Shendi University
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Effect of *Cuminum Cyminum* oil on the edible oil (Sunflower oil) properties and antibacterial activities

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

Submitted by:

Amina Abdelrhim Belal Mohamed

B.Sc. (Science, Chemistry and Zoology, 2011)

Supervisor:

Dr. Faroug Bakheit Mohamed Ahmed Elsonni

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بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخۡرَجۡنَا بِهِۦ نَبَاتَ كُلِّ شَىۡءٖ فَأَخۡرَجۡنَا مِنۡهُ خَضِرٗا نُّخۡرِجُ مِنۡهُ حَبّٗا مُّتَرَاكِبٗا وَمِنَ ٱلنَّخۡلِ مِن طَلۡعِهَا قِنۡوَانٞ دَانِيَةٞ وَجَنَّٰتٖ مِّنۡ أَعۡنَابٖ وَٱلزَّيۡتُونَ وَٱلرُّمَّانَ مُشۡتَبِهٗا وَغَيۡرَ مُتَشَٰبِهٍۗ ٱنظُرُوٓاْ إِلَىٰ ثَمَرِهِۦٓ إِذَآ أَثۡمَرَ وَيَنۡعِهِۦٓۚ إِنَّ فِي ذَٰلِكُمۡ لَأٓيَٰتٖ لِّقَوۡمٖ يُؤۡمِنُونَ ﴿٩٩﴾ ﴾

صدق الله العظيم

سورةالأنعام الآية (99)

DEDICATION

- *To my parents who taught me that the means to ends is patience.*

- *To all who stood beside me and pushed me ahead particularly my sisters*

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LIST OF ABBREVIATIONS

AOAC Association of Official Analytical Chemists
ASTM American society for testing and materials
AV Acid value
BPCBritish 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
NTP National toxicology program
PVPeroxide value
NRA National renderers association
SV Saponification value

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ABSTRACT OF THE STUDY

This study was designed to determine the chemical profile of *Cuminum Cyminum L*(green cumin) oil and its effect on the physical and chemical properties of sunflower oil and detection the antibacterial activity of this oil. Laboratory investigation; oil extraction, gas chromatography analysis, physiochemical assess and antibacterial activity were done to fulfillment of the study objectives.

Green cumin oil was extracted from its mature seeds by water-steam distillation process and the extracted oil was analyzed by GC-MS to determine its chemical profile. The GC-MS analysis of cumin oil showed that eleven constituents were identified; seven hydrocarbon monoterpens (33.09%) and four oxygenated monoterpens (66.91%). The monoterpens were α-Thujene 0.41%, α-pinene 0.90%, β-pinene 10.72%, β-myrcene1.27%, α-phellandrene 1.18%, *p*-cymene 3.54% and γ-terpinene 15.07%, and oxygenated monoterpens identified were cumin aldehyde 21.10%, Carboxaldehyde 5.34%, 2-Caren-10-al 17.74% and cumin alcohol 22.65%.

The physiochemical properties of sunflower oil were tested before and after addition of extracted cumin oil.The cumin oil had positive effect on the physiochemical properties values; specific gravity, viscosity and color, and peroxide, acidity, free fatty acids and iodine value. There were significant differences for the these properties; ($p < 0.05$) for physical properties and $p < 0.001$ for peroxide value, $p < 0.05$ for acid and free fatty acid value, and $p < 0.005$ for iodine value. The study was attributed the high effect of cumin oil on the physical and chemical properties mainly to antioxidant constituents in the extracted cumin oil such as monoterpens. Cumin oil had high impact on the chemical properties of reused oil which was appeared clear significant difference ($p < 0.001$) between chemical properties of reused edible oil before and after addition of cumin oil.

To assessment of the antibacterial activity of cumin oil, six types of bacteria (four gram positive and two gram negative) were prepared in the laboratory of Shendi University. The screening was carried outusing the cup-plate agar diffusion method at four different concentrations (12.5%, 25%, 50% and 100%). The results showed that all tested concentrations of cumin oil showed antibacterial activity against gram positive and gram negative bacteria. The most susceptible bacteria

strains was *Salmonella typhi*with highest inhibition zone values (30mm) at concentration 25% and 100%.

Cumin oil contains number of antioxidants compounds that will make it good source in preservation of foods and oils against rancidity oxidative durin storage. Cumin oil also contains antiseptic, analgesic anti-inflammatory and anti-bacterial constituents so that can be used in the preparation of medicinal drugs, principally, the study was showed it has high impact on the bacteria that causes typhoid disease.

ملخص الدراسه

صممت هذه الدراسة لتحديد المكونات الكيميائية للزيت المستخلص من نبات الكمون الأخضر ، وتأثير هذا الزيت على الخواص الفيزيائية والكيميائية لزيت زهرة الشمس، وكذلك الكشف عن نشاط زيت الكمون كمضاد للبكتيريا. تم إستخلاص الزيت من بذور الشمار الناضجه بواسطة عملية التقطير بالماء والبخار وأجريت بعد ذلك عملية تحليل للزيت الناتج بواسطة جهاز التحليل الكروماتوغرافى الغازى.

أوضح التحليل أن الزيت المستخلص من الشمار يتكون من أحد عشر مركباً سبعة منها عبارة عن تربينات أحاديه كانت نسبتها (33.09%) وتشمل: الفا ثيوجين ، ألفا بينين ، بيتا بينين ، بيتا ميرسين ، الفا فيلاندرين ، بارا سيمين و قاما تربينين. أما المركبات الأربعة الأخرى فكانت عبارة عن تربينات أحاديه أكسجينية كانت نسبتها (66.91%) وتشمل ألدهيد الكيومين ، ألدهيد كاربوكسيلى ، كارين-2-أل-10 وكحول الكيومين .

إختبرت الخواص الفيزيائيه والكيميائيه لزيت زهرة الشمس قبل وبعد إضافة زيت الكمون وكان لزيت الكمون تأثير إيجابي على الخواص الفيزيائيه (الثقل النوعي ، معامل الانكسار ،اللزوجه) والكيميائيه (رقم البيروكسيد ، الحمضيه ، والأحماض الدهنية الحره ورقم اليود) وقد وجد أن هنالك فرق معنوي في هذه الخواص حيث كانت ($P< 0.05$) للخواص الفيزيائيه ، اما الكيميائية فقد كانت للبيروكسيد ($P < 0.001$) والحمضية والأحماض الدهنية الحره ورقم اليود ($P < 0.05$). وأعزت الدراسة التأثير الواضح لمستخلص زيت الكمون الأخضر – على الخصائص الفيزيائية والكيميائية – في المقام الأول إلى مركبات مضادات الأكسدة الموجودة في الزيت المستخرج من الكمون مثل التربينات الأحادية. كما كان لزيت الكمون الأثر العالى على الخصائص الكيميائية للزيت المكرر إستخدامه فى عمليات طهى متعدده حيث أظهرت الدراسه فرقاواضحا بينقيم الخواص الكيميائية لزيت الطعام المكرر الاستخدام قبل وبعد إضافة زيت الكمون ($p < 0.001$).

لتقييم النشاط البكتيري لزيت الكمون(الشمار الأخضر) تم تجهيز ستة أنواع من البكتيريا (أربعة موجبة صبغ جرام و إثنتين سالبة صبغ جرام) وأجري الفحص بواسطة طريقة الأجار باستخدام أربعة تراكيز مختلفة (12.5٪، 25٪، 50٪ و 100٪). وأظهرت النتائج أن جميع تراكيز زيت الكمون الأخضر لها نشاط مضاد للبكتيريا التي تم إختيارها كعينة للدراسة.

زيت الكمون الأخضر يحتوي على عدد من المركبات المضاده للاكسدة والتي تعمل كمصدر جيد لحفظ الاطعمه والزيوت طوال فترة التخزين ، أيضا يحتوي على على مضادات الالتهاب والفيروسات والبكتيريا والتي يمكن إستخدامها في تحضير الأدويه، حيث أوضحت الدراسه أن للزيت المستخلص تاثيرا كبيرا على البكتيريا المسببة لمرض التايفويد .

CHAPTER ONE

1. Introduction and literature review

1.1. Introduction:

Cuminum Cyminum L (Green cumin) is an herbaceous and medicinal crop and one of the oldest and popular seed spice worldwide after black pepper (Divakara and Anandara, 2013). The term ‘spice’ originated from the Latin word ‘species’, meaning of specific kind. A closely related term, ‘herb’, is used to distinguish plant parts finding the same uses but derived from leafy or soft flowering parts. The two terms may be used for the same plants in which the fresh leaves are used as herbs, while other dried parts are used as spices. (Nazeeem, 1995).

Green cumin is popularity spread from Latin America to Africa and all over Asia (Anonymous, 2008). Although cumin was originally cultivated in Iran, where it is one of the most important export crops, and Mediterranean region but today it is also grow in Uzbekistan, Tajikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Bulgaria, Cyprus and Chile, where India is the largest producer and consumer of cumin seed in the world (Elkamali, 1991).The estimated world production is around 300,000 tons (Divakara and Anandara, 2013). In fact, green cumin is one of the most important crops in terms of exports, income, water use efficiency and reclamation of the arid and semiarid regions (Tuncturk and Tuncturk 2006).

In Sudan, cumin spread up the Nile valley where it continues to be sown by stallholders in the winter season to provide flavoring and other parts of the country. The production is between 0.9 – 1.4 tons in 2013 – 2014 according to ministry of agricultural statistic report, 2014. In the Sudan, Despite the relative importance of this medicinal plant in crop rotation, it has not adequately studied and there is no much information on potential yield of the current cultivated plant worldwide (Katan, *et al*., 2011).

Spices are generally composed of fiber, carbohydrate, fat, sugar, protein, gum, ash, volatile (essential oils), and other nonvolatile components. All of these components impart each spice’s particular flavor, color, nutritional, health, or

preservative effects. The essential oils in spices are generally composed of terpenes or terpene derivatives, oxygenated derivatives of hydrocarbons, benzene compounds and nitrogen- or sulfur-containing compounds (Raghavan, 2007). Cumin seed is generally used as a spicy food in the form of powder for imparting flavor to different food preparations and it also has a variety of medicinal properties (Kafie, *et al*., 2002).

Sunflower oil is nonvolatile oil compressed from the sunflower seeds (*Helianthus Annuus*). In recent years, there has been an increase in demand for sunflower crops such as sunflower oil. Measures such as the development of hybrid sunflowers to increase oil production have been introduced to meet this demand (Christov, 2012).

Different physical and chemical parameters of edible oil were used to monitor the compositional quality of oils (Ceriani, *et al*., 2008). These physicochemical parameters include iodine value, acid value, free fatty acid, viscosity, color, refractive Index and peroxide value (Mousavi, *et al*., 2012). Edible oils are one of the main constituents of the diet used for cooking purposes. Several researchers studied the impact of temperature on the stability, viscosity, peroxide value, and iodine value to assess the quality and functionality of the oil (Li, *et al*., 2011).

The analysis of essential oils was developed in parallel with the technological developments in gas chromatography. The most outstanding improvements in the determination of the composition of extracted oil came from the introduction of tandem techniques involving prior or further chromatography or spectroscopy. The amount of information on the application of gas chromatography and hyphenated to essential oils has led to much research in this field.

Objectives of the study:

The present study aims to:

- Determine the chemical profile of *Cuminum Cyminum L.* oil.
- Determine the effect of oil of *Cuminum Cyminum L.* on the physical and chemical properties of Sunflower oil.
- Detection the antibacterial activity of cumin oil.

1.2. Literature review

1.2.1: *Cuminum Cyminum L.*:

1.2.1.1: Nomenclature and Classification:

Nomenclature:

Cuminum Cyminum L. is commonly known as cumin. The word cumin in English is derived from the Latin Cuminum, which itself was derived from Greek 'kyminon' (Rai, *et al*., 2012). Cumin is known under various names in the East Asia countries; e.g. jeera (term used in India), Safaid jeera (Bengali), Zeera (Punjabi) (Agarwal, 1996). The common names of the plant were: Arabic: Kamoun, Kamun; Roman caraway; French: Cumin, Cumin de Malte; German: Kreuzkümmel; Italian: Cumino; Japanese: Hime unikyoo; Portuguese: Cominho; Russian: Kmin; Spanish: Comino; Swedish: Spiskummin (Al-Snafi, 2015).

Classification of green cumin:

Kingdom: Plantae.
Subkingdom: Viridiplantae.

Infrakingdom: Streptophyta.

Superdivision: Embryophyta.

Division: Tracheophyta.

Subdivision: Spermatophytina.

Class: Magnoliopsida.

Superorder: Asteranae.

Order: Apiales.

Family: Apiaceae.

Genus: Cuminum.

Species: Cuminum Cyminum.

1.2.1.2: Plant Description and distribution:

Cumin is a small flowering herbaceous plant belonging to the Apiaceae, in the genus. The cumin plant grows to 30–50 cm tall and is harvested by hand. It is an annual plant, with a slender, branched stem 20–30 cm tall. It has blue-green

linear leaves and finely separated. The white or pink flowers are borne in small compound umbels (Weiss, 1996).

Cumin is grown from seed and a hot climate is ideal, but it can be grown in cooler regions if started under glass in spring. Sandy soil is most excellent; when the seedlings have hardened, transplant carefully to a sunny aspect, planting out 15 cm apart. The plants bloom in June and July. The seeds are normally ready four months after planting. Cut the plants when the seeds turn to brown, thresh and dry (Cupboard, 2004). The major constraints facing the production of cumin worldwide are losses caused by diseases, insects, and weeds (Chand, *et al*., 1999).

Figure (1-1): Seeds of green cumin (Agrawal, *et al*., 2001)

1.2.1.3: Physicochemical characteristics:

The main physiochemical characteristics of green cumin are; moisture content: 8%, PH: 7.3, total ash: 7.5, acid insoluble ash: 18%, alcohol soluble extractive: 6.58%, water soluble extractive: 138% and ether soluble extractive: 11.44 ± 0.20 and 12.36 ± 0.23% in the wet and dry fruits (Rai, *et al*., 2012). Physical properties of the essential oil of cumin seeds: extraction percentage; 2.3-5.7 %, color: colorless or pale yellow, refractive index (20 ºC): 1.47-1.50 and density (20 ºC): 0.90 - 0.94 (Gohari and Saeidnia, 2011). The chief components of the characteristic aroma of unheated whole seeds are *p*-menthen-7al and cuminaldehyde in combination with other related aldehydes (Wang and Jones, 2004).

1.2.1.4. Chemical constituents of green cumin:

Green cumin seeds yield, crude protein 18.40 ± 0.16 and 19.88 ± 0.20%, crude fibers 21.82 ± 0.13 and 23.57 ± 0.13%, total carbohydrate 55.58 and 60.05% in the wet and dry fruits respectively (Moawad, *et al*., 2015). In addition, the seeds yield about 22% fats, numerous free amino acids, and a variety of flavonoidglycosides, including derivatives of apigenin and luteolin (Ishikawa, *et al*., 2002). Cumin seeds contain up to 5% of a volatile oil composed primarily of aldehydes up to 60%. The cuminaldehyde content varies considerably, depending on the source of the oil. Monoterpene hydrocarbons are another major component of the oil; sesquiterpenes are minor constituents (Gagandeep, *et al*., 2003 and Takayanagi, *et al*., 2003).

Phytochemicals analysis showed that *Cuminum Cyminum* contained: alkaloid, anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid (Rai, *et al*., 2012). Cumin also contains very good amounts of B-complex vitamins such as thiamin, vitamin B6, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C. The seeds are rich source of many flavonoid phenolic anti-oxidants such as carotenes, zeaxanthin and lutein (Chand, *et al*., 1999). It also contains safrole, a mutagen, which is degraded by cooking(Li and Jang, 2004). Organic acids (aspartic, citric, malic, tartaric, propionic, ascorbic, oxalic, maleic and fumaric acids) were isolated from seeds of cumin (Hashum and Al-Hashemi, 2014).

In more detail, nutrient contents of cumin (in 2 g of seeds) are included: water 0.16 g; some calories: protein 0.36 g, carbohydrates 0.88 g, dietary fiber 0.22 g, total fat 0.44 g, saturated fat 0.04 g, monounsaturated fat 0.28 g, polyunsaturated fat 0.06 g, , Ash (g) 0.16, some vitamins such as vitamin A 25.40 IU, thiamin (B_1) 0.02 mg, niacin (B_3) 0.10 mg, niacin equiv 0.10, vitamin C 0.16 mg, vitamin E 0.02 mg, folate 0.20µg and vitamin K 0.11µg; in addition it contained some minerals such as calcium 18.62 mg, copper 0.02 mg, iron 1.32 mg, magnesium 7.32 mg, manganese 0.06 mg, phosphorus 9.98 mg, potassium 35.76 mg, selenium 0.10µg, sodium 3.36 mg and zinc 0.10 mg. (Parthasarathy, *et al*., 2008).

The constituents of cumin oil were differing according to the area from which the *cuminum cyminum* samples were taken. The major compounds in the

Turkish cumin seed oil were cuminaldehyde (19.25-27.02%), p-mentha-1,3-dien-7-al (4.29 - 12.26%), *p*-mentha-1,4-dien-7-al (24.48 - 44.91%), γ-terpinene (7.06 - 14.10%), *p*-cymene (4.61 - 12.01%) and β-pinene (2.98 - 8.90%) (Baser, *et al*., 1992). Cuminaldehyde, γ-terpinene, *o*-cymene, limonene and β-pinene were determined to be the major constituents of Syrian cumin (Rihawy, *et al*., 2014).The major compounds in cumin oil of Egyptian cultivars were cumin aldehyde (35.25%), tetradecene (12.25%), γ-terpenene (12%), β-cimene (9.72%), *p*-mentha-2-en-ol (9%), α-terpinyl acetate (5.32%), α- terpinolene (3%), lmonine (0.5%), myrcene (0.2%), β-pinene (0.9%) and α-pinene (0.19%) (Moawad, *et al*., 2015). Tunisian variety of *cuminum cyminum* contained cuminlaldehyde (39.48%), gamma-terpinene (15.21%), *o*-cymene (11.82%), β-pinene (11.13%), 2-caren-10-al (7.93%), trans-carveol (4.49%) and myrtenal (3.5%) as major components (Hajlaoui,2010). Analysis of the fruit oil of *cuminum cyminum* from Delhi showed that the major constituents were transdihydrocarvone (31.11%), γ-terpinene (23.22%), p-cymene (15.8%), α- phellandrene (12.01%) and *p*-menth-2-en-7-ol (3.48%) and cuminlaldehyde constituted only 0.58% (Chaudhary, 2014). Analysis of cumin oil samples from four different German regions showed that the major compounds in all samples were monoterpenes β -pinene, *p*-cymene, γ-terpinene, the terpenoid aldehydes, cuminic aldehyde and the isomeric menthadien carboxaldehydes (Wanner, *et al*., 2010).

1.2.1.5. Uses and Pharmacological Activities of green cumin:-

Cumin had some reputation as a drug but its chief medicinal use now days in veterinary medicine. This spice is also used as a homeopathic treatment for a variety of conditions. Due to its numerous medicinal properties, cumin is used as an ingredient in many home remedies and ayurvedic preparations (Clark, 1998). In traditional herbal medicine cumin was used to treat hoarseness, jaundice, dyspepsia and mixed with other ingredients to treat diarrhea and colic (Parthasarathy, *et al*., 2008).

In America and Africa the cumin is used as an abortive and as an emmenagogue. In Indonesia, it was used in cases of bloody diarrhea and headache (paste is applied to the forehead). It was also taken orally for rheumatic ailments. In India, cumin was used as an abortifacient, for kidney and bladder stones, chronic diarrhea, leprosy and eye disease (Nitin, *et al*., 2012). In Unani system of medicine, the cumin fruits were used for the treatment of corneal

opacities, ulcers and to relieve cough and inflammation (Shivakumar, *et al*., 2010). In Sudan, although cumin is a medicinal plant, used in food industries, drinks, cosmetics and soap (Carvalho, 2004).

Oral administration of cumin for 6 weeks to diabetic rats resulted in significant reduction in blood glucose and body weight (Katan, *et al*., 2011). Cumin supplementation was found to be more effective than glibenclamide in the treatment of diabetes mellitus. In a glucose tolerance test conducted in rabbits cumin significantly increased the area under the glucose tolerance curve and hyperglycaemic peak. The pharmacologically active constituent of cumin seed oil was characterized as cuminaldehyde which inhibited aldose reductase and alpha-glucosidase isolated from rat(Srinivasan, 2005).

Cumin decreased aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activities and decreased the tissue (liver and kidney) levels of cholesterol, triglycerides and phospholipids and prevented the changes in the composition of fatty acids (Razzaghi, *et al*., 2009).

The cancer chemoprevention potential of cumin seed could be attributed to its ability to alter carcinogen metabolism (Shirke, 2008). The detoxification and chemo-preventive properties increase secretion of anti-carcinogenic enzymes from the glands. The antioxidants like eugenol and limonene present in cumin have strong anti-tumor properties. Recent research has also publicized that cumin may prevent the growth of breast and colon cancer cells (Jansen, 1981 and Weiss, 2002). The cumin oil exhibited high antioxidant activity which has been attributed largely to the presence of monoterpene alcohols, flavonoids and other polyphenolic compounds (Najda, *etal*., 2008).

Cumin seeds are reported to be estrogenic. The presence of phytoestrogens in cumin has been shown and also related to its anti-osteoporotic effects. In the animals receiving a methanolic extract of cumin, a significant reduction in urinary calcium excretion and augmentation of calcium content and mechanical strength of bones was found. Animals showed greater bone and ash densities and improved micro architecture, with no adverse effects like body weight gain and weight of atrophic uterus (Prajapati, *et al*., 2003). Cumin is a stimulant as well as a relaxant at the same time. This property cannot be attributed to a single component alone, but studies show that a proper intake of vitamins (particularly

B-complex) and a good digestion help induce a sound sleep, cumin helps both of these (Schmutterer, 2002). Cumin has vitamin-E in large quantity. Vitamin-E is good for skin and keeps the skin young and gleaming, so cumin helps to treat skin problems (Skrinjar, *et al*., 2009).

Cumin helps in digesting food properly. It is one of the best herbs for digestive sluggishness; it helps in the cure of digestion related problems that is due to cuminaldehyde which stimulate our salivary glands and this enables the primary digestion of food (Agarwal, 1996). Thymol is another compound present in cumin that stimulates the glands secreting digestive acids to bring about complete digestion of food. Moreover, it relieves for gas troubles, bloating and gurgling. The active principles in the cumin may increase the motility of the gastro- intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions (Weiss, 1996).

Cumin is very rich in iron (above 66 mg. in each 100 grams) which is more than 5 times the daily requirement of iron for an adult. This iron is the main constituent of haemoglobin in the red blood corpuscles of blood (Albert, *et al*., 1980). Haemoglobin transfers oxygen (as iron oxide) to the body cells and whose deficiency causes anaemia. Therefore cumin can be a nutritious additive to daily diet for anemic people. Cumin iron also is very good for lactating mothers as well as women who are undergoing menses or who are pregnant, since they are more in need of iron than others (Kafie, *et al*., 2002). Moreover, cumin help ease and increase secretion of milk in lactating women due to presence of thymol, which tends to increase secretions from mammary glands. According to Ministry of agricultural statistic report 2014, cumin calcium is an important constituent of milk and hence cumin is very good for lactating mothers.

Cumin has a distinctive aroma, which adds spice to the dishes that are being cooked. Cumin seeds were highly honored as a culinary seasoning in both ancient Greek and Roman kitchens (Elkamali, 1991). The seeds are used as a spice for flavoring food of various kinds like bread, cheese and curry powder. It is also used in other food products, backed food, meat and meat products, pickles, vegetables soups and gravies. The oil of cumin was used sausages and chutneys (Farrell, 1985).

The absolute is superior to the oil in flavoring is cuminaldehyde, the chief constituent of cumin oil. This will not only add taste in food, but will also act as one of the herbal remedies against many health disorders (Al-Bataina, *et al*., 2003).

1.2.1.6. Antimicrobial activities of green cumin:

An anti microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoa (Levey, 1994). The history of antimicrobials began with the observations of Pasteur, who discovered that one type of bacteria could prevent the growth of another (Bolin, *et al*., 1977). Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth of another microorganism (Aberoum and Deokule, 2008). Of course, today common usage the term antibiotic is used refer to almost any drug that attempts to ride your body of bacterial infection (Wang and Jones, 2004).

The old anti microbial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and / or heavy metals (Level, 1994). Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine, and the presence of numerous drug resistant strains poses a new challenge. Herbal drugs have been extensively used in this field for many centuries (Hajlaoui, *et al*., 2010). Essential oils of plant are known for their antimicrobial capability and have the potential to control plant diseases caused by bacteria and, in particular, eradicate bacteria from seeds (Matsubara, *et al*., 2000).

Recently, there has been a growing interest in natural products due to their availability, fewer side effects or toxicity as well as better biodegrade ability as compared to the available antibiotics and preservatives (Kalemba and Kunicka, 2003). The exploration of naturally-occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives (Abbas, 2010).

Cumin contains fatty oil and has an antimicrobial effect. A powder suspension of the cumin has diverse inhibitory effects; it inhibits mycelium

growth, toxin production or α-toxin production in aspergillus ochraceus, C. versicolor, and C. flavus (Agarwal, 1996). Numbers of investigations have shown the antimicrobial activity of cumin, the antibacterial action was assessed against a range of useful and pathogenic gram-positive and gram-negative bacteria strain (Farag,*et al*., 1989). Cumin seed oil and alcoholic extract inhibited the growth of Klebsiella pneumoniae and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity (Skrinjar, 2009).

The cumin antimicrobial activity was attributed to cuminaldehyde, carvone, limonene and linalool, whereas limnonene, eugenol, α-pinene and some other minor constituents have been suggested to contribute to the antimicrobial activity of cumin oil (Razzaghi, *et al*., 2009). Antifungal activity of cumin is recorded against soil, food, animal and human pathogens, including yeasts, aflatoxins and mycotoxin producers (Hajlaoui, *et al*., 2010).

1.2.2. Sunflower oil:

1.2.2.1. Plant Description:

Sunflowers are tall annuals. Modern cultivated varieties of sunflower reach a plant height of between 1.5 and 2.5 m at flowering and have strong taproots, from which deeply-penetrating lateral roots develop(Alfred, 2002). There is one apical inflorescence on a stem of 20-30 leaves. Leaves are large, dark green and roughly heart shaped, they have a wrinkled surface and prominent veins. The flower head typically has a maximum diameter of 15-30 cm. The flowers tend to be cross-pollinating and the best temperature range for the production of seed is 20-25°C (Lide, 1991).

1.2.2.2: Sunflower oil:

Sunflower oil is the non-volatile oil expressed from sunflower seeds. The oil content of the seed ranges from 22% to 36% (average, 28%): the kernel contains 45–55% oil. Seed and oil yield are reduced under conditions of stress. Oilseed producing varieties have a 1000 seed weight of 40 to 60g and non-oilseed varieties have a 1000 seed weight of sometimes over 100g. The expressed oil is of light amber color with a mild pleasant flavor (Christov, 2012).

1.2.2.3: Composition of sunflower oil:

Sunflower oil is mainly triglycerides (fats), typically derived from the fatty acids linoleic acid and oleic acid(Alfred, 2002). The British pharmacopoeia (BPC) list of sunflower oil is the following profile:

- Palmitic acid: 4 - 9%.
- Stearic acid: 1 - 7%.
- Oleic acid: 14 - 40%.
- Linoleic acid: 48 - 74%.
- Average protein content of the seed: 20-30%.
- Sunflower oil also contains lecithin, tocopherols, carotenoids, waxes and high vitamin E content.

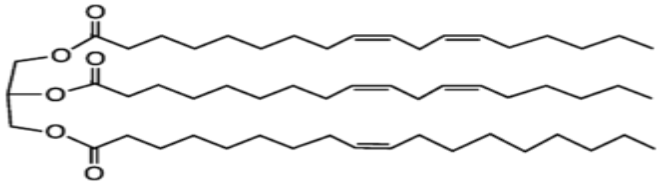


Figure (1-2): The chemical structure of sunflower oil (BPC, 2005)

1.2.2.4: Types of sunflower oil:

There are several types of sunflower oils:

- High linoleic: it typically has at least 69% linoleic acid
- High oleic: it has at least 82% oleic acid.
- Mid oleic.

High oleic sunflower oils are classified as having monounsaturated levels of 80% and above. In general, sunflower oil is a combination of mono-unsaturated and polyunsaturated fats with low saturated fat levels (NRA, 2008).

1.2.2.5: Uses of sunflower oil:

***i- As frying oil*:**

Sunflower oil behaves as a typical vegetable triglyceride. Linoleic sunflower oil is common cooking oil that has high levels of the essential fatty acids called polyunsaturated fat.

***ii- In cosmetics*:**

Sunflower oil has smoothing properties and is considered noncomedogenic. The high-oleic variety possesses shelf life sufficient for commercial cosmetic formulation (USDA, 1998).

iii- In cardiovascular benefits:

Sunflower oil of any kind has been shown to have cardiovascular benefits as well. Diets combined with a low fat content and high levels of oleic acid have been suggested to lower cholesterol which, in turn, results in a smaller risk of heart disease (Johnson, 2014).

***iv- As skin protection*:**

Sunflower oil, like other oils, can retain moisture in the skin. It may also provide a protective barrier that resists infection in pre-term infants (Skoric,*et al*., 2008).

***v- Negative health effects*:**

A high consumption of omega-6 polyunsaturated fatty acids, which are found in most types of vegetable oil including sunflower oil, may increase the likelihood that postmenopausal women will develop breast cancer. Similar effect was observed on prostate cancer. Other analysis suggested an inverse association between total polyunsaturated fatty acids and breast cancer risk uses as frying oil (USDA, 1998).

1.2.3: Characterization of edible oils:

Seed oils are important sources of nutritional oils, industrial raw materials and nutraceuticals. The characteristics of oils from different sources depend mainly on their compositions; no oil from a single source can be suitable for all purposes thus the study of their constituents is important (Aberoum and Deokule, 2008). Many consumers are looking for variety in their diets and aware of the health benefits of fresh fruits and vegetables and of special interest are food sources rich in antioxidants (Simon, *et al*., 1995)

Oils in the diet are available to the body as fatty acids. Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism as major metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators (Rustan and Drevon, 2005).The total energy intake from oils for a normal healthy adult is approximately 30 energy percent

and that in the western diets is about 40 energy percent. High fat diets enhance the incidence of coronary heart disease (Roman, *et al*. 1995).

1.2.3.1. General properties:

a. Physical properties:

The common physical properties of such oils are that they float on water but are not soluble in it; they are greasy to touch, and have lubricating properties; they are not readily volatile and may be burned without leaving any residue (Nouredini, *et al*., 1992). Physical properties of oil according to Lewis (1990), colour, refractive index and viscosity play an important role in determining the quality of any oil because they give physical specification for description of the oil.

i- Specific Gravity (SG):

The specific gravity of oil is the ratio of the weights of given volume of oil to that of equal volume of distilled water same temperature, all weighting being done in absence of air and it is expressed without units. Less than SG 1.0 floats on water, greater than SG 1.0 sinks in water, majority of oils "float on water". In general, specific gravity of spilled oil will increase over time (Tcheknavorian, 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 (Kumar and Tripathi, 2011).

ii- 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 (Kumar and Tripathi, 2011). Usually refractive index reading is taken at 270 ^{0}C except for those, which are not liquid at this temperature, in which case a high temperature (300^{o}C) depending on the melting point of the material shall be used (Tcheknavorian, 1993).

iii- Viscosity:

Viscosity is defined as the resistance of liquid to flow (Nouredini, *et al*., 1992). High viscosity implies a high resistance to flow while a low viscosity indicates a

low resistance to flow. 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). One of the most common instruments for measuring kinematic viscosity is the glass capillary viscometer (Kim, *et al*., 2010). Viscosity is independent of pressure (except at very high pressure) and decreasing temperature increases viscosity (Nouredini, *et al*., 1992).

vi-Colour:

Color of oil comes from natural coloring matters; carotene, xanthophyll and chlorophyll (Adikini, 2002). Exposing plant leaves to hot air drying may cause quality degradation due to color reactions and decomposition of active ingredients (Fennell, *et al*., 2004). 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 (Garcia and Yousfi, 2005; Maskan, 2003; Sinnecker, *et al*., 2002).

Colour is measured quantitatively using such equipment as spectrophotometers or tristimulus colorimeters. Usually the colour is quantified using the units proposed by the Commission International d'Eclairage (CIE). The colours are thus defined by their tristimulus values (X, Y, and Z), which may be used to calculate the respective derivative values, L*, a*, and b* colour space coordinates (Calvo, 2004). The lightness value (L*) indicates how light or dark a colour is. The a* and b* values indicate the locations along the respective red-green and yellow-blue axes. Colour is quite stable for the oil stored in darkness, as one would expect from visual inspection of the respective absorption spectra. On the other hand, the colour parameters of the samples stored under visible irradiation change continuously and almost monotonously during the storage period (Psomiadou and Tsimidou, 2002).

b. Chemicals properties:-

i- Rancidity:

Fats undergo changes during storage which result in the production of an unpleasant taste and odor, which is commonly referred to as rancidity is brought about by the action of air (oxidative rancidity) or by microorganisms (ketonic

rancidity) (weybridge and surry 1970). Oxidation primarily occurs with unsaturated fats. Via a free radical process, the double bonds of an unsaturated fatty acid can undergo cleavage, volatile aldehydes and ketones. There are some factors influencing fat oxidation (Sebranek and Neel, 2008):

Temperature:The rate of fat oxidation is highly dependent on temperature. Considerable improvement in storage stability can therefore be gained by lowering the storage temperature. As an example, it has been found that the storage time for frozen raw, lean meat can be extended approximately by 3 times by lowering the temperature from -15 to -25°C(Peter, 2000).

Oxygen:Oxygen in the air may be displaced by an inert gas such as nitrogen or carbon dioxide to retard oxidative rancidity, or the products may be packed under vacuum. These methods require the use of packaging materials with low oxygen permeability(Kumar and Tripathi, 2011).

Type of fat: In general, the softer the fat, the more unsaturated are the fatty acids and the more susceptible they are to oxidation and oxidative rancidity. However, vegetable fats, although unsaturated, are usually more stable than animal fats because they contain natural antioxidants. The most common antioxidant found in vegetable fats is vitamin E(Weybridge and Surry 1970).

Light:Packages that exclude light can be used to protect the products against fat oxidation.

Metals:Metals such as copper, iron, manganese, and chromium increase rate of fat oxidation. As a result, the preferred storage containers are steel drums, tin, or nonmetallic materials such as plastic.

Products from fat oxidation:Traces of oxidized fat in ingredients can accelerate oxidative rancidity in the remainder of the products. Steam treatment under vacuum conditions has been effective in removing products of deterioration (odorous substances) from some oils and fats.

Depending on the type of oil, its age, storage conditions, etc., peroxide value, UV Coefficientstests or combined are good indicators of oil rancidity (NRA, 2008).

ii-Peroxide value (PV):

The PV method measures their formation by determining the amount of iodine liberated from their reaction with potassium iodide and expressing the result in milli-equivalents per kilogram (meq/kg). Hydroperoxides are further oxidized to aldehydes and ketones which are responsible for the changes in odor and flavor of rancid fats (NRA, 2008). The peroxide number gives information about the number of peroxide compounds in the oil and hence of the age and quality of the edible oil. The lower the peroxide numbers the better and/or newer the oil. The peroxide value test is a commonly requested test used to measure the stability of various food products including pet foods, feeds, and human foods (AOAC, 1990).

The peroxide value is the primary measurement of oils rancidity and it gives us an idea of oils' freshness and storage conditions. It is a commonly used as indicator of the shelf life of a product because an elevated peroxide value will accompany disagreeable odors. Nonetheless, it is expected that fresh and well processed oils should show peroxides value less than 12 (NRA, 2008). The human threshold for detecting these changes seems to correspond to a peroxide value 40 meq/kg. If a fat has a peroxide value of < 40 meq/kg and does not smell rancid, it is most likely in the initial stages of oxidation and can readily be used in feed ration. If the peroxide value is > 40 meq/kg and the fat smells rancid, it is likely in its later stages of oxidation (NIS, 1992).

iii- Iodine value (IV):

The iodine value of an oil or fat is defined as the weight of iodine grams absorbed by 100 part of weight of the sample. 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 (Asuquo,*et al*., 2012). The calculated iodine value is not meant to be a rapid method, but instead gives two results (iodine value of triacylglycerols and free fatty acids) from one analysis (Keefe and Pike, 2009).

Iodine value can be used to estimate fat structure and unsaturation: saturation (U/S) ratios. Unsaturated fats have higher IV's than saturated fats, so the higher the IV, the softer the fat (Knothe, 2003). High iodine value indicates high unsaturation of fats and oils, it should also be noted that the less unsaturated fats with low iodine values are solid at room temperature or conversely, oil that are

more highly unsaturated are liquids showing there is real (Kyriakidis and Katsiloulis, 2000). As a general rule, if the iodine number is in the range of 0-70, then you are looking at a "fat"; if the iodine number is greater than 70, you are looking at oil (Knothe, 2003).

iv- Acid value:

The acid value or acid number of oil is defined as number of milligrams of potassium hydroxide required to neutralize the free acids contained in 1 mg of the perfumery material (Harold, *etal.*, 1981). The acid value is often used as quality indicator in frying oils, where a limit of 2mg KOH/g oil is sometimes used. In addition to free fatty acids, acid phosphates and amino acids also can contribute to acidity (food).The acid value corresponds to the amount of carboxylic acid groups in fatty acids. The older oil is the higher the acid value as triglycerides are converted into fatty acids and glycerol upon aging (ISO, 1983).

The acid value is measure of extent to which the glycerides in the oil have been decomposed by lipase action. The decomposition is accelerated by heat and light as rancidity is usually compared by free fatty acid formation. The determination is often used as general indication of the condition and edibility of oils (Dorodo, *et al*., 2002). The maximum levels for acid value of edible fats and oils were established by the ministry of public ealth 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 (ISO, 1983).

The acid value of oil often increases as the oil ages, especially if the oil is improperly stored. Processes such as oxidation of aldehydes and hydrolysis of esters increase the acid value. Oils which have been thoroughly dried and which are protected from air and light show little change in the amount of free acids (Kumar and Tripathi, 2011).

v- Saponification value (SV):

The saponification value (ester value) may be defined as the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1 gm of the perfumery materials (Denniston,*et al*., 2004). The higher the saponification value, the lower the mean chain length of the triglycerides (RNA, 2000). The ester of fatty acids of low molecular

weight requires the most alkali for saponification so that mean of the saponification value is inversely proportional to the mean of the molecular weights of the fatty acid in the glycerides present. The saponification value is not in general, as useful for identification purposes as the iodine value (Knothe, 2002).

The saponification value indicates the ability of the oil to make soap. High saponification value indicates that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries. The saponification values of the oils analyzed are in the range of 192.0 to 247 (Kyriakidis and Katsiloulis, 2000).

1.2.4. Antioxidants:

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 (Chen and Huang, 1998). These oxidative reactions result in a partial loss of minor constituents, primary cause of the health-promoting effects of oil consumption (Visoli and Galli, 1998). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells (Manna, *et al*., 1997).

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. 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 (Bjelakovic,*et al*., 2013; Abner,*et al* , 2011**).**

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid oxidation (Sies, 1997). The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and

enzyme systems having synergistic and interdependent effects on one another (Abner, *et al*., 2011).

The major active phytochemicals responsible for the antioxidant activity of plant derivatives are polyphenols, flavonoids, phenolic diterpenes and tannins. Moreover, the essential oils of herbs and spices are widely known for their strong antioxidant, antimicrobial and antifungal activities in foods (Hygreeva, *et al*., 2014). The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts (Abner, *et al*., 2011).

Food antioxidants are compounds that increase the resistance of fats to oxidation and consequent deterioration or rancidity (Sebranek and Neel, 2008). Natural antioxidants have also been shown to effectively reduce oxidative rancidity in ground meat products while providing additional sources of nutrients and flavor (Bilancia,*et al*., 2006). It is important to note that antioxidants can't be expected to stop rancidity. Their effectiveness lies only in slowing down the rate of oxidation. Natural antioxidants, such as those contained in some spices, such as rosemary, sage, and marjoram, have met acceptance for the retardation of rancidity in meat products (Morello, *et al*., 2004).

1.2.5: Distillation of essential oils:

Early efforts at extraction used alcohol and a fermentation process. New methods of essential oils extraction are entering the mainstream of aromatherapy, offering new choices in oils never before available. Extraction of oils from plant is generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing (De Silva, 1995). Distillation is a method of separating liquids in a solution by using the differences in their boiling points of the individual components. Also, depending on the concentrations of the components present, the liquid mixture will have different boiling point characteristics (Boelens, 1990).

The preparation of the material for distillation varies. Some materials, and in particular flowers, should be distilled as quickly as possible. Many herbs are

left to wilt, or are dried before distillation while barks, seeds and roots can be dried and stored for several months prior to distillation (Ryman and Daniele 1984). As oil may be lost during drying care needs to be taken and low temperatures used. Allowing leaves to dry in the shade or partial shade will result in less loss than direct sun drying. Cumin oil is usually obtained by steam distilling the milled spice, although hydro-diffusion gives a higher yield, and more recently by supercritical gaseous extraction, which is claimed to give oil closer to the aroma and taste of the spice (Simon, 1987).

1.2.5.1: Steps of Distillation:

The distillation process includes five steps as following (Kumar and Tripathi, 2011):

i- Boiling*:*

The boiling point of a pure liquid is defined as the temperature at which the vapor pressure of the liquid exactly equals the pressure exerted on it by the atmosphere and is one of its characteristic physical properties.

ii- Collection:

The gas of the more-volatile substance is carried away so that it can't mix in with the remaining liquid. It may then be condensed and cooled back into a separate liquid.

iii- Considerations*:*

A liquid does not immediately turn into a gas once its boiling point is reached. The vapor that is produced by heating the solution will actually contain both substances, although it will have a higher concentration of more-volatile substance.

iv- Purification:

Distillation would not produce components of extremely high purity by itself. This requires chemical separation techniques to be performed on the distilled product.

1.2.5.2: Types of Distillation

i- Simple distillation:

In simple distillation, all the hot vapors produced are immediately channeled into a condenser that cools and condenses the vapors. Therefore, the distillate will not

be pure. It is usually used only to separate liquids whose boiling points differ greatly or to separate liquids from in volatile solids or oils (Denny, 1991).

ii- Fraction distillation*:*

Fractional distillation is the separation of a mixture into its component parts, or fractions, such as in separating chemical compounds by their boiling point by heating them to a temperature at which several fractions of the compound will evaporate (Vacchiano, 1992). The highest boiling or least volatile liquid tends to condense more. Because the vapor pressures of the monoterpene hydrocarbons are very close, it is not possible through simple fractionation (Simon, 1987).

iii- Steam distillation:

This process involves bubbling steam through a heated mixture of the raw material. Steam distillation is employed in the manufacture of essential oils, for instance, perfumes. In this method, steam is passed through the plant material containing the desired oils. It is also employed in the synthetic procedures of complex organic compounds(Kumar and Tripathi, 2011).

v- Water distillation:

Water distillation is the simplest and cheapest distillation method. The plant material is totally immersed in water and boiled. The steam and oil vapor is condensed and the oil is separated from the water using the separation system. Water distillation remains the recommended method for barks, such as cinnamon and sandalwood and certain flowers (Vacchiano, 1992).

vi- Water – steam distillation:

Water-steam distillation is an improvement of simple water distillation. The charge of plant material is supported on a mesh or grill above boiling water. In steam and water distillation, the plant material cannot be in direct contact with the fire source and greatly reduces local overheating and burning of the charge. The advantage of steam and water distillation over water distillation is higher oil yield (Simon, 1987). .

1.2.5.3. Extraction solvent:

There are two types of extraction solvent:

i- Single solvent extraction:

It is well known that mono and sesquiterpenes are much less soluble in alcohol than the oxygenated constituents; with the solubility of these latter compounds increasing in the following order; ether and oxides < esters < ketones < aldehydes < alcohols. As a result, alcohol washing can be used to remove the oxygenated compounds of oil (Boucard and Serth, 1991).

ii- Two-phase solvent system:

During extraction, the hydrocarbons of the oil dissolve in the pentane and the oxygenated compounds dissolve in the alcohol. Each fraction must then be concentrated to remove the solvent. This is done by fractional distillation. The oils produced this way are free from monoterpene and sesquiterpene hydrocarbons (Laurence,*et al*., 1989).

1.2.5.4. Oil separator:

The final step in the distillation of essential oils is the separation from the water from the condenser using a special flask called a Florentine (Baser, 1992). Oils separate from water according to their density because they are immiscible or only sparingly soluble. If their density is less than 1.00, then they will float and are called "lighter than water" oils, whereas if their density is greater than 1.00, then they will sink and are referred to as "heavier than water" oils. Most of the oils from herbaceous plants and leaves are lighter than oils, while only a few of the wood and root oils are heavier than water oils. Because of this difference, different oil separators have to be used for the two types of oil (Ferrer and Matthews, 1987).

At the end of the distillation the oil and water in the special flask is placed in a large laboratory separating funnel and allowed stand for several hours after which the water can be run off (Vacchiano, 1992). At is stage a small plug of cotton wool is often placed in the outlet of the funnel. The oil runs through the plug any final traces of water are removed by the cotton wool (Kumar and Tripathi, 2011). Separation of oils whose density is very close to water or if one of the major components of the oil has a density greater than 1.00, while all of the others have a density less than 1.00, is more difficult. To complete separations of such oils the temperature of the condensate must be increased so that the temperature in the oil separator is sufficiently high for separation (Porter and Lammerink, 1994). According to Denny (1991), as the temperature in the

separator increases, the density of the oil (and its components) decreases. This decrease in oil density is far greater for the oil than it is for water, so separation of hard to separate oils can be achieved if temperatures are of 50°C or more

1.2.6: Essential oils:

Essential oils are volatile oils distilled from plant materials and represent the typical flavour and aroma (the essence) of a particular plant. Essential oils are also known as volatile oils, ethereal oils or simply as the oil of the plant from which they were extracted (Agarwal, 1996). These oils are found in special cells, glands or ducts located in different parts of a plant such as the leaves, barks, roots, flowers and fruits and sometimes in just one or two parts. The oils are usually present in very small amounts and comprise only a tiny fraction of the entire plant material. The oils are produced during some metabolic processes of the plant and are secreted or excreted as odoriferous by-products (Ryman and Daniele, 1984).

Oil is essential in the sense that it contains the essence of the plant's fragrance the characteristic fragrance of the plant from which it is derived. The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism (Albert, *et al*., 1980). The extracted oil content of plant material is low, typically 1 to 3% of the plant weight. They are thus low-volume, very high value products. This makes them attractive crops for remote smallholders where high transport costs prevent the transport of lower value cash crops. The quality of the oil obtained from a particular species will be influenced by where it is grown and how it has been processed (Kumar and Tripathi, 2011).

1.2.6.1: Chemistry of essential oils:

Extracted oils are complex mixtures of sometimes hundreds of chemical compounds. Pure essential oils can be essentially classified into two groups (Oprean, *et al*., 1998)

a- Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

b-Nonvolatile residue:It comprises 1–10% of the oil containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoid.

Most of these compounds can be grouped into a few major classes but there are also many components of essential oils that bear little resemblance to these classes. In the overview of important and characteristic components given below, compounds are classified into four major groups: aliphatic compounds, terpenes and terpene derivatives, benzene derivatives and miscellaneous compounds (Tezel, *et al*., 2000)

i- Hydrocarbon:

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic hydrocarbon found in plants is isoprene having the following structure.

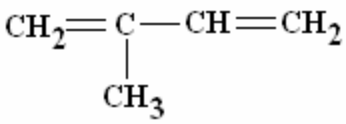


Figure (1-3). Isoprene chemical structure (Baser and Demirci 2007)

ii- Terpenes:

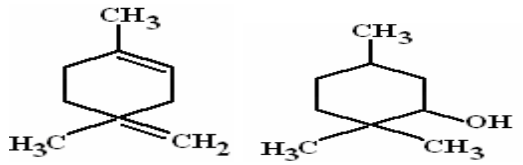
- The terpenoids are the most important group of natural products as essential oils are concerned.
- Generally have names ending in "ene." For examples: Limonene, Pinene, Piperene, Camphene, etc.
- Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal (Baser and Demirci 2007).
- Terpenes can be further categorized in monoterpenes, sesquiterpenes and Diterpenes, referring back to isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes (Oprean, et al., 2011).

Monoterpenes **[$C_{10}H_{16}$]**

- Monoterpenes are Analgesic, Bactericidal, Expectorant, and Stimulant.

- Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10). But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids are known as monoterpenoids (Kumar and Tripathi, 2011).

- Monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (α and β) – Pine genera as well.



Limonene Menthol

Figure (1-4). The structures of some monoterpenes (Tezel, *et al*., 2000)

Sesquiterpenes

- Sesquiterpenoids contain 15 carbon atoms and this result in their having lower volatilities and hence higher boiling points than monoterpenoids (Baser and Demiric, 2007).

- Sesquiterpenes are anti-inflammatory, antiseptic, analgesic, antiallergic.

- Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic (Kumar and Tripathi, 2011).

Diterpenes

- Diterpenes are antifungal, expectorant, hormonal balancers, hypotensive.
- Diterpenes are made up of four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils.
- Diterpenes occur in all plant families and consist of compounds having a C20 skeleton.
- There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives.

- Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) (Kumar and Tripathi, 2011).

iii- Alcohols

- Alcohols are antiseptic, antiviral, bactericidal and germicidal.
- Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester.
- When the terpene is monoterpene, the resulting alcohol is called a monoterpenol.
- Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use (Kumar and Tripathi, 2011).

iv- Aldehydes

- Aldehydes are antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal, disinfectant and sedative.
- Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections(Baser and Demiric, 2007).

v-Acids

- Acids are anti-inflammatory.
- Organic acids in their free state are generally found in very small quantities within essential oils.
- Plant acids act as components or buffer systems to control acidity (Oprean, *et al*., 2011).

vi- Esters

- Esters are formed through the reaction of alcohols with acids.
- Essential oils containing esters are used for their soothing, balancing effects.
- Because of the presence of alcohol, they are effective antimicrobial agents.
- Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system(Tezel, *et al*., 2000).

vi- Ketones:

- Ketones are anticatarrhal, cell proliferant, expectorant, and vulnerary.

- Ketones often are found in plants that are used for upper respiratory complaints because they assist the flow of mucus and ease congestion.
- Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue.
- Ketones are usually (not always) very toxic (Oprean, *et al*., 2011).

xi- Lactones

- Lactones are known to be particularly effective for anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions.
- Lactones have an even stronger expectorant action then ketones (Kumar and Tripathi, 2011).

1.2.6.2: Applications of essential oils:

Essential oils are very concentrated, about 75 to 100 times more concentrated than the fresh spice. Unlike fatty oils, these "essential" oils are volatile and highly concentrated. They do not have the complete flavor profile of ground spices, but they are used where a strong aromatic effect is desired. Essential oils are used at a very low level of 0.01% to 0.05% in the finished product. They can be irritating to the skin, toxic to the nervous system if taken internally (by themselves), and can cause allergic reactions and even miscarriages (Raghavan, 2007).

Commercial essential oils are required to comply with sets of standards. Such standards and specifications are set out in monographs published by standards organizations such as international standards organization (ISO), British standards (BS), essential oil association of USA (EOA), American society for testing and materials (ASTM), Turkish standards institute (TSE), Indian standards (IS), etc., or in pharmacopoeias or codices such as British Pharmacopoeia (BP), US Pharmacopoeia (USP) and European Pharmacopoeia (EP) (Eman, *et al.*, 2013).

There are about three hundred essential oils in general use today by professional practitioners. With the continual bombardment of viral, bacterial, parasitic and fungal contamination in our world, essential oils are a great benefit to help protect our bodies and homes from this onslaught of pathogens (Porter and Lammerink, 1994). Immune systems need support and essential oils can give

it. Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile and highly concentrated (Baser, 1992).

Essential oils have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes (Ministry of agricultural statistic, 2014). On oral application the following effects are observed: expectorating, appetite stimulating, choleretic, cholekinetic, carminative, spasmolytic, antiinflammatory, antiseptic, diuretic, sedative and circulation stimulating (Kumar and Tripathi, 2011).

Essential oils or volatile or ethereal layer of oils, find as wide and varied application in many industries for the scenting and flavoring of all kinds of finished products, some of them luxuries, most of them necessities in our advanced civilization. Many of these products contribute directly to our health, happiness and general well being. Most obvious role of essential oils is as fragrance materials but they are equally important as flavoring materials and in medicine. They are used in perfumes,cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products (Carvalho, 2004).

Perfumes are often used to favorably influence mood; aromatherapy goes further by exploiting this to create a soothing, tranquillizing or healing effect on a patient. Spices, which have volatile oil as their flavour principles, have long been used as flavoring materials but few of us realize that they are actually indispensable in bringing about proper digestion of food (Baser and Demirci, 2007). The market of cosmetics where essential oils are used in insignificantly small quantities has been established for a long time in Europe and America Carvalho, 2004).

1.2.6.3. Storage of essential oils:

It the storage of essential oils is one of the most important aspects in the pathway of essential oil production. Very less is known about the processes which cause the spoilage of essential oils such as oxidation, resinification, polymerization, hydrolysis of esters and interaction of functional groups. These

processes are activated by these attributes; heat, air (oxygen), moisture and light. To prevent this following aspects are to be taken into consideration; protection from sun light, storage in a cool place, storage in dry environment, stored in an amber colored bottles and bottle should be filled up to brim

Oils containing alcohol like sandalwood, Geranium are quite stable and can withstand prolonged storage (Denny, 1991). In order to remove moisture which is one of the worst factors in the spoilage of essential oil the smaller lots can be made free from moisture by addition of anhydrous sodium sulphate, by thoroughly shaking, keeping aside and then filtering (Baser, 1992). In case of viscous oil problem, moisture could be tackled by addition of common salt and then allowing mixture to stand until supernatant oil has become clear. The lower layer could be filtered. Centrifuging in high-speed centrifuges (rpm greater than 15,000) is an excellent mode of clarifying the oils (Kumar and Tripathi, 2011).

1.2.6.4. Safety of Essential Oils

Chronic studies have been performed on more 30 major chemical constituents found in many essential oils. The majority of these studies were hazard determinations that were sponsored by the National Toxicology Program (NTP). Even at these high intake levels, the majority of the constituents show no carcinogenic potential (Smith et al., 2005). Likewise, dietary toxicity and carcinogenicity data (WHO, 2001)for cinnamyl alcohol, cinnamaldehyde, cinnamyl acetate, and other members of the congeneric group of 3-phenyl-1-propanol derivatives show similar toxic and carcinogenic endpoints A comparison of the oral toxicity data (WHO, 2004) for limonene, myrcene, pinene, and other members of the congeneric group of terpene hydrocarbons show similar low levels of toxicity with the same high-dose target organ endpoint (kidney).

The safety data for the congeneric chemical groups that are found in vast majority of essential oils have been reviewed (Adams,*et al*., 2007; Smith *et al*., 2002; WHO, 2004). The second key factor in the determination of safety is the level of intake of the congeneric group from consumption of the essential oil. Intake of the congeneric group will, in turn, depend on the variability of the chemical composition of the essential oil in the marketplace and on the conditions of use. Hypothetically, a congeneric group of increased toxic potential

that accounts for only 5% of the essential oil may be prioritized higher than a congeneric group of lower toxic potential accounting for 95% (Newberne,*et al*., 1999).

The overall objective of the guide is to organize and prioritize the chemical constituents of an essential oil in order that no reasonably possible significant risk associated with the intake of essential oil goes unevaluated (Baser and Buchbauer, 2010). The main side effects of essential oils are allergic reactions; some oils have phototoxic effects, only a few essential oils show necrotic, narcotic, nephrotoxic, hepatotoxic and cancerogenic actions. In many cases the side effects are purely toxic effects caused by misuse of essential oils (Kumar and Tripathi, 2011).

1.2.7: Gas Chromatography (GC):

Gas chromatography is a technique for separating volatile substance by percolating a gas stream over a stationary phase. This depends upon the adsorptive properties of the column packing to separate sample (Joulain, 1994). The potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known (Vekey, 2001). The basic parts of the GC-MS as following (Scotte, 1996):

- Cylinder of Gases.
- Flow controller and pressure regulator.
- Injection part (sample inlet)
- Column.
- Detector (With necessary electronics).
- Recorder.
- Thermostat for injector, column and detector.

Common packing used is silica gel, molecular sieve, and charcoal. The liquid is spread as a thin film over an inert solid and the basis for components to the separated are carried through the column by an inert gas (carrier gas) (Vekey, 2001). The sample mixture is partitioned between the carrier gas and a

nonvolatile solvent (Stationary phase) supported on an inert size graded solid (solid support). The solvent selectively separates the sample components, according to their distribution coefficient; until they form separate bands leaving the column in the gas stream and recorded as a function of time by a detector (Kumar and Tripathi, 2011).

All gas chromatographs are designed to operate over a wide range of temperatures. Consequently, in order to avoid solute condensation in the detector or detector-connecting tubes the detector should be capable of operating at least 20°C higher than the maximum column temperature (Scotte, 1996). Essential oils are complex mixtures of volatile compounds whose separation depends upon the skill of the analyst to optimize the conditions of analysis, such as the selection of column type, stationary phase (Costa, *et al*., 2007).

In modern systems, analyze quantization is performed by a computer-based data processor called "integrator". It has been shown that, for a given compound, quite different quantitative results can be obtained using different integrators and detectors (Divakara and Anandara, 2013). Therefore, prior standardization or validation of the GC equipment is necessary to achieve reliable and reproducible results. Accurate quantitative results can be obtained only by the use of an internal standard or calibration using reference (Anonymous, 2008). Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds (Lafferty, *et al*., 1999).

CHAPTER TWO

2. Materials and Methods

2.1. Materials

2.1.1. Samples:

- Green cumin.
- Sunflower oil.
- Six types of bacteria.

2.1.2. Reagents:

- Sulfuric acid.
- Potassium iodide.
- Sodium thiosulfate.
- Glacial acetic acid.
- Ethanol.
- Sodium hydroxide.
- Phenolphthalein solution.
- Potassium iodide.
- Thiosulphate.
- Glacial acetic acid.
- Iodine trichloride.
- Carbon tetrachloride.
- Starch.
- Mueller–Hinton agar
- Normal saline.
- Positive control (Gentamicine).
- Dimethyl sulphoxide (DMSO).

2.1.2 Equipments:

- Conical flask
- Burette
- Propeller Stirrer
- Conical flask
- Burette
- Propeller Stirrer.
- Beaker.
- Pipette.
- Burette.
- Glassware.
- Needles.
- Sterilized swab.

2.1.3. Apparatus:

- Gas chromatography mass spectroscopy.
- Refractometer.
- Tintometer.
- Viscometer.

2.2. Study Methods:

2.2.1. Samples preparation:

Cumin seeds were purchased from Shendi local market obtained in the crop season 2015 - 2016, while the sunflower oil sample was purchased from Arabian oils company, Khartoum.Six types of bacteria (two gram positive and four gram negative) were prepared in the laboratory of medical laboratories collage, Shendi University.

2.2.2. Experiments and tests:

The determination of chemistry profile of cumin oil was carried out at laboratory of science faculty, university of Khartoum. The physical and chemical properties of oils were carried out at researches center and industrial consulting, university of Sudan, Shambat, Khartoum. Antibacterial activity of cumin oil was done at the medical laboratories college, Shendi University.

2.2.2.1. Extraction of cumin oil:

*Cumin Cyminum L.*oil was extracted by steam distillation and water. Mature cumin seeds were put into distillation apparatus over water, then the water was heated, the steam passed through the cumin seeds, vaporizing the volatile compounds. The vapors flowed through a coil, where they were condensed back to liquid, which is then collected in the receiving vessel. At the end of the distillation process cumin oil was separated from water depending on the difference in their density by using laboratory separating funnel. The cumin oil was collected in dark pure bottle and had been ready to tests.

2.2.2.2. Determination of cumin oil components:

Cumin oil was analyzed by using a gas chromatography mass spectroscopy (GCMS- QP 2010 plus) equipped with selective detector mass spectroscopy. Operating condition for GC were: injection temperature 250 C^0, pressure 61.8 kpa, total flow 364 ml/min, column flow 1.2 ml/min, linear velocity 39.4 cm/sec and oven temperature 35.0 C^0 for 3 minutes and held 280 C^0 for 4 minutes; mass spectroscopy operating parameters were: ion source temperature 250 C^0, interface temperature 250 C^0, solvent cut time 3.50 minutes, detector gain mode relative, threshold 0; and mass spectroscopy table scan m/z range from 35 – 800.

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 (NIST147 & WILEY7).

2.2.2.3. Determination the physiochemical properties of oils:

2.2.2.3.1. Physical properties (Weybridge and surry, 1970):

i- Determination of Color:-

The color intensively was read using Lovibond tintometer; units of red, yellow and blue were measured according to a AOCSmethod sample of oil was

placed in a standard sized glass cell. The instrument was switched on and looked through the eye piece and visually compared with red, yellow, blue, and neutral color standards. Results are expressed in terms of the numbers associated with the color standards.

ii- Determination of refractive index:

Abbey refractometer was used in this determination. A drop of the sample was transferred into a glass slide of the refractometer. Water at 30 C^{o} was circulated round the glass slide to keep its temperature uniform. Through the eye piece of the refractometer, the dark portion was viewed and adjusted in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value noted and recorded as the refractive index.

iii-Specific gravity:

Specific gravity was determined by simplified method buthas higher precision in determination of specific gravity of liquids. The method depends on the comparison of the weight of the same volumes (sample and water) at specific temperature (30^{0}C). The vial was cleaned and dried then was weighed empty (A). The vial was filled with prepared sample in such manner to prevent entrapment of air bubbles then the vial was weighed within oil sample (B). The previous steps were repeated with pure water (C).

Calculation:-

Specific gravity at $30C^{0}$ = B-A/ C-A

Where:

A: weight in gm of empty vial at 30 C^{0}.

B: weight in gm of vial with oil at 30 C^{0}.

C: weight in gm of vial with water at 30 C^{0}.

iv- Determination of Viscosity:

The viscosity of the oil sample was measured according to Cock and Van Red (1992). The viscometer was suspended in the constant temperature bath

($35C^0$) so that the capillary was standing vertical. The instrument exactly filled to the mark at the top of a bower reservoir with the oil by means of pipette inserted to side arm of the tube, the oil moved in to the top the upper reservoir, then liquid was allowed to a flow freely through the tube and the time required for meniscus to pass from the work above the supper reservoir to that at bottom of the upper reservoir was recorded, the flow time of distilled water measured by following the same steps above. The oil viscosity was calculated from the following equation:

$$\text{Relative viscosity} = \frac{T - T_0}{T_0}$$

Where:

T: flow time of the oil.

T_0: flow time of distilled water.

2.2.2.3.2. Determination of chemical properties:

i- Determination of Peroxide value (PV):-

Principle of reaction*:*

PV is defined as the milliequivalents of peroxide per kg of sample. It is a redox titrimetric determination. The assumption is made that the compounds reacting under the condition of the test are peroxides or similar product of lipid oxidation. Addition of excess potassium iodide reacts with the peroxide, iodine is produce. Through titration process, iodide reacts with standardized sodium thiosulfate using a starch indicator (Budavari, 1996).

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

$$I_2 + \text{starch } (\textit{blue}) + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6 + \text{starch } (\textit{colorless})$$

The method:

Oil sample (3g) was weighed into a 250 ml brown glass beaker and placed onto the sample rack. 20 ml solvent mixture [ethanol, acetic acid 3:2] and 1 ml

concentrated potassium iodide were added then the beaker was closed and kept for 5 minutes. After that 1 ml of starch and 80 ml distilled water were added and the solution was titrated with $Na_2S_2O_3$ (0.001 mol/l) until the end point (Weybridge and Surry, 1970) .

Calculation:

$$\text{Peroxide value} = \frac{(S-B) \times N \times 1000}{W}$$

Where:

S: volume of titrant (ml) for sample.

B: volume of titrant (ml) for blank.

N: normality of $Na_2S_2O_3$ solution (mEq/ml).

1000: conversion of units (g/kg).

ii- Acid value:

Principle:

Acid value of oil is the number of mg of potassium hydroxide necessary to neutralize the free acid in 1g of the sample. The reaction is accelerated by heat and light (WhitePJ, 1991).

$$R\,COOH + KOH \xrightarrow[\text{Light}]{\text{Heat}} RCOOK + H_2O$$

The method:

25 ml diethyl ether was mixed with 25 ml ethanol and 1 ml phenolphthalein solution 1% and then carefully neutralized with 0.1N alkali. Oil sample was dissolved in the neutral solvent, the mixture was shaking constantly until a pink color which persisted for 15 second was obtained and then titrated with aqueous sodium hydroxide (0.1 N)(Weybridge and Surry ,1970) .

Calculation:

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

Where:-

V: volume in ml of potassium hydroxide solution.

N: normality of the potassium hydroxide solution.

M: mass in gm of the material taken for the test.

56.1: molecular weight of potassium hydroxide.

iii- Free fatty acid:

Principle:

Measure of fat acidity is normally reflecting the amount of fatty acids hydrolyzed from triacylglycerols. In samples containing no acids other than fatty acids (no acid phosphates or amino acids), free fatty acid and acid value may be converted from one to the other using conversion factors (White, 1991). The free fatty acid of sunflower oil was calculated from this formula:

Free fatty acid = Acid Value / 2

iv- Determination of iodine Value (IV).

Principle:

Iodine value is grams of fat or oil absorbed per 100g of sample. Oil reacts with iodine or some other halogen such as ICl, and then potassium iodide is react to reduce excess ICl to free iodine. The liberate iodine is titrate with sodium thiosulphatestandard using a starch indicator (IUPAC, 1987).

$$ICl + R\text{-}CH = CH - COOH \longrightarrow R\text{-}CHI\text{-}CHCl\text{-}COOH + ICl\ (\textit{remaining})$$

$$ICl + 2KI \xrightarrow{} KCl + KI + I_2$$

$$I_2 + \text{starch}\ (\textit{blue}) + 2Na_2S_2O_3 \xrightarrow{} 2NaI + Na_2S_4O_6 + \text{starch}\ (\textit{colorless})$$

The method:

Wijs solution: 8g iodine trichloride was dissolved in 200 ml tetrachloride then the solution was mixed and diluted to 1000 ml with glacial acetic acid.

To oil sample 20 ml of Wijs solution were added. The reaction was allowed to stand in the dark for 30 minutes, after that15 ml of potassium iodide solution 10% and 100 ml water were added and the mixture was then titrated with 0.1 N thiosulphate solution that by using starch as indicator just before the end point (titration = *a* ml). A blank was carried out at the same time (titration = *b* ml) (Weybridge and surry, 1970) .

Calculation:

$$\text{Iodine value} = \frac{(B-S)\times N\times 126.9}{W\times 100}$$

Where:

B: volume of titrant (ml) for blank

S: volume of titrant (ml) for sample

N: normality of $Na_2S_2O_3$ (mol/1000ml)

126.9: MW of iodine (g/mol)

W: sample mass (g).

2.2.2.4. Antibacterial activity of cumin oil:

To assessment of the antibacterial activity of cumin oil, six types of bacteria (four gram positive and two gram negative) were prepared in the medical laboratories collage of Shendi University. The cup plate agar diffusion method was adopted to assess the antibacterial activity of the extracted oil (Ibeawuchi and Mbaata, 2002)

The method:

The medium was cool to 45 – 50 C^0 and poured into the plates, then was allowed to set on a level surface to a depth of approximately 4mm. The

antibacterial discs stocks were kept at -20 C^0. A supply of cottonwool swabbed on wooden applicator sticks was prepared.

2 ml of the standardized bacterial suspension were mixed thoroughly with 250 ml nutrient agar at 45 ^{0}C. Aliquots of the inoculated agar (20 – 25 ml) were distributed into steril petri dishes. The agar was left to solidify and 4 wells (7 mm in diameter) were made using a sterile cork bore (No 7). Tow concentrations (12.5, 25, 50 and 100 %) were made for extracted oil of cumin, as well, by dissolving the extracts and fractions in dimethyl sulphoxide (DMSO). The effects of the cumin oil were considered after 18 – 24 hours by measuring the inhibition zone diameter of each treatment. Three replicates were carried out for each extract/ fractions and control against the test organisms. The relative percentage inhibition of the test with respect to positive control was calculated by using the following formula:

$$\text{Relative percentage inhibition of the test extract} = \frac{(X-Y)\times 100}{(Z-Y)}$$

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 (Okeke2003).

2.2.3. Statistical analysis:

The data after collected, Statistical analysis was done by using SPSS programme under windows (IBM) computer system. Mean was calculated for comparison between different groups. The confidence limit was 95%, the p value was considered to be significant at value of ≤ 0.05.

CHAPTERTHREE

3. Results

3.1. GC-MS result:

The GC- MS analysis of cumin oil showed that eleven constituents were identified; seven hydrocarbon monoterpens (33.09%) and four oxygenated monoterpens (66.92%). The monoterpens were α-Thujene 0.41%, α-pinene 0.90%, β-pinene 10.72%, β-myrcene1.27%, α-phellandrene 1.18%, *p*-cymene 3.54% and γ-terpinene 15.07%, and oxygenated monoterpens identified were cumin aldehyde 21.10%, Carboxaldehyde 5.34%, 2-Caren-10-al 17.74% and cumin alcohol 22.65%. Table 3-1& Figure 3-1 represented the results of GC-MS analysis.

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

Terpene Type	Constituent	Percentage %	Retention Time	Base peak	Mass Peak
Monoterpens	α-Thujene	0.41	10. 56	93.10	383
	α-Pinene	0.90	10.783	93.10	396
	β-Pinene	10.72	12.275	93.10	417
	β-Myrcene	1.27	12.750	93.10	418
	α- Phellandrene	1.18	13.175	93.10	348
	p-Cymene	3.54	13.875	119.15	389
	γ-Terpinene	15.07	15.042	93.10	325
Oxygenated monoterpens	Cumin aldehyde	21.10	20.708	133.15	380
	Carboxaldehyde	5.43	19.225	109.10	392
	2-Caren-10-al	17.74	21.958	97.10	382
	Cumin alcohol	22.65	22.175	107.10	389

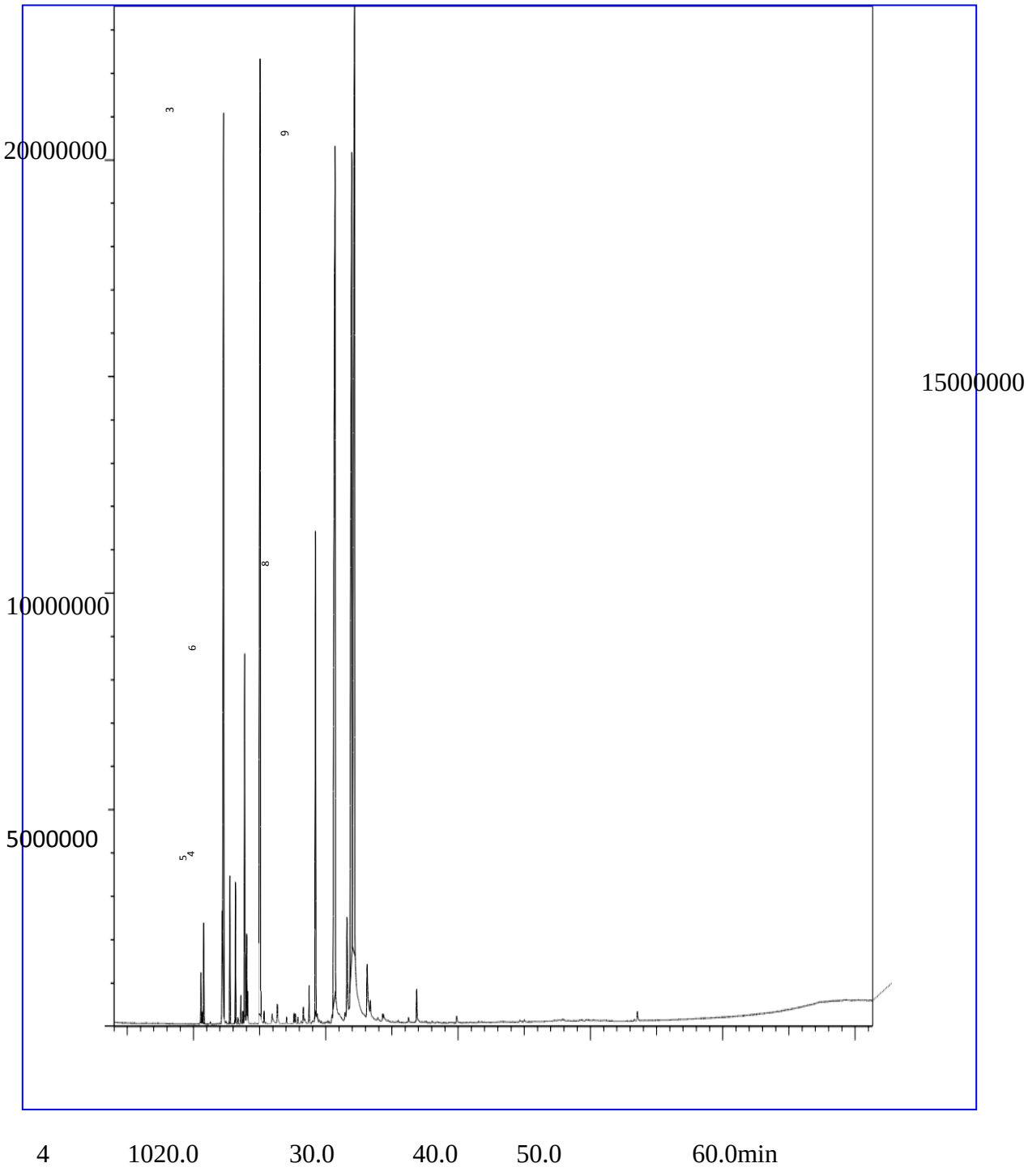


Figure (3-1):Chemical profile of cumin oil by GC-MS

3.2. Effect of cumin oil on the sunflower oil properties:

3.2.1. Physical properties:

A modern produced sample of sunflower oil and other sample of extracted cumin oil were subjected to determine their physical properties; viscosity, refractive index, specific gravity and color. Table (3-2)

Table (3-2): The physical properties of oil samples

Physical property	Viscosity	Refractive Index	Specific gravity	Color		
				Blue	*Red*	*Yellow*
Cumin oil	9.77	1.48	0.90	0.00	1.7	25.35
Sunflower	20.30	1.47	0.92	0.0	0.6	0.5

An other sample of sunflower oil was subjected also to determine the previous physical properties during storage intervals of 15 day over period of 10 weeks. Table (3-3)

Table (3-3): The physical properties of new sunflower sample at the beginning of storage and after five intervals of storage

Physical property	Viscosity	Refractive Index	Specific Gravity	Color		
				Blue	*Red*	*Yellow*
New sample	20.30	1.47	0.92	0.0	0.6	0.5
After 1st s. p.	46.65	1.47	0.91	0.1	0.7	11.35
After 2nd s. p.	48.71	1.47	0.92	0.1	0.8	11.8
After 3rd s. p.	49.06	1.47	0.92	0.1	0.9	11.9
After 4th s. p.	51.28	1.47	0.93	0.2	0.9	12.25
After 5th s. p.	50.84	1.47	0.94	0.2	1.05	12.55
p value	< 0.05	-	= 0.005	< 0.05	< 0.05	< 0.05

s. p.: *storage period* - : *no significant difference*

For two equal samples of sunflower oil, two volumes 0.1ml and 0.5 ml of cumin oil were added separately so as to explain the effect of cumin oil volume on the physical property, and then the two mixtures of oils were subjected to determine the above properties at the beginning and during the same storage periods. Tables (3-4, 3-5, 3-6 and 3-7)

Table (3-4): The viscosity property of sunflower oil and mixture oil during five intervals of storage

Storage period	Viscosity property		
	Sample of Sunflower Oil	*Mixture oil*	
		Sample +0.1 ml	*Sample +0.5 ml*
Before storage	20.30	20.32	20.42
After 1st s. p.	46.65	47.25	47.60
After 2nd s. p.	46.65	47.25	47.76
After 3rd s. p.	49.06	47.70	47.98
After 4th s. p.	51.28	49.43	48.70
After 5th s. p.	50.82	49.80	48.20
p. value	< 0.05	< 0.05	<0.05

Table (3-5): The refractive index property of sunflower oil and mixture oil during five intervals of storage

Storage period	Refractive index property		
	Sample of Sunflower	*Mixture oil*	
		Sample + 0.1 ml	Sample + 0.5 ml
Before storage	1.47	1.47	1.47
After 1st s. p.	1.47	1.47	1.47
After 2nd s. p.	1.47	1.47	1.47
After 3rd s. p.	1.47	1.47	1.47
After 4th s. p.	1.47	1.47	1.47
After 5th s. p.	1.47	1.47	1.47
After 6th s. p.	1.47	1.47	1.47
p. value	-	-	-

Table (3-6): The specific gravity property of sunflower oil and mixture oil during five intervals of storage

Storage period	Specific gravity property		
	Sample of Sunflower	*Mixture oil*	
		Sample + 0.1 ml	*Sample + 0.5 ml*
Before storage	0.90	0.90	0.91
After 1st s. p.	0.91	0.92	0.92
After 2nd s. p.	0.91	0.92	0.92
After 3rd s. p.	0.92	0.92	0.92
After 4th s. p.	0.93	0.93	0.92
After 5th s. p.	0.94	0.93	0.93
Mean of storage	0.94	0.93	0.93
p. value	= 0.005	< 0.05	< 0.05

Table (3-7): The color property of sunflower oil and mixture oil during five intervals period

Color	Sample sunflower oil			Sample+ 0.1 ml			Sample+ 0.5 ml		
	B	*M*	*P value*	*B*	*M*	*P value*	*B*	*M*	*P value*
Red	0.5	0.9	> 0.05	0.4	0.74	< 0.05	0.00	0.7	< 0.05
Blue	0.5	0.1	< 0.05	0.4	0.1	< 0.05	0.3	0.12	< 0.05
yellow	0.4	11.87	< 0.05	0.4	11.52	< 0.05	0.3	11.74	< 0.05

B: before storage *M: mean of storage periods*

3-2.2: Chemical properties:

Chemical properties of oil; peroxide value (PV), acid value (AV), free fatty acid (FFA) and iodine value (IV) were investigated for new sample of sunflower oil and cumin oil, Table (3-8). Then the volumes 0.1 ml , and 0.5 ml were added separately for two samples of edible oil (sunflower), triple samples, (edible and two mixture oil) were subjected again to assess the previous properties during five intervals of storage (each 15 day) for 10 weeks. Tables (3-9, 3-10, 3-11 and 3-12)

Table (3-8): The chemical properties of new sunflower oil and cumin oil

Oil type	PV	AV	FFA	IV
Sunflower oil	2.00	0.93	0.47	90.00
Volatile cumin oil	1.69	0.17	0.08	97.68

Table (3-9): The peroxide value property of sunflower oil and mixture oil during five intervals of storage

Storage period	Peroxide value		
	Sample of Sunflower Oil	*Mixture oil*	
		Sample +0.1 ml	*Sample + 0.5 ml*
Before storage	2.00	1.3	1.00
After 1st s. p.	5.04	3.88	3.28
After 2nd s. p.	6.80	5.04	3.52
After 3rd s. p.	8.36	5.13	3.90
After 4th s. p.	9.92	7.30	5.34
After 5th s. p.	10.72	8.40	8.10
p. value	= 0.001	= 0.001	< 0.05

Table (3-10): The acid value property of sunflower oil and mixture oil during six intervals period

Storage period	Acid value property		
	Sample of Sunflower Oil	*Mixture oil*	
		Sample ml	*Sample + 0.5 ml*
Before storage	0.93	0.17	0.17
After 1st s. p.	0.47	0.47	0.38
After 2nd s. p.	0.60	0.44	0.35
After 3rd s. p.	0.85	0.51	0.41
After 4th s. p.	1.04	0.57	0.48
After 5th s. p.	1.83	0.68	0.61
p. value	< 0.05	< 0.001	< 0.005

Table (3-11): The free fatty acid property of sunflower oil and mixture oil during five intervals period

Storage period	Free fatty acids property		
	Sample of Sunflower oil	*Mixture oil*	
		Sample + 0.1 ml	*Sample +0.5 ml*
Before storage	0.47	0.67	0.67
After 1st s. p.	0.24	0.24	0.19
After 2nd s. p.	0.30	0.22	0.18
After 3rd s. p.	0.43	0.26	0.21
After 4th s. p.	0.52	0.29	0.24
After 5th s. p.	0.92	0.34	0.31
p. value	< 0.05	< 0.05	< 0.005

Table (3-12): The iodine value property of sunflower oil and mixture oil during five intervals period

Storage period	Iodine value property		
	Sample of Sunflower Oil	*Mixture oil*	
		Sample + 0.1 ml	*Sample + 0.5 ml*
Before storage	90.00	97.68	97.68
After 1st s. p.	117.61	115.64	116.61
After 2nd s. p.	122.35	117.32	117.92

After 3rd s. p.	123.90	118.27	118.90
After 4th s. p.	126.18	119.30	119.73
After 5th s. p.	129.35	120.14	121.39
p. value	< 0.001	< 0.001	< 0.001

3.2.3. Effect of cumin oil on re-used oil properties:

To determine the effect of cumin 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 physical and chemical properties before and after addition (0.1ml and 0.5ml) of volatile oil of cumin. Tables (3-13 & 3-14)

Table (3-13): Physical properties of reused oil before and after addition of cumin oil

Physical property		*Reused sunflower oil*	*Mixture oil*		*P value*
			Sample + 0.1 ml	*Sample + 0.5 ml*	
Viscosity		46.72	46.97	47.41	< 0.05
Refractive index		1.47	1.47	1.47	-
Specific gravity		0.92	0.92	0.89	< 0.05
Color	*Red*	2.7	1.60	1.70	< 0.05
	Blue	0.00	0.00	0.00	
	Yellow	20.1	4.20	5.10	

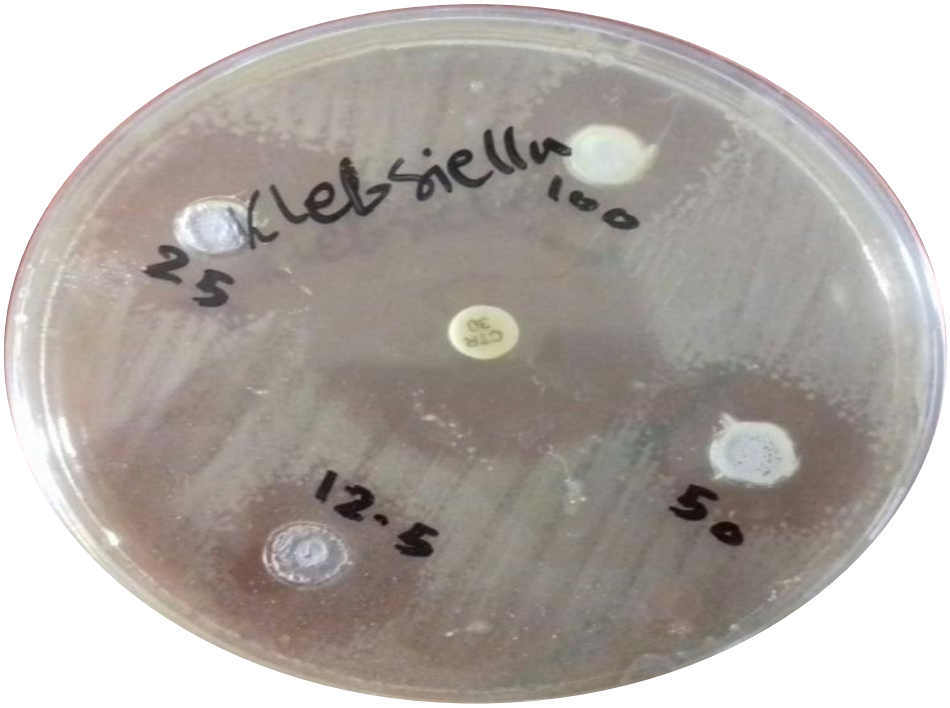
Table (3-14): Chemical properties of new sample, reused oil before and after addition of extract oil of cumin

Sample of oil	Chemical properties			
	POV	AV	FFA	IV
Fresh sample oil	2.00	0.93	0.47	90.00
Reused oil	36.00	0.54	0.27	99.25
Reused oil + 0.5 ml	9.00	0.26	0.13	101.50
P value	< 0.05	< 0.05	< 0.05	< 0.05

3.3. Antibacterial activity of volatile cumin oil:

The extracted oil of *Cumin Cyminum* was used to assess its effectiveness as antibacterial that through testing on six types of bacteria; two of them were bacteria gram-negative (*Escherichia Coli– Salmonella Typhi*) and the remainders were bacteria gram-positive (*Proteus Vulgaris*, *Klebsiella Pneumonae*, *Enterococcus Feacalis* and *Staphylococcus Aureus*) agar and nutrient broth. The screening was carried outusing the cup-plate agar diffusion method at four different concentrations (12.5%, 25%, 50% and 100%). The oil was dissolved in dimethyl sulfoxide (DMSO). The commercial antibiotic, gentamicin (10µg) was used as the positive control. The obtained results were represented in figures (3-2, 3-3, 3-4, 3-5, 3-6 and 3-7).

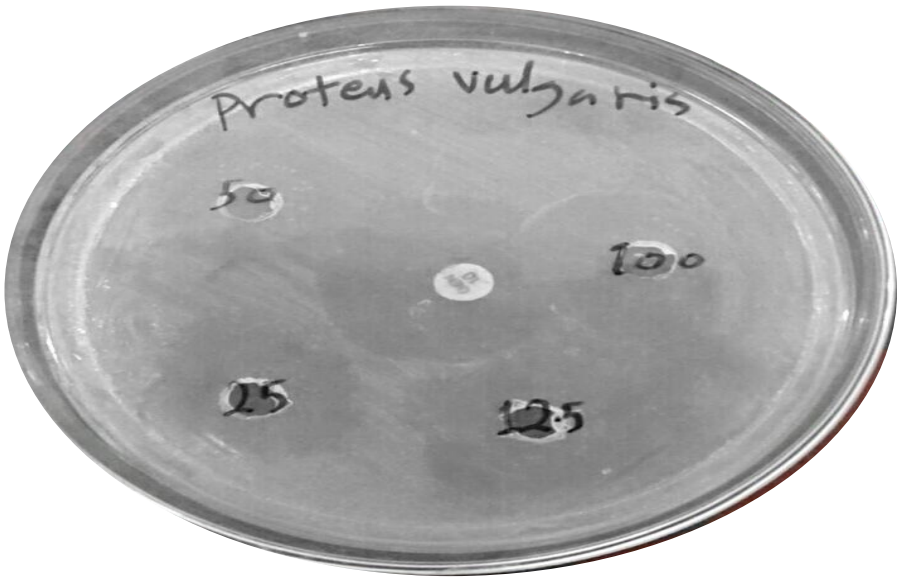
Figures (3-2): The effect of cumin oil on the*Staphylococcus Aureus*



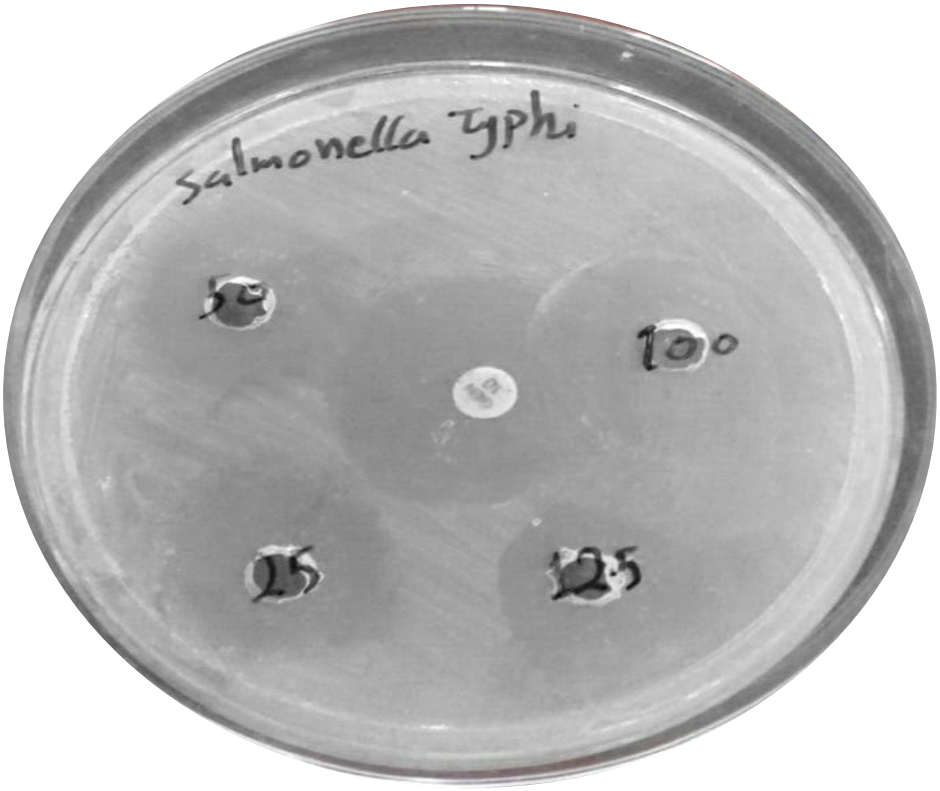
Figures (3-3): The effect of cumin oil on the*Klebsiella Pneumonae*

Figures (3-4): The effect of cumin oil on the*Escherichia Coli*

Figures (3-5): The effect of cumin oil on the*Enterococcus Fecalis*



Figures (3-6): The effect of cumin oil on the*Proteus Vulgaris*



Figures (3-7): The effect of cumin oil on the*Salmonella Typhi*

The inhibition area and minimum inhibition zones diameters (MIZD) according to different concentration (12.5%, 25%, 50% and 100%) were measured in mm. Tables 3-15 & 3-16.

Table (3-15): Inhibition area of antibacterial activity of different concentration of cumin oil on the some types of bacteria

Test Bacteria	Type of Bacteria	MIZD in mm			
		12.5%	**25%**	**50%**	**100%**
Enterococcus fecalis	*Gram negative*	-	-	10	10
Klebsiella pneumonae	*Gram positive*	18	25	18	20
Proteus vulgaris	*Gram positive*	25	20	15	13
Staphylococcus aureus	*Gram positive*	08	09	11	15
Escherichia coli	*Gram positive*	10	12	13	16
Salmonella typhi	*Gram negative*	23	30	20	30

Table (3-16): Mean of minimum inhibition zones diameters (mm) compared with positive and negative control

Test bacteria	Type of bacteria	Mean of MIZD	Gentamici	DMSO
Enterococcus fecalis	*Gram-negative*	05	30	0
Klebsiella pneumonae	*Gram positive*	20.02	32	0
Proteus vulgaris	*Gram positive*	19.50	40	

Staphylococcus aureus	*Gram positive*	10.75	34	
Escherichia coli	*Gram-positive*	15.75	30	
Salmonella typhi	*Gram-negative*	25.75	40	

DMSO: *Dimethyl sulfoxide*

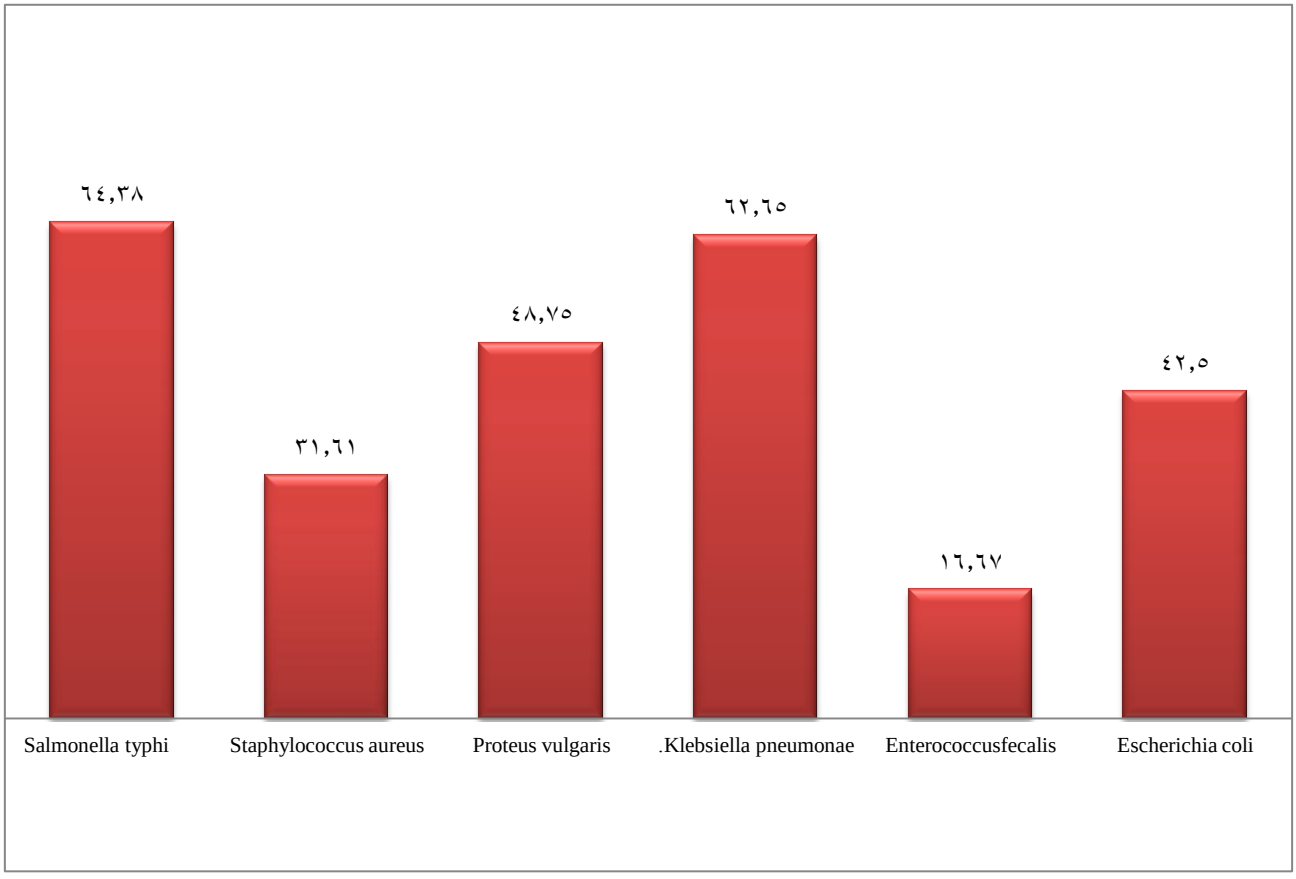


Figure (3-8): illustrate the relative percentage inhibitionof volatile cumin oil compare to standard antibiotic

CHAPTER FOUR

4. Discussion, conclusion and recommendations

4.1. Discussion:

4.1.1. Chemical profile:

Due to the enormous amount of raw product used to make wholly natural essential oils, it is important to study the chemical composition of the cumin oil fraction once the essential oil is extracted. This fraction is characterized by the complexity in the separation of its components, which belong to various classes of compounds and which are present in a wide range of concentrations. Therefore it is complicated to establish a composition profile of essential oils. The gas chromatographic method is almost exclusively used for the qualitative analysis of volatiles.

Green cumin oil was extracted from its mature seeds by water-steam distillation process and the extracted oil was analyzed by GC-MS to determine its chemical profile. The analysis showed that the chemical constituents of oil were; monoterpens percentage was (33.09%) and oxygenated percentage was 66.91%, table (3-1). Comparison of our data with data from other studies, cuminaldehyde of our result (21.10%) was lower than that results reported by Baser, et al., 1992 in Turkey, Maowad, *et al*., 2005 in Egypt and Hajlaoui, 2010 in Tunisia who found cuminaldehyde percentage was 25%, 35.25% and 39.48% respectively and higher than that reported by Chaudhary, 2014 in Delhi which found it 0.33% only. The possible explanation of the varieties in the previous percentages may be due to differences in soil type, climate, agriculture and irrigation system, and distillation process.

4.1.2: physical properties:

The present study was revealed that there was clear increase on the values of physical properties (viscosity, specific gravity and color) when we compared between values of sample before storage, during and at the end of storage period. Whereas there was significance difference between the values of above three periods; p values were; < 0.05, = 0.005 &< 0.05 for viscosity, specific gravity

and color respectively which was indicated to the effect of storage, table (3-2) (temperature, light and oxygen), this might be explained due to oxidative or microorganism rancidity which responsible for this differences in above values of physical properties of edible oil. Oxidation is the predominant cause of oil quality deterioration during storage (Morello, *et al*., 2004).

After addition of cumin oil to sunflower oil, our study showed that there is direct clear increase in values of two physical properties (specific gravity and viscosity) during early storage periods, and then the values were decreased in reverse proportion with along storage periods compared with values of edible oils alone , tables (3-6, 3-4). This could be attributed to that the cumin oil itself had specific gravity and viscosity that may be increasing the both physical properties values of edible oil. After storage the rancidity reactions were take place and then were reflected in the decrease of two properties values.

Refractive index had constant value 1.47 that was not affected by the storage periods or addition of cumin oil, table (3-5) while the color property in general it has different values because its measured was influenced by the measurements of sub color; red, blue and yellow and any increase in one of this three colorsfollowed by decrease in other color, table (3-7). However, according to national renderers association (2008), the measurement of oil color is depends mainly on red color value which should be less than 0.05. The storage had significant difference on the oil red color (< 0.05); there was increase in red color during storage from 0.5 to 0.9. Directly after the addition of cumin oil the red color value was declined from 0.5 to 0.0, other decline was appeared in the red color values of storage mean for pure sample compared with storage mean of mixture oil (0.9 to 0.7) that was also companying with significant difference ($p < 0.05$).

4.1.3: Chemical properties:

Peroxide value of sunflower oil had been increasing with storage periods (from 2.00 at the beginning of storage to 10.72 at the end of storage) in which indicated the occurrence of oxidation process as a result of storage (previously mentioned causes). 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 cumin oil (0.1 ml) the present study appeared that there was clear decreased in peroxide value from 1.69 to 1.30 at the beginning of storage and from 10.72 to 8.40 at the end storage period with presence of significant difference ($p = 0.001$), this result was reflected high influence of cumin oil on the peroxide value of edible oil (sunflower oil). On the other hand, the study also revealed that the increase in the addition volume of cumin oil was followed by more decrease in the peroxide value ($p < 0.05$), table (3-9). That was proved clear effect of cumin oil on the peroxide value which could be explained by the presence of chemical constituents of cumin oil that have antioxidant role such as monoterpens and oxygenated monoterpens which they reacted with formed peroxides that produced in the edible oil due to oxidation process. The major active phytochemicals responsible for the antioxidant activity of plant derivatives are polyphenols, flavonoids and terpenes. Moreover, the essential oils of herbs and spices are widely known for their strong antioxidant in foods (Hygreeva, *et al*., 2014).

Acid value and free fatty acids values are depend on the carboxyl group in the fat. In general our study was represented that the values of both chemical properties (acid and free fatty acids value) of pure sunflower oil were increasing with storage period 0.93& 0.47 at the beginning storage to 1.83 & 0.92 at the end storage period respectively. When both volume (0.1 ml and 0.5) of cumin oil were separately added, similar values as pure sunflower oil sample were observed at the beginning of storage for two properties. All the values of pure and tow mixtures samples were declined after the first period of storage to half the value or less of that at the beginning of storage then along with storage periods the values of both properties had been increasing until the end of storage period. Likewise, the increase of cumin volume was resulting in more decreasing on the two chemical properties values compared with pure sample and the two mixtures 0.1 ml and 0.5 ml was appeared significant difference $p < 0.001$ and $p < 0.001$ for acid value, and $p < 0.05$ and $p < 0.005$ for free fatty acids correspondingly in tables (3-10, 3-11). The predictable explanation here depend on the presence of cumin oil components which might be react with carboxyl group of free fatty acids and prevent or delay the analysis of triacylglycerol to

free fatty acids that result in decrease of carboxyl groups concentration and then decrease the acid and free fatty acids value. After the initial storage and upon storage extension until the end the environmental factors may take place and decomposition and analysis of esters was occurred which liberated carboxyl group and then increase free fatty acids of the purified and two mixtures oil resulted in an increase in the value of two properties.

Iodine value principally depends on the presence of double bonds on the oil structure either from free fatty acids or acids including in triacylglycerol residues. Our study showed that the iodine values were increasing with storage periods and after addition of two volume of cumin oil (two mixtures) and there was cleared significant difference ($p < 0.001$) for three samples, table (3-12). This an increasing could be attributed to the effect of air action on the sunflower oil which were lead to formation of new compounds contains double bonds or due to unsaturated compound that found in cumin oil. Otherwise, evident impact showed when the extracted oil of cumin was added, there was clear decreasing in iodine values compared with those before addition. The probability explanation might be due to interaction between components of cumin oil with double bonds of edible oil compounds which resulted in decreasing in double bonds number and then the iodine values along with storage periods.

4.1.4. Reused oil:

Several studies utilizing herbs, spices, fruits and vegetable extracts, and have shown that addition of these extracts to raw and cooked meat products decreased lipid oxidation, improved color stability and total antioxidant capacities which are important characteristics for shelf stable meat products (Zhang et al., 2010). The arrival of oil frying to the boiling point and then cooled and re-used it again that is threat human health because of the chemical changes was occurred as a result of oxidation during boiling and decomposition of food. The higher temperature oil the higher greater degree of oxidation and thus affected food stuff that fry in this oil. The study showed a definite impact of extracted oil of cumin on the sunflower oil that was used several times in cooking ($p < 0.05$) for physical properties and ($p < 0.001$) for chemical properties, table (3-13).

There was an apparent drop in values after the addition of cumin oil, compared to the values of the reused oil before the addition, especially in the chemical properties except iodine value, where the values before and after the addition were; (36.00 – 9.00), (0.54 – 0.26), (0.27 – 0.13) & (99.25 – 101.50) for peroxide value, acid value, free fatty acids value and iodine value respectively in table (3-14). This was reflected in high impact of the cumin oil on the reused oil and improves its chemical properties and thus reduces the excess severity on human health. That may be due to some compounds were found in the cumin oil which were detected in this study by gas chromatography mass spectrometer such as α-pinene and other terpenes. Pinene has been used as anti-cancer agent in Traditional Chinese medicine, also for its anti oxidant, anti-inflammatory, antiseptic, expectorant and bronchodilator properties (Neuenschwander, 2010).

4.1.5. Evaluation the antibacterial activity of the oil extracts:

Microorganisms can cause diseases to human being Infectious diseases are the world's leading cause of premature deaths and killing thousands of people (Piddock and Wise, 1989). Thus, the control of microorganisms is crucial in prevention and curing of diseases caused by their actions. An antimicrobial agent is a substance that kills or inhibits the growth or prevents damage due to the action of infectious microorganisms (Baron *et al.*, 1994).

The extract oil of cumin was investigated for *in vitro* antibacterial activity against six bacteria strains, which were *Escherichia coli, Klebsiella Pneumoniae* , *Staphyllococcus Aureus,Enterococcus Fecalis,Proteus Vulgaris* and*Salmonella Typhi*; The motivation of choosing these bacterial species had been justified by the possibility to demonstrate the different antibacterial activity, generated by different wall structure when the Gram-positive bacteria is compared with Gram-negative bacteria.

The results showed that all tested concentrations of cumin oil antibacterial activity against gram positive and gram negative bacteria, only *Enterococcus*

fecalis was appeared resistanceat the concentrations 12.5% and 50%. In addition the concentration 100% inflicted the highest antibacterial activities except in the cases of *Klebsiella pneumonia* and *Proteus vulgaris*. The most susceptible bacteria strains was *Salmonella typhi*with highest inhibition zone values (30mm) at concentration 25% and 100%. On the other hand, the tested concentrations 12.5% and 25% of volatile oil showed high inhibition zone against *Klebsiella pneumoniae* and *Proteus vulgaris* (25mm) compared with tested concentrations 50% and 100%. Previous study had shown that cuminoil caused highest inhibitory zones (23mm) against *Salmonella sp* compared with *E. coli* and *P. aeruginosa* (18 and 10 mm) respectively (Sepehri, *et al.,* 2014) .

Generally all of the cumin oil concentrations were found to be active against the gram positive bacteria. *Enterococus fecalis* revealed the weakest results (10mm) at the higher concentration, followed by *Staphyllococcus aureus* which caused inhibition zone, ranged from lower to moderate (8-15mm), while *Klebsiella pneumoniae* and *Proteus vulgaris* revealed moderate to high inhibition zone (18 - 25 mm and 13 - 25 mm) respectively. In contrast the results obtained by gram negative bacteria clearly showed that *Salmonella typhi* had the highest inhibition zone (20 - 30 mm) compared with *Escherichia coli* which was caused inhibition zone, ranged from 10 - 16 mm(In table 3-15).These results were showed that extracted oil of *cumin cyminum* has activity against gram positive and gram negative bacteria. The results of the present investigation was agree with those of Sheikh *et al*, (2010), who reported that the cumin oil of *C. Cyminum*showed an antibacterial activity against gram-positive and gram-negative bacteria.

These results were comparable with standard antibiotic (Gentamicin) which was showed inhibition zone ranged from 30-40mm (In table 3-16). The highest sensitive of bacteria compared with standard antibiotic gentamicin is

Salmonella typhi (64.38%)followed by*Klebsiella pneumonia* (62.65%)while*Enterococcus fecalis* (16.67%)revealed high resistance compared with antibiotic (In figure 3-8). According to Lorenzetti, *et al.* (1991) myrcene has an analgesic effect and is likely to be responsible for the medicinal properties of lemon grass tea. It has anti-inflammatory properties through prostaglandin E_2. *p*-Cymene may have a potential anti-inflammatory action (Zhong,*et al.*, 2013). Both compounds (myrcene and *p*-cymene) were detected in the volatile oil of cumin oil of this study. The essential oils of herbs and spices are widely known for their strong antioxidant, antimicrobial and antifungal activities (Hygreeva*et al*., 2014).

4-2. Conclusion

Cumin oil was extracted and analyzed and chemical constituents were monoterpens and oxygenated compounds. Physical and chemical properties of sunflower oil were studied, before and after addition of cumin oil at the beginning and during of storage periods every 15 days for 75 days. The study found that cumin oil had clear impact on the physiochemical properties of the Sudanese edible oil (Sunflower oil), and also had high effect on the reused oil sample. Antibacterial activity of extracted cumin oil was studied by selecting six types of bacteria (two gram negative and four gram positive) and the study was revealed that the oil has an evident effect on bacteria. Higher effect of cumin oil was appeared on *Salmonella typhi*and less impact was on *Enterococcus Fecalis*. The overall evaluation of this study concludes that the cumin have a good antioxidant and antibacterial potential.

4.3. Recommendations:

Based on data obtained from the present study, the following recommendations are suggested:

- *Cumin Cyminum* oil contains number of antioxidants compounds that will make it good source in preservation of foods and oils against rancidity oxidative during storage.
- Fast food is becoming prevalent because it possess grainy flavor for young and old alike. Parents should be attention to the damage on their families as a result of eating fast food which was cooked in oil used more than once in frying.
- Cumin oil also contains antiseptic, analgesic, anti-inflammatory and anti-bactericidal constituents so that can be used in the preparation of medicinal drugs, principally, the study was showed it has high impact on the bacteria that cause typhoid disease.
- Further studies in extracted oil of cumin should be done to detect its role as antioxidant and antimicrobial in foods.

CHAPTER FIVE

5. References and Appendixes

5.1. References:

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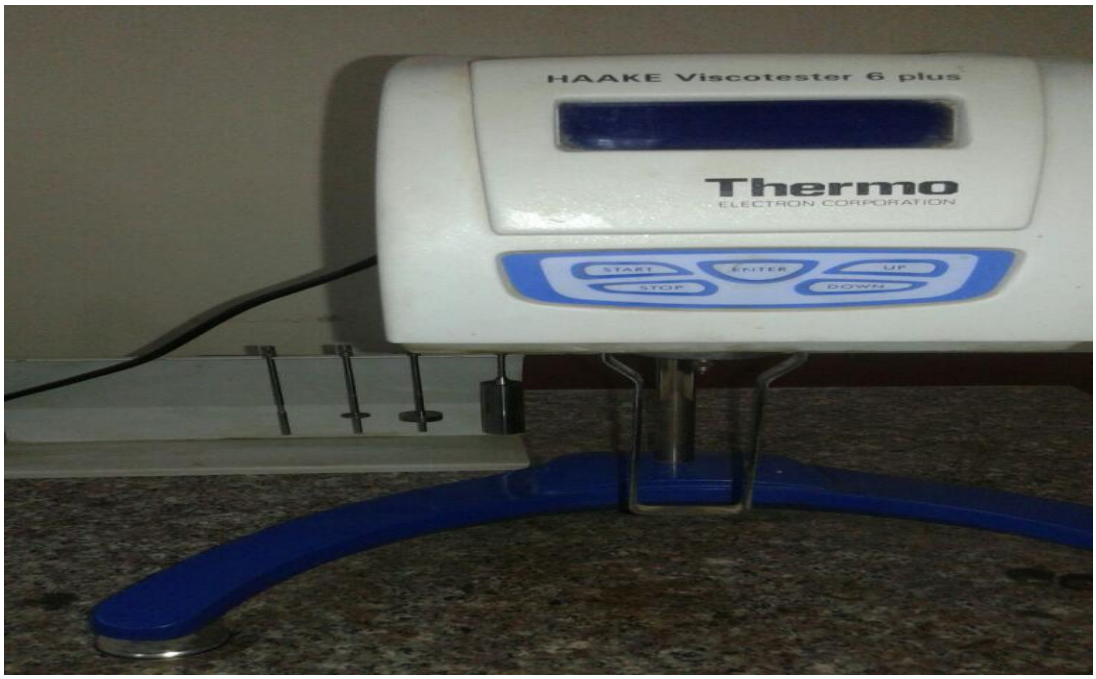
5.2. Appendixes:

5.2.1. Standard values:

Standard values of physical and chemical properties of Sunflower oil

Refractive index	Specific gravity	Red Color	Peroxide value	Iodine Value	Acid Value	Free fatty acid	REFERENCE
1.467- 1.469	-	-	≤10	110-143	≤ 0.6	0.085	FAO / WHO
1.465	918-923	-	<10	110-143	< 0.6	< 0.3	Weighbridge and Surrey
-	-	< 0.5	< 10	-	-	-	NRA (2008)

5.2.2. Detectors



Viscometer used to determine the viscosity

GC-MS used to determine the chemical compound

Refractometer to determine the refractive index

Tintometer to determine the color