Shendi University
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Influence of *Cinnamomum zeylanicum* extract on the physiochemical properties of storage of sunflower oil

A thesis submitted in fulfilment of the requirements for the degree of M. Sc. (Chemistry)

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بسـم الله الرحمـــن الرحيـم

قال تعالى:

(يَٰبُنَيَّ إِنَّهَآ إِن تَكُ مِثۡقَالَ حَبَّةٖ مِّنۡ خَرۡدَلٖ فَتَكُن فِي صَخۡرَةٍ أَوۡ فِي ٱلسَّمَٰوَٰتِ أَوۡ فِي ٱلۡأَرۡضِ يَأۡتِ بِهَا ٱللَّهُۚ إِنَّ ٱللَّهَ لَطِيفٌ خَبِيرٞ ﴿١٦﴾ يَٰبُنَيَّ أَقِمِ ٱلصَّلَوٰةَ وَأۡمُرۡ بِٱلۡمَعۡرُوفِ وَٱنۡهَ عَنِ ٱلۡمُنكَرِ وَٱصۡبِرۡ عَلَىٰ مَآ أَصَابَكَۖ إِنَّ ذَٰلِكَ مِنۡ عَزۡمِ ٱلۡأُمُورِ ﴿١٧﴾ وَلَا تُصَعِّرۡ خَدَّكَ لِلنَّاسِ وَلَا تَمۡشِ فِي ٱلۡأَرۡضِ مَرَحًاۖ إِنَّ ٱللَّهَ لَا يُحِبُّ كُلَّ مُخۡتَالٖ فَخُورٖ ﴿١٨﴾)

صدق الله العظيم

سورة لقمان

Dedication

This thesis is dedicated to:

My loving parents who gave me faith and love.

My supportive husband and sons.

My beloved siblings.

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Firstly, I would like to express my extreme gratitude to Allah for His blessings without which I would not have been able to complete this thesis. My sincere appreciation and thanks to my supervisor, Dr. Faroug Bakheit Elsonni, Faculty of Sciences and Technology, University of Shendi, for his academic input and support throughout this experience. I am indebted to Dr. Ibrahim Kabashi, Dept. of Statistical Sciences, Faculty of Sciences and Technology, University of Shendi for his assistance in statistical analysis. I would like to take this opportunity to thank Mr. Khalid Saeed, Medical Laboratories College, Shendi University, the laboratory staff of the Center for Research and Industrial Consulting, Shambat, Khartoum, and those of the Department of Chemistry, University of Shendi, for their invaluable assistance. My appreciation also goes to my respected colleague, Abdel Muhaymin Gabralla, who has rendered significant help and advice.

ABSTRACT

Ceylon cinnamon has been used for centuries around the world with numerous benefits attributed to it including antioxidant properties of the bark oil. The present study aimed to determine the chemical profile of *Cinnamomum zeylanicum* (Ceylon cinnamon) oil, and its effects on the physical and chemical properties of sunflower oil, in storage, and to detect its antibacterial activity. Cinnamon bark oil was extracted by steam distillation and the extracted oil was analyzed via GC-MS to determine its chemical profile. The GC-MS. analysis of cinnamon oil was identified 11 unique compounds; six sesquiterpenes (α-copaene, α-amorphene, β-cedrene, α-muurolene, β-bisabolene, and δ-cadinene), three aldehydes (benzaldehyde, benzenepropanal, and cinnamaldehyde), an alkene (styrene) and a monoterpene alcohol (L-borneol). The physiochemical properties of sunflower oil were tested before and after addition of extracted cinnamon oil, during five intervals of storage. Cinnamon oil had an overall positive effect on the physiochemical properties, namely specific gravity and viscosity, peroxide value, acid value and saponification value and a slight effect on the color and refractive index. These findings can be linked to the antioxidant compounds present in the cinnamon bark oil such as α-copaene, cinnamaldehyde and benzaldehyde. The antibacterial activity of cinnamon bark oil was assessed via six species of bacteria (three gram positive and three gram negative) using the cup-plate agar diffusion method at five different concentrations (6.25%, 12.5%, 25%, 50% and 100%). The results showed that the tested concentrations of cinnamon oil show significant antibacterial activity against both gram positive and gram negative bacteria. Cinnamon oil contains a number of antioxidants compounds that will make it a useful additive in the edible oil industry for longer shelf life and likewise, in the production of antibacterial medical formulations.

ملخص البحث

تم إستخدام القرفة الهندية لعدة قرون في جميع أنحاء العالم وذلك للفوائد العديده التى تمتلكها مثل إحتوائها على مركبات مضادة للأكسدة. هدفت الدراسة الحالية إلى إستخلاص زيت القرفه ومن ثم تحديد مكوناته الكيميائية ، آثاره على الخصائص الفيزيائية والكيميائية لزيت عباد الشمس خلال عملية التخزين، وإختبار نشاطه كمضاد للبكتيريا. تم إستخراج زيت اللحاء من القرفة بواسطة التقطير البخاري وتم تحليل الزيت المستخرج عبر كروماتوغرافيا الغاز-مطياف الكتلة . حدد تحليل كروماتوغرافيا الغاز-مطياف الكتلة لزيت القرفة احدى عشرمركب صنفت كالتالى ستة منها سسكوتربينات هى (α-كوباين و α-أمورفين و β-سيدرين و α-مورولين و β-بيسابولين و δ-كادينين) وثلاثة ألدهيدات (بنزالدهيد و بنزينبروبانال و سينامالدهيد) ، مركب واحد عباره عن ألكين (ستيرين) وكحول مونوتربين (L-بورنيول). تم إختبار الخصائص الفيزيوكيميائية لزيت عباد الشمس قبل وبعد إضافة زيت القرفة المستخرج، وعند بدء وأثناء وعند نهاية عملية التخزين (75 يوم) وذلك خلال خمس فترات من التخزين (كل 15 يوم). أظهرت الدراسه أن لزيت القرفة تأثير واضح على الخصائص الفيزيوكيميائية، وهي الثقل النوعي واللزوجة، وقيمة البيروكسيد، وقيمة الحموضة و قيمة التصبن و تأثير طفيف على اللون و معامل الإنكسار. تعزى الدراسه هذه النتائج بصوره أساسيه إلى وجود المركبات المضادة للأكسدة في الزيت المستخلص من نبات القرفة مثل α-كوباين، سينامالديهيد والبنزالديهيد. تم تقييم النشاط المضاد للبكتيريا لزيت لحاء القرفة من خلال ستة أنواع من البكتيريا (3 موجبة الغرام و 3 سالبة الغرام) باستخدام طريقة الإنتشارعلى اطباق بتري أجار و خمسة تراكيز مختلفة لزيت القرفه (6.25٪، 12.5٪، 25٪، 50٪، 100٪). بينت النتائج أن التراكيز المختبرة من زيت القرفة لها نشاط مضاد البكتيريا الإيجابية والسالبة لصبغة غرام. وخلصت الدراسه إلى أن زيت القرفة يحتوي على عدد من المركبات المضادة للأكسدة والتى تجعل منه مادة فعاله ومفيدة في المحافظه على زيت الطعام لمدة صلاحية أطول وكذلك الإستفادة من الزيت لإنتاج عقاقير طبية مضادة للأمراض البكتيرية.

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AV	Acid Value
BPC	British Pharmacopoeia Commission
CVMP	Committee for Veterinary Medicinal Products
DMSO	Dimethyl sulfoxide
ESCOP	European
FFA	Free fatty acid
GC-MS	Gas Chromatography-Mass Spectrometry
HV	Hydroxyl Value
IUPAC	International Union of Pure and Applied Chemistry
IV	Iodine value
IZD	Inhibition Zone Diameter
JSA	Japanese Standards Association
MIZD	Minimum Inhibition Zone Diameter
MUFA	Monounsaturated fatty acids
NIST	National Institute of Standards and Technology
NRA	National Renderers Association
OGG	Oil Gas Glossary
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
RD	Relative Density
SCO_2	Supercritical carbon dioxide
SG	Specific Gravity
SV	Saponification Value
UNFAO	United Nations Food and Agricultural Organization
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
WHO	World Health Organization

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CHAPTER ONE

1. Introduction and Literature Review

1.1. Introduction:

Cinnamomum zeylanicum (cinnamon) is one of the oldest spices known and was used in ancient Egypt not only as a beverage flavoring and medicine, but also as an embalming agent. It was so highly treasured that it was considered more precious than gold. For the Romans, cinnamon was just as sacred and important. It was burned at funerals and it is said that the Emperor Nero burnt a whole year's supply at the funeral of his wife Poppaea (Pushpitha, 2006). Around this time, cinnamon also received much attention in China, which is reflected by its reference in one of the earliest books on Chinese botanical medicine, dated around 2,700 BC cinnamon's popularity continued throughout history (Maheshwari, *et al.*, 2013).

Cinnamon was one of the first spices to be traded between Asia and Europe and the fact that Venetian merchants controlled the entire cinnamon trade in Europe fueled other European explorers to travel afar in search of other ways of obtaining the precious spice. After the Portuguese discovered Sri Lanka (Ceylon) at the end of the 15th century and took control of the cinnamon trade there, the Dutch removed them from power a century later, followed by the British in 1796. Since then the importance of the cinnamon trade declined, as cinnamon began to be produced and cultivated in other areas (Maheshwari, *et al.*, 2013).

Cinnamon became one of the most relied upon spices in Medieval Europe. Due to its demand, cinnamon became one of the first commodities traded regularly between the Near East and Europe. There is a written report on the use of cinnamon in an Arabic source from around 1275. In 1310, Johannes of Montevino confirmed the existence of cinnamon trees in Ceylon. About 100 years later, Nicolo de Conti accommodated an accurate description of the cinnamon tree (Theodora, 2017).

The cinnamon tree is popular for its distinct fragrance. The bark of the tree has always enjoyed an unparalleled demand due to its essential oil that is aromatic, making a great flavoring agent. The oil is extracted by pounding the bark, macerating it and finally distilling the essence. Cinnamon has a characteristic odor and a pungent taste which are a result of cinnamaldehyde

aging in the presence of oxygen. Cinnamon bark is used around the world as a spice for flavor and can be consumed directly. The powder is used in a variety of soups and desserts. Cinnamon is associated with a number of health benefits. The volatile oil extracted from its bark is a trusted cure for common colds and diarrhea. The extract is believed to be an antioxidant that also has antimicrobial properties, making the spice a preferred preservative (Khan, *et al.*, 2003).

Objectives of the study:

This study aimed to achieve the following objectives:

- To determine the chemical profile of *Cinnamomum zeylanicum* via Gas Chromatography-Mass Spectrometry.
- To determine the influence of *Cinnamomum zeylanicum* on physical and chemical properties of sunflower oil.
- To determine the antibacterial activity of *Cinnamomum zeylanicum* oil.

1.2. Literature Review

1.2.1. Cinnamon:

1.2.1.1. Common name:

The cinnamon name was derived from the Greek word ‘kinnamon’. The name cinnamon is correctly used to refer to Ceylon cinnamon, also known as “true cinnamon” (Ravindran, *et al.*, 2003).

1.2.1.2. Classification of the cinnamon plant:

Cinnamomum is a genus of evergreen trees and shrubs and this genus contains about 250–350 species worldwide, distributed in tropical and subtropical regions of North America, Central America, South America, Southeast Asia, and Australia.

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Laurales
Family: Lauraceae
Genus: Cinnamomum
Species: *Cinnamomum zeylanicum* (Jayaprakasha, *et al*., 2007).

1.2.1.3. Origin of Cinnamon:

The cinnamon tree is reported to have originated in the central hills of Sri Lanka (Ceylon). Toponymical evidences as well as the archaeological and historical findings prove that cinnamon grew in the north and north central provinces during the period of the Aryan settlers who had then established the Anuradhapura kingdom (Ravindran, *et al.*, 2003).

1.2.1.4. Morphology of cinnamon:

Cinnamomum zeylanicum belongs to the diminutive evergreen tree in the Laurel (Lauraceae) family. The tree can grow up and reach a height in a range 6 to 12 m. The stem is robust with its diameter ranging between 30 – 60 cm diameters. Mature trees have a thick bark of brown or gray and have many low branches with tapered and rounded leaves. The exterior and interior color of cinnamon bark is light brown. Cinnamon is spicy, slightly sweet, warm and fragrant (Sulaiman, 2013). Its bark is black-brown, and the inner bark has a cinnamic aldehyde flavor. Young branchlets are gray, and somewhat tetragonous, white-maculate. The buds are sericeous puberulent. The leaves are usually opposite; with petiole around 2 cm, glabrous; and the leaf blade is greenish white abaxially, green and shiny adaxially, ovate or

ovate-lanceolate, 11–16 × 4.5–5.5 cm, leathery or subleathery, glabrous on both surfaces. The flowers are yellow, gray and puberulent outside. The fruit is ovoid, 10–15 mm, and black when mature; the perianth cup in fruit is cupuliform, dilated, dentate, teeth truncate or acute at apex (Li, *et al.*, 2008).

Figure (1-1): Leaves of *Cinnamomum zeylanicum* (Sulaiman, 2013)

Figure (1-2): Bark of *Cinnamomum zeylanicum* (Cinnamon sticks) (Sulaiman, 2013)

1.2.1.5. Types of cinnamon:

There are two main types of cinnamon that are used in cooking today, although around one hundred different types of cinnamon trees actually exist. Ceylon cinnamon (true cinnamon) using only the thin inner bark, has a finer, less dense, and more crumbly texture, and is considered to be less strong (Maheshwari, *et al.*, 2013). It has a lighter, sweeter and more delicate flavor. This sweet cinnamon is used in all types of cakes, biscuits, crumbles and breads. It is very popular in savory dishes, such as curries. Ceylon cinnamon is produced in Sri Lanka, India, Madagascar, Brazil and the Caribbean (Charles, 1999).

The other main type of cinnamon is obtained from the cassia tree, which is found in Southeast Asian countries such as Indonesia, Burma, China and Vietnam. Cassia tree (Chinese cinnamon) is less expensive than true cinnamon, and perhaps spicier and more pungent. It is therefore preferred in exotic meat dishes, curries and other savory foods. Cassia has a much stronger (somewhat harsher) flavor than true cinnamon. Furthermore, it is generally a medium to light reddish brown, hard and woody in texture, and thicker (2–3 mm thick), as all of the layers of bark are used. Cassia cinnamon is darker and harder than Ceylon variety (Archer, 1988).

1.2.1.6. Forms of cinnamon:

Cinnamon is available in two forms, the cinnamon stick or ground cinnamon powder. To obtain the spice, the inner bark is stripped from the tree and allowed to dry in the sun. Whilst drying, it rolls up into a quill known as a cinnamon stick. Some of the quills are then ground down into a powder and is known as ground cinnamon (Maheshwari, *et al.*, 2013).

1.2.1.7. Pharmacology and usage of Cinnamon:

Cinnamon has been used for many purposes since early times. Cinnamon has been used as a cooking spice and in preventing food from going stale since the 16thcentury. Cinnamon was also used as flavoring in cookies, biscuits and cakes. It is also widely used in traditional and modern medicine, perfumes and aromatherapy. Cinnamon was used as a stomachic, in cases of nerve weakness and for the treatment of diarrhea, dyspepsia, hyperacidity, reflux, vomiting and bloating (Onderoglu, *et al.*, 1999).

Due to the presence of blood thinning compounds, cinnamon helps to alleviate pain and ensure the oxygen supply to the body cells leading to higher metabolic activity. Cinnamon can also be used to increase the appetite. In addition, it is used as a treatment against respiratory ailments

(Blumenthal, 1998). Cinnamon bark may possess a potentiating effect on insulin which is a water soluble polyphenol compound and can be used in the treatment of type 2 diabetes as well as to lower triglyceride levels and serum cholesterol (Khan, *et al*., 2003).

The utilization of cinnamon extract has proved to be more effective than thyme extract in inhibiting both *in vitro* growth and urease activity of *Helicobacter pylori* (Tabak, *et al*., 1999). Cinnamon is also used in poultices to treat minor bacterial and fungal infections of the skin externally and as a rub in aromatherapy to promote blood circulation (Tisserand and Young, 2014). The essential oil of cinnamon contains both antifungal and antibacterial principles that can be used to prevent food spoilage due to bacterial contamination (Fabio, 2003). Furthermore, it is also proven that cinnamon oil is effective against some species of toxicogenic fungi (Anjorin, *et al*., 2013; Abd El-Aziz, *et al*., 2015)

Cinnamon can be used as perfume, room fresheners and can also be added in potpourris because of its pleasant smell and refreshing aroma that can soothe and relax the mind and body (Broadhurst, *et al.*, 2000).

The work of Jayaprakasha and others, 2007 had shown that the fruit of cinnamon which is an underutilized and unconventional part of the plant contains a significant amount of phenolics and antioxidants to counteract the damaging effects of free radicals and may protect against mutagenesis. Antioxidants are often added to food to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process (Shahidi and Wanasundara, 1992).

Studies have reported that methanolic extracts of cinnamon contain a number of antioxidant compounds which can effectively scavenge reactive oxygen species, including superoxide anions and hydroxyl radicals, as well as other free radicals under *in vitro* conditions (Mathew and Abraham, 2006).

Toxicity studies on cinnamon conclude that the extracts were low to moderate in toxicity (Anderson, 2008; Shah, 1998) and cinnamon bark extracts below 0.5 g/kg were safe for use in efficacy studies (Ahmad, et al., 2015). Prior to that, studies have reported that cinnamon is safe for consumption in medicinal amount (Dugoua, *et al.*, 2007) or amounts commonly found in food (USFDA, 2017), and that the cinnamaldehyde amount consumed should not exceed 700µg/kg (Newall, *et al.*, 1996).

In summary of most studies, cinnamon finds many uses from cooking to perfumery and its essential oils are a potent medicine for many ailments, possessing significant antioxidant activity, and are safe with little to moderate toxicity.

1.2.2. Essential oils:

Essential oils are fragrant, concentrated hydrophobic oils, obtained from the flowers, leaves, seeds, bark, wood, or roots of plants, carrying the characteristic scent and flavor of the particular plant (Burt, 2004). For a thousand years ago, essential oils have played an important role in our daily lives (Sulaiman, 2013). The first people who used the essential oils were the Egyptians and they applied them to medical practice, beauty treatment, food preparations and also in religious ceremony. Meanwhile, the Greeks used the essential oils in therapeutic massages and aromatherapy practices and the Romans used the essential oils to promote health and personal hygiene (Doterra, 2017).

Currently, essential oils are used in perfumery, cosmetics and bath products (e.g. agarwood and tea tree oil), in food and beverages, in household cleaning products (e.g. pine oil), insecticides and repellents (e.g. lemongrass) and aromatherapy (e.g. ylang-ylang) (Tan, *et al*., 2015; Maia and Moore, 2011).

1.2.2.1: Volatile fractions and residues of essential oils:

Extracted oils are complex mixtures of sometimes hundreds of chemical compounds. Pure essential oils can be essentially classified into two groups; namely volatile fractions and nonvolatile residues (Oprean, *et al*., 2006).

1.2.2.1.1. *Volatile fraction:*

The volatile fraction constitutes 90–95% of the oil and contains the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

1.2.2.2.2. *Nonvolatile residue:*

Nonvolatile residues comprise 1–10% of the oil and include hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoid (Tezel and Hortacsu, 2000).

1.2.2.2. Chemical groups in essential oils:

Most of the 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, compounds are classified into four major groups; aliphatic compounds, terpenes and terpene derivatives, benzene derivatives and miscellaneous compounds (Tezel and Hortacsu, 2000).

1.2.2.2.1. Hydrocarbons

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. A basic hydrocarbon found in plants is isoprene, having the following structure (Husnu, *et al.*, 2007).

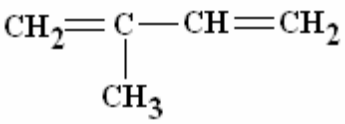


Figure (1-3). Chemical structure of isoprene (Husnu, *et al.*, 2007).

1.2.2.2.2. Terpenes:

The terpenoids are the most important group of natural products where essential oils are concerned. They generally have the suffix (-ene), such as limonene, pinene, piperene, and camphene. Terpenes, in general, possess anti-inflammatory, antiseptic, antiviral, and bactericidal properties (Husnu, *et al.*, 2007). Terpenes can be further categorized into monoterpenes, diterpenes and sesquiterpenes. Referring to the isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, a sesquiterpene is formed and four linked isoprene units form diterpenes (Oprean, *et al.*, 2006).

i- Monoterpenes **[$C_{10}H_{16}$]:**

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10). Some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids. Monoterpenes are analgesic, bactericidal, expectorant, and stimulant. Monoterpenes can be cyclic molecules (menthol – monocyclic; camphor – bicyclic; pinenes (α and β) – Pine genera as well (Tezel and Hortacsu, 2000).

Limonene Menthol

Figure (1-4). The structures of some monoterpenes (Tezel and Hortacsu, 2000)

ii-Sesquiterpenes:

Sesquiterpenes are biogenetically derived from farnesyl pyrophosphate, and in structure may be linear, monocyclic or bicyclic (Rao and Pandey, 2007). Sesquiterpenoids contain 15 carbon atoms and this results in their having lower volatilities and hence higher boiling points than monoterpenoids (Husnu, *et al.*, 2007). Sesquiterpenes are anti-inflammatory, antiseptic, analgesic, and antiallergenic.

iii- Diterpenes:

Diterpenes occur in all plant families and consist of compounds having a C20 skeleton. Diterpenes are made up of four isoprene units, and since this molecule is too heavy to allow for evaporation with steam in the distillation process, it is rarely found in distilled essential oils. 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 are antifungal, expectorant, hormonal balancers, hypotensive, but have limited therapeutic importance and are used in certain sedatives (coughs) (Rao and Pandey, 2007).

1.2.2.2.3. Alcohols*:*

Alcohols exist naturally, either as free compounds, or combined with terpenes or esters. Alcohols are antiseptic, antiviral, bactericidal and germicidal. Alcohols have a very low or totally absent toxic reaction in the body or on the skin and are therefore considered safe to use (Rao and Pandey, 2007).

1.2.2.2.4. Aldehydes:

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

1.2.2.2.5. Acids:

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. Acids possess anti-inflammatory activity (Oprean, *et al.*, 2006).

1.2.2.2.6. Esters:

Esters are formed through the reaction of alcohols with organic 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 and Hortacsu, 2000).

1.2.2.2.7. Ketones:

Ketones are anti-catarrhal, cell proliferate, expectorant, and vulnerary. Ketones are often 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 very toxic (Oprean, *et al.*, 2006).

1.2.2.2.8. 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 (Rao and Pandey, 2007).

1.2.2.3. Chemical components of cinnamon:

Volatile oils are products from the distillation process of the bark, leaves, flowers, or buds of a plant. The chemical composition of these oils varies depending on the part of the plant used for the distillation process. Cinnamon bark and leaf oils are steam distillation products obtained from the inner bark and leaves respectively (Sulaiman, 2013). A commercial sample of essential oils from *Cinnamomum zeylanicum* contained approximately 63% cinnamaldehyde, 8% limonene, 7% eugenol, 5.5% cinnamaldehyde propylene, and 1-2% of a variety of terpenoid compounds which are pinene and camphene. Cinnamon also contains β-caryophyllene (1-4%), linalool (1-3%) and 1.8-cineole (1-2%) (ESCOP, 2003).

According to Wang, *et al.*, 2009, the main constituents of the *Cinnamomum zeylanicum* leaf are 79.75% eugenol, 16.25% trans-cinnamaldehyde, 0.14% linalool, neohexane, cinnamyl alcohol and other compounds groups such as aldehydes, esters, alcohols, ketones and alkanes. Cinnamon bark contains up to 4% essential oil consisting primarily of cinnamaldehyde (60-75%), cinnamyl acetate (1-5%), and eugenol (1-10%) (Sulaiman, 2013). Other constituents are oligopolymeric, procyanidins, cinnamic acid, phenolic acids, pentacyclic diterpenes and their acetyl derivative, cinnzeylanine, and the sugars mannitol, L-arabino-D-xylanose, L-arabinose, D-xylose, α-D-glucose as well as mucilage polysaccharides (Hansel, *et al.*, 1992).

1.2.2.3.1. Main constituents of cinnamon bark:

The main constituent of cinnamon bark oil is cinnamaldehyde, while eugenol is the main component of cinnamon leaf oil. The essential oil of the cinnamon bark is described in a summary report in the European Pharmacopoeia, Committee for Veterinary Medicinal Products, 2009 according to this information, the cinnamon oil mainly contains cinnamaldehyde (55 –76%), eugenol (5-18 %) and safrole (up to 2%). This document refers also to human use (CVMP, 2000).

1.2.2.3.1.1. *Cinnamaldehyde:*

The scientific name of α-Cinnamaldehyde is 3-Phenyl-2-propenal.

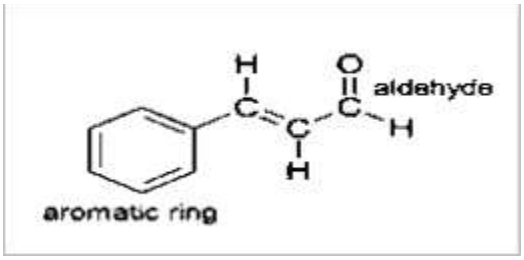


Figure (1-5): Chemical structure of cinnamaldehyde (Sulaiman, 2013)

Cinnamaldehyde is a pale yellow, liquid with a warm, sweet and spicy odor, and pungent taste. It is found naturally in the essential oils of Chinese cinnamon (75–90%) and Ceylon cinnamon (60–75%) as the primary component in the steam distilled oils. Cinnamaldehyde is used in a large range of products including bakery goods, confection, and beverages as well as in toothpastes, mouth washes and chewing gum. Furthermore, it is used effectively in air fresheners for odor neutralization and in manufacturing protocols for the preparation of natural benzaldehyde through a retro-aldol process (Sulaiman, 2013).

1.2.2.3.1.2. *Eugenol:*

Eugenol is a phenylpropene, an allyl chain-substituted guaiacol, and a member of the phenylpropanoids class of chemical compounds.

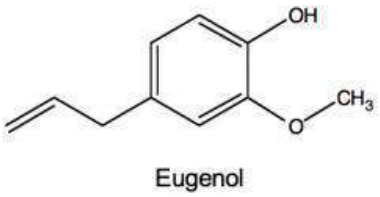


Figure (1-6): Chemical structure of Eugenol (Sulaiman, 2013).

The name is derived from the scientific name for clove, *Eugenia aromaticum* or *Eugenia caryophyllata*. It is a clear to pale yellow oily liquid

extracted from certain essential oils, especially from clove oil, cinnamon, basil and bay leaf. It is slightly soluble in water, and soluble in organic solvents and has a spicy clove-like aroma. Eugenol is used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic. It was used in the production of vanillin though most vanillin is now produced from phenols or from lignin. Eugenol is also used in formulating insect attractants and UV absorbers, and analgesics. Eugenol possesses significant antioxidant, anti-inflammatory and cardiovascular properties, in addition to analgesic and local anesthetic activity (Pramod, *et al.*, 2010).

1.2.2.3.1.3. Safrole*:*

Safrole is a member of the methylenedioxybenzene group of compounds.

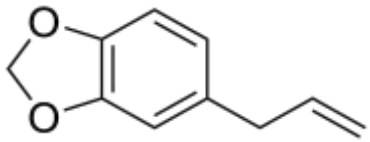


Figure (1-7): Chemical structure of safrole (Barbosa, et al., 2012)

Safrole is a clear, colorless or slightly yellow liquid (density 1.09g/cm^3). Like many compounds from the methylene dioxybenzene group, it is a precursor in the synthesis of the insecticide synergist piperonyl butoxide and the recreational drug Ecstasy (Barbosa, *et al.*, 2012). Safrole was formerly used as a food flavor. Safrole is also present in root beer, and has been used as an additive in chewing gum, toothpaste, soaps and certain pharmaceutical preparations. It is a slight carcinogen of the liver and is banned by the United States Food and Drug Administration (USFDA) for use in food. Safrole has also been shown to exhibit anti-angiogenic properties and antibacterial activity (Kamdem and Gage, 1995).

1.2.2.4. Methods of obtaining the essential oil:

There are various types of extraction that are used to obtain essential oils, such as distillation (e.g lavender, peppermint, and eucalyptus oil), cold pressing or mechanical expression (citrus peel oils and olive oil), solvent extraction (using hexane or supercritical carbon dioxide) and florasol extraction (Pramod, *et al.*, 2010).

1.2.3. Distillation process:

Distillation is a process of separating the component or substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of the mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction (Kravchenko, 2014).

1.2.3.1. Types of distillation:

There are several types of distillation used in the oil extraction, as discussed in the following:

1.2.3.1.1. Simple distillation

In simple distillation, the vapor is immediately channeled into a condenser. Consequently, the distillate is not pure but rather its composition is identical to the composition of the vapors at the given temperature and pressure. That concentration follows Raoult's law. As a result, simple distillation is effective only when the liquid boiling points differ greatly (rule of thumb is 25 °C) or when separating liquids from non-volatile solids or oils. For these cases, the vapor pressures of the components are usually different enough that the distillate may be sufficiently pure for its intended purpose (IUPAC, 2017).

1.2.3.1.2. Cold pressing (mechanical expression):

Cold pressing is used to extract the essential oils from the rinds of citrus fruit such as orange, lemon, and grapefruit. This method involves the simple pressing of the rind at about 120 ºF to extract the oil. The rinds are separated from the fruit, and then ground or chopped and pressed. The result is a mixture that will separate over time. The essential oils extracted this way retain their scent and do not undergo much change. However, oils extracted this way have a relatively short shelf life (Rao and Pandey, 2007).

1.2.3.1.3. Fractional distillation:

For many cases, the boiling points of the components in the mixture will be sufficiently close that Raoult's law must be taken into consideration. Therefore, fractional distillation must be used in order to separate the components by repeated vaporization-condensation cycles within a packed fractionating column. This separation, by successive distillations, is also referred to as rectification (Perry and Green, 1984).

1.2.3.1.4. Steam distillation:

Steam distillation is a method for distilling compounds which are heat-sensitive. The temperature of the steam is easier to control than the surface of a heating element, and allows a high rate of heat transfer without heating at a very high temperature. This process involves bubbling steam through a heated mixture of the raw material (Harwood and Moody, 1989). By Raoult's law, some of the target compound will vaporize (in accordance with its partial pressure). The steam becomes charged with the essence. The vapor mixture is cooled and condensed, usually yielding a layer of oil and a layer of water, which can then be separated and bottled. Steam distillation of various aromatic herbs and flowers can result in two products; an essential oil as well as a watery herbal distillate (Harwood and Moody, 1989).

1.2.3.1.5. Vacuum distillation:

Some compounds have very high boiling points. To boil such compounds, it is often better to lower the pressure at which such compounds are boiled instead of increasing the temperature. Once the pressure is lowered to the vapor pressure of the compound (at the given temperature), boiling and the rest of the distillation process can commence. This technique is referred to as vacuum distillation and it is commonly found in the laboratory in the form of the rotary evaporator (Harwood and Moody, 1989). This technique is also very useful for compounds which boil beyond their decomposition temperature at atmospheric pressure and which would therefore be decomposed by any attempt to boil them under atmospheric pressure. Molecular distillation is vacuum distillation below the pressure of 0.01 torr (Furniss, *et al.*, 1989).

1.2.3.1.6. Air-sensitive vacuum distillation:

Some compounds have high boiling points as well as being air sensitive. A simple vacuum distillation system as exemplified above can be used, whereby the vacuum is replaced with an inert gas after the distillation is complete. However, this is a less satisfactory system if one desires to collect fractions under a reduced pressure. The Perkin triangle, has means via a series of glass or Teflon taps to allow fractions to be isolated from the rest of the still, without the main body of the distillation being removed from either the vacuum or heat source, and thus can remain in a state of reflux. To do this, the sample is first isolated from the vacuum by means of the taps, the vacuum over the sample is then replaced with an inert gas (such as nitrogen or argon) and can then be stoppered and removed. A fresh

collection vessel can then be added to the system, evacuated and linked back into the distillation system via the taps to collect a second fraction, and so on, until all fractions have been collected (Leonard, *et al.*, 1994).

1.2.3.1.7. Short path distillation:

Short path distillation is a distillation technique that involves the distillate travelling a short distance, often only a few centimeters, and is normally done at reduced pressure. A classic example would be a distillation involving the distillate travelling from one glass bulb to another, without the need for a condenser separating the two chambers. This technique is often used for compounds which are unstable at high temperatures or to purify small amounts of compound. The advantage is that the heating temperature can be considerably lower (at reduced pressure) than the boiling point of the liquid at standard pressure, and the distillate only has to travel a short distance before condensing (Harwood and Moody, 1989).

1.2.3.1.8. Zone distillation:

Zone distillation is a distillation process in long container with partial melting of refined matter in moving liquid zone and condensation of vapor in the solid phase at condensate pulling in cold area. The process is worked in theory. When zone heater is moving from the top to the bottom of the container then solid condensate with irregular impurity distribution is forming. Then most pure part of the condensate may be extracted as product. The process may be iterated many times by moving (without turnover) the received condensate to the bottom part of the container on the place of refined matter. The irregular impurity distribution in the condensate (that is efficiency of purification) increases with number of repetitions of the process (Kravchenko, 2011; Kravchenko, 2014).

1.2.3.1.9. Solvent extraction:

In solvent extraction, a hydrocarbon (for example, hexane) is added to the plant material to help dissolve the essential oils. When the solution is filtered and concentrated by distillation, a substance containing resins or a combination of wax and essential oil remains. From the concentrate, pure alcohol is used to extract the oil. After the alcohol evaporates, the oil is left behind. Solvent extraction is suitable for expensive, delicate and thermally-unstable materials such as jasmine and hyacinth. Since solvents may leave a small amount of residue in the essential oils, solvent extraction is not considered the best method for extraction (Rao and Pandey, 2007).

1.2.3.1.10. Carbon dioxide extraction:

Carbon dioxide (CO_2) and supercritical carbon dioxide (SCO_2) extraction use carbon dioxide as the solvent that elutes the essential oils away from the raw plant material. The lower pressure CO_2 extraction involves chilling carbon dioxide to between 35 and 55 ºF and pumping it through the plant material at around 1000 psi pressure. The carbon dioxide is condensed to a liquid, under these conditions. Supercritical carbon dioxide extraction involves heating the carbon dioxide to 87 ºF and pumping it through the plant material at around 8000 psi. Under these conditions, the carbon dioxide is like a dense fog. With release of pressure in both these processes, the carbon dioxide escapes in its gaseous form leaving behind the essential oil, which may then be further extracted by steam distillation. By applying low pressure, and then low temperature, a good quality of essential oil can be obtained (Rao and Pandey, 2007).

1.2.3.1.11 Enfleurage:

Enfleurage is an intensive way of extracting oil from flowers such as jasmine and tuberose flowers. It involves layering fat over flower petals and after the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. Then, the alcohol is evaporated and the essential oil is collected (Rao & Pandey, 2007).

1.2.3.2. Applications of distillation:

Commercially, distillation has many applications, for example:

- In the fossil fuel industry distillation is a major class of operation in obtaining materials from crude oil for fuels and for chemical feed stocks.
- Distillation permits separation of air into its components notably oxygen, nitrogen, and argon for industrial use.
- In the field of industrial chemistry, large amounts of crude liquid products of chemical synthesis are distilled to separate them, either from other products, or from impurities, or from unreacted starting materials.
- Distillation of fermented products produces distilled beverages with a high alcohol content, or separates out other fermentation products of commercial value.
- Distillation is a very old method of artificial desalination (Khawaji, *et al.*, 2008).

1.2.3.3. Distillation of cinnamon:

Cinnamon essential oil is obtained by steam distillation (water or solvent) and soxhlet extraction. In the soxhlet extraction method, cinnamon sticks are mashed and placed inside a thimble of thick filter paper and loaded into the soxhlet extractor. The extraction solvent is then heated to reflux for hours or days, and after that the product is collected and purified using a rotary evaporator. Then the solvent is evaporated from the oil (Wong, *et al.*, 2014).

1.2.4. Edible oils:

Cooking oil is plant, animal, or synthetic fat that is used in frying, baking, and other types of cooking. It is also used in food preparation and flavoring as salad dressings and bread dips, and in this sense might be more accurately termed edible oil. Cooking oil is typically a liquid at room temperature, although some oils that contain saturated fat, such as coconut oil, palm oil and palm kernel oil are solid (Medline Plus, 2017).

About 79% of the over 100 million tons of edible oils and fats produced worldwide annually are derived from plant sources and are referred to as vegetable oils. Vegetable oils play important functional and sensory roles in food products, and they act as carriers of fat soluble vitamins A, D, E, and K. They also provide energy and essential linoleic and linolenic acids responsible for growth, and they are one of the main ingredients used to manufacture soaps, cosmetics, and pharmaceutical products. Vegetable oils are mostly used for cooking and frying of foods and snacks (Fasina and Colley, 2008).

There is a wide variety of cooking oils from plant sources such as olive oil, palm oil, soybean oil, canola oil (rapeseed oil), corn oil, peanut oil and other vegetable oils, as well as animal-based oils like butter and lard. Oil can be flavored with aromatic food stuffs such as herbs, cinnamon or garlic. Heating an oil changes its characteristics. Oils that are healthy at room temperature can become unhealthy when heated above certain temperatures, so when choosing a cooking oil, it is important to match the oil's heat tolerance with the cooking method (USDA, 2005).

All oils degrade in response to heat, light, and oxygen. To delay the onset of rancidity, a blanket of an inert gas, usually nitrogen, is applied to the vapor space in the storage container immediately after production, a process called tank blanketing. In a cool, dry place, oils have greater stability, but may thicken, although they will soon return to liquid form if they are left at room temperature. To minimize the degrading effects of heat and light, oils should be removed from cold storage just long enough for use (Kochhar and Henry, 2009).

1.2.4.1. General properties of edible oils

1.2.4.1.1. Physical properties:

1.2.4.1.1.1. *Color:*

Color measurement is very important in the edible oil industry, to analyze product purity, composition and degree of degradation. For

vegetable oils, each type of oil will have its own “sell by color” specification. Therefore, color measurement is used to ensure quality, to check final product conformance to predetermined color tolerance and compliance with customer specifications. The Lovibond color method is a widely used method that measures color in the edible oil industry by means of a tintometer. A Lovibond tintometer consists of red, yellow and blue glass standards (Tan, *et al*., 2004).

1.2.4.1.1.2. *Viscosity:*

Viscosity can be defined as the measurement of a fluid’s internal resistance to flow at a specified temperature. It is a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress. In everyday terms (and for fluids only), viscosity is "thickness" or "internal friction". Thus, water is “thin", having a lower viscosity, while honey is "thick", having a higher viscosity. Put simply, the less viscous the fluid is, the greater its ease of movement (fluidity) (Meyer, 2007).

All real fluids (except super fluids) have some resistance to stress and therefore are viscous, but a fluid which has no resistance to shear stress is known as an ideal fluid or in-viscid fluid. The study of flowing matter is known as rheology, which includes viscosity and related concepts. There are two ways to measure a fluid’s viscosity, i.e. Dynamic (Absolute) viscosity and kinematic viscosity (Meyer, 2007). Any significant change detected in the measured viscosity requires prompt action and could be indicative of severe degradation of oil, cross contamination, water ingression or other factors that can be confirmed by measuring other parameters (i.e. water content) (Fitch, 2001).

The absolute viscosity of fluids is an important property needed in fluid flow and heat transfer unit operations. This includes pumping, flow measurement, heat exchange, sterilization, freezing, and many other operations. In a study, all of the vegetable oils studied were found to be Newtonian fluids. Rice bran oil was the most viscous (0.0398 Pa.s at 38°C) while walnut oil was the least viscous (0.0296 Pa.s) (Diamante and Lan, 2014).

1.2.4.1.1.3. *Density:*

The mass density or density of a material is defined as its mass per unit volume. The symbol most often used for density is ρ (the Greek letter rho). In the United States’ oil and gas industry density is also defined as its weight per unit volume although this quantity is more properly called

specific weight. Different materials usually have different densities, so density is an important concept regarding buoyancy, purity and packaging. Osmium is the densest known substance at standard conditions for temperature and pressure (OGG, 2010).

The reciprocal of the density of a substance is called its specific volume, a representation commonly used in thermodynamics (Cengel and Boles, 2008). Density is an intensive property in that increasing the amount of a substance does not increase its density; rather it increases its mass. Mathematically, density is defined as mass divided by volume:

$$\rho = \frac{M}{V}$$

where ρ is the density, m is the mass, and v is the volume. From this equation, mass density must have units of a unit of mass per unit of volume. As there are many units of mass and volume covering many different magnitudes there are a large number of units for mass density in use. The density of oils varies with each type and temperature. The range is from 0.91 to 0.93 g/cm3 between the temperatures of 15 °C and 25 °C. Comparing to water, whose density is 1.00 g/ml, cooking oil is less dense (Dorfman, 2000).

Less dense fluids float on more dense fluids if they do not mix. This concept can be extended, with some care, to less dense solids floating on more dense fluids. If the average density (including any air below the waterline) of an object is less than water (1.0 g per ml) it will float in water and if it is more than water's it will sink in water. In some cases density is expressed as the dimensionless quantities specific gravity (SG) or relative density (RD), in which case it is expressed in multiples of the density of some other standard material, usually water or air/gas. The mass density of a material varies with temperature and pressure, this variance is typically small for solids and liquids and much greater for gasses. Increasing the pressure on an object decreases the volume of the object and therefore increase its density (OGG, 2010).

Increasing the temperature of a substance (with some exceptions) decreases its density by increasing the volume of that substance. In most materials, heating the bottom of a fluid results in convection of the heat from bottom to top of the fluid due to the decrease of the density of the heated fluid. This causes it to rise relative to more dense unheated material (Cengel and Boles, 2008).

1.2.4.1.1.4. *Refractive index:*

Refractive index is a dimensionless physical quantity, which is specific for a certain medium, and its value characterizes the speed of light in this medium. We distinguish between the relative and absolute refractive index. The absolute refractive index is defined as a ratio of the speed of light in vacuum and in selected medium. Relative refractive index is defined as a ratio of speeds of light in two different media. Usually it characterizes properties of an interface between these media. Generally, the refractive index depends on the wavelength of incident light (Tcheknavorian, 1993).

1.2.4.1.2. Chemical properties:

Lipids in edible oils are susceptible to photo-oxidation and auto-oxidation during processing and storage, which is a major problem for the oil industry. Oxidation may cause undesirable flavors and taste, decomposing the nutritional quality, and leading to production of toxic compounds. Oxidation of oils may be influenced by different factors such as the degree of unsaturation, heat, light, oil processing, antioxidants and transition metals (Choe and Min, 2006). Another important factor is the reusing of fried oils. This practice is not only restricted to roadside food stalls, and reputable food outlets in large cities also use this technique to lower their costs. The repeated heating of cooking oil result in oil that is more prone to lipid peroxidation (Jaarin, *et al.*, 2011). Furthermore, repeated frying of oils darken the oil at different rates, and the oil is discarded only when the oil becomes foamy or smelly (Azman, *et al.*, 2012).

Photo-oxidation is a much faster reaction that involves attack at double bond (Lawson, 1997). Rancidity of food items can be the result of both auto and photo-oxidation, which are natural oxidation and chemical degradation processes of edible oils, where fatty acid esters of oils are converted into FFA giving a smell observed in many vegetable oils (Anwar, *et al*., 2003). Thus, indicators of poor oil quality include elevated FFA, low smoke point, change of color, low iodine value, peroxide value, total polar material, high foaming properties and increased viscosity. To investigate oxidative initial rancidity of an oil, its PV value is determined (Kheang, *et al*., 2006).

The quality of an oil is dependent on its chemical compositions, which is affected by oxidation, and this can be measured via various parameters such as peroxide value, acid value, and iodine value (Kheang, *et al*., 2006).

1.2.4.1.2.1. *Peroxide value*:

The peroxide value (PV), which depends on temperature, time and light measures the extent of primary oxidation of oils (rancidification). Rancidity of oils can produce potentially toxic compounds associated with long term health effects such as neurological disorders, heart and cancer. Oils with a high degree of unsaturation are highly susceptible to oxidation as compared to saturated oils. Moreover, vegetable oils become rancid much faster than animal oils. Oils also become susceptible to microbial rancidity, in which microorganisms such as bacteria and yeast use their enzymes to break down chemical structures in the oil, leading to the production of unwanted odors and flavors (List, *et al*., 2005).

For instance, subjecting oils to two different frying temperatures: 130°C and 170°C, using the same frying time (20 minutes), was noticed to cause changes in the PV of pure oil, though in many cases the PV value was constant after 130°C. These results show that it is important to add antioxidants such as vitamin E or C as preservatives in vegetable oils to delay or down slow the development of rancidity (Kaleem, *et al.*, 2015).

1.2.4.1.2.2. *Acid value:*

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of potassium hydroxide (KOH) in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is and indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol (Kardash and Turyan, 2005).

Free fatty acids are a source of flavors and aromas. On one side, we have short chain FFA which tend to be water soluble and volatile with characteristic smell. On the other side, we have long chain saturated and unsaturated fatty acids. The later are more prone to oxidation in their free form and their breakdown products (aldehydes, ketones, alcohols, and organic acids) provide characteristic flavors and aromas. In most cases these flavors and aromas are considered a defect in oils, fats, and foods that contain them. However, there are instances where hydrolysis of triglycerides

and oxidation of FFA are key in the development of desirable flavor and aroma in foods. This is the case of aged cheeses and some processed meat (Cao, *et al.*, 2008).

1.2.4.1.2.3. *Saponification value*:

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the substance. The esters of the fatty acids of low molecular weight require the most alkali for saponification, so that the saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present. The saponification value is not, in general, as useful for identification purposes as the iodine value. The saponification value is of most use for detecting the presence of coconut oil (SV 255), palm-kernel oil (SV 247) and butter fat (SV 255), which contain a high proportion of the lower fatty acids (JSA, 1992).

Unsaponifiable matter can be defined as the material present in oils and fats which after saponification of the oil or fat by caustic alkali and extraction by a suitable solvent remains non-volatile on drying at 80^{o} C. Wax esters and hydrocarbons affect the saponification values of fatty compounds (Knothe, 2002).

1.2.4.1.2.4. *Iodine value*:

Iodine value (IV) is a measure of the total number of double bonds present in fats and oils. It is expressed as the "number of grams of iodine that will react with the double bonds in 100 grams of fats or oils". The determination of iodine value can be done by Wijs iodine. Fatty acids in oil or fat react with a halogen (iodine) resulting in the addition of the halogen at the double bond site (C=C) (Thomas, 2002). The higher the iodine number, the more C=C bonds are present in the fat. Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Accompanying materials such as carotenes and squalene mainly affect the iodine value of fatty compounds (Knothe, 2002).

1.2.4.1.2.5. *Hydroxyl value*:

Hydroxyl value is the number in mg of potassium hydroxide required to neutralize any acid when combined by acylation in 1 g of the substance under examination and therefore equivalent to the hydroxyl content of 1.0 g of the substance (WHO, 2006). The hydroxyl value (HV) is applicable to fatty compounds (or their mixtures) containing hydroxyl groups. The

hydroxyl value can be influenced by the presence of accompanying materials such as mono-acylglycerols and diacylglycerols (Knothe, 2002).

1.2.4.2. Sunflower oil:

Sunflower oil is the non-volatile oil obtained from the seeds of *Helianthus annuus*.

1.2.4.2.1. Morphology of the sunflower:

The sunflower plant is an annual, erect, drought-tolerant crop that reaches a height of between 1.5 and 2.5 m at flowering and has strong tap roots, from which lateral roots develop. There is one apical inflorescence on a stem of 20-30 leaves. Stems are usually round early in the season, angular and woody later in the season, and normally unbranched. The leaves are large, dark green and roughly heart shaped and they have a wrinkled surface and prominent veins. The leaves are individually stalked and arranged round the stem to maximize light interception (Martinez-Force, *et al*., 2015).

The sunflower head is made up of 1,000 to 2,000 individual flowers joined at a common receptacle. The flower head typically has a maximum diameter of 15-30 cm which consists of mostly yellow and sterile ligulate or ray flowers, and central disk flowers which mature into seeds. The flowers tend to be cross pollinating and the best temperature range for the production of seeds is 20-25°C. The expressed oil is of light amber color with a mild and pleasant flavor, whilst the refined oil is a pale yellow color (Murphy, 1994).

1.2.4.2.2. World production of sunflower oil:

The world's largest sunflower oil producers are Russia, Ukraine and Argentina. The crop originated in subtropical and temperate zones, but through selective breeding has been made highly adaptable, especially to warm temperate regions. Sunflowers are adapted to a range of soil conditions, but grows best on well- drained, high water holding capacity soils with a near neutral pH (Murphy, 1994).

1.2.4.2.3. Methods of sunflower oil extraction:

Sunflower oil can be extracted by use of chemical solvents (e.g., hexane), or cold (expeller) pressing (i.e., by crushing the seeds and then pressing them). Cold-pressing sunflower seed oil under low-temperature conditions is a preferred method, for those seeking an extraction process that doesn't involve chemical solvents. Refining sunflower oil through solvent extraction, de-gumming, neutralization, and bleaching can make it more heat-stable and suitable for high-temperature cooking, but will also remove

some of the oil's nutrients, free fatty acids, phospholipids, polyphenols, phytosterols, flavor, and color (Murphy, 1994).

1.2.4.2.4. Characteristics of sunflower oil:

According to WHO (2006), sunflower oil has the following characteristics:

Property	Value
Density	918.8 kg/m3
Color	yellow
Viscosity	0.04914 kg/(m.s) at 25 °C
Refractive index	~ 1.4735
Saponification value	188-194
Iodine value	120-145
Unsaponifiable matter	1.5-2%

1.2.4.2.5. Types of sunflower oil:

There are several types of sunflower oils, namely; high linoleic sunflower oil and this type typically has at least 69% linoleic acid and high oleic sunflower oil, which has at least 82% oleic acid and mid oleic sunflower oil. 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. Most sunflower oil used today, whether in food or cosmetics, is high-oleic. Its higher stability allows for longer product shelf life with lower risk of spoilage (NRA, 2008).

Modern oil seed biotechnology has targeted the production of fats enriched in saturated fatty acids mainly stearic acid, in common oil crops. In response, several sunflower mutants with high stearic acid have been isolated by classical breeding and mutagenesis techniques. Some of these sunflower mutants express the high-stearic trait on a high-oleic background, and so their oil is enriched in triacylglycerols which are typically present in cocoa butter (Anushree, *et al*., 2017).

1.2.4.2.6. Constituents of sunflower oil:

Sunflower oil is a monounsaturated (MUFA), polyunsaturated (PUFA) mixture of mostly oleic acid (omega-9)-linoleic acid (omega-6) group of oils. Sunflower oil is mainly triglycerides (fats), typically derived from the fatty acids linoleic acid (which is doubly unsaturated) and oleic acid (Thomas, 2002).

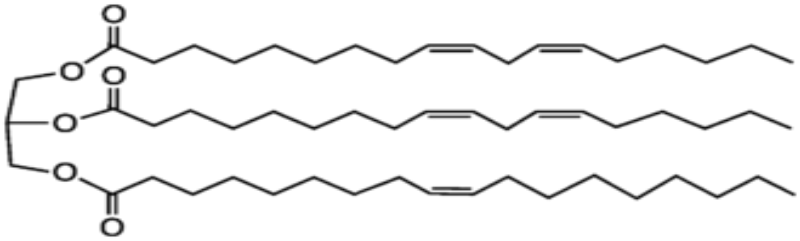


Figure (1-8). Structure of sunflower oil (Thomas, 2002)

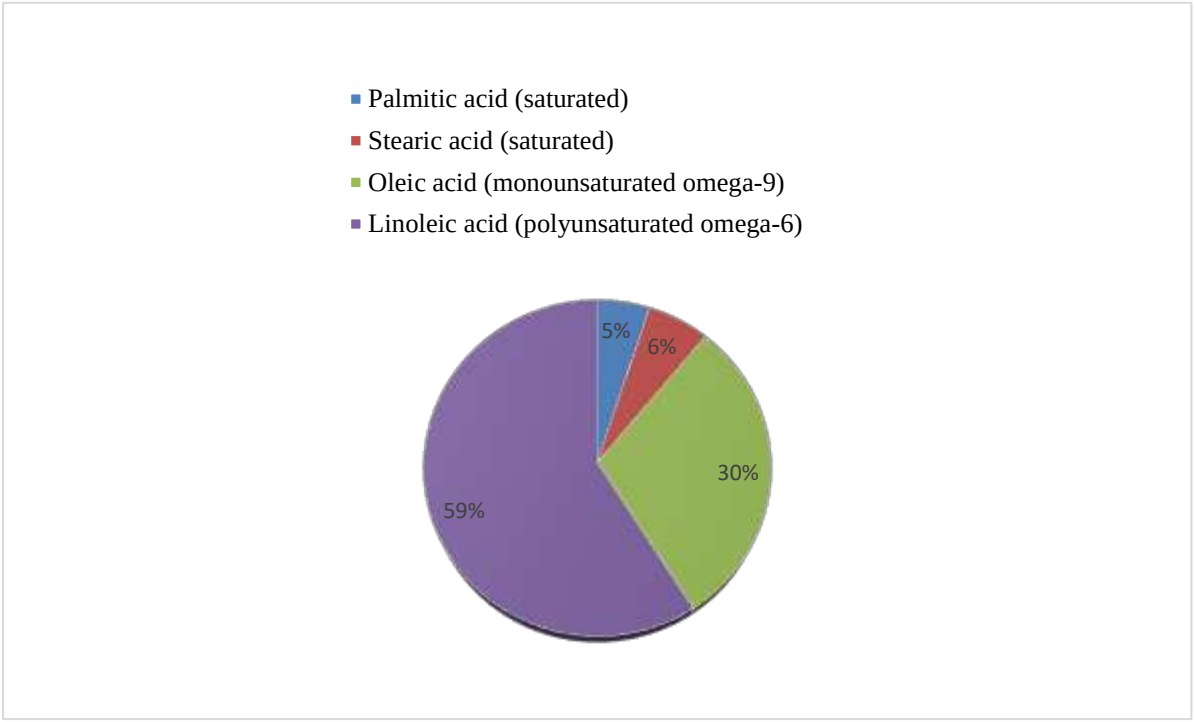


Figure (1-9): The constituents of sunflower oil (BPC, 2005)

The average protein content of the seed is around 20-30%. The oil contains appreciable quantities of vitamin E, sterols, squalene, and other aliphatic hydrocarbons. Sunflower oil also contains the phosphatides lecithin and cephalin, tocopherols, carotenoids and waxes (Murphy, 1994).

Sunflower oil is particularly susceptible to degradation by heat, air, and light, which trigger and accelerate oxidation. Keeping sunflower oil at

low temperatures during manufacture and storage can help minimize rancidity and nutrient loss as can storage in dark glass bottles or plastic that has been treated with an ultraviolet light protectant (Cox, 1979).

1.2.4.2.5. Uses of Sunflower Oil:

1.2.4.2.7.1. *As frying oil:*

Refined sunflower oil is used for low-to-extremely-high-temperature cooking. As a frying oil, it behaves as a typical vegetable triglyceride. Unrefined sunflower oil is a traditional salad dressing in Eastern European cuisines and an ingredient in sunflower butter (USDA, 1998). Some common snack foods, such as potato chips, contain sunflower oil.

1.2.4.2.7.2. *As skin moisturizer:*

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). Sunflower oil has smoothing properties and is considered noncomedogenic, meaning that it does not block pores. The high-oleic variety possesses shelf life sufficient enough for commercial cosmetic formulations (USDA, 1998).

1.2.4.2.7.3. *Cardiovascular benefits:*

Sunflower oil of all types 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 reduces the risk of heart disease (Johnson, *et al.*, 2005).

1.2.4.2.7.4. *As a fuel:*

Sunflower oil can also be used to run diesel engines when mixed with diesel in the tank (Johnson, *et al.*, 2005).

1.2.5. Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one being a stationary bed of large surface area, and the other a gas that percolates through the stationary bed. More often, the stationary phase is a porous solid covered with an absorbing liquid. A sample of the analyte is introduced by syringe injection into the heated injector tube, where it is vaporized and mixed with carrier gas (Prathap, *et al.*, 2013).

As the sample vapor is carried through the column by the carrier gas, the analyte partitions between the gas and liquid phases according to the analyte components' solubility in the liquid at the column operating temperature. This equilibrium partitioning continues as the sample is moved through the column by the carrier gas. Each component travels at a characteristic rate, and if the column has sufficient length and resolving power, the sample will be completely separated by the time it reaches the detector. The rate of movement depends on the sample’s solubility in the stationary phase, the carrier gas flow rate, and the temperature (Prathap, *et al.*, 2013).

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty. These sensitive devices were bulky, fragile, and originally limited to laboratory settings. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample (Gohlke and McLafferty, 1993).

In 1996 the top of the line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first generation GC/MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields. The GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the

substance, this could lead to false positive identification (Goesmann, *et al.*, 2005).

1.2.5.1. GC-MS Instrumentation:

The GC-MS is composed of two major building blocks, the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenylpolysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column (Niemann, *et al.*, 2005).

The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone (Prathap, *et al.*, 2013).

The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer. Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample (Niemann, *et al.*, 2005).

Samples are introduced to the column via an inlet. This inlet is typically injection through a septum. Once in the inlet, the heated chamber acts to volatilize the sample. In a split system, a constant flow of carrier gas moves through the inlet. A portion of the carrier gas flow acts to transport the sample into the column. Another portion of the carrier gas flow gets directed to purge the inlet of any sample following injection (septum purge).

Yet another portion of the flow is directed through the split vent in a set ratio known as the split ratio. In a splitless system, the advantage is that a larger amount of sample is introduced to the column. However, a split system is preferred when the detector is sensitive to trace amounts of analyte and there is concern about overloading the column (Niessen, 2001).

1.2.5.2. Applications of GC-MS:

1.2.5.2.1. In analysis of compounds:

GC-MS, in general, is used for the analysis of unknown organic compound mixtures. For instance, GC-MS can determine the composition of bio-oils processed from raw biomass. Analysis of aromatic compounds such as esters, fatty acids, alcohols, aldehydes and terpenes, present in food, beverage and perfumes can also be analyzed by means of GC-MS (Prathap, *et al.,* 2013).

1.2.5.2.2. In scientific research:

In the field of research, GC-MS is used to provide a profile of plant extracts in a variety of solvents. In metabolomics studies, GC-MS provides a profile of the volatile metabolites contributing to plant aroma, plant defense responses, and other processes. The main advantages of using GC-MS for metabolomics include its high separation power, high peak capacity, reproducible retention times, robust quantification, high selectivity and high sensitivity, and fast compound identification (Dettmer, *et al.*, 2007).

1.2.5.2.3. In medical field:

In medicine, GC-MS made it possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth. This has helped in earlier diagnosis and treatment of these diseases. Similarly, in anti-doping laboratories, GC-MS can be used to analyze athlete's urine samples for performance-enhancing drugs.

1.2.5.2.4. Security and chemical warfare agent detection:

GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification (Adams, 2007).

1.2.5.2.5. Environmental monitoring and cleanup:

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies. There are some compounds for

which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective (Bliesner, 2006).

1.2.5.2.6. Forensic and criminal cases:

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate (Prathap, *et al.*, 2013).

CHAPTER TWO

2. Materials and Methods

2.1. Materials:

2.1.1. Samples:

- *Cinnamomum zeylanicum* bark.
- Sunflower oil.
- Six species of bacteria.

2.1.2. Reagents and solutions:

- Diethyl ether.
- Dimethyl sulfoxide (DMSO).
- Distilled Water.
- Ethanol.
- Gentamicin.
- Glacial acetic acid.
- Hydrochloric acid.
- Mueller–Hinton agar
- Normal saline.
- Phenolphthalein solution.
- Potassium hydroxide.
- Potassium iodide.
- Sodium hydroxide.
- Sodium thiosulfate.
- Starch solution.
- Sulfuric acid.

2.1.3. Apparatus:

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

2.1.4. Instruments:

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

2.2. Methods:

2.2.1: Samples preparation:

Cinnamomum zeylanicum bark was purchased from Shendi local market, while the sunflower oil sample was purchased from Arabian Oils Company, Khartoum. Six types of bacteria (three gram positive and three gram negative) were prepared in the Laboratory of Medical Laboratories College, Shendi University. The cinnamon sticks were mashed prior to use.

2.2.2. Experiments and tests:

The determination of the chemical profile of cinnamon oil was carried out at the laboratory of the Faculty of Science, University of Khartoum. The physical and chemical properties of oils were carried out at Researches Center and Industrial Consulting, Shambat, Khartoum. Antibacterial activity of cinnamon oil was done at the Medical Laboratories College, Shendi University.

2.2.2.1. Extraction of cinnamon oil:

Cinnamomum zeylanicum essential oil was obtained by steam distillation and soxhlet extraction. In steam distillation, mashed cinnamon sticks were placed into a distillation flask and connected to a steam generator via a glass tube, and condensor. The essential oils were extracted by boiling water for four hours. The recovered mixture was allowed to settle and the oil was collected. The oil yield was 0.5%. The separation process of cinnamon oil from water was mainly dependent on the difference in their densities by using the laboratory separating funnel. The cinnamon oil was kept in a clean, dark bottle and was ready for tests.

2.2.2.2. Determination of cinnamon oil components:

Cinnamomum zeylanicum oil was analyzed by using a gas chromatography mass spectroscopy (GCMS- QP 2010 plus) equipped with selective detector mass spectroscopy. Operating conditions for the gas chromatography are shown in Appendix I. The identification of oil constituents was carried out by peak matching library research using the standard mass library (NIST147 & WILEY7).

2.2.2.3. Determination of physiochemical properties:

2.2.2.3.1. Physical properties:

I- ***Determination of Color:-***

The color was intensively read using a Lovibond tintometer; and units of red, yellow and blue were measured according to the AOCS method (1989). The sample of oil was placed in a standard sized glass cell. The instrument was switched on and viewed through the eye piece and visually compared with red, yellow, blue, and neutral color standards. The results are expressed in terms of the numbers associated with the color standards.

II- ***Determination of refractive index:***

Abbey refractometer was used in this procedure. A drop of the sample was transferred into a glass slide of the refractometer. Water at 30 °C was circulated around 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 a simplified method that has 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 ºC). The vial was cleaned and dried, and then was weighed empty (A). The vial was then filled with prepared sample in such a manner as to prevent entrapment of air bubbles and then the vial was weighed with the oil sample (B). The previous steps were repeated with pure water (C).

Calculation:

$$\text{Specific gravity at 30 ºC} = \frac{B-A}{C-A}$$

where:

A: weight in gm of empty vial at 30 ºC.

B: weight in gm of vial with oil at 30 ºC.

C: weight in gm of vial with water at 30 ºC.

IV- ***Determination of Viscosity:***

The viscosity of the oil sample was measured according to Cocks and Van Rede (1966). The viscometer was suspended in the constant temperature bath (35 ºC) so that the capillary was standing vertical. The instrument exactly filled to the mark at the top of the lower reservoir with

the oil by means of a pipette inserted to the side arm of the tube, the oil moved in to the top of the upper reservoir. Then liquid was allowed to flow freely through the tube and the time required for meniscus to pass from the work the top of the upper reservoir to the bottom of the upper reservoir was recorded, and the flow time of distilled water measured in the same way.

Calculation:

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

where:

T: flow time of the oil.

T_0: flow time of distilled water.

2.2.2.3.2. Chemical properties:

I- ***Determination of Peroxide value*** **(PV)*:-***

Principle of reaction*:*

PV is a redox titrimetric determination. The assumption is made that the compounds reacting under the condition of the test are peroxides or similar products of lipid oxidation. By addition of excess potassium iodide which reacts with the peroxides, iodine is produce. In the titration process, iodine 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 it was closed and kept for 5 minutes. Then, 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) (Pearson, 1970).

Calculation:

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

w*here:*

S: volume of titrant (ml) for sample.

B: volume of titrant (ml) for blank.

N: normality of $Na_2S_2O_3$ solution.

1000: conversion of units (g/kg).

W: weight of sample (g)

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 (White, 1991).

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

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. The oil sample was dissolved in the neutral solvent, the mixture was shaken constantly until a pink color which persisted for 15 second was obtained and then titrated with aqueous sodium hydroxide (0.1 N) (Pearson ,1970) .

Calculation:

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

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- ***Saponification value:***

Principle of reaction:

The saponification value of an oil or fat is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the sample.

$$C_3H_5(C_{17}H_{35}COOH)_3 + 3KOH \longrightarrow C_3H_5(OH)_3 + 3C_{17}H_{35}COOK$$

The method:

35g potassium hydroxide was dissolved in 20 ml water and diluted to 1 liter with alcohol (95%) and the stood overnight and decanted off the clear liquid. 2 g of the oil sample was weighed into a conical flask and 25 ml alcoholic potassium hydroxide solution was added. A reflux condenser was attached, and the flask was heated in boiling water for 1 hour, and was shaken frequently. 1 ml of phenolphthalein (1%) solution was added and the excess alkali titrated with 0.5 N hydrochloric acid. A blank was carried out at the same time.

Calculation:

$$\text{Saponification value} = \frac{(b-a)\times 28.05}{wt}$$

where:

a: titration volume of sample test in ml.

b: titration volume of blank in ml.

wt: sample weight in g.

28.05: concentration conversion coefficient in mg/ml

2.2.2.4. Antibacterial activity of cinnamon oil:

To assess the antibacterial activity of cinnamon oil, six types of bacteria (three gram positive and three gram negative) were prepared. The cup plate agar diffusion method was adopted to assess the antibacterial activity of the extracted oil (Kavanagh, 1972).

The method:

The medium was cooled to 45–50ºC and poured into plates, and then allowed to set, on a level surface, to a depth of approximately 4mm. The antibacterial disc stocks were kept at -20 ºC. A supply of cotton wool swabs on wooden applicator sticks was prepared. Two milliliters of the standardized bacterial suspension were mixed thoroughly with 250 ml nutrient agar at 45 ºC. Aliquots of the inoculated agar (20 – 25 ml) were distributed into sterile petri dishes. The agar was left to solidify and 4 wells (each 7 mm in diameter) were made using a sterile cork bore (No. 7). Four concentrations of cinnamon oil extracts (6.25, 12.5, 25, 50 and 100 %) were prepared by dissolving the extracts in dimethyl sulfoxide (DMSO). The effects of the cinnamon oil were considered after 24 hours by measuring the inhibition zone diameter (IZD) after each treatment. Three replicates were carried out for each extract and control against the test organisms. The positive control used was 30 µL gentamycin, a commercial antibiotic. The negative control used was 100% DMSO. 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.

2.3. Statistical analysis:

After collection, the data was analyzed using SPSS statistical analysis software, version 16.0, on Windows 7 Ultimate operating system. The results were considered to be significant when p value was less than or equal to 0.05.

CHAPTER THREE
3. Results and Discussion

3.1. Chemical profile of cinnamon oil:

Cinnamon oil was analyzed using gas chromatography-mass spectrometry (GC-MS) to obtain its constituents. The cinnamon bark oil chromatogram shows 13 peaks and the analysis gave 11 distinct compounds. The compounds were; styrene, benzaldehyde, benzenepropanal, L-borneol, cinnamaldehyde, α-copaene, α-amorphene, β-cedrene, α-muurolene, β-bisabolene, and δ-cadinene. The findings are similar to those obtained in a study on cinnamon essential oil where cinnamaldehyde, limonene and copaene dominated the oil obtained by steam distillation (Saleem, *et al.*, 2015). In the present study, cinnamaldehyde also dominated, followed by δ-cadinene. The results are shown in Tables (3-1), (3-2), and Figure (3-1).

Table (3-1): The chemical constituents of cinnamon oil as analyzed by GC-MS

Peak	Constituent	Percentage	Retention Time	Base Peak	Mass Peak
1	Styrene	0.68	9.393	104.05	383
2	Benzaldehyde	0.46	11.788	106.05	390
3	Benzenepropanal	0.52	18.296	91.05	395
4	L-borneol	1.16	18.422	95.10	413
5	E-Cinnamaldehyde	78.86	20.011	131.05	402
6	E-Cinnamaldehyde	3.06	21.771	131.10	443
7	Cinnamaldehyde	7.92	21.986	131.05	409
8	α-copaene	0.81	24.315	119.10	418
9	α-amorphene	0.80	26.892	161.10	436
10	β-cedrene	1.89	26.983	119.10	441
11	α-muurolene	1.89	27.481	105.05	438
12	β-bisabolene	0.67	27.613	69.10	442
13	δ-cadinene	3.02	28.041	95.10	433

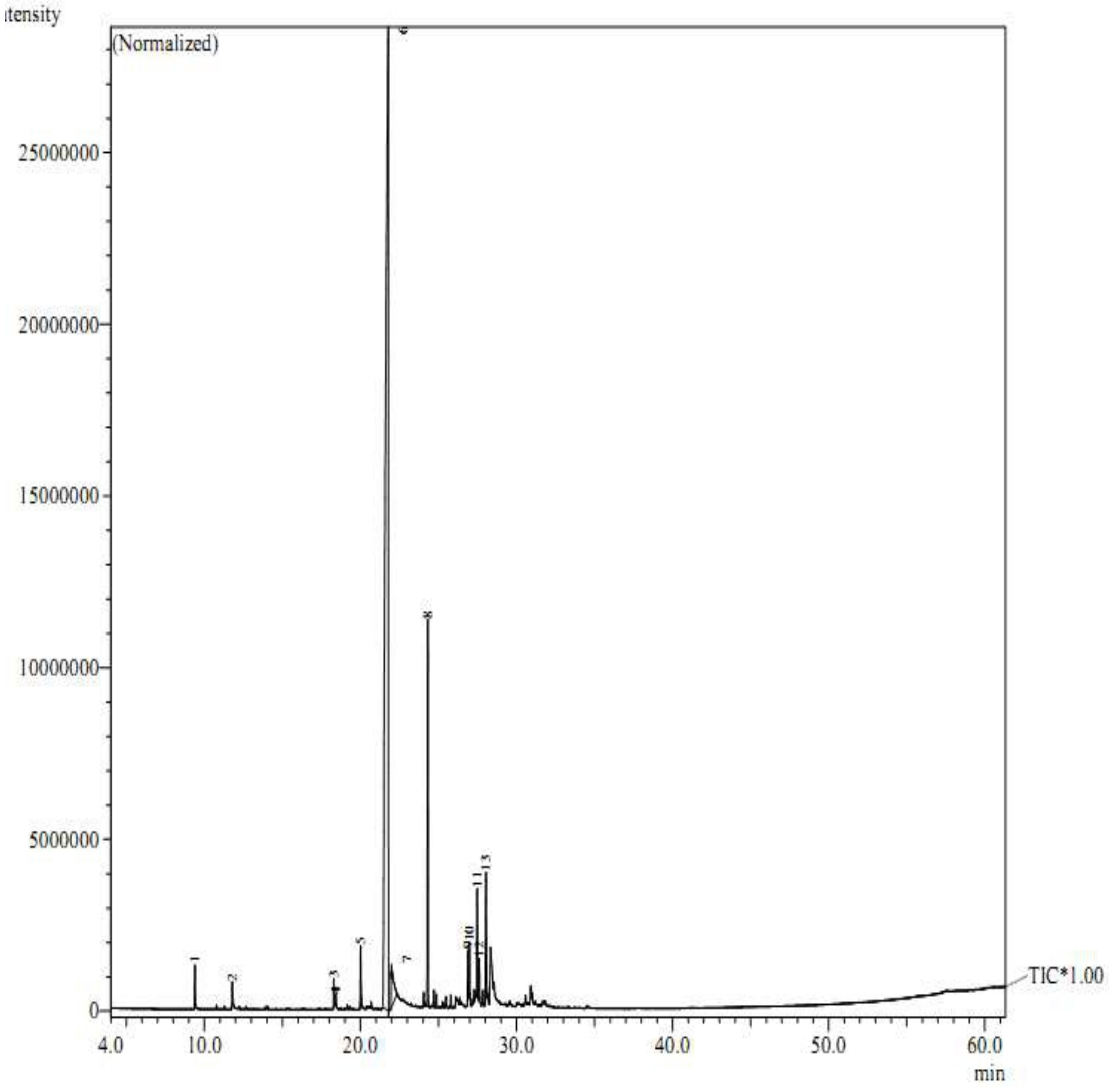
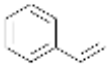
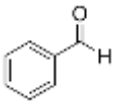
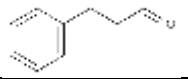
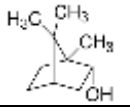
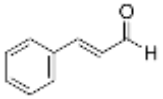
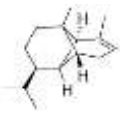
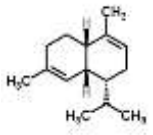
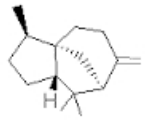
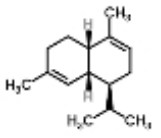
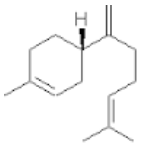
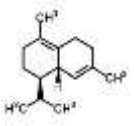


Figure (3-1): Chemical profile of extracted cinnamon oil obtained from GC-MS

Table (3-2). Compounds obtained from GC-MS of cinnamon essential oil

Compound	Compound group	Molec. Formula	Structure
Styrene	alkene	C_8H_8	
Benzaldehyde	aldehyde	C_7H_6O	
Benzenepropanal	aldehyde	$C_9H_{10}O$	
L-borneol	monoterpene alcohol	$C_{10}H_{18}O$	
Cinnamaldehyde	aldehyde	C_9H_8O	
α-copaene	sesquiterpene	$C_{15}H_{24}$	
α-amorphene	sesquiterpene	$C_{15}H_{24}$	
β-cedrene	sesquiterpene	$C_{15}H_{24}$	
α-muurolene	sesquiterpene	$C_{15}H_{24}$	
β-bisabolene	sesquiterpene	$C_{15}H_{24}$	
δ-Cadinene	sesquiterpene	$C_{15}H_{24}$	

3.2. Physiochemical properties:

The effect of cinnamon oil on the physiochemical properties of sunflower oil was assessed by determine the effect of storage and increasing of added oil volume. This was achieved by determining the values of newly-produced samples of sunflower oil and three mixtures of the two oils. The mixtures were composed from the same three sunflower sample plus different three volume of cinnamon oil separately; 0.1 ml, 0.3ml and 0.5ml, and then the values at the beginning, during and at the end of storage were compared.

3.2.1. Physical properties:

3.2.1.1. *Refractive index:*

The refractive index of sunflower oil is 1.47. During the storage period, it did not change, and when 0.1, 0.3 and 0.5ml cinnamon oil were added there was no observable change in refractive index of sunflower oil, as can be seen from Figure (3-2).

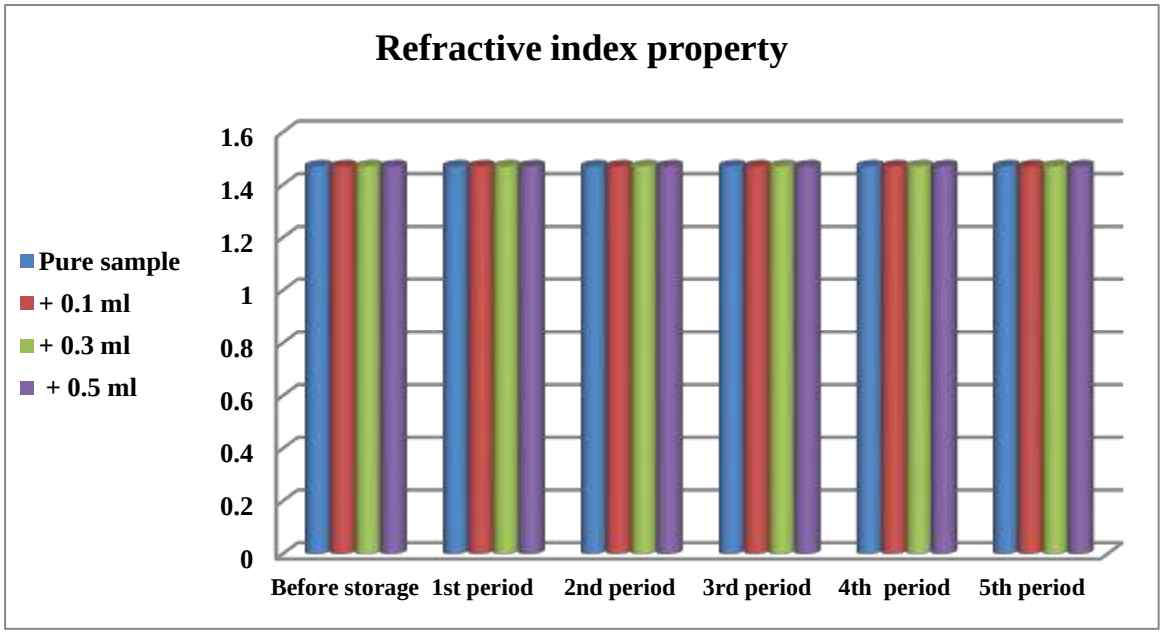


Figure (3-2): Refractive indices of pure sunflower oil and the mixtures of sunflower oil and cinnamon oil

The oil refractive index is a physical property that depends mainly on oil density. Since the density of the new sample and mixtures oil compared with the end storage values did not vary significantly, this explains why the refractive index remained constant throughout the storage.

3.2.1.2. *Viscosity:*

The viscosity of fresh sunflower oil was 43.9 and it increased with storage to 50.78. Also the increase was observed after three different volumes of cinnamon oil were added, illustrated by Figure 3-3.

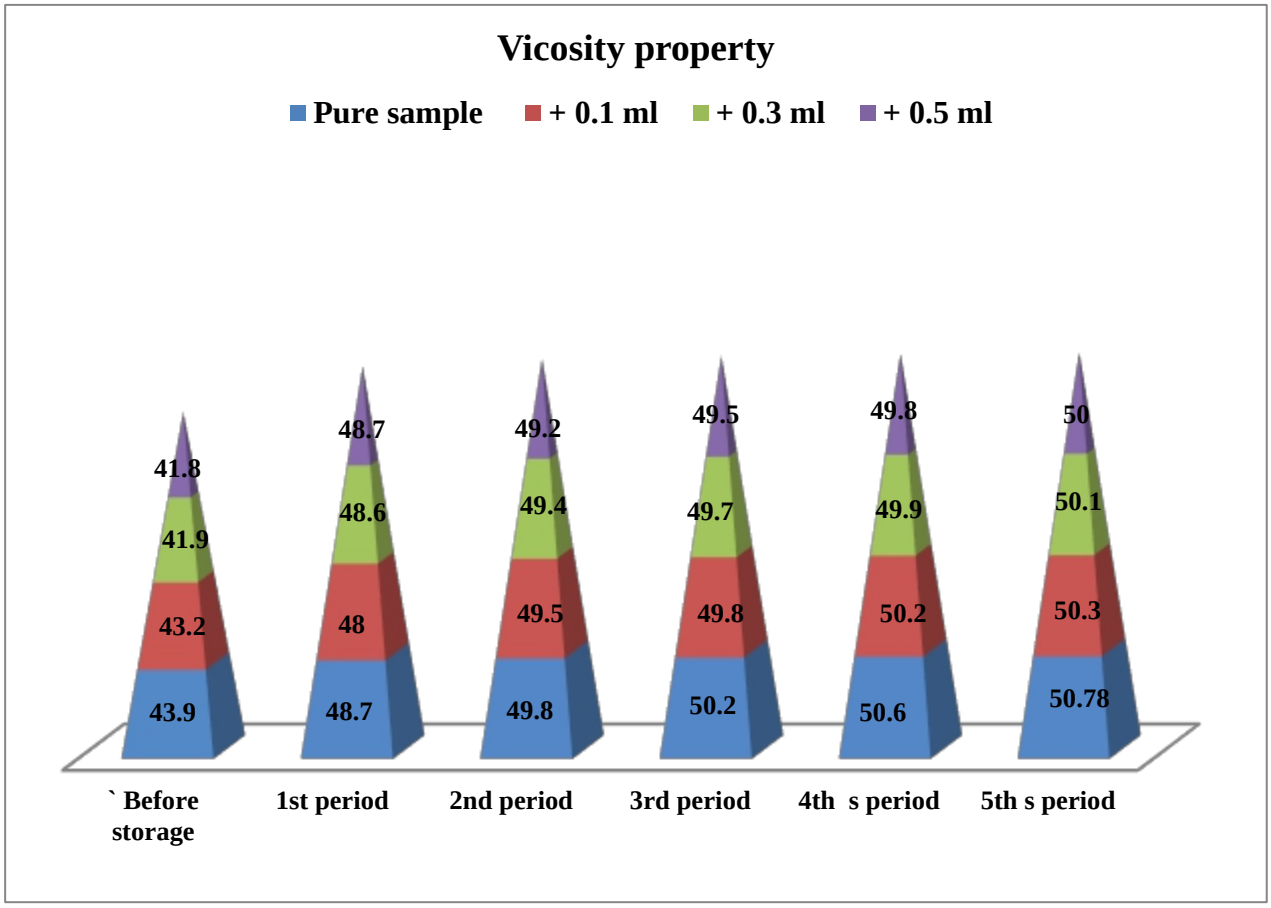


Figure (3-3). The effect of extracted cinnamon oil on the sunflower oil viscosity.

The viscosity of edible oils, in general, depends on the molecular structure of the oil and the nature of the triacylglycerols present in the oil, the temperature and on shear stress. The increase indicates the storage effect on the viscosity of sunflower oil via oxidation process. Oxidation changes the chemical composition of oils and hence it alters the viscosity of the oil. The values were analyzed statistically and the P-value was found to be less than 0.05, making the results statistically significant.

3.2.1.3. *Specific gravity*

The specific gravity of sunflower oil is less than the specific gravity of water, thus it floats on the water and its value is 0.91. At the end of storage, the specific gravity of the oil had gradually increased and reached 0.94. In general, the specific gravities of a fresh sample of sunflower oil and three mixtures increased with storage, as shown in Figure (3-4).

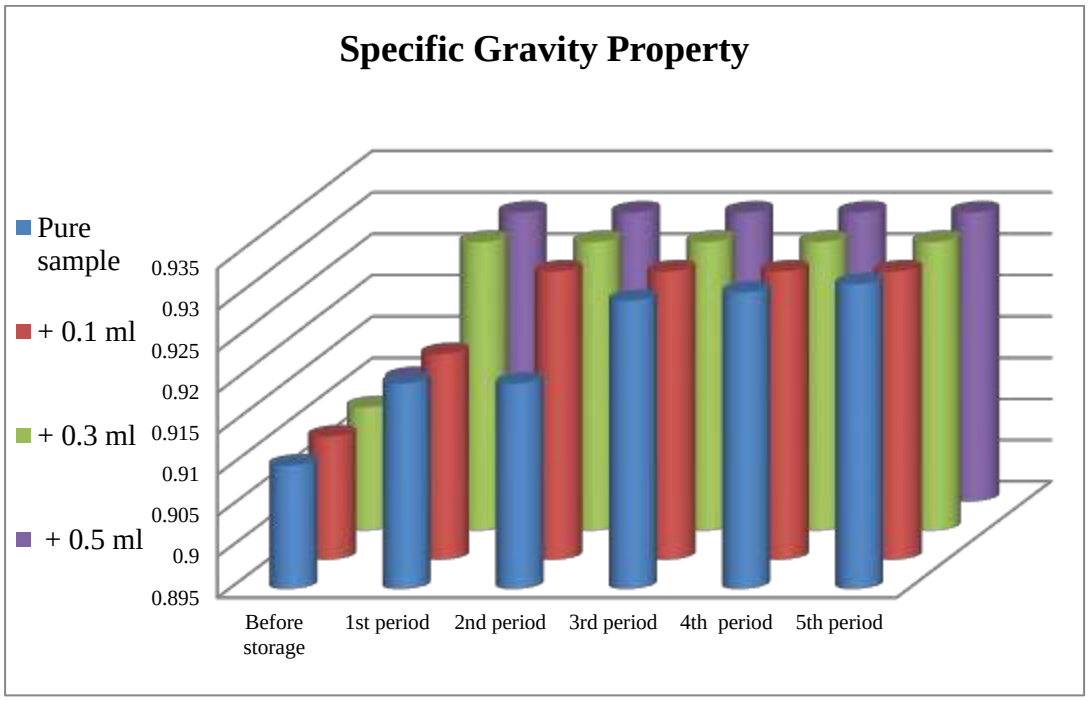


Figure (3-4): The effect of cinnamon oil on sunflower oil specific gravity

The specific gravity ranged between 0.92 - 0.93. This means that the sunflower oil density increased gradually with storage duration. When cinnamon oil was added to the sunflower oil in the range of 0.1, 0.3 and 0.5 ml, the average readings were all found to be 0.93. Even though, the average specific gravities appear to be the same, the trend for the specific gravity of sunflower oil alone increased with storage duration. On the other hand, the specific gravity of the sunflower oil reached a constant value of 0.93, at all three levels, which suggests that the cinnamon oil prevents further increase in the density of the sunflower oil. In fact, part of the increase in density can be attributed to the cinnamon oil itself, which is denser than the sunflower oil and water.

3.2.1.4. *The color:*

According to the National Renderers Association (2008), the red color is more significant in studies to determine the oil color since red color is the standard dye found in oils. The effect of cinnamon oil on the sunflower oil red color was investigated for newly-produced samples and three mixtures. The study compared the values at the initial of storage period with the mean value of five intervals of storage, Figure (3-5) shows that.

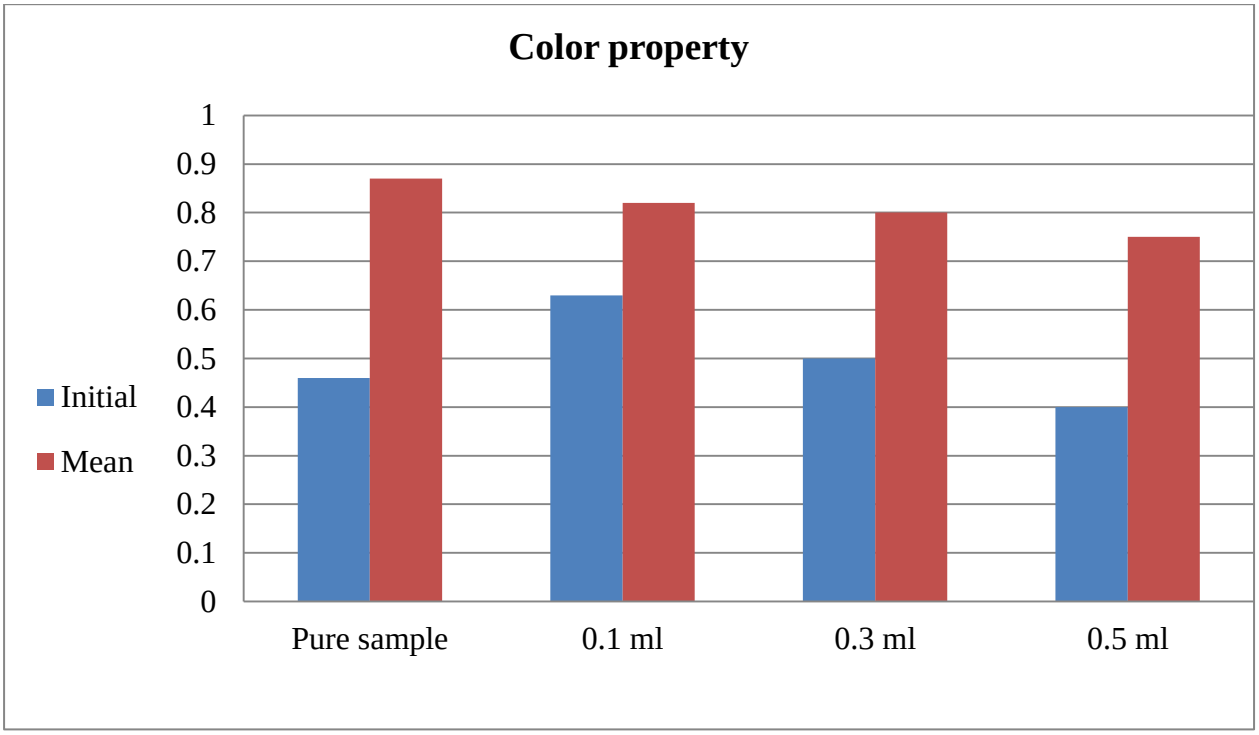


Figure (3-5). The red color of pure and three mixtures oils

The red color value of new sunflower oil sample was 0.46 and increased during storage period to 0.87 that represents the average value of five interval storage. The values were analyzed statistically and the P-value was found to be less than 0.05, making the results statistically significant. At this level the study revealed that the storage had a noticeable effect on the red color of the pure sample and this could be explained due to the storage factors i.e. temperature and light. The added volume of cinnamon oil appeared to have a clear drop in the mean values of red color with increasing volume when it is compared with the beginning and the end of storage of pure sample values, and this might be due to variety in cinnamon oil itself or due to different reactions between the two oils.

3.2.2. Chemical Properties

3.2.2.1. *Peroxide value:*

The effect of addition of cinnamon oil to sunflower oil was studied by measuring the peroxide value for sample without cinnamon oil as well as three mixtures throughout the storage periods. The peroxide value of the fresh sample of sunflower oil was revealed to be gradually increasing during the five storage periods; 2.8, 5.0, 6.8, 8.5, 10 and 10.8 respectively. Instead, the peroxide values of the three mixtures at the end of storage were less than those of the pure sunflower oil sample which were 8.9, 8.7 and 8.5 for the 0.1, 0.3 and 0.5 ml added cinnamon oil respectively. These results reflect the effects of cinnamon oil addition itself and the added volume. When 0.5ml cinnamon oil is added, the PV does not go beyond 8.5, which is attained by the pure sunflower oil from just 3 weeks of storage, as seen in Figure (3-6).

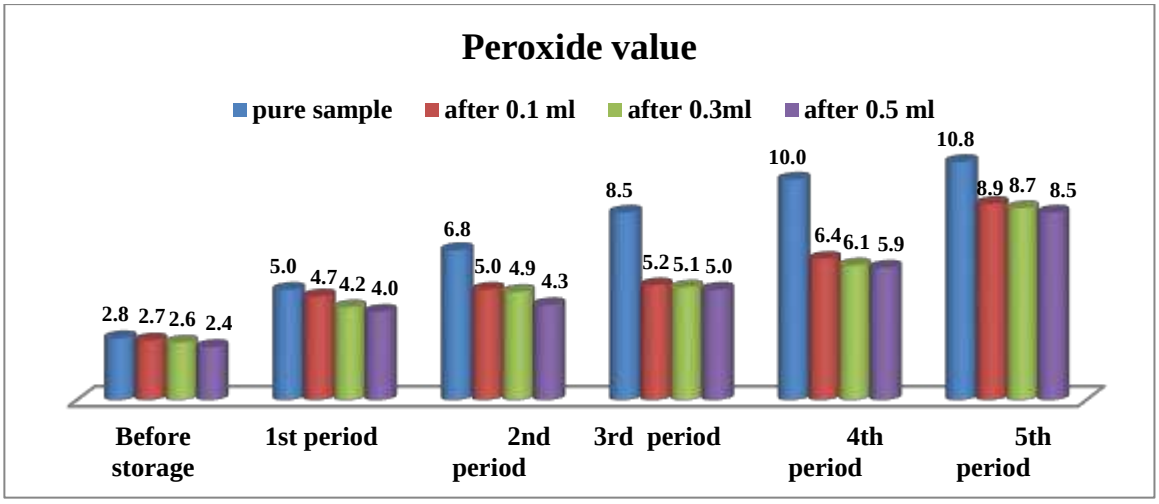


Figure (3-6): The effect of cinnamon oil on the sunflower oil peroxide value

Peroxide is the main product that gives rise to objectionable flavor in food products, and it proceeds through the free radical chain reaction, where it attacks the double bond at room temperatures. Thus, the double bonds found in fats and oils play an essential role in autoxidation. Our findings may be due to the effect of temperature of storage, humidity and oxygen which collectively cause oil oxidation and in turn result in elevation of peroxide value that continued to increase throughout the storage periods. However, when cinnamon oil is added, less oxidation takes place and therefore a clear reduction in peroxide value is noted since the cinnamon oil has multiple antioxidants compounds such as α-copaene, cinnamaldehyde and benzaldehyde. The values were analyzed statistically and the P-value was found to be 0.01, making the results statistically significant.

3.2.2.2. Acid value:

The study shows that the acid value of the pure sample and three mixtures increased along with storage. The acid values before and at the end of storage were: (0.43 – 1.57), (0.43 – 0.67), (0.4 – 0.64), and (0.4 – 0.61) for the pure sample and three mixture respectively. The values were analyzed statistically and the P-value was found to be 0.04, making the results statistically significant. Figure (3-7) illustrates these findings.

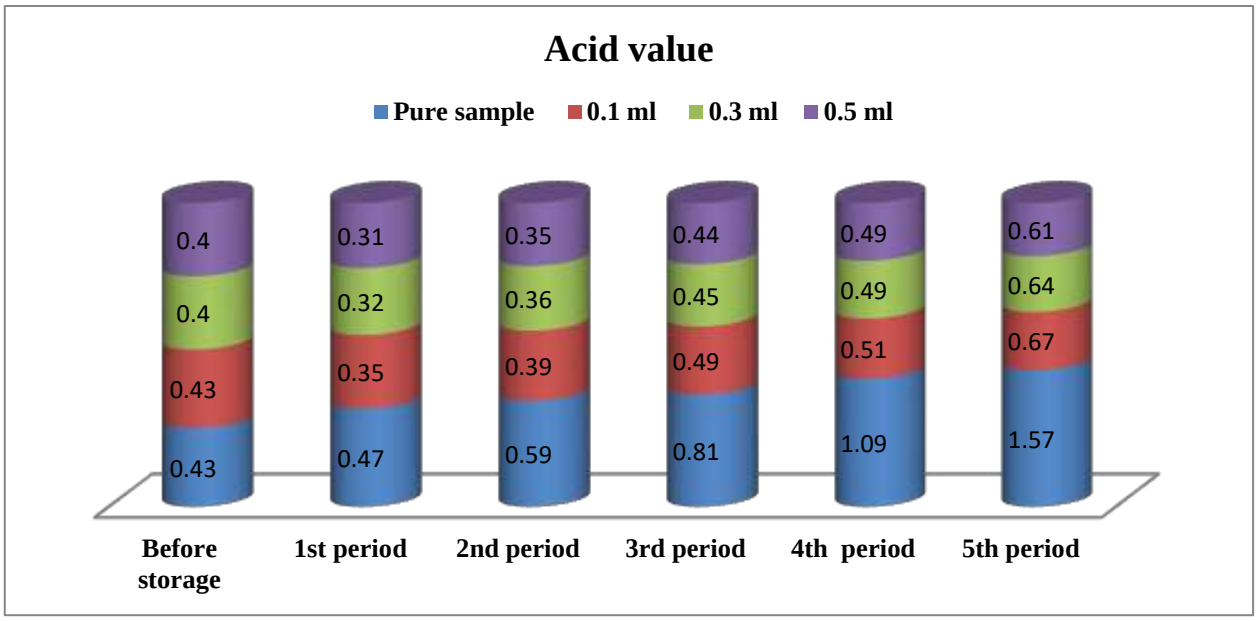


Figure (3-7): Acid value of sunflower oil and oil mixtures

The oils, during the storage period, were exposed to the degradation of esters by oxidation and rancidity process leading to release of free fatty acids and thus increasing the carboxyl groups, which result in increasing of the oils' acid value. Our study elucidated a big drop in the acid value after the first period of storage for the three added mixtures, and then the values started to increase until the end of storage. The average of acid values for pure sunflower oil during the storage period was 0.91, and when cinnamon oil is added at the range of 0.1ml, 0.3ml, and 0.5ml, the average readings obtained were 0.48, 0.45, and 0.44 respectively. This could be due to the reaction occurring in the three mixtures between carboxyl groups of free fatty acids in sunflower oil with hydroxyl groups of alcoholic compounds in the extracted cinnamon oil which lead to ester formation that is responsible for a decrease in free carboxyl groups and thus the acid value.

3.2.2.3. Saponification value:

The fresh sample of sunflower oil gave a saponification value of 184.6 and during the five storage periods, this value increased to 191.5, 199.2, 213.9, 220.7 and finally 229.9. When cinnamon oil was added as 0.1, 0.3 and 0.5ml, the saponification values before storage were 184.6, 184.29 and 184.2, respectively. At the end of storage, these values had all increased to 222.6, 219.3 and 210.8 respectively. These findings shown in Figure (3-8).

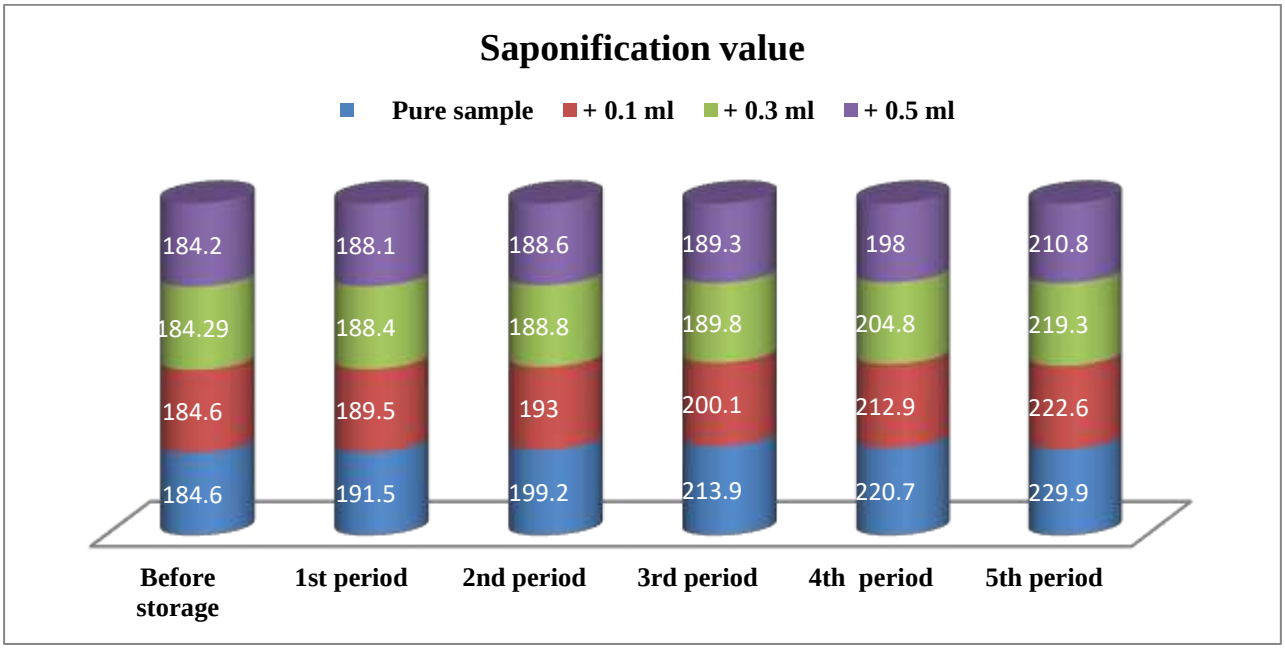


Figure (3-8): The effect of cinnamon oil on the sunflower oil saponification value

During the storage period, the saponification value increased due to the increase in the esters being broken down into alcohols and free fatty acids. Plenty of fatty acids were neutralized by titration with potassium hydroxide and thus result in an increase in the saponification value. When cinnamon oil is added in varying amounts, as in the figure above, there is a decrease in the oxidation of the oil, which might be due to the presence of antioxidants which act as reducing agents and reduce the ester breakdown.

On the other hand, the fatty acids in the cinnamon oil may have higher molecular weight than the fatty acids of sunflower oil, so they consume less alkali and cause a decline in the saponification values as it is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present. The values were analyzed and the P-value was found to be 0.02, thus the results are statistically significant.

3.3. Antibacterial activity of volatile cinnamon oil

The antibacterial activity of the extracted of C*innamomum zeylanicum* was investigated via the cup-plate agar diffusion method on six different

types of bacteria. Three of the bacteria were Gram positive; *Enterococcus fecalis*, S*taphylococcus aureus*, *and bacillus cereus*. The other three were Gram negative bacteria, i.e. *Escherichia coli*, *pseudomonas aeruginosa*, and *Salmonella typhi*. The cinnamon oil was prepared at five different concentrations (6.25%, 12.5%, 25%, 50%, and 100%). DMSO (Dimethyl sulfoxide) was used as the solvent, and as a negative control. For the positive control, Gentamicin, a commercial antibiotic was used.

From the results of the minimum inhibition zone diameters (MIZD), in mm, of all six species of bacteria, it can be noted that the extracted cinnamon oil was effective against all six types of bacteria. Figures (3-9) - (3-15) and Tables (3-3) – (3-5) illustrate the antibacterial activity results of cinnamon oil.

Figure (3-9): The effect of cinnamon oil on *Staphylococcus aureus*

Figure (3-10): The effect of cinnamon oil on *Bacillus cereus*

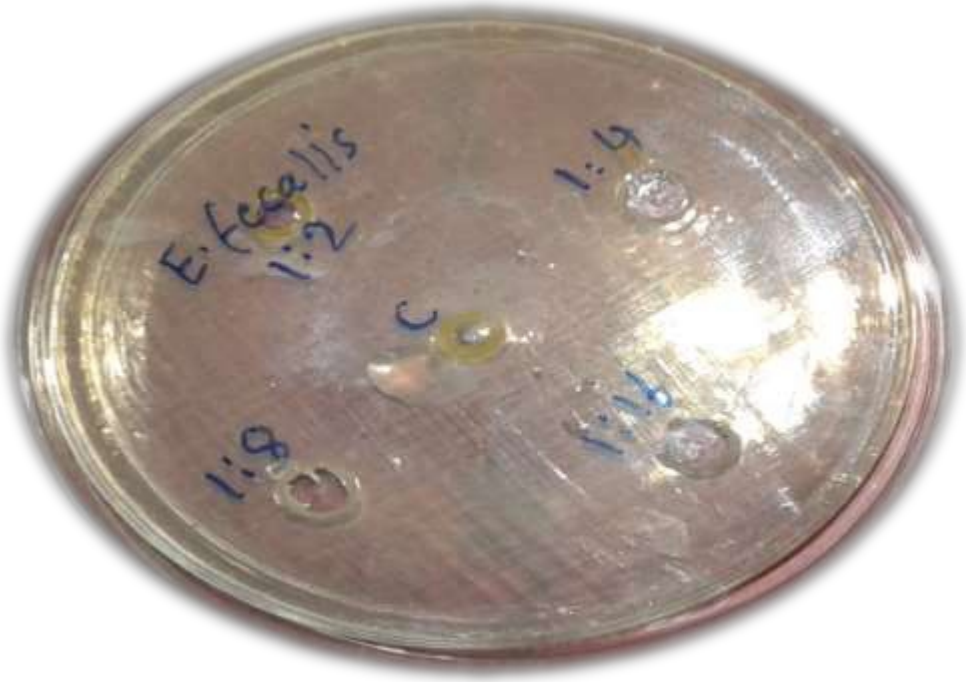


Figure (3-11): The effect of cinnamon oil on *Enterococcus fecalis*

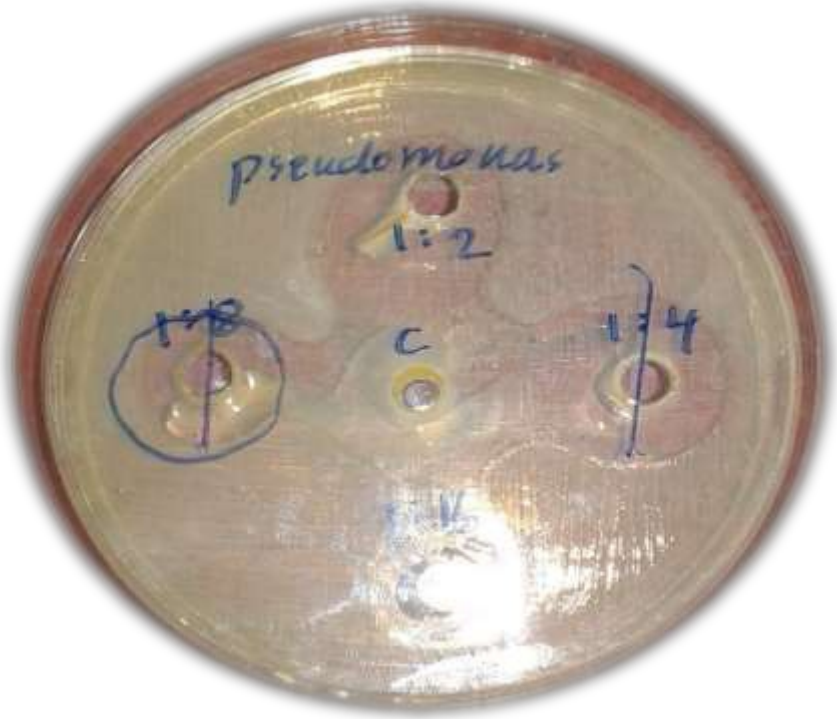


Figure (3-12): The effect of cinnamon oil on *Pseudomonas aeruginosa*

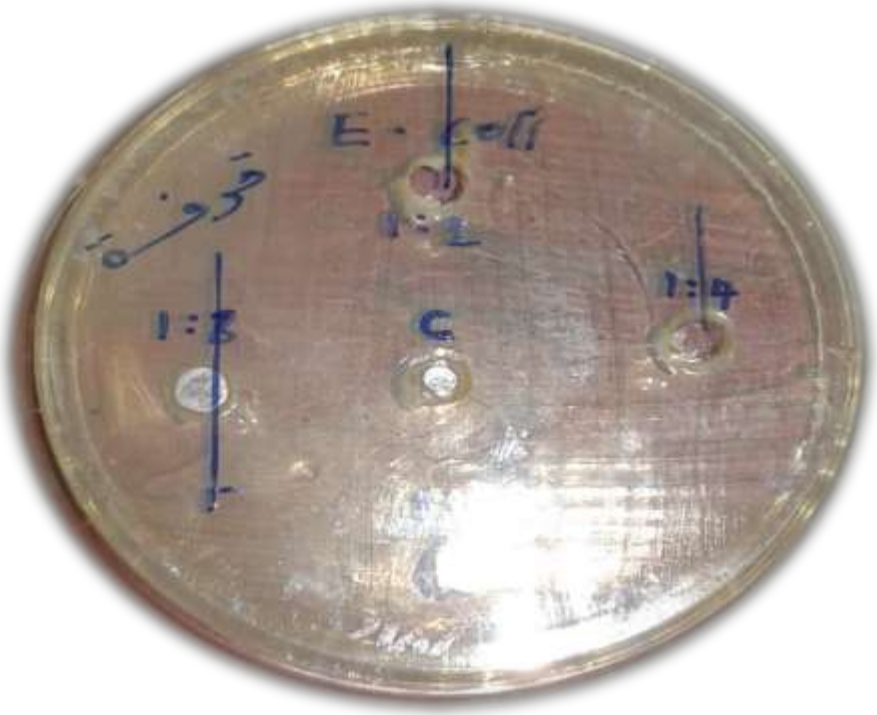


Figure (3-13) The effect of cinnamon oil on *Escherichia coli*

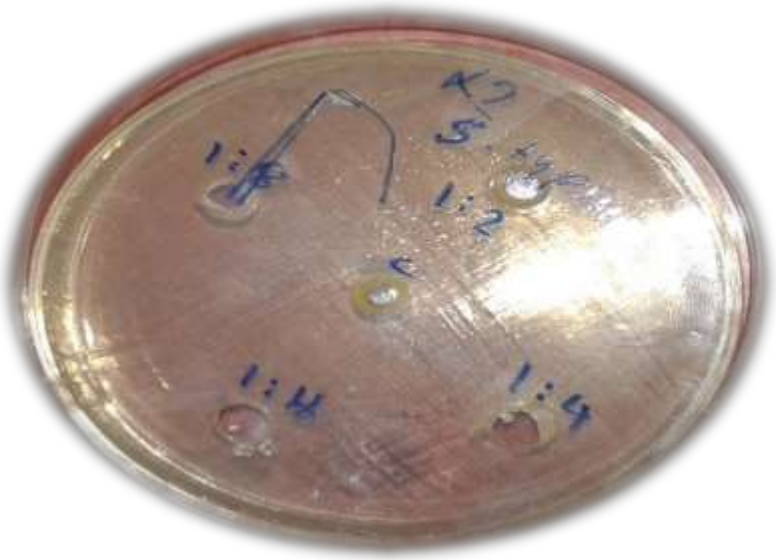


Figure (3-14): The effect of cinnamon oil on *Salmonella typhi*

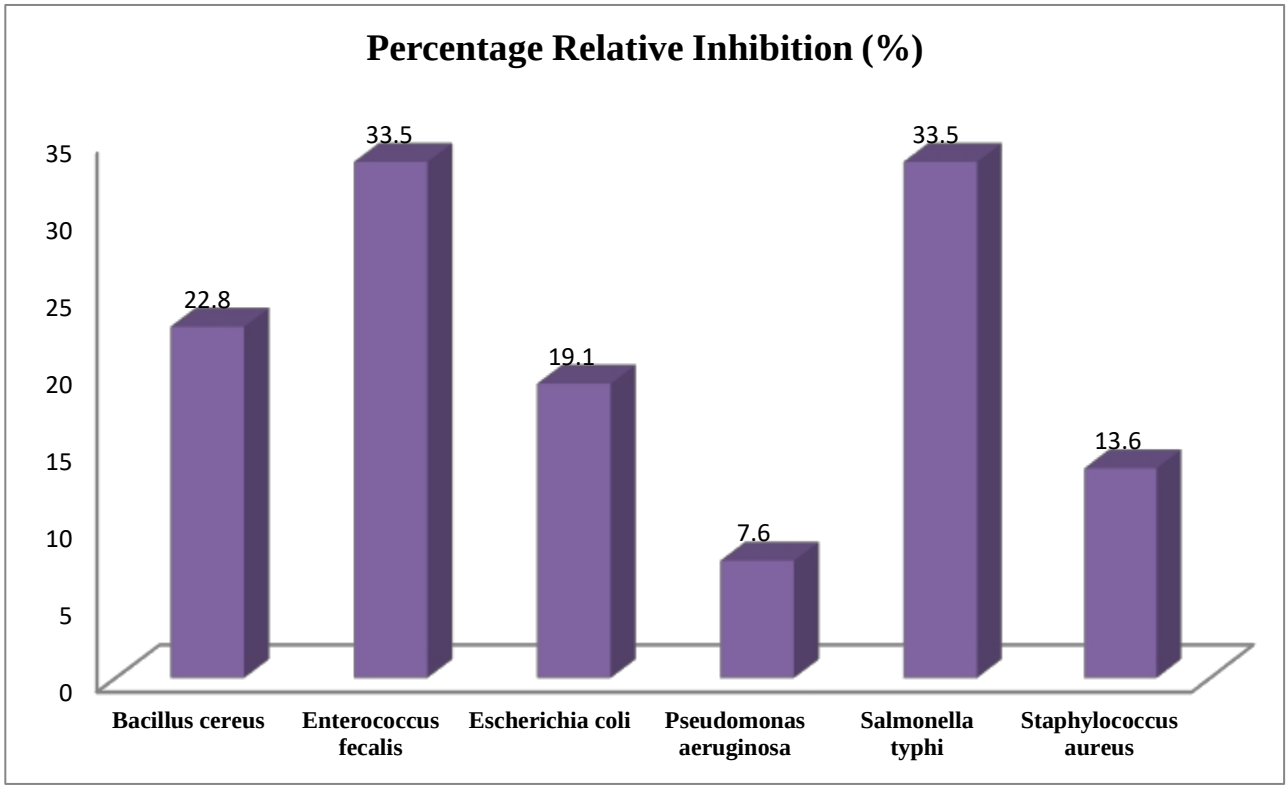


Figure (3-15). Relative percentage inhibition of cinnamon oil as antibacterial activity

Table (3-3). Mean inhibitory zone diameters for gram positive and negative bacteria

Bacteria	Mean (mm)
Gram positive	40
Gram negative	32

Table (3-4) Antibacterial activity of different concentrations of cinnamon oil compared to positive and negative controls (Gentamicin & DMSO)

Species	Type of Bacteria	MIZD in mm (%)						
		6.25	12.5	25	50	100	Gentamicin	DMSO
S. aureus	Gram positive	40	40	40	40	40	37	0
Bacillus cereus	Gram positive	40	40	40	40	40	22	0
E. fecalis	Gram positive	40	40	40	40	40	15	0
P. aeruginosa	Gram negative	16	17	20	22	25	20	0
Escherichia coli	Gram negative	30	30	32	34	40	19	0
Salmonella typhi	Gram negative	40	40	40	40	40	15	0

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The MIZD values of three gram positive species; *S. aureus*, *B. cereus*, and *E. fecalis* were all 40mm which mean that a minimum concentration of 6.25% is sufficient to produce a high antibacterial effect. This result indicated that the cinnamon oil has a significant inhibitory effect on gram positive bacteria, as shown by the higher value of the mean inhibition zone diameter for the gram positive bacteria, 40mm, when compared to that of the gram negative bacteria. Smith-Palmer, *et al*., (2001) found that the gram positive bacteria are generally more sensitive to essential oils and this could be due to the lack of thickness of the bacterial wall, which makes it more permeable to the hydrophobic essential oils than gram negative bacteria.

In the case of the three species of gram negative bacteria, i.e. *P. aeruginosa*, *E. coli* and *S. typhi*, it can be noted that the diameter

increased with increasing cinnamon oil concentration. This suggests that the cinnamon oil contains antibacterial compounds and its antibacterial activity is dependent on the concentration of these compounds. The negative control, as expected, did not have any antibacterial activity. The values were analyzed statistically and the P-value was found to be less than 0.05, making the results statistically significant.

All these findings on the antibacterial activity of cinnamon bark oil are in congruence with the results of previous studies using cinnamon essential oil on bacteria responsible for respiratory infections (Fabio, *et al.*, 2007) and food-borne pathogens (Smith-Palmer, *et al.*, 1998). The anti-microbial action is due to the potential of hydrophobic essential oils to disrupt the bacterial cell membrane and its structures leading to ion leakage. It was found that cinnamaldehyde is the primary compound responsible for major antibacterial activity. Trans-cinnamaldehyde is also known to inhibit bacterial acetyl-CoA carboxylase (Ranasinghe, *et al.*, 2013).

CHAPTER FOUR
4. Conclusion and Recommendations

4.1 Conclusion:

This study aimed to determine the chemical profile of *Cinnamomum zeylanicum* oil via Gas Chromatography-Mass Spectrometry, to determine its influence on some physical and chemical properties of sunflower oil, as well as its antibacterial activity. Regarding the first objective, a chemical profile of the extracted oil of *Cinnamomum zeylanicum* was obtained, via gas chromatography-mass spectrometry and it was found that the major constituent of the oil was cinnamaldehyde. Besides that, many terpenes were found such α-copaene, δ-cadinene and α-amorphene. The physical and chemical properties of the sunflower oil were studied, after the addition of cinnamon oil. It was found that cinnamon oil helped the sunflower oil retain its properties during storage and decreased the oxidation and rancidification. The color of sunflower oil was slightly altered by the cinnamon oil, but the refractive index remained unchanged. The change in density observed was minute. The viscosity of the oil during the storage did not increase much, when cinnamon oil was added. As for the chemical properties, the peroxide value, acid value and the saponification value of the sunflower oil after addition of cinnamon oil were determined. Overall, the results on the chemical and physical properties of sunflower oil before and after storage, with and without addition of cinnamon oil, suggest that cinnamon oil enhances the properties of sunflower oil and prevents its deterioration during storage. Finally, the antibacterial activity of the extracted cinnamon oil was investigated using the cup-plate method. The oil showed significant antibacterial activity against all six species of bacteria investigated, and inhibited the gram positive bacteria more significantly than the gram negative bacteria. the mean inhibitory zone diameters at 50% concentration of cinnamon oil, for each bacteria were as follows; *S. typhi* (40mm), *P. aeruginosa* (22mm), *S. aureus* (40mm), *B. cereus* (40mm), *E. coli* (34mm) and *E. fecalis* (40mm). Thus, it can be concluded that cinnamon oil has a high potential as an antibacterial.

4.2. Recommendation:

- *Cinnamomum zeylanicum* bark oil has potential as an antioxidant for applications in the food industry to protect oils and other food from oxidative degradation.
- It is recommended that further studies on the antibacterial, antifungal, antiviral and antioxidant activity of the cinnamon oil should be studied.

  The following specific recommendations are suggested:
- For the antibacterial activity, a wider range of species of bacteria can be taken, and for the Gram positive bacteria specifically, we can look at smaller concentrations of cinnamon oil to determine the minimum concentration that is effective in inhibiting the bacteria.
- From the perspective of food applications, studies can be done to investigate the effect of cinnamon oil on reused cooking oil and on the physiochemical properties of other edible oils such as sesame oil, peanut oil, palm oil, coconut oil and corn oil.
- Finally, antioxidant activity suggests that cinnamon oil may have some anticancer potential. This, too, can be investigated.

CHAPTER FIVE

5. References and Appendices

5.1. References:

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5.2. APPENDICES

Appendix I

5.2.1. GC-MS Settings

GCMS system	GCMS-QP2010 Plus (GCMS EI)
Column	Restek Rtx-5MS (5% diphenyl, 95% dimethylpolysiloxane)
[GC-2010]	
Column Oven Temp.	35.0 ºC
Injection Temp.	250.00 ºC
Injection Mode	Split
Flow Control Mode	Linear Velocity
Pressure	61.8 kPa
Total Flow	364.2 mL/min
Column Flow	1.20 mL/min
Linear Velocity	39.4 cm/sec
Purge Flow	3.0mL/min
Split Ratio	300.0
High Pressure Injection	OFF
Carrier Gas Saver	OFF
Splitter Hold	OFF

Oven Temp. Program

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

[GCMS-QP2010 Plus]	
Ion source Temp.	200.00 ºC
Interface Temp.	250.00 ºC
Solvent Cut Time	3.50 min
Detector Gain Mode	Relative
Detector Gain	0.00 kV
Threshold	0
[MS table]	

--Group 1 –Event 1—

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

Sample Inlet Unit	GC

Appendix II

5.2.2. Standard Values:

Table (5-1). Standard values of sunflower oil physiochemical properties

Refractive Index	Specific Gravity	Red Color	Peroxide Value	Iodine Value	Acid Value	FFA	Source
1. 467 - 1.469	-	-	≤10	≤10	≤ 0.6	0.085	WHO, 2006
1.465	0.918 – 0.923	-	<10	<10	< 0.6	< 0.3	Pearson, 1970
-	-	< 0.5	< 10	< 10	-	-	NRA, 2008

Appendix III

5.2.3. Equipment:

Figure (5-1). Viscometer to measure oil viscosity

Figure (5-2). GC-MS system to detect the compounds in essential oil

Figure (5-3). Lovibond tintometer to measure oil color

Figure (5-4). Refractometer to determine refractive index of oil

Appendix IV

RESULTS FOR ANTIBACTERIAL ACTIVITY OF GENTAMICIN

Figure (5-5). Antibacterial activity of gentamicin on *S. aureus* and *E. Fecalis*

***Figure (5-6). Antibacterial activity of gentamicin on* B. cereus *and* E. coli**

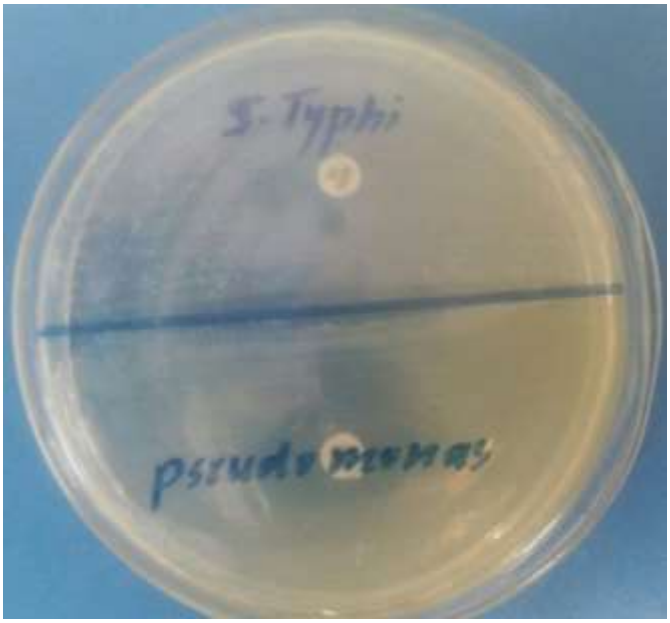

Figure (5-5). Antibacterial activity of gentamicin on *S. typhi* and *P. aeruginosa*