (European pharmacopoeia,2004).The maximum levels for acid value of edible fats and oils were established by the ministry of public health at 0.6 mg KOH/1 g oil for reused fats and reused oils or mixed fats/oils, 1.0 mg KOH/1 g oil for mixed fats and mixed oils, and 4.0 mg KOH/1g oil for natural fats and natural oils or mixed fats/oils (J.I.S. K 0070-1992).

1.2.4.2.2.4.*Iodine Value:*

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of the oil/fat, when determined by using Wijs solution. The determination of the iodine value is based on the addition of iodine to the double bonds of unsaturated fatty acids. It is constant for a particular oil or fat, but the exact figure obtained on the particular technique employed .The oil/fat sample taken in carbon-tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wijs solution).The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulfate solution (J.I.S. K 0070-1992).

1.2.4.3. Auto oxidation and oxidative stability in edible oils:

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: 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 (Mochida, and, Nakamura, 2006).

By definition, the oxidative stability of oil is a measure of the length of time taken for oxidative deterioration to commence. On a general level, the rates of reactions in auto oxidation schemes are dependent on the hydrocarbon structure, heteroatom concentration heteroatom speciation, oxygen concentration, and temperature (Cichelli, et al,2004). If untreated, oils from vegetable origin oxidize during use and polymerize to a plastic like consistency (Redd, and, Marzouky,2005).Even when they are not subjected to the intense conditions of industrial applications, fats and oils are liable to rancidity. This happens more so in fats that contain unsaturated fatty acid radicals (Sharma,et,al,2007).

To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase). Produced internally or the dietary antioxidants: vitamin A, vitamin C, and vitamin E. Antioxidant dietary supplements do not improve health nor are they effective in preventing diseases as shown by randomized clinical trials including supplements of beta-carotene, vitamin A, and vitamin E singly or in different combinations having no effect on mortality rate (Bjelakovic,et al, 2013).

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors. Consequently, packaging of fresh fruits and vegetables contains an 8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food (Vertuani,et al, 2004).

1.2.4.4. Uses of edible oils:

1.2.4.4.1. ***Culinary uses:***

Many edible oils are consumed directly or indirectly as ingredients in food, this role they share with some animal fats, including butter and ghee.

1.2.4.4.2.*Industrial uses:*

Edible oils are used as an ingredient or component in many manufactured products. Many edible oils are used to make soaps, skin products candles, perfumes and other personal care and cosmetic products. Some oils are particularly suitable as drying oils, and are used in making paints and other wood treatment products. Dammar oil (a mixture of linseed oil and dammar resin) as example is used almost exclusively in treating the hulls of wooden boats. Edible oils are increasingly being used in the electrical industry as insulators(Hossain,and, Amjad 2012).

1.2.4.4.3. ***As fuel:***

Edible oils are also used to make biodiesel, which can be used like conventional diesel. Some edible oil blends are used in unmodified vehicles but straight edible oil, also known as pure plant oil, needs specially prepared vehicles which have a method of heating the oil to reduce its viscosity. The edible oil economy is growing and the availability of biodiesel around the world is increasing. It is believed that the total net greenhouse gas savings when using edible oils in place of fossil fuel based alternatives for fuel production range from 18 to 100% (Bunkyakiat, et al. (2006).

1.2.5. Sunflower oil:

1.2.5.1. Description of plant:

Sunflowers are botanically classified as helianthus annuus. The sunflower plants reach various heights, but most are from 1.52–2.1 m tall. The diameter of the flower heads is relatively large, typically between 7.62 -15.24 cm, although some can measure more than 30 cm. an exception is the dwarf varieties, which are only 0.91–1.22 m high and have smaller flower heads. A common characteristic of sunflowers is a tendency for their flowering heads to follow the movement of the sun during the day. This phenomenon, called heliotropism, has the benefit of reducing damage from birds and preventing the development of disease (Jean,and,Roger 1994).

Sunflowers are a large plant and are grown throughout the world because of their relatively short growing season. Domesticated sunflowers typically have a single stalk topped by a large flower.This is significantly different from the smaller, multiply branched wild sunflower. During the growing season, the individual flowers are each pollinated. Seed development then begins moving from the outer rim of the flower toward the centre. It generally takes 30 days after the last flower is pollinated for the plant to mature(Motloch, and, John ,2000).

1.2.5.2. Sunflower oil extraction:

Sunflower oil is generally extracted in two stages. Initially, a fraction of the oil is mechanically extracted by screw- press expelling. The cake obtained from this pressing stage, containing 15–20% of oil, is later solvent extracted, usually with hexane. Oils obtained by pressing are considered to be of better nutritional quality than those obtained by solvent extraction. However, both fractions are generally blended prior to storage (Cox,and, Jeff ,1979).

1.2.5.3. Sunflower oil extraction characteristics:

Sunflower oil has the following characteristics(Chu,and, Michael,2004):

- Liquid at room temperature.
- Smoke point of refined oil: 232 °C.
- Smoke point of unrefined oil: 227 °C.
- Density (25 °C): 917 kg/m^3.
- Refractive index (25 °c): ≈1.473.
- Viscosity (25 °C, unrefined): 0.04914 kg/(m*s).
- Refined sunflower oil is pale yellow in colour.
- Slightly fatty odour.

1.2.5.4. Composition of sunflower oil:

Sunflower oil contains predominantly linoleic (48–7%), oleic (14–40%), palmitic (4–9%) and stearitic (1–7%) there are several types of sunflower oils produced, such as high linoleic, high oleic and mid oleic. high linoleic sunflower oil typically has at least 69% linoleic acid while high oleic sunflower oil has at least 82% oleic acid. The variation in the unsaturated fatty acids profile is strongly influenced by both genetics and climate(Alfred,and,Thomas,2002). In the last decade, high stearic lines of sunflower oil have been developed in Spain to avoid the use of hydrogenated vegetable oils in the food industry. The conventional sunflower oil (high linoleic) is used for home cooking oil and margarine and for industrial use (paint, etc). The high oleic sunflower oil is used for cosmetics, gasoline blend and other purposes. Sunflower oil also contains lecithin, tocopherols, carotenoids, waxes and high vitamin E content (Skorić,et al,2008).

1.2.5.5. Modified properties of sunflower oil:

Like other edible vegetable oils, sunflower oil may be chemically modified by hydrogenation or by interesterification with other vegetable and animal fats.A relatively good oxidative stability of regular sunflower oil results from the insignificant content of linolenic acid. To increase the stability of vegetable oils having a higher linolenic acid content, light hydrogenation is required to reduce the level of linolenic acid, while avoiding the formation of significant amounts of trans isomers. In producer countries, regular sunflower oil is partially hydrogenated for use in the manufacture of shortenings and margarines (U,F,A,D,A, 2011).

The behavior of sunflower oil in the hydrogenation process is similar to that of soybean oil, and does not require special reaction conditions. Nor does it contain any compounds that may interfere with the hydrogenation reaction, unlike the case with rapeseed oil. However, the use of partially hydrogenated sunflower oil for the manufacture of margarine spreads may lead to the appearance of graininess during storage, resulting from a strong tendency of partially hydrogenated sunflower oil to formcrystals (T,T,F,2006).

1.2.5.6. Oxidative stability of commercial sunflower oil:

Good oxidative stability results in a good storage quality of sunflower oil and related oilbased products. The oxidation reactions leading to the deterioration of both sunflower oil and related products during their useful life are the same as for other edible vegetable oils (Toshiyuki, et, al,1999).

1.2.5.7. Uses of sunflower oil in foods:

Both regular and high- oleic sunflower oil may be used for the preparation of several food products, although the former is generally used because of the price difference between the two types. Other specific uses of sunflower oil are associated with the particular properties of different sunflower oil types, which differ in oil composition. In countries where sunflower oil is common edible oil, it is mainly used as salad dressing and cooking oil.Industrially, sunflower oil is used in frying applications and in the manufacture of mayonnaise and oil based dressings. Although high- oleic sunflower oil is also suitable for these applications, it is used predominantly as frying oil(N,S,A,2011).

Both continuous and discontinuous deep- fat frying is normally used for the preparation of food products. Discontinuous frying is used according to consumer demand. Frying oil is exposed to the air as it is heated for relatively short and sometimes occasional cooking periods, and most often with infrequent oil replenishment. Continuous frying, used for the industrial processing of fried and pre- fried foods, the steam generated during the frying process constitutes a protective barrier against air exposure and oxidation. Oil decomposition products absorbed with the frying oil by the product affect not only the sensory and nutritional quality but also the useful life of fried foods such products include polar compounds such as triacylglycerol dimers and polymers, di- and mono acyl glycerols, free fatty acids and peroxidised compounds (Tautorus, et al,2006).

1.2.6. Gas chromatography- Mass Spectrometer (GC-MS):

1.2.6.1. History of GC:

Chromatography dates to 1903 in the work of the Russian scientist, Mikhail Semenovich Tswett. German graduate student Fritz Prior developed solid state gas chromatography in 1947. Archer John Porter Martin, who was awarded the Nobel Prize for his work in developing liquid–liquid (1941) and paper (1944) chromatography, laid the foundation for the development of gas chromatography and he later produced liquid-gas chromatography (1950). Erika Cremer laid the groundwork, and oversaw much of Prior's work. Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture (Schug and Kevin,1974).

1.2.6.2. Phases of GC:

Gas chromatography composes from two phases, mobile and stationary phase.In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. Helium remains the most commonly used carrier gas in about 90% of instruments although hydrogen is preferred for improved separations. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness (Murry, and, John,2011).

1.2.6.3. Principle of GC:

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. First, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid. (Hence the full name of the procedure is "Gas–liquid chromatography", referring to the mobile and stationary phases, respectively.) Second, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column

chromatography (typically) has no such temperature control. Finally, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas (Pavia,et al,2006).

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale. Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas–liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently used in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors(Grob, and, Konrad,1997).

1.2.6.4. Method of GC:

Two valves are used to switch the test gas into the sample loop. After filling the sample loop with test gas, the valves are switched again applying carrier gas pressure to the sample loop and forcing the sample through the column for separation.The method is the collection of conditions in which the GC operates for a given analysis. Method development is the process of determining what conditions are adequate and/or ideal for the analysis required (Harris,and,Daniel,1999). Conditions which can be varied to accommodate a required analysis include inlet temperature, detector temperature, column temperature and temperature program, carrier gas and carrier gas flow rates, the column's stationary phase, diameter and length, inlet type and flow rates, sample size and injection technique. Depending on the detector(s) (see below) installed on the GC, there may be a number of detector conditions that can also be varied. Some GCs also include valves which can change the route of sample and carrier flow. The timing of the opening and closing of these valves can be important to method development (Grob, and, Robert L.2004).

In general, substances that vaporize below 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process. Professionals working with GC analyze the content of a chemical product, for example

in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water (Higson, et al, 2004).

GC is used for two type of analysis methods:

1.2.6.4.1.*Qualitative Analysis:*

Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. Retention time can be used to identify analytes if the method conditions are constant. Also, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analytes. However, in most modern applications, the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analytes represented by the peaks (Harris,and,Daniel,1999).

1.2.6.4.2.*Quantitative Analysis:*

The area under a peak is proportional to the amount of analyte present in the chromatogram. By calculating the area of the peak using the mathematical function of integration, the concentration of an analyte in the original sample can be determined. Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analyte, or by determining the relative response factor of an analyte. The relative response factor is the expected ratio of an analyte to an internal standard (or external standard) and is calculated by finding the response of a known amount of analyte and a constant amount of internal standard.In most modern GC-MS systems, computer software is used to draw and integrate peaks, and match MS spectra to library spectra (Harris,and,Daniel,1999).

1.2.6.5. Gas Chromatography–Mass Spectrometry (GC-MS):

GC-MS is properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. 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 (Sahil, 2011). GC can well separate complex mixtures, and MS can detect these compounds. The combination of the two has a more favorite place, for example, both GC and MS can run in the gaseous state; thus, they can be connected directly, and the interface is very simple. Simply speaking, the performance of GC/MS is stable, and the reproducibility is good (Linde AG.2012).

1.2.6.6. Applications of GC:

GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples. Gas chromatography is used extensively in forensic science. Disciplines as diverse as solid drug dose (pre-consumption form) identification and quantification, arson investigation, paint chip analysis, and toxicology cases, employ GC to identify and quantify various biological specimens and crime-scene evidence (Amirav, et al,2008).

The Supersonic GC-MS can be applied with few major advantages to practically all GC-MS analysis types. The main features of enhancedmolecular 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 Supersonic GC-MS in broad range of areas including (Alon, and ,Amirav,2006):

- Petrochemical and hydrocarbons analysis.
- Geochemical research.
- Forensic (arson, explosives, drugs, unknowns).
- Environmental analysis.
- Pesticide analysis and food safety.
- Pharmaceutical and drug analysis.
- Clinical toxicology.
- Food and fragrance.
- High end research GC-MS.
- Service and institution GC-MS analyze.

CHAPTER TWO

CHAPTER TWO

2. Materials and Methods

2.1. Materials:

2.1.1. Chemicals and reagents:

- Acetic acid
- Chloroform solvent
- Potassium iodide solution
- Sodium thiosulphate
- Starch solution
- Ethyl alcohol
- Phenolphthalein indicator solution
- Potassium hydroxide.
- Alcoholic potassium hydroxide
- Hydrochloric acid
- Mueller–Hinton agar
- Normal saline
- Gentamicine (Positive control)
- Dimethyl sulphoxide (DMSO)

2.1.2. Equipments:

- Standard thermometer
- Conical flasks
- Burette
- Separating funnel
- Propeller Stirrer
- Glass cells
- Needles
- Sterilized swab

2.1.3. Apparatuses:

- Gas chromatography-mass spectrometer (GC-MS)
- Distillation apparatus
- Lovibond tintometer

- Refractometer Abbe
- Pycnometer
- Balance
- Water bath

2.2. Methods

2.2.1. Samples Collect:

The dried sample of coriander fruits were purchase from the local market of Shendi in the Rive Nile state, from season harvest 2015 – 2016. The sunflower oil was purchased from Arabian oils company in Khartoum state. Six species of bacteria (three gram-positive and three gram-negative) were prepared in the laboratory of medical laboratories collage, Shendi University.

2.2.2. Experiments and tests:

The extraction of*Coriandrum Sativum L* oil and determination of chemical properties of oils were carried out in researches center and industrial consulting, Khartoum, whilst the determination of chemical profile of coriander oil was performed at laboratory of science faculty, university of Khartoum. Antibacterial activity of coriander oil was done at the medical laboratories college, Shendi University.

2.2.2.1. Extraction of Coriander oil:

Coriander oil was extracted by steam distillation and water method. Mature coriander fruits were put into distillation instrument over water, then the water was heated and the steam passed through the coriander fruits and vaporized the volatile compounds. The vapors were flowed through a coil, where they were condensed back to liquid, which then was collected in the receiving vessel. At the end of the distillation process coriander oil was separated from water depending on the difference in their density by using laboratory separating funnel. Theoilof coriander was collectedand kept at suitable temperature in dark pure bottle and had been ready for chemical profile analysis and to determine the chemical and physical tests.

2.2.2.2. Determination of coriander oil components:

Coriander oil was analyzed by using a gas chromatography mass spectrometry(GCMS-QP 2010 plus), equipped with selective detector mass spectrometry. The procedure and working mode of GC-MS are shown in appendix (1).The identification of oil constituents was carried out by comparing retention times with those of authentic reference compounds, or peak matching library research using the standard mass library (Pino, J.A.1996).

2.2.2.3. Determination of chemical properties(Toshiyuki, C. 1999):

2.2.2.3.1. *Determination of Peroxide value (PV):*

Principle:

$$ROOH + KI \xrightarrow{H^+,\ heat} ROH + KOH + I_2$$

$$I_2 + starch\ (blue) + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6 + starch\ (colorless)$$

Procedure:

5g of sample were weighed into a 250 ml stopper conical flask, 30 ml acetic acid and chloroform solvent mixture in the ratio of 3:2 were added and swirled until was dissolved, then 0.5 ml saturated potassium iodide solution was added with a Mohr pipette. The mixture solution was stand for 1min in dark with occasional shaking, after that about 30 ml of distilled water were added and also about 0.5 ml starch solution was added as indicator and then the reaction was initiated and continued with shaking vigorously until all iodine (I_2) was released from chloroform ($CHCl_3$) layer. The liberated iodine was slowly titrated with 0.1 N sodium thiosulphate solution or (0.001 mol/l) $Na_2S_2O_3$, with vigorous shaking until yellow colour was a disappeared). Chakrabarty,et,al,2011).

Calculation:

$$\text{Peroxide value} = \frac{\text{titer} \times N \times 100}{\text{sample weight}}$$

Where,

Titre = ml of sodium thiosulphate used (blank corrected)

N = normality of sodium thiosulphate solution.

2.2.2.3.2.*Determination of acid value:*

Principle:

The acid value is determined by directly titrating the oil in an alcoholic medium against standard potassium hydroxide solution.The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture and temperature.

$$RCOOH + KOH \xrightarrow[Heat]{Light} RCOOK + H_2O$$

Procedure:

A accurately appropriate amount of the cooled oil sample was weighed in a 250 ml conical flask, 50 ml of freshly neutralized hot ethyl alcohol was added and one ml of

phenolphthalein indicator solution. The mixture was boiled for five minutes and titrated against standard alkali solution 0.100N or 0.001M with vigorously shaking during the titration.

Calculation:

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where,

V = volume in ml of standard potassium hydroxide used.

N = normality of the potassium hydroxide solution solution.

W = weight in g of the sample.

2.2.2.3.3. *Determination of Saponification Value:*

Principle:

The oil sample is saponify by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid.

Procedure:

A portion(2g) of the sample was measured into a flask and 25ml of alcoholic potassium hydroxide solution was added to it. A reflux condenser was attached to the flask and the solution heated for one hour with occasional shaking. A 3 drops of phenolphthalein solution was added and titrated with 0.5m HCL. A blank was carried out with water and recorded as

Calculation:

$$\text{Saponification Value} = \frac{(b-a) \times 28.05}{W}$$

Where,

b = volume in ml of standard hydrochloric acid required for the blank.

a = volume in ml of standard hydrochloric acid required for the sample.

W = weight in gm of the oil/fat taken for the test.

2.2.2.4. Antibacterial activity of coriander oil:

To evaluate the antibacterial activity of coriander oil, six types of bacteria (three grams positive and three gram negative) were selected in the clinical laboratory of the University of Shendi. The agar plate method was adopted to evaluate the antibacterial activity of the extracted oil (Telci, 2006).

Procedure:

The medium was cool to 45-50 C^0 placed in the plates, then allowed to adjust the surface level to a depth of about 4mm. Antibiotic drug stocks were kept at -20C^0. Cotton wool swab supplies are prepared on wooden rod sticks.2 ml mixture of bacterial suspension was prepared thoroughly uniformed with 250 mL nutrient agar at 45 C^0 .Agar inoculation (20-25 ml) was distributed vouchers in sterile Petri dishes. Leaves were left to harden and 4 wells (7mm in diameter) were made using sterile cork bore. Grains (12.5, 25, 50 and 100%) were prepared for oil extracted, as well as by dissolving the extracts and fractures in dimethyl sulfoxide (DMSO).The effects of coriander oil were considered after 24-48 hours by measuring the diameter of the inhibition region of each treatment. Three replicates were performed for each extract / fracture and control of living organisms tested.

Calculation:

The relative percentage of test inhibition with respect to positive control using the following law:

Percentage of relative inhibition of test extraction

$$= \frac{(X-Y)}{(Z-Y)} \times 100$$

Where:

X: Total area of inhibition of the test extract.

Y: Total area of inhibition of the solvent.

Z: Total area of inhibition of the standard drug.

2.2.7. Statistical Analysis:

Data was collected and entered into the statistical analysis program (SPSS) using the Windows computer system (IBM). The P value was considered significant at 0.05 and the maximum confidence was 95%.

CHAPTER THREE

CHAPTER THREE

3. Results and Discussion

3.1. GC-MS result:

The coriander oil obtained by steam distillation which was colorless to pale yellow liquid and then was analyzed by GC-MS. The constituents of coriander oil were; hydrocarbons, monoterpene, aldehydes,alcohols, phenols, acids andesters. The following tables (3-1), (3-2) and figure (3-1) represent GC-MS analysis of coriander oil.

Table (3-1): The chemical constituents of coriander oil analyzed by gas chromatography

Peak	Constituent	Percentage %	Retention Time	Base peak	Mass Peak
1	Heptanal	0.62	9.745	70.10	403
2	α-Thujene	0.19	10.554	93.10	397
3	α-pinene	1.76	10.769	93.10	410
4	Camphene	0.15	11.267	93.10	407
5	β-Phellandrene	0.57	12.147	93.10	410
6	β-pinane	7.18	12.240	93.10	427
7	p-Cymene	3.18	13.856	119.10	395
8	γ-Terpinene	10.96	15.014	93.10	418
9	1-Octanol	3.82	15.478	56.10	443
10	Linalool	13.98	16.440	71.10	392
11	Camphor	2.12	17.723	95.10	412
12	Trimethy-cyclohexene carboxaldehyde	5.00	19.209	109.10	423
13	Decanal	7.23	19.496	57.10	425
14	Cuminaldehyde	9.16	20.663	133.10	393
15	*cis*-2-Decenal	7.11	21.144	70.05	416
16	Thymol	9.62	21.930	79.10	419
17	1-Propanol, 2-methyl-1-phenyl	8.02	22.082	107.10	425
18	*trans*-2-dodecenal	1.39	26.521	70.05	423
19	Tetradecanoic acid	1.00	33.426	73.05	440
20	Phthalic acid	1.22	35.558	149.00	420
21	Dibutyl phthalate	4.17	37.455	149.00	478
22	Tetradecanoic acid	0.94	44.580	57.10	473
23	Diisobutyl phthalate	0.40	47.719	149.05	441
24	Palmitic acid	0.20	48.065	57.10	430

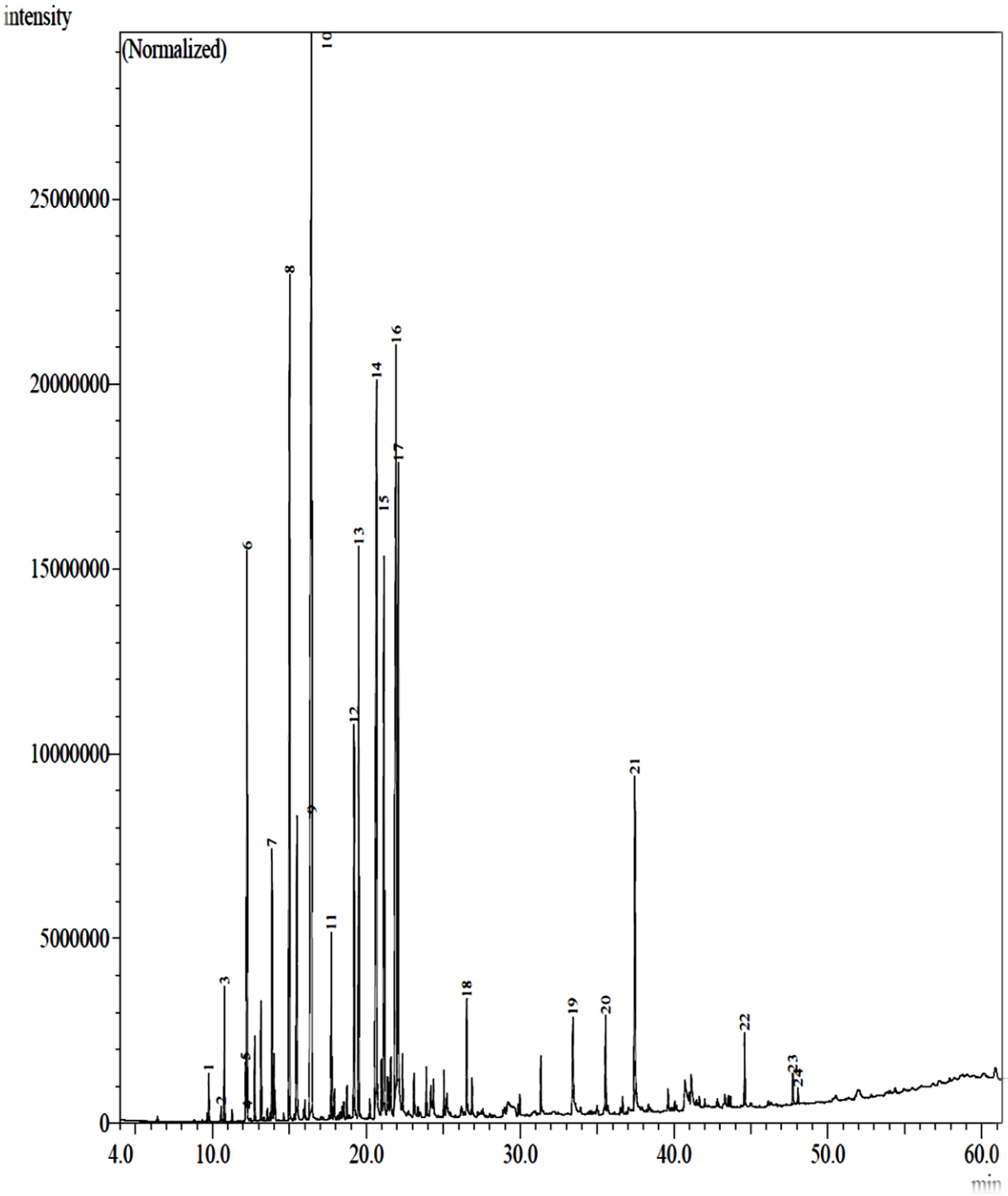
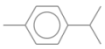
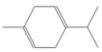
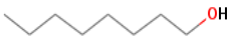
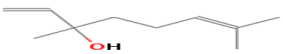
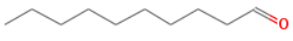
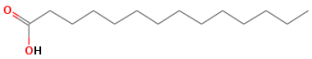
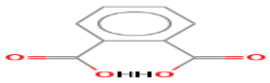
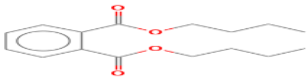
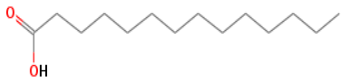
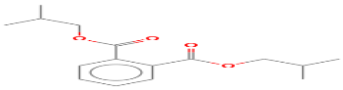
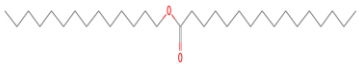


Figure (3-1): The GC- MS peaks of coriander oil

Table (3-2): The structures of coriander constituents

Constituent	Formula	Structure
Heptanal	$C_7H_{14}O$	
α-Thujene	$C_{10}H_{16}$	
(R)-α-pinene	$C_{10}H_{16}$	
Camphene	$C_{10}H_{16}$	
β-Phellandrene	$C_{10}H_{16}$	
Pinane	$C_{10}H_{18}$	
p-Cymene	$C_{10}H_{14}$	
γ-Terpinene	$C_{10}H_{16}$	
1-Octanol	$C_8H_{18}O$	
Linalool	$C_{10}H_{18}O$	
d-camphor	$C_{10}H_{16}O$	
Trimethyl-3-cyclohexene-1-carboxaldehyde	$C_{10}H_{16}O$	
Decanal	$C_{10}H_{20}O$	
Cuminaldehyde	$C_{10}H_{12}O$	
2-Decenal	$C_{10}H_{18}O$	
Thymol	$C_{10}H_{14}O$	
1-Propanol, 2-methyl-1-phenyl	$C_{10}H_{14}O$	
***trans*-2-dodecenal**	$C_{12}H_{22}O$	
Tetradecanoic acid	$C_{14}H_{28}O_2$	

Constituent	Formula	Structure
Phthalic acid	$C_8H_6O_4$	
Dibutyl phthalate	$C_{16}H_{22}O_4$	
Tetradecanoic acid	$C_{14}H_{28}O_2$	
Diisobutyl phthalate	$C_{16}H_{22}O_4$	
Palmitic acid	$C_{30}H_{60}O_2$	

This study was detected camphor (2.12%) which was lower than that reported by Ramdan and Abd Algader, they were studied Sudanese *Coriander sativum* oilsand found its percentage (15.5%), and the both studies have similar α pinene percentage (1.76%). The present study also was revealed lower percentage of γ-terpinine (10.96%) and linalool (13.98%) when it compared with those percentages of Baba, 1991 who was studied Bangaldish coriander oil and reported (14.4%) and (37.7%) for two compounds respectively. the chemical composition of essential oil can vary according to the geo-climatic location and growing conditions (soil type, climate, altitude and amount of water available), season (for example or after flowing), and time of day when harvesting is achieved. The chemical composition of oil, both quantitative and qualitative, differs according to the extraction technique for example steam distillation method oils rich in terpene hydrocarbons, in contrast the supercritical extracted oils contained a higher parcentage of oxygenated compounds (Donelian,*et al*., 2009).

3.2. Effect of coriander oil on chemical properties:

3.2.1: Pure sunflower oil

3.2.1.1. Comparison between sunflower and coriander oil:

The chemical properties; peroxide acid (PV), acid value (AV) and saponification value (SV) of extracted oil and sunflower oil were evaluated, table (3-3) show that.

Table (3-3): Chemical properties values of sunflower and coriander oil

Oil type	PV	AV	SV
Sunflower oil	2.82	0.93	184.60
Coriander oil	0.42	2.84	187.28

The coriander oil has acid and saponification value higher than that of sunflower that may be attributed to plenty of free fatty acids and double bonds respectively in coriander oil rather than sunflower oil. On the other hand sunflower has peroxide value higher than coriander oil and this could be explained due to presence of antioxidant compounds in the coriander oil.

3.2.1.2. Effect of coriander oil on the chemical properties:

The effect of coriander oil on the chemical properties of sunflower oil was determined by studied the storage (75 day) and added coriander oil factors. To done that the following samples were prepared; pure sun flower oil sample and three mixtures oils which were composed of same three samples of pure sunflower sample plus coriander oil 0.1, 0.3 and 0.5 ml separately and respectively. Then the chemical properties were assessed at the beginning, during (every 15 days) and at the end of storage for four samples that mentioned before.

3.2.1.2.1.Peroxide Value:

Effect of coriander extracts on the primary oxidation of sunflower oil was measured by determined of peroxide value. Four samples were subjected to storage process and the peroxide value was investigated at the beginning, during (five intervals) and at the end of storage, table (3-4) illustrates that.

Table (3-4): Peroxide value of four samples on storage period

Storage period	**Peroxide value**			
	Sunflower oil	***Mixture oil***		
		Sample +0.1 ml	**Sample + 0.3 ml**	**Sample + 0.5 ml**
Before storage	2.82	2.74	2.54	2.48
After 1st s. p.	4.96	4.70	4.42	4.13
After 2nd s. p.	6.82	5.30	5.00	4.80
After 3rd s. p.	8.53	5.91	5.74	5.20
After 4th s. p.	10.05	6.73	6.48	6.20
After 5th s. p.	10.76	8.95	8.50	8.26
p. value	< 0.05	< 0.05		

s.p.= storage period

Peroxide value of sunflower oil had been significant increasing ($P<0.05$) with storage periods from 2.82 at the beginning of storage to 10.76 at the end of storage which indicated the occurrence of oxidation process as a result of storage. Sunflower oil is softer oil and more susceptible to oxidation because it contains double bond of unsaturated fatty acids that became aldehyde, ketones and peroxides, and when peroxides concentration reached a certain level, complex chemical changes occurred and volatile oil products were formed.

After addition of coriander oil (0.1 ml) the present study appeared that there was clear decreased in peroxide value from 2.82to 2.74 at the beginning of storage and from 10.76to 8.95 at the end storage period, this result was reflected high influence of coriander oil on the peroxide value of edible oil (sunflower oil). On the other hand, the study also revealed that the increase in the addition (0.3 and 0.5ml) volume of coriander oil was followed by more decrease in the peroxide value (2.82, 2.74, 2.54 and 2.48) and (10.76, 8.95, 8.50 and 8.26) at the beginning and the end respectively with presence of significant difference (p □ 0.05) for added volume. These results could be explained due to that coriander oil is good source of high antioxidant activity compounds such as monoterpene, alcohols, polyphenols and phytochemicals in coriander oil which serve as potential antioxidants.

3.2.1.2.2.Acid value:

Acid value is a very important parameter for evaluating the quality of oils, as it represents the free content of fatty acids due to enzymatic activity.

The acid value of four samples was determine through the storage period and our results were appeared the following in table (3-5).

Table (3-5): Acid value of four samples during storage period

Storage period	Acid value			
	Pure SunflowerOil	*Mixture oil*		
		Sample + 0.1 ml	**Sample + 0.3 ml**	**Sample + 0.5 ml**
Before storage	0.93	0.17	0.16	0.15
After 1st s. p.	0.45	0.38	0.33	0.31
After 2nd s. p.	0.59	0.45	0.39	0.38
After 3rd s. p.	0.81	0.50	0.48	0.41
After 4th s. p.	1.09	0.52	0.51	0.49
After 5th s. p.	1.57	0.62	0.61	0.60
p. value	< 0.05	< 0.05		

This parameter can be used to verify the level of oxidizing degradation of oil from enzymatic or chemical oxidation. Acid value depends on the carboxyl group in the fat. In general our study was found that the acid value of pure sunflower oil was increasing with storage period from (0.93) at the beginning to (1.57) at the end of storage period. When the three different volumes were added, the three mixtures values were reduced at the beginning until the end of storage (0.17, 0.16 and 0.15) and (1.57, 0.62, 0.61 and 0.60) for 0.1, 0.3 and 0.5 ml respectively. The effect of added volume was observed on clear reduction that appeared with increasing of coriander oil

volume. This may be attributed to the storage effect during which the oxidation process was occurred in sunflower oil result in increasing of free fatty as well as acid value. Free fatty acids were reacted with alcoholic and phenolic compounds in coriander oil to esters formation that may be decline free fatty acids and then acid value.

3.2.1.2.3.Saponification value:

Our study showed that the saponification value of pure sunflower oil sample was 184.00 and after addition of three volumes of coriander oil (0.1, 0.3 and 0.5) the saponification value became 189.95, 190.65 and 193.68 respectively, these values revealed the effect of volume addition which act to increasing the saponification value. At the end of storage the saponification value were 229.93, 200.19, 200.79 and 200.79 for pure and mixtures respectively. There was clear increasing when were compared with beginning values. Significant difference (0.05) was calculated for storage and added volume, table (3-6) represent that.

Table (3-6): Effect of coriander oil on saponification value of sunflower oil

Storage period	**Saponification value**			
	Sample of Sunflower	***Mixture oil***		
		Sample + 0.1 ml	**Sample + 0.3 ml**	**Sample +0.5 ml**
Before storage	184.00	189.95	190.65	193.68
After 1st s. p.	188.98	190.66	191.91	194.00
After 2nd s. p.	199.17	19433.	195.33	196.36
After 3rd s. p.	217.25	196.33	196.66	197.37
After 4th s. p.	220.73	208.33	210.12	210.71
After 5th s. p.	229.93	200.19	200.68	200.79
p. value	< 0.05	< 0.05		

This might be explained due to the fact that coriander oil components as alcohols, phenols and acids were reacted with carboxylic and hydroxylic compounds in edible oil respectively which increase the triacylglycerol and so the saponification value. On the other hand the free fatty acids of coriander oil may have lower molecular weight than free fatty acids of sunflower oil.

3.2.2.Effect of coriander oil on re-used oil:

To determine the effect of coriander oil on the reused oil properties, new sample of sunflower oil was subjected for repeated cooking processes (reused oil), then the sample was prepared to assess its chemical properties before and after addition 0.5ml (mixture). Table (3-7) illustrated the chemical properties of pure sunflower, reused and mixture oil.

Table (3-7):Effect of coriander oil on the reused oil

Sample of oil	Chemical properties		
	POV	**AV**	**SP**
Fresh sample oil	2.64	0.42	184.60
Reused oil	3.59	0.51	187.28
Reused oil + 0.5 ml	2.83	0.44	184.51
P value	□ 0.05	□ 0.05	□ 0.05

One of the major problems in high lipid product in food industry is rancidity resulting in undesirable flavor changes and decline in nutrients leading to change in their texture and appearance. Lipid peroxidation causes oxidative stress, resulting in the development of rancidity, unpleasant taste and odors as well as changes in color and losses related to nutritional value, use of antioxidants reduces oxidative rancidity (Bhanger,*et al.*, 2007).The study showed a definite impact of extracted coriander oil on the sunflower oil that was used several times in cooking (p □0.05). There was an apparent drop in chemicals properties values of reused oil after the addition of coriander oil when it compared with reused oil values before the addition, the values before and after the addition were;(3.59- 2.63), (0.51-0.44), (187.28-184.51) for peroxide value, acid value, and saponification value respectively, this was mainly attributed to the antioxidant compounds in the coriander oil so that little coriander oil addition may greatly serve the purpose. Darughe,*et al*. 2012,were studied the antioxidant effects of coriander oil in cake, they were found that antioxidant effect may be due to the presence of terpenoid components (camphor, limonene, α-pinene and geraniol). This result was reflected the high impact of coriander oil on the decline reused oil (especially peroxide value) and improves its chemical properties and thus reduces the excess severity on human health.

3.3. Antibacterial activity of coriander oil:

The antibacterial activity of coriander oil was tested according to (Teshale,2013). with miner modification against six bacterial strains.The anitibacterial activity include three gram-positive reference strains were(*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923and *Enterococcus faecalis* ATCC 29212), whilst three gram-negative reference strains were (*Escherichia coli* ATCC 25922,*Salmonella typhimurium* ATCC 13311 and *Pseudomonas aeruginosa* ATCC 27853).

All bacterial strains were stored in Brain Heart Infusion (BHI) broth with 20 % (v/v) glycerol at 280^{0}C. Prior to susceptibility testing, each strain was inoculated on BHI agar to ensure optimal growth and purityplating suitable. Four different concentrations (12.5%, 25%, 50% and 100%) of the oil were dissolved in dimethyl sulfoxide (DMSO)in triplicates using nutrient agar medium. The pH was adjusted to (7.4 - 7.6) for bacteria. The plates were incubated at 37 ^{0}C for 24 hour, and then the commercial antibiotic, gentamicin (10µg) was used as the positive control. The diameter of inhibition zones were measured, the obtained results were represented in figures (3-2, 3-3, 3-4, 3-5, 3-6 and 3-7) and table (3-8).

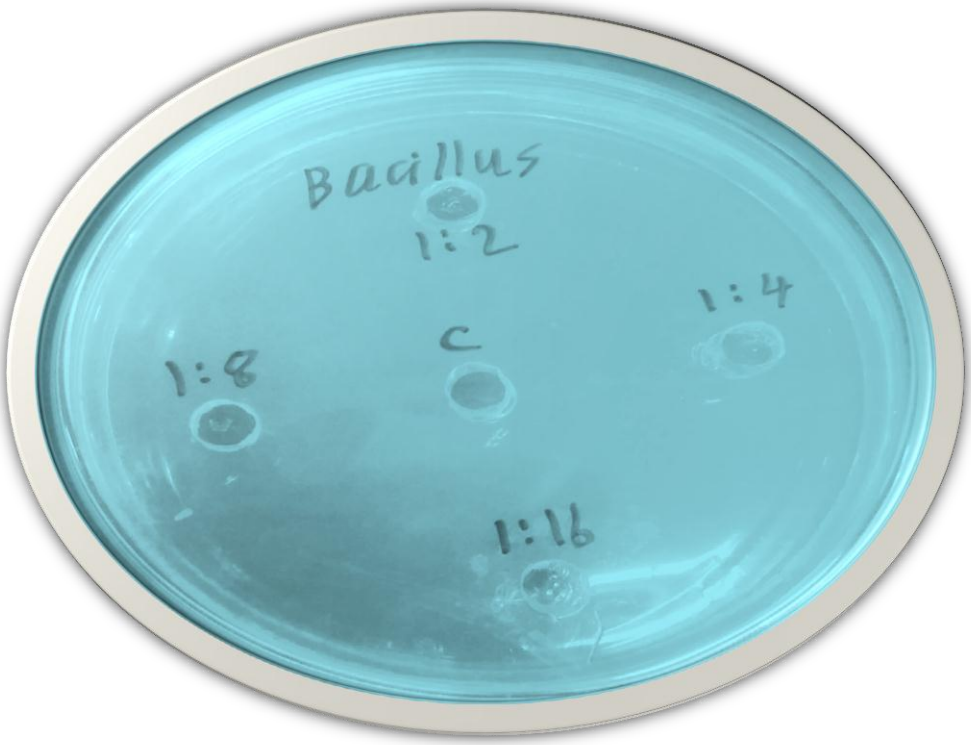


Figure (3- 2): Effect of coriander oilon *Bacillus cereus* ATCC 11778

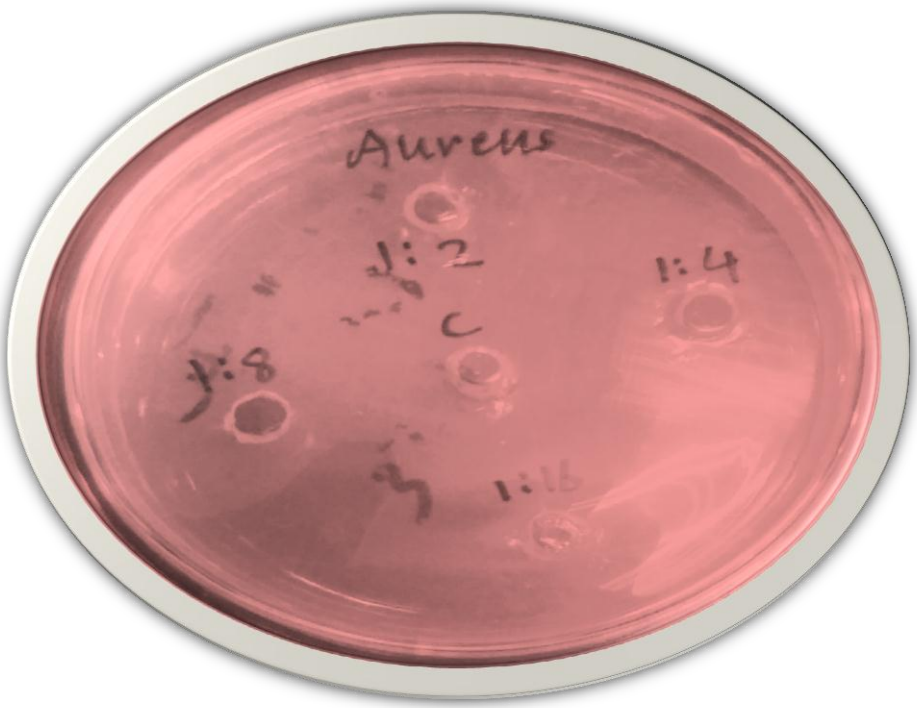


Figure (3- 3): Effect of coriander oilon *Staphylococcus aureus* ATCC 25923

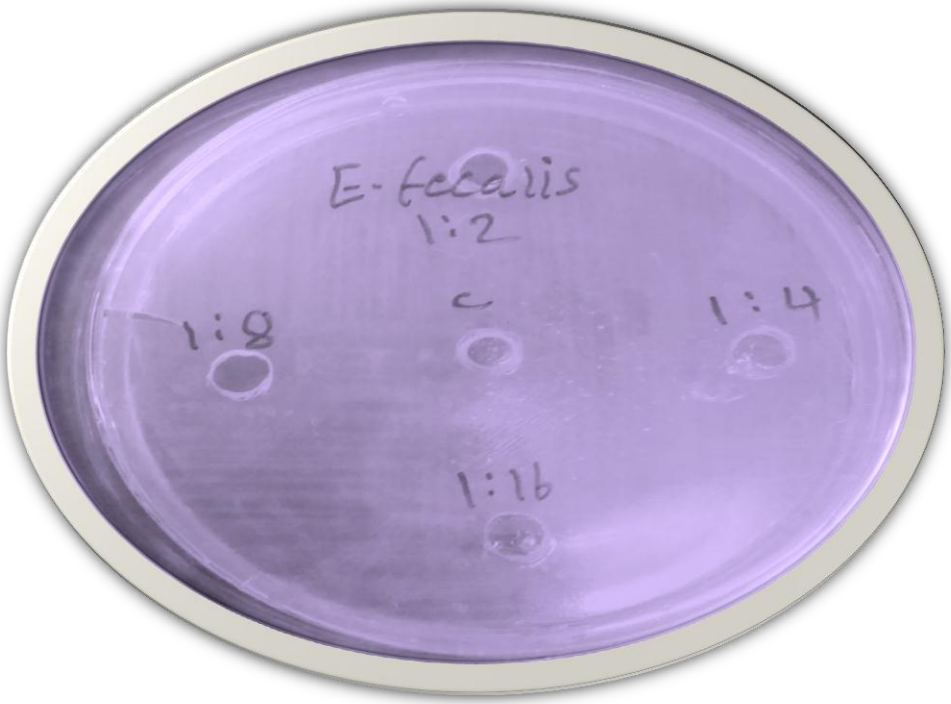


Figure (3- 4): Effect of coriander oilon *Enterococcus faecalis* ATCC 29212

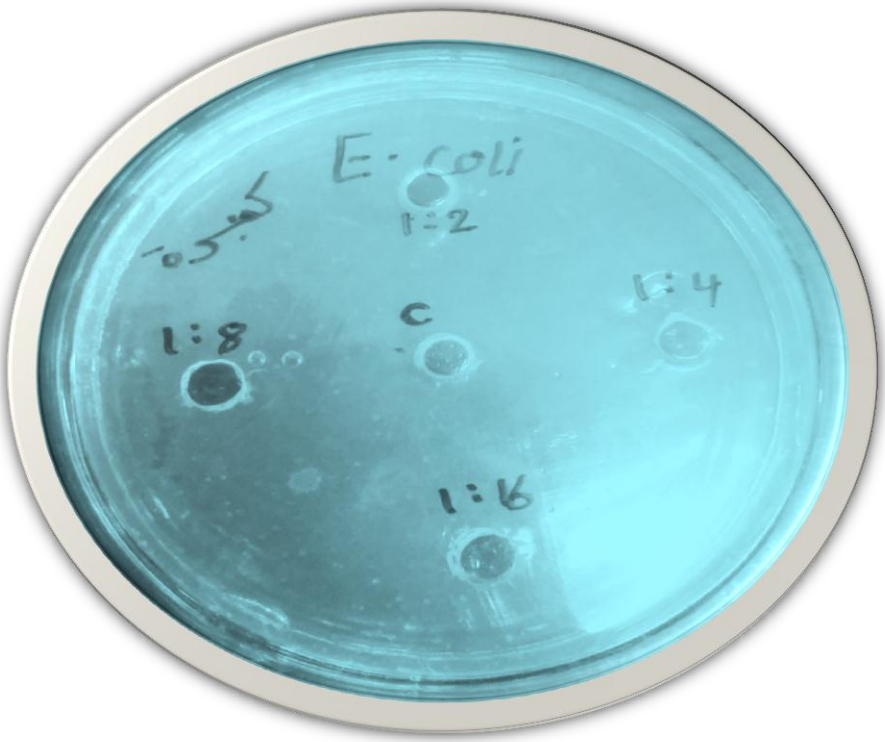


Figure (3- 5): Effect of coriander oilon *Escherichia coli* ATCC 25922

Figure (3- 6): Effect of coriander oilon *Pseudomonas aeruginosa*ATCC 27853

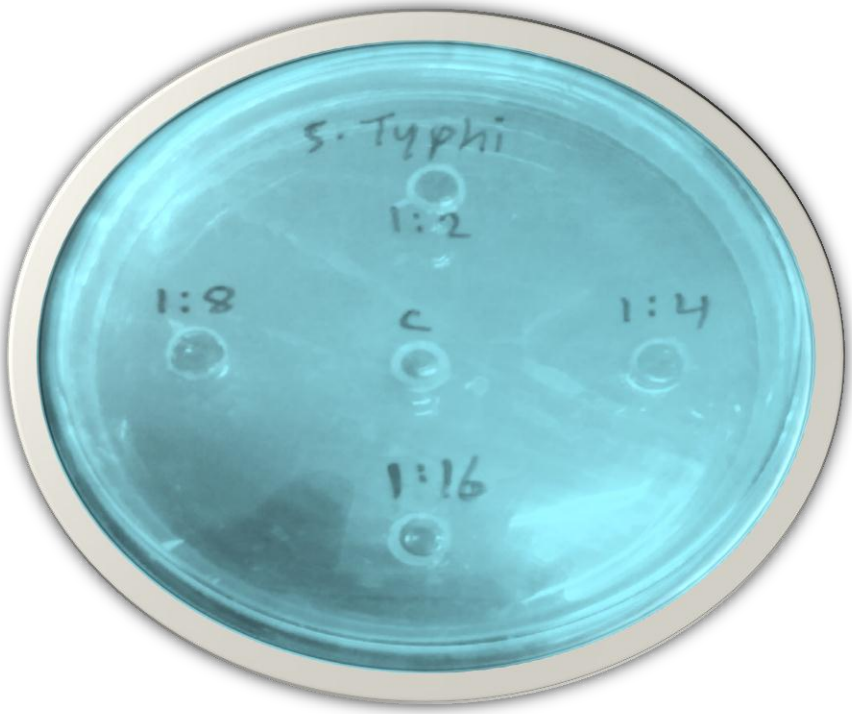


Figure (3- 7): Effect of coriander oilon *Salmonella typhimurium* ATCC 13311

Table (3-8): Mean Inhibition zones diameter MIZD in mm of coriander oil against six bacteria types with positive control Gentamicin

Tested Bacteria	Type of bacteria	MIZD in mm				
		12.5%	25%	50%	100%	Gentamicin
Bacillus cereus	**Positive**	18	20	23	27	30
Staphylococcus aureus	**Positive**	33	36	39	40	32
Enterococcus faecalis	**Positive**	0	14	17	20	25
Escherichia coli	**Negative**	15	18	21	23	27
Salmonella typhimurium	**Negative**	0	13	18	20	30
Pseudomonas aeruginosa	**Negative**	18	19	21	23	35

In general, the was revealed that the coriander oil appeared higher influence on the positive gram strain rather than negative gram strain, this might be due to that the gram positive strains are more sensitive and potential of hydrophobic essential oils to disrupt the bacterial cell membrane and its structure leading to ion leakage. The antibacterial avtivity of coriander oil showed highly activity against *Staphylococcus aureus* at all used concentration 12.5% ,25%,50% ,100% with mean of inhibition zone diameter (33,36,39,and 40) mm respectivlly compared with the antibiotic gentamicin. the crud extrated oil of coriander oil showed variable and good results against the used refernce bacterial strains. The results showed that all tested concentrations of coriander oil as antibacterial activity against gram positive and gram negative bacteria; *Salmonella typhimurium*and*Enterococcus fecalis*respectively were appeared resistance at the concentrations 12.5% .In addition the concentration 100% inflicted the highest antibacterial activities. The most susceptible bacteria strains was *Staphylococcus aureus* with highest inhibition zone values (33mm) at concentration 25% and 100% (40mm).On the other hand, the tested concentrations 25% and 50% of volatile oil showed high inhibition zone against*Bacilluscereus* and *Proteus vulgaris* (20mm)and (23mm) compared with tested concentrations. Previous study had shown that coriander oil caused highest inhibitory zones (23mm) against Salmonella sp compared with E. coli and P. aeruginosa (18 and 10 mm) respectively (Wangensteen, (2004).

CHAPTER FOUR

CHAPTER FOUR

4. Conclusion and Recommendations

4.1. Conclusion:

This study was designed to determine the effect of coriander extract on the chemical properties of storage and reused sunflower oil. Coriander oil was extracted by water steam distillation process then GC-MS was used to determine the chemical profile of coriander oil which revealed that the coriander seeds oil contains 24 compounds were;Heptanal, α-Thujene, α-pinene, Camphene, β-Phellandrene, β-pinane, p-Cymene, γ-Terpinene, 1-Octanol,Linalool, Camphor, Trimethy-cyclohexene carboxaldehyde, Decanal, Cuminaldehyde, *cis*-2-Decenal, Thymol, 1-Propanol, 2-methyl-1-phenyl, *trans*-2-dodecenal, Tetradecanoic acid, Phthalic acid, Dibutyl phthalate, Tetradecanoic acid, Diisobutyl phthalate,Palmitic acid. The chemical properties (peroxide, acid and saponification value) of sunflower oil were examined before, during and at the end of storage, likewise after addition of coriander oil. The coriander oil was appeared significant clear effect (p 0.05) on chemical properties of both storage and reused sunflower oil .The study was attributed that the coriander extract isrichly oil in antioxidants such as monoterpens and sesquiterpenes which serves to decline the high autoxidation of sunflower oil. The study also was upon to detect the antibacterial activity of coriander oil through investigation of six types of bacteria (three positive grams strain and three negative grams strain). The study found that coriander oil has positive effect on bacteria especially gram-positive strain, *Staphylococcus aureus* mainly,thus coriander oil can be used in pharmaceutical products (therapeutic work). In general, natural products of coriander and their extracts can be used widely as an antioxidant to autoxidation of oils and fats in foods.

4.2. Recommendations:

By studying the effect of coriander oil, the present study recommends the following:

- Coriander oil is a good source for preservation of fats and oil due to the plenty of antioxidant compounds.
- Education and awareness community about seriousness of reused oil in cooking foods especially fast food.
- Coriander has antibacterial activity, thus can apply this advantage in antibiotic production against bacterial inflammatory.
- Support the related research of aromatic plants due to their importance in human life.
- In the light of our result of this study further research can be conducted in this field to detect other benefits of coriander extract.

CHAPTER FIVE

5. References and Appendix

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5.2. Appendices

Appendix 1: GC-MS mode

Un Of KH
GCMS-QP2010Plus
Sample Scan by GC-MS EI

Sample Information

Analyzed	: 2/18/2016 9:54:12 AM
Sample Type	: Unknown
Sample Name	: 1
Data File	: C:\GCMSsolution\shendi_feb\Sample 1_18FEB.qgd
Method File	: C:\GCMSsolution\shendi_feb\essential_oil.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\17_feb 2016.qgt
Admin	

(1)

Method

[Comment]

——— Analytical Line 1 ———

[GC-2010]

Column Oven Temp.	:35.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:61.8 kPa
Total Flow	:244.2 mL/min
Column Flow	:1.20 mL/min
Linear Velocity	:39.4 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:200.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	35.0	3.00
5.00	240.0	0.00
3.00	280.0	4.00

< Ready Check Heat Unit >

Column Oven	: Yes
SPL1	: Yes
MS	: Yes

< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >

SPL1 Carrier	: Yes
SPL1 Purge	: Yes

< Ready Check APC Flow >
< Ready Check Detector APC Flow >

External Wait	:No
Equilibrium Time	:3.0 min

[GCMS-QP2010 Plus]

IonSourceTemp	:200.00 °C
Interface Temp.	:250.00 °C
Solvent Cut Time	:3.50 min
Detector Gain Mode	:Relative
Detector Gain	:0.00 kV
Threshold	:0

[MS Table]

--Group 1 - Event 1--

Start Time	:4.00min
End Time	:61.33min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1666
Start m/z	:35.00
End m/z	:800.00

Sample Inlet Unit :GC

Appendix 2: apparatus

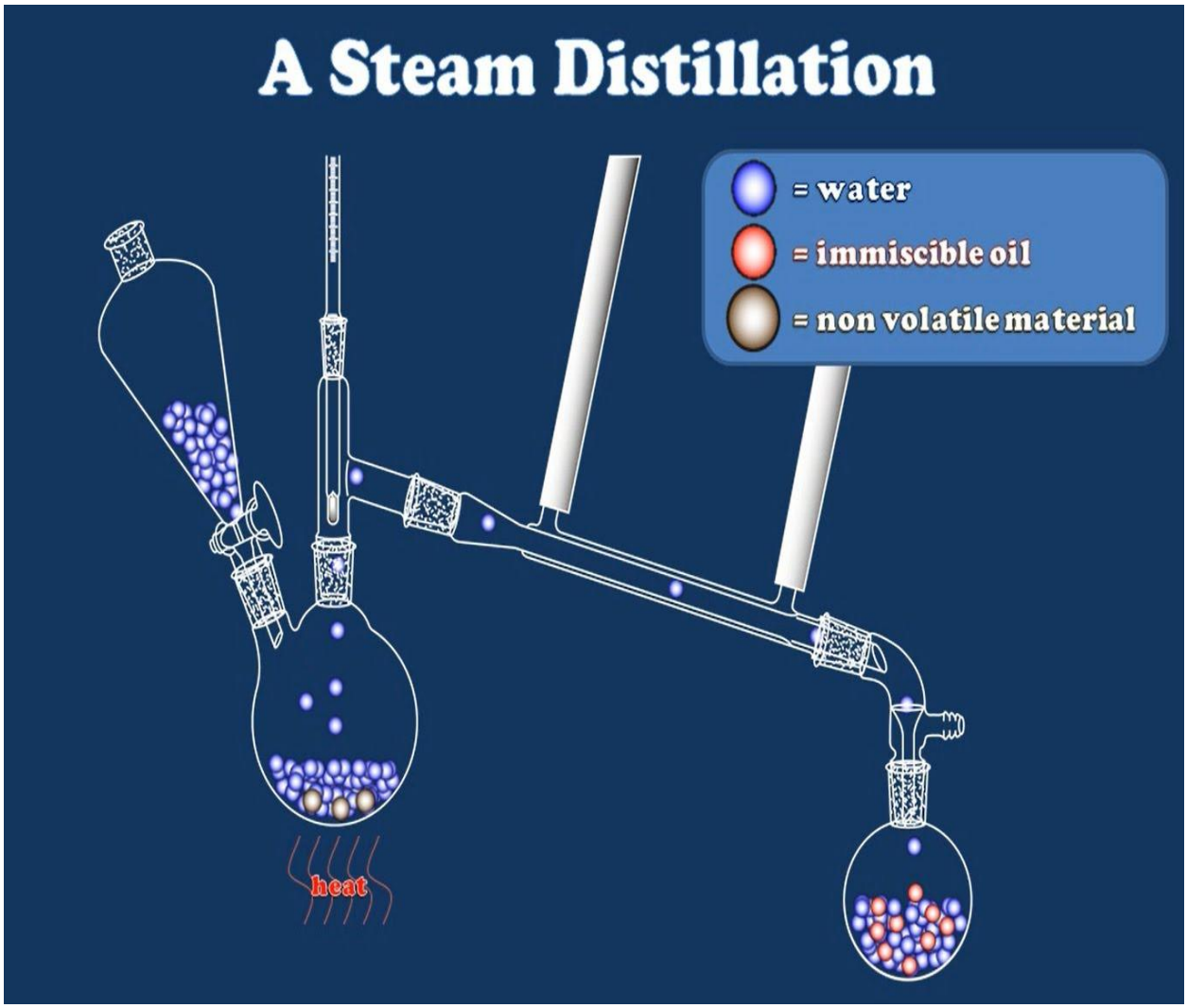

Figure (5-1). Distillation apparatus

Figure (5-2). GC-MS apparatus