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Physicochemical Characterization Biological Activity and Antioxidant Effect of Oil Extracted form Cinnamon Bark

A thesis Submitted in Partial Fulfillment for the Requirement of the Degree of Bachelor

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الآيـة

قال تعالى:

الَّذَي جَعَلَ لَكُلُم مِّنَ الشَّجَرِ الأَخْضَرِ نَارِإِ ذَالفَّانَةُ مَّذَ لَهُ وُق ِدُ ون)

سورة يس الاية 80

Dedication

We are dedicating this work to our families and our teachers in chemistry department.

Acknowledgement

All thank to almighty Allah for giving us health and patience to accomplish this work.

We would like to express our deepest gratitude and respect to our supervisor Dr.Amira Abd elazeez for helping us during this study and providing us with the scientific support and professional idea with enthusiasm ,patience, and a lot of extremely useful discussion.

Our gratitude goes to Dr. Amira Abdazez to help us to accomplish this work.

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Abstract

Cinnamon oil sample was authenticated by evaluating its extraction, physical and chemical properties and analysis of the extracted oil, the result show that the density (0.90392 g\ml) viscosity was (4.133cps) color was (30 and 1.7) refractive index (1.5)the acid value was (0.419)and peroxide value was(1.5). The sample was analyzed by using it as anti bacterial agent and it was found has anti bacterial effect against many type of bacteria and fungi ,cinnamon oil also has anti oxidant effect and it was detected by using of sun flower oil (yarah) and it was play very effective role as antioxidant.

مستخلص البحث

تم إستخلاص زيت القرفة ودراسة الخواص الفيزيائية والكيميائية للعينة ووجد ان الكثافة (0.90392)اللزوجة (4.133)،اللون (30,17) ،معامل الإنكسار (1.50) ،ووجد ان رقم الحموضة للزيت (0.491)،رقم البيروكسيد(1.5)حللت العينة بإستحدامها كعامل مضاد للبكتيريا ووجد ان لديها تأثير عالي ضدالعديد من البكتيريا والفطريات،زيت القرفة ايضا" له تأثير كمضاد الكشف عن هذا التأثير بإستحدام زيت زهرة الشمس(يارا) ولعب دور فعال كمضاد للأكسدة. للاكسدة وتم

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Chapter One

1.1 Introduction

Cinnamon:

Cinnamon is a spice obtained from the inner bark of several trees from the genus *Cinnamomum* that is used in both sweet and savoury foods. The term "cinnamon" also refers to its mid-brown colour. While *Cinnamomum verum* is sometimes considered to be "**true cinnamon**", most cinnamon in international commerce is derived from related species, which are also referred to as "**cassia**" to distinguish them from "true cinnamon".[1][2]

Cinnamon is the name for perhaps a dozen species of trees and the commercial spice products that some of them produce. All are members of the genus *Cinnamonum* in the family Lauraceae. Only a few of them are grown commercially for spice.

1.2 History:

In classical times, four types of cinnamon were distinguished (and often confused):

- True cinnamon, the bark of C. verum (also called C. zeylanicum) from Sri Lanka
- Malabathrum or malobathrum (from Sanskrit literally "dark-tree leaves"), several species including *C. tamala* from the north of India
- Serichatum, C. cassia from Seres, that is, China
- Cassia the bark of *Cinnamomum iners* from Arabia and Ethiopia, literally "the peel of the plant" which is scraped off the tree[6]

Cinnamon has been known from remote antiquity. It was imported to Egypt as early as 2000 BC, but those who report it had come from China confuse it with cassia.[7] Cinnamon was so highly prized among ancient nations that it was regarded as a gift fit for monarchs and even for a god; a fine inscription records the gift of cinnamon and cassia to the temple of Apollo at Miletus.[8] Though its source was kept mysterious in the Mediterranean world for centuries by the middlemen who handled the spice trade, to protect their monopoly as suppliers, cinnamon is native to Bangladesh, Sri Lanka, the Malabar Coast of India, and Burma.[9]

The first Greek reference to *kasia* is found in a poem by Sappho in the seventh century BC. According to Herodotus, both cinnamon and cassia grew in Arabia, together with incense, myrrh, and ladanum, and were guarded by winged serpents. The phoenix was reputed to build its nest from cinnamon and cassia. Herodotus mentions other writers who believed the source of cassia was the home of Dionysos, located somewhere east or south of Greece.

The Greeks used *kásia* or *malabathron* to flavour wine, together with absinth wormwood (*Artemisia absinthium*). While Theophrastus gives a good account of the plants, he describes a curious method for harvesting: worms eat away the wood and leave the bark behind.

Egyptian recipes for *kyphi*, an aromatic used for burning, included cinnamon and cassia from Hellenistic times onward. The gifts of Hellenistic rulers to temples sometimes included cassia and cinnamon, as well as incense, myrrh, and Indian incense (*kostos*), so one might conclude that the Greeks used it for similar purposes.

The Hebrew Bible makes specific mention of the spice many times: first when Moses is commanded to use both sweet cinnamon (Hebrew: קּבָּמוֹן, qinnāmôn) and cassia in the holy anointing oil;[10] in Proverbs where the lover's bed is perfumed with myrrh, aloes, and cinnamon;[11] and in Song of Solomon, a song describing the beauty of his beloved, cinnamon scents her garments like "the smell of Lebanon".[12] Cassia was also part of the *ketoret*, the consecrated incense described in the Hebrew Bible and Talmud. It was offered on the specialized incense altar in the time when the Tabernacle was located in the First and Second Jerusalem temples. The *ketoret* was an important component of the temple service in Jerusalem. Psalm 45:8 mentions the garments of the king (or of Torah scholars) that smell of myrrh, aloes, and cassia.

Pliny[13] gives an account of the early spice trade across the Red Sea that cost Rome 100 million sesterces each year. Cinnamon was brought around the Arabian peninsula on "rafts without rudders or sails or oars", taking advantage of the winter trade winds.[14] Pliny also mentions cassia as a flavouring agent for wine.[15]

According to Pliny, a Roman pound (327 grams (11.5 oz)) of cassia, cinnamon, or serichatum cost up to 300 *denarii*, the wage of ten months' labour. Diocletian's Edict on Maximum Prices[16] from 301 AD gives a price of 125 *denarii* for a pound of cassia, while an agricultural labourer earned 25 *denarii* per day. Cinnamon was too expensive to be commonly used on funeral pyres in Rome, but the Emperor Nero is said to have burned a year's worth of the city's supply at the funeral for his wife Poppaea Sabina in AD 65.[17]

Malabathrum leaves (*folia*) were used in cooking and for distilling an oil used in a caraway sauce for oysters by the Roman gourmet Gaius Gavius Apicius.[18] Malabathrum is among the spices that, according to Apicius, any good kitchen should contain.

The famous Commagenum unguent produced in Commagene, in present-day eastern Turkey, was made from goose fat aromatised with cinnamon oil and spikenard. Malobathrum from Egypt (Dioscorides I, 63) was based on beef fat and contained cinnamon, as well; one pound cost 300 *denarii*. The Roman poet Martial (VI, 55) made fun of Romans who drip unguents, smell of cassia and cinnamon taken from a bird's nest, and look down on a man who does not smell at all. Through the Middle Ages, the source of cinnamon was a mystery to the Western world. From reading Latin writers who quoted Herodotus, Europeans had learned that cinnamon came up the Red Sea to the trading ports of Egypt, but where it came from was less than clear. When the Sieur de Joinville accompanied his king to Egypt on crusade in 1248, he reported – and believed

– what he had been told: that cinnamon was fished up in nets at the source of the Nile out at the edge of the world (i.e., Ethiopia). Marco Polo avoided precision on the topic.[19] Herodotus and other authors named Arabia as the source of cinnamon: they recounted that giant cinnamon birds collected the cinnamon sticks from an unknown land where the cinnamon trees grew and used them to construct their nests, and that the Arabs employed a trick to obtain the sticks. Pliny the Elder wrote in the first century that traders had made this up to charge more, but the story remained current in Byzantium as late as 1310.

The first mention that the spice grew in Sri Lanka was in Zakariya al-Qazwini's *Athar al-bilad wa-akhbar al-'ibad* ("Monument of Places and History of God's Bondsmen") about 1270.[20] This was followed shortly thereafter by John of Montecorvino in a letter of about 1292.[21]

Indonesian rafts transported cinnamon directly from the Moluccas to East Africa (see also Rhapta), where local traders then carried it north[22][23][24] to Alexandria in Egypt. Venetian traders from Italy held a monopoly on the spice trade in Europe, distributing cinnamon from Alexandria. The disruption of this trade by the rise of other Mediterranean powers, such as the Mamluk sultans and the Ottoman Empire, was one of many factors that led Europeans to search more widely for other routes to Asia.

When Portuguese traders landed in Ceylon (Sri Lanka), they restructured the traditional production and management of cinnamon by the Sinhalese. They established a fort on the island in 1518 and protected Ceylon as their cinnamon monopoly for over 100 years. Later, Sinhalese held the monopoly for cinnamon in Ceylon.

Dutch traders finally dislodged the Portuguese by allying with the inland Kingdom of Kandy. They established a trading post in 1638, took control of the manufactories by 1640, and expelled the remaining Portuguese by 1658. "The shores of the island are full of it," a Dutch captain reported, "and it is the best in all the Orient. When one is downwind of the island, one can still smell cinnamon eight leagues out to sea." [25]:15 The Dutch East India Company continued to overhaul the methods of harvesting in the wild and eventually began to cultivate its own trees.

During the 1500s, when the Spanish sent an expedition out from New Spain (Mexico) and arrived at the Philippines, they found that cinnamon was growing in the island of Mindanao near the Rajahnate of Butuan.[26] The species of cinnamon was *Cinnamomum mindanaense* which was closely related to *C. zeylanicum* and was found to be just as good as the cinnamon found in Sri Lanka. This cinnamon was often mixed with the chocolate the Spanish discovered from the Aztecs to sweeten it. This alternative Mindanao cinnamon to the one found in Sri Lanka, which was controlled by the Portuguese, supplied Spanish needs and this cinnamon traveled the route through the Americas and eventually to Spain, where it competed with Sri Lankan cinnamon.[27]

In 1767, Lord Brown of the British East India Company established Anjarakkandy Cinnamon Estate near Anjarakkandy in Cannanore (now Kannur) district of Kerala, and this estate became Asia's largest cinnamon estate. The British took control of Ceylon from the Dutch in 1796. However, the importance of the monopoly of Ceylon was already declining, as cultivation of the cinnamon tree spread to other areas, the more common cassia bark became more acceptable to consumers, and coffee, tea, sugar, and chocolate began to outstrip the popularity of traditional spices.

1.3 Cultivation:



Figure 1-1: Leaves from a wild cinnamon tree

Aggregate annual production of cinnamon and cassia amounts to 27,500–35,000 tons, worldwide. Of this, *C. verum* accounts for 7,500–10,000 tons of production, with the remainder produced by other species.[1] Sri Lanka produces 80–90% of the world's supply of *C. verum*, but that is the only species grown there; *C. verum* is also cultivated on a commercial scale in Seychelles and Madagascar.[1] Global production of the other species averages 20,000–25,000 tons, of which Indonesia produces around two-thirds of the total, with significant production in China. India and Vietnam are also minor producers.[1]

Cinnamon is cultivated by growing the tree for two years, then coppicing it, i.e., cutting the stems at ground level. The following year, about a dozen new shoots form from the roots, replacing those that were cut. A number of pests such as *Colletotrichum gloeosporioides*, *Diplodia* spp., and *Phytophthora cinnamomi* (stripe canker) can affect that growing plants, sometimes leading to death.[28]

The stems must be processed immediately after harvesting while the inner bark is still wet. The cut stems are processed by scraping off the outer bark, then beating the branch evenly with a hammer to loosen the inner bark, which is then pried off in long rolls. Only 0.5 mm (0.02 in) of the inner bark is used; [citation needed] the outer, woody portion is discarded, leaving metre-long cinnamon strips that curl into rolls ("quills") on drying. The processed bark dries completely in

four to six hours, provided it is in a well-ventilated and relatively warm environment. Once dry, the bark is cut into 5- to 10-cm (2- to 4-in) lengths for sale. A less than ideal drying environment encourages the proliferation of pests in the bark, which may then require treatment by fumigation. Fumigated bark is not considered to be of the same premium quality as untreated bark.

Sri Lanka cinnamon has a very thin, smooth bark with a light-yellowish brown colour and a highly fragrant aroma. In recent years in Sri Lanka, mechanical devices have been developed to ensure premium quality and worker safety and health, following considerable research by the universities in that country, led by the University of Ruhuna.

1.4 Grading:

The Sri Lankan grading system divides the cinnamon quills into four groups:

- Alba, less than 6 mm (0.24 in) in diameter
- Continental, less than 16 mm (0.63 in) in diameter
- Mexican, less than 19 mm (0.75 in) in diameter
- Hamburg, less than 32 mm (1.3 in) in diameter

These groups are further divided into specific grades. For example, Mexican is divided into M00 000 special, M000000, and M0000, depending on quill diameter and number of quills per kilogram.

Any pieces of bark less than 106 mm (4.2 in) long are categorized as quillings. Featherings are the inner bark of twigs and twisted shoots. Chips are trimmings of quills, outer and inner bark that cannot be separated, or the bark of small twigs.

1.5 Species

- Cinnamomum cassia (cassia or Chinese cinnamon, the most common type)
- C. burmannii (Korintje, Padang cassia, or Indonesian cinnamon)
- C. loureiroi (Saigon cinnamon, Vietnamese cassia, or Vietnamese cinnamon)
- C. verum (Sri Lanka cinnamon or Ceylon cinnamon)
- C. citriodorum (Malabar cinnamon)
- C. tamale (Indian cinnamon)

Cassia is the strong, spicy flavour associated with cinnamon rolls and other such baked goods, as it handles baking conditions well. Chinese cinnamon is generally a medium to light reddish brown, hard and woody in texture, and thicker (2–3 mm (0.079–0.118 in) thick), as all of the layers of bark are used. Ceylon cinnamon, using only the thin inner bark, has a lighter brown

color, a finer, less dense and more crumbly texture, and is considered to be subtler and more aromatic in flavour than cassia, losing much of its flavour during cooking.

Levels of the blood-thinning agent coumarin in Ceylon cinnamon are much lower than those in cassia.[30][31]

The barks of the species are easily distinguished when whole, both in macroscopic and microscopic characteristics. Ceylon cinnamon sticks (quills) have many thin layers and can easily be made into powder using a coffee or spice grinder, whereas cassia sticks are much harder. Indonesian cinnamon is often sold in neat quills made up of one thick layer, capable of damaging a spice or coffee grinder. Saigon cinnamon (*C. loureiroi*) and Chinese cinnamon (*C. cassia*) are always sold as broken pieces of thick bark, as the bark is not supple enough to be rolled into quills. The powdered bark is harder to distinguish, but if it is treated with tincture of iodine (a test for starch[32]), little effect is visible with pure Ceylon cinnamon, but when Chinese cinnamon is present, a deep-blue tint is produced.[33][34]

1.6 Flavor, aroma, and taste:



Figure 2-1: verum bark essential oil

The flavour of cinnamon is due to an aromatic essential oil that makes up 0.5 to 1% of its composition. This essential oil is prepared by roughly pounding the bark, macerating it in sea water, and then quickly distilling the whole. It is of a golden-yellow colour, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent taste and scent come from cinnamic aldehyde or cinnamaldehyde (about 90% of the essential oil from the bark) and,

by reaction with oxygen as it ages, it darkens in colour and forms resinous compounds. Other chemical components of the essential oil include ethyl cinnamate, eugenol (found mostly in the leaves), beta-caryophyllene, linalool, and methyl chavicol. [citation needed]

1.7 Food uses:

Besides use as flavourant and spice in foods, cinnamon-flavored tea, also flavored with cardamom, is consumed as a hot beverage in Bangladesh, India, and Pakistan.

Cinnamon bark is used as a spice. It is principally employed in cookery as a condiment and flavouring material. It is used in the preparation of chocolate, especially in Mexico, which is the main importer of cinnamon.[35] It is also used in many dessert recipes, such as apple pie, doughnuts, and cinnamon buns, as well as spicy candies, coffee, tea, hot cocoa, and liqueurs. In the Middle East, cinnamon is often used in savory dishes of chicken and lamb. In the United States, cinnamon and sugar are often used to flavor cereals, bread-based dishes, such as toast, and fruits, especially apples; a cinnamon-sugar mixture is even sold separately for such purposes. It is also used in Turkish cuisine for both sweet and savory dishes. Cinnamon can also be used in pickling. Cinnamon powder has long been an important spice in enhancing the flavor of Persian cuisine, used in a variety of thick soups, drinks, and sweets.[36]:10–12

Use as an alcohol flavorant:

Cinnamon is a popular flavoring in numerous alcoholic beverages.[37] Fireball Cinnamon Whisky is a good example. There are many similar products throughout the world.

Cinnamon brandy concoctions, called "cinnamon liqueur" and made with distilled alcohol, are popular in parts of Greece. In Europe, popular examples of such beverages are *Maiwein* (white wine with woodruff) and $\dot{Z}ubr\acute{o}wka$ (vodka flavored with bison grass).

1.8 Benefits of Cinnamon Oil

The cinnamon plant is used in a few different ways to produce medicinally beneficial products. For example, you're probably familiar with common cinnamon spice that's sold in nearly every grocery store in the U.S. Cinnamon oil is a bit different because it's a much more potent "extract" form of the plant that contains special compounds not found in the dried spice.

There are two primary types of cinnamon oils available on the market: cinnamon bark oil and cinnamon leaf oil. While they have some similarities, they're different products with somewhat separate uses. Cinnamon bark oil is extracted from the outer bark of the cinnamon tree. It's considered very potent and has a strong, "perfume-like" smell, almost like taking an intense whiff of ground cinnamon. Cinnamon bark oil is usually more expensive than cinnamon leaf oil.

Cinnamon leaf oil has a "musky and spicy" smell and tends to have a lighter color. While cinnamon leaf oil might appear yellow and murky, cinnamon bark oil has a deeper redbrown color that most people usually associate with cinnamon spice. Both are beneficial, but cinnamon bark oil may be more potent.

Many of the benefits of cinnamon bark oil have to do with its ability to dilate blood vessels. Cinnamon bark can help enhance nitric oxide function, which causes increased blood flow and lower levels of inflammation.

Some of the most researched health benefits of cinnamon oil include:

- * Decreases inflammation
- * Increases circulation
- * Fights viruses
- * Fights free radicals
- * Relieves depression
- * Stimulates the immune system
- * Stimulates libido
- * Fights parasites

1.9 Traditional medicine:

Cinnamon has a long history of use in traditional medicine, but no evidence indicates it is useful to treat any medical condition.[38]

1.10 Toxicity:

The European Food Safety Authority in 2008 considered toxicity of coumarin, known to cause liver and kidney damage in high concentrations and a significant component of cinnamon, and metabolic effect on humans with CYP2A6 polymorphism, and confirmed a maximum recommended tolerable daily intake (TDI) of 0.1 mg of coumarin per kg of body weight.[39][40] The European Union set a guideline for maximum coumarin content in foodstuffs of 50 mg per kg of dough in seasonal foods, and 15 mg per kg in everyday baked foods.[41]

According to the maximum recommended TDI of 0.1 mg of coumarin per kg of body weight, which is 5 mg of coumarin for a body weight of 50 kg:

- **1.11 Antioxidants**: are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include
 - * Beta-carotene
 - * Lutein
 - * Lycopene
 - * Selenium
 - * Vitamin A
 - * Vitamin C

* Vitamin E

Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines. To minimize risk, tell you of your health care providers about any antioxidants you use.

ACID VALUE:

In chemistry, acid value (or "neutralization number" or "acid number" or "acidity") is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance.[1] The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds. In a typical procedure, a known amount of sample dissolved in organic solvent (often isopropanol), is titrated with a solution of potassium hydroxide (KOH) with known concentration and with phenolphthalein as a color indicator.

The acid number is used to quantify the amount of acid present, for example in a sample of biodiesel. It is the quantity of base, expressed in milligrams of potassium hydroxide, that is required to neutralize the acidic constituents in 1 g of sample.

```
 A \ N = (V \ e \ q \ - b \ e \ q) \ N \ 56.1 \ W \ o \ i \ l \ \{\displaystyle \ AN=(V_{eq}-b_{eq})N\{\frac \ \{56.1\}\{W_{oil}\}\}\} \ AN=(V_{eq}\}-b_{eq}\})N\{\frac \ \{56.1\}\{W_{oil}\}\}\}
```

Veq is the volume of titrant (ml) consumed by the crude oil sample and 1 ml of spiking solution at the equivalent point, beq is the volume of titrant (ml) consumed by 1 ml of spiking solution at the equivalent point, and 56.1 is the molecular weight of KOH. WOil is the mass of the sample in grams.

The molar concentration of titrant (N) is calculated as such:

```
N = 1000 \ W \ K \ H \ P \ 204.23 \ V \ e \ q \ \{\displaystyle \ N = \{\frac \ \{1000W_{KHP}\}\} \{204.23V_{eq}\}\}\} \\ N = \{\frac \ \{1000W_{\{KHP\}\}} \{204.23V_{eq}\}\}\}
```

In which WKHP is the mass (g) of KHP in 50 ml of KHP standard solution, Veq is the volume of titrant (ml) consumed by 50 ml KHP standard solution at the equivalent point, and 204.23 is the molecular weight of KHP.

There are standard methods for determining the acid number, such as ASTM D 974 and DIN 51558 (for mineral oils, biodiesel), or specifically for biodiesel using the European Standard EN 14104 and ASTM D664 are both widely used worldwide. Acid number (mg KOH/g oil) for biodiesel should to be lower than 0.50 mg KOH/g in both EN 14214 and ASTM D6751 standard fuels. This is since the FFA produced may corrode automotive parts and these limits protect vehicle engines and fuel tanks.

As oil-fats rancidify, triglycerides are converted into fatty acids and glycerol, causing an increase in acid number. A similar observation is observed with biodiesel aging through analogous

oxidation processes and when subjected to prolonged high temperatures (ester thermolysis) or through exposure to acids or bases (acid/base ester hydrolysis)

Nutritional information:

Ten grams (about 2 teaspoons) of ground cinnamon contain:[43]

• Energy: 103.4 kJ (24.7 kcal)

• Fat: 0.12 g

• Carbohydrates: 8.06 g (of which - fibers: 5.31 g, sugars: 0.2 g)

• Protein: 0.4 g

Cinnamon oil:

The component:

Name cassia oil; cassia bark oil

Is containing:

Saturated fatty: 0.3

Sodium: 10 mg

Potassium: 413 mg

Sugar: 2.2 g

Protein: 4g

Magnesium: 60 mg

Iron: 8.3

Calcium: 1.002

Vitamins:

(A)2951I

(B)0.2 mg

(C) 3.8 mg

1- Cinnamaldehyd:

(2E)_3_phenyl prop-2-eno

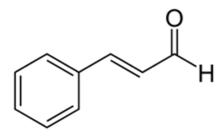


Figure 3-1: cinnamal dehyd

Name: cinnamic aldehyde Chemical formula: c9H8o Molar mass: 132.16 g/mol

Density: 1.0497 g/ml

2- Eugenol:

4_allyl_2_methoxyphenol or 2_methoxy_4_ (2_prpenyl)phenol

Name: Eugenic acid

Chemical formula: C10H12O2 Molar mass: 164.20 g/mol-1

Density: 1.06 g/cm3

Properties	
Chemical formula	С9Н8О
Molar mass	132.16 g/mol
Appearance	Yellow oil
Odor	pungent, cinnamon-like
Density	1.0497 g/mL
Melting point	−7.5 °C (18.5 °F; 265.6 K)
Solubility in water	Slightly Soluble
Solubility	soluble in ether, chloroform
	insoluble in petroleum ether
	miscible with alcohol, oils
Refractive index (nD)	1.6195

Decomposition:

when heated yo decomposition its emits acrid smoke and irritating fumes optical rotation:

-2.5 to 2 20 deg(cinnamon leaf oil) ;-2 to 0 at 20 deg (cinnamon bark oil) .

Index of refraction = 1.6 to 1.5910 at 25 deg (cassia oil)

1.12 Distillation:

is a process of separating the component 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 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.

Isolation of cinnamon oil:

Commercial cinnamon consists of dried, ground bark from the cinnamon tree, and contains about 2% cinnamaldehyde, which is responsible for its distinct flavor and odor.

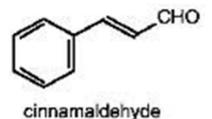


Figure 9-1: cinnamaldehyde

The isolation will be accomplished by steam distillation. This means that the solid cinnamon will be boiled in water, and the steam will be condensed and collected. Since cinnamaldehyde is soluble in steam (but not in water), it will be carried up with the distillate and form a finely distributed emulsion, which will appear milky upon cooling. Many other essential oils can be isolated in this way – anisole from anise, camphene from nutmeg, carvone from caraway and spearmint, cuminaldehyde from cumin, eugenol from cloves, safrole from sassafras, and limonene from citrus peel.

A common way of isolating cinnamon oil along with cinnamaldehyde from cinnamon bark, even in industrial scale, is through steam distillation. The cinnamon oil isolated through steam distillation contains roughly around 90% trans-cinnamaldehyde. Cinnamaldehyde contains a formylgroup, and is therefore an aldehyde. Its structure has a phenyl group attached to an unsaturated aldehyde. It named through IUPAC nomenclature as 3-phenyl-2-Propenal. The experiment aimed to isolatecinnamon oil from cinnamon bark by steam distillation. From cinnamon oil, cinnamaldehyde could be extracted by multiple extractions using DCM as a solvent, through aqueous dispersion. In the experiment done, cinnemaldehyde was analyzed by

subjecting it to Tollen's test andPhenylhydrazone test, both of which test forthe presence of aldehydes

Proceuder:

Steam distillation of the cinnamon:

- Obtain a 100 ml Erlenmeyer flask with a 14/20 ground glass joint from the instructor.
- Add 15 ml of distilled water, 2 drops of Triton X-100 (a surfactant which reduces foaming), 2.0 g of cinnamon, and a long stir bar.
- Attach a Hickman still to the flask, then top it with a reflux condenser. Attach the reflux condenser to the water hoses and turn on the water. Insulate the top of the flask below the neck of the still with aluminum foil.
- Turn on the stirrer and begin to heat the cinnamon mixture slowly until it begins to boil.
- If it foams up into the still, you are heating too quickly if the foam gets into the lip of the still, you'll have to take it off and clean it before continuing.
- Remove the distillate with a pipet as it collects and place it in a beaker or flask.
- If, after collecting some distillate, the still begins to look dry, add up to 1 ml of water (no more!). Try not to bake the cinnamon onto the glassware, as it is hard to clean out.
- You should collect about 5 ml of distillate; once it no longer turns milky on cooling, most of the cinnamaldehyde has been removed.

Isolation of the cinnamaldehyde from the distillate:

- Place a sep funnel on the clamp and put a beaker underneath it. After making sure the stopcock is closed, transfer the distillate to the sep funnel.
- Extract it by adding about 5-10 ml of dichloromethane, shaking, allowing it to separate, and draining off the dichloromethane.

- Repeat two more times, combining all of the dichloromethane layers that you drain off.

 Don't throw away anything until you are sure you have what you want!
- Dry the dichloromethane solution by adding sodium sulfate until it is free flowing.
- Transfer the solution to a tared (preweighed) round bottom flask and rinse the solid sodium sulfate with a little more dichloromethane. Evaporate the solution on the rotovap.
- Observe the product that you have obtained and record your observation. Authentic cinnamaldehyde is a clear, slightly yellow liquid with a strong odor of cinnamon.
- When the flask is cool, obtain the mass of the cinnamon oil that you have extracted.

 Calculate the % recovery of cinnamaldehyde.
- Discuss the odor, appearance, mass, and percent recovery of the cinnamaldehyde in your

1.13 IR Spectrophotometry

Infrared Spectrophotometry is designed to identify or determine the sample by measuring absorption of infrared radiation of wavenumbers in a region of 4,000 to 400 cm-1, at various wavenumbers, when it passes through the sample. This method uses the property that the infrared absorption spectrum of a substance is characteristic of its chemical structure. Infrared spectra are shown in charts drawn by plotting the wavenumbers on the abscissa and the transmittances or absorbance on the ordinate. Unless otherwise specified, when the spectrum of the sample is similar in the intensity of absorption at the same wavenumber to the spectrum of the Reference Standard or the corresponding Reference Spectrum given in section 10 of REGENTS, SOLUTIONS AND OTHER REFERNCE MATERIALS, the sample is identified as concordant with the expected substance. When the spectrum of the sample measured in a solid state is different in the position and relative intensity of the absorption maximum from the Reference Standard spectrum or the Reference Spectrum, treat both the sample and the Reference Standard under the same conditions as directed in the individual monograph, then record the spectra. When comparing the two spectra, care should be taken to allow for the possibility the two spectra are different in resolving power because different instruments are used to measure sample spectrum and the Reference Spectrum. The greatest variations based on the difference in resolving power between two instruments are likely to occur between 4000 cm-1 and 2000 cm-1. For Fourier-transform infrared spectrophotometers, the precision of wavenumbers is invariable through the total scanning region because the resolving power of them is constant, regardless of wavenumber Infrared Reference Spectra ranging between 4000 cm-1 and 400 cm-1 are given in section 10 in the heading of REAGENT, SOLUTIONS, OTHER REFERNCE MATERIALS for the substances for which identification tests are specified in the individual monographs.

1.14 Ultraviolet and visible spectrometers

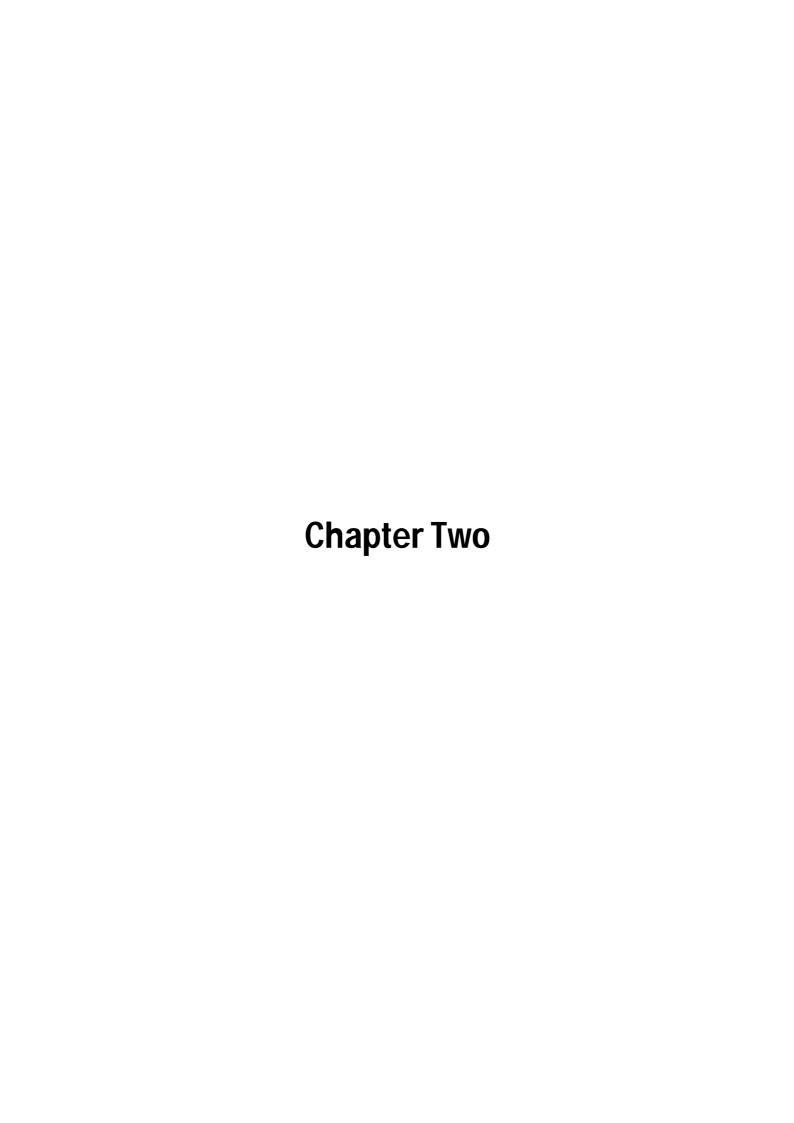
Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness. This description outlines the basic principles for those new to UV-Visible spectrometry. It is intended purely as a brief introduction to the technique and it is Thermo Spectronic's policy to continually add to this range of documentation for further details, as they become available.

Definitions and Units Radiation is a form of energy and we are constantly reminded of its presence via our sense of sight and ability to feel radiant heat. It may be considered in terms of a wave motion where the wavelength, λ , is the distance between two successive peaks. The frequency, ν , is the number of peaks passing a given point per second. These terms are related so that: $c = \nu\lambda$ where c is the velocity of light in a vacuum. Figure 1 The wavelength λ of electromagnetic radiation The full electromagnetic radiation spectrum is continuous and each region merges slowly into the next. For spectroscopy purposes, we choose to characterize light in the ultraviolet and visible regions in terms of wavelength expressed in nanometers. Other units which may be encountered, but whose use is now discouraged, are the Angstrom (Å) and the millimicron (m μ). 1nm = 1m μ = 10Å = 10-9 meters

Objective of Research

The objectives of this project are:

- To extraction of cinnamon oil from cinnamon bark.
- To determine physical and chemical properties of essential cinnamon oil.
- To make analysis of extracted cinnamon oil.



2.1. Materials

Purified water, chloroform, diethyl ether (99), Glacial acetic acid, Chloroform, Potassium Iodide, anhydrous sodium thio sulfate

2.2. Equipments

Conical flask, Burette, pipette, Beaker, Sensitive balance, Baknometer

2.3. Apparatus

Viscometer, Lovibond (color meter), IR instrument, UV instrument

2.4 Method

2.4.1 Physical properties of cinnamon oil:

1. Viscosity:

Viscosity of oil was determined with the viscometer, the sample was flowed in the apparatus, the viscosity of oil was calculated as follow:

Viscosity=t (of flow sample)\t(of flow water).

The average time of flowed sample=124sec.

Time of flow water=30sec.

Viscosity= $124\30 = 4.133$ cp.

2. Color:

found The color of `oils are usually compared in alovibond Tintometer, using 1 in or 5 1\4 in cell.

The apparatus was giving a result: Yellow at 30°

Red at 1.7°

Blue nil

The color of cinnamon oil was to be (yellowish red color).

Refractive index:

The refractive index of oils is applied at standard temperatures for the expression of result: 20C oils The refractive index of cinnamon oil = 1468. (Standard refractive index =1.54).

4. density:

Determination of weight per ml (apparent density) of oils by pyknometer usually at 20°c.

Suitable amount of cinnamon oil was taken the weight of sample is 4.5196.

Specific density = weight \volume = $4.5196\5 = 0.90392$ g\ml.

Relative density = weight of sample \weight of water.

 $= 4.55196 \ 4.9681 = 0.9097 \ \text{g} \ \text{m}$

2.4.2 Chemical properties of cinnamon oil:

1. Acid value:

. Acid Value Of oil was determined by addition di-methyle ether to sample, and titrated against sodium hydroxide (0.1N)

Blank (ether+NaoH) = 0.3ml.

(Sample + ether + NaoH) = 0.6ml.

Viscosity=t (of flow sample)\t(of flow water).

 $AC = 0.3*0.1*56.1\2.002$

AC = 0.419.

F.F = 0.419

2. Peroxide value:

Mixture of oil and glacial acetic acid and chloroform was prepared, and saturated potassium iodide was added then, it was titrated against sodium thio _sulfate.

Blank (glacial acetic acids chloroform) with NaOH=0.1ml.

Sample +NaOH=0.4ml.

Peroxide value=V*N*1000\W (sample)

Peroxide value = $(0.4-0.1)*0.01*1000\2 = 1.5$.

2.5 Analysis of cinnamon oil:

. Anti bacterial effect of cinnamon oil :

Methods:

2.5.1 Preparation of the test organisms:

2.5.2 Preparation of bacterial suspensions:

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

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

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

2.5.3 Preparation of fungal suspension:

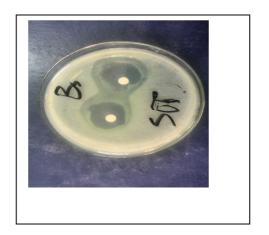
The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.5.4 Testing of antibacterial susceptibility

Disc diffusion method

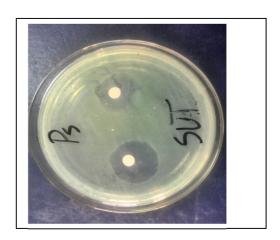
The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculums was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 μ l of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

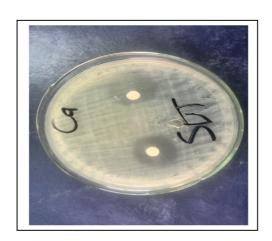
- 1- National Committee for Clinical Laboratory Standards (NCCLS) (1999). Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania document M100-S9, Vol.19.
- 1- The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while 13-18mm as active and >18mm as very active.
- **2-** The results were expressed in terms of the diameter of the inhibition zone:< 9 mm, inactive; 9-12 mm, partially active,13-18 mm, active.

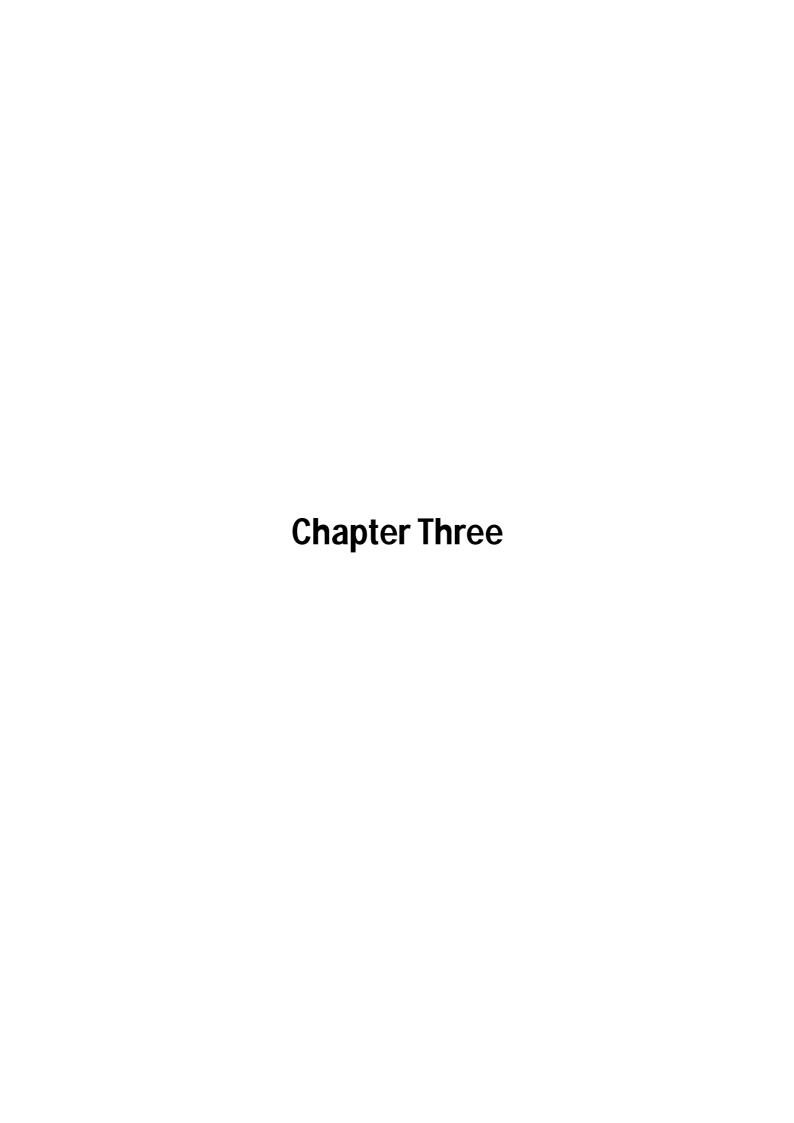












Results

3.1. Physical properties:

Number	Test	Result
1.	Viscosity	4.133cps
2.	Density	0.90392g\ml
3.	Color	30,1.7
4.	Refractive index	1.5

3.2. Chemical properties:

Number	Test	Result
1.	Acid value	0.419
2.	Peroxide value	1.5

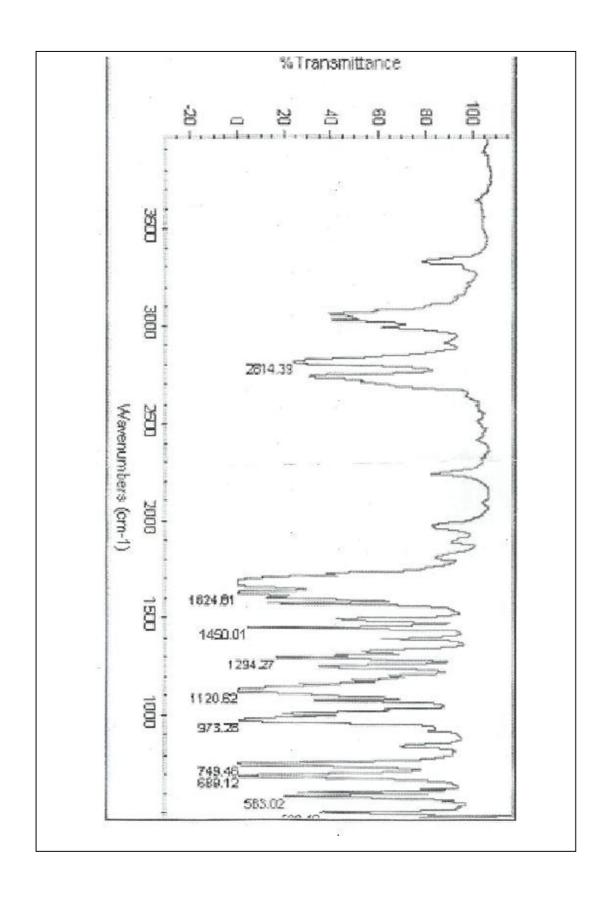
3.3Analysis of cinnamon oil:

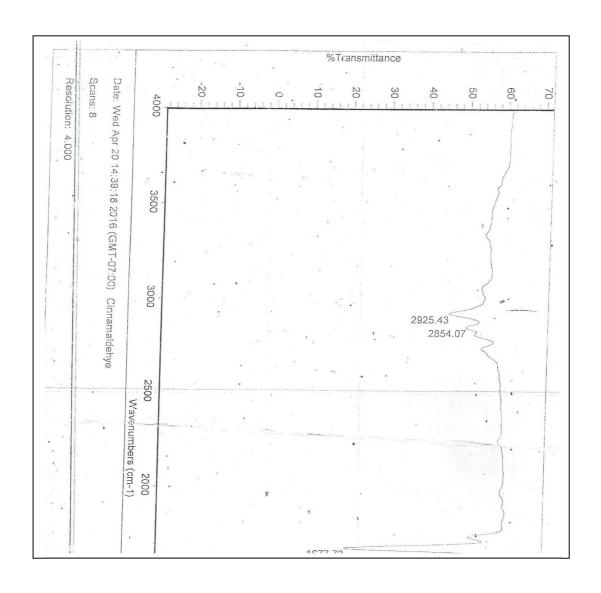
3.3.1 Anti oxidant effect of cinnamon oil:

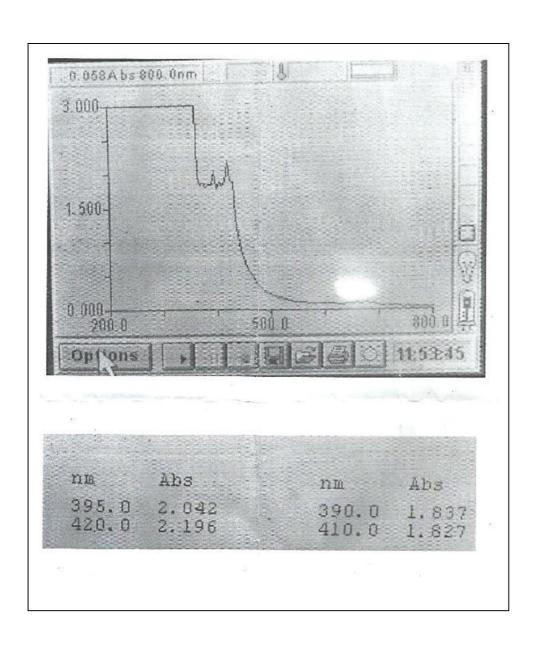
Refractive	Y	<u>A</u>	<u>B</u>	Density	Viscosity	Peroxide	Acid	Name of
<u>index</u>						<u>value</u>	<u>value</u>	<u>sample</u>
1.4632	8.6	0.7	0	0.9121	45.57	4.22	0.471	Blank at
								zero time
1.4629	8.7	0.3	0	0.9028	45.87	3.99	0.462	
1.4615	8.5	0.3	0	0.9031	45.39	3.18	0.431	
1.4677	8.9	0.3	0	0.9138	46.32	4.97	0.531	Blank after
								15 day
1.4675	9.0	0.4	0	0.9122	46.22	4.89	0.521	
1.4673	9.1	0.7	0	0.9121	46.17	4.77	0.500	
1.4610	8.8	0.4	0	0.9081	45.97	3.88	0.471	Sun flower
								oil+0.4ml of
								cinnamon
								oil
1.4623	9.1	0.4	0	0.9071	45.89	4.00	0.460	
1.4650	8.7	0.3	0	0.9121	45.98	4.11	0.438	

1.4631	8.4	0.3	0	0.9011	45.87	3.99	0.430	Sun flower oil after 15 day +0.3ml cinnamon oil
1.4623	8.5	0.2	0	0.9121	45.75	3.97	0.4150	
1.4610	8.6	0.3	0	0.9121	45.49	3.38	0.448	
1.4621	8.2	0.3	0	0.9211	45.82	3.77	0.438	Sun flower after 15days+0.5 ml cinnamon oil
1.4613	8.9	0.3	0	0.9211	45.39	3.82	0.470	
1.4621	8.8	0.3	0	0.9201	45.71	3.78	0.478	
1.4678	8.9	0.3	0	0.9231	46.22	4.10	0.460	Blank after 30day
1.4629	8.9	0.5	0	0.9231	46.25	4.09	0.458	
1.4621	8.9	0.7	0	0.9219	46.35	4.00	0.460	
1.4617	8.6	0.5	0	0.91001	45.80	4.11	0.487	Sun flower after 30 day +0.1ml cinnamon oil
1.46781	8.9	0.4	0	0.9231	46.87	4.22	0.511	
1.4691	8.7	0.4	0	0.9211	46.71	4.18	0.499	
1.4678	8.9	0.3	0	0.9231	46.22	4.10	0.46	Sun flower oil after30day+ 0.3ml cinnamon oil
1.4629	8.9	0.5	0	0.9231	46.25	4.09	0.458	
1.4621	8.9	0.7	0	0.9219	46.35	4.00	0.460	
1.4632	8.7	0.3	0	0.9187	46.37	4.08	0.440	Sun flower after30 day +0.5ml cinnamon oil
1.4647	8.9	0.3	0	0.91271	46.63	4.10	0.453	
1.4639	8.9	0.3	0	0.9210	46.55	4.18	0.460	

1.4778	10. 1	1.2	0.2	0.9381	47.27	9.87	0.781	Blank after 45 day
1.4867	10. 2	1.3	0.1	0.9321	47.37	9.59	0.791	
1.4671	10. 3	1.2	0.3	0.9197	47.86	9.78	0.787	
1.46631	8.9	0.8	0	0.9217	46.97	5.63	0.590	Sun flower after 45 day +0.1ml cinnamon oil
1.4637	9.0	0.7	0	0.9331	46.99	5.98	0.587	
1.4671	9.1	0.8	0	0.9319	46.99	5.77	0.592	
1.46511	9.0	0.6	0	0.9318	46.72	5.58	0.549	Sun flower oil +0.3ml cinnamon oil
1.46171	9.0	0.6	0	0.9321	46.71	5.98	0.587	
1.46217	9.0	0.9	0	0.9311	46.58	5.77	0.592	
1.456321	9.0	0.9	0	0.9219	46.22	5.30	0.522	Sun flower oil after 45 day+0.5ml cinnamon oil
1.46761	8.9	0.7	0	0.9319	46.39	5.22	0.522	
1.46701	8.9	0.5	0	0.9317	46.87	5.11	0.563	







3.3.2 Anti bacterial:

Antibacteial Activty									
EC	EC PS Sa BS Ca								
	-VE	+V]	Е	F					
20	22	30	20	20					

3.4 DISCUSSION:

3.4.1 Physical properties:

Refractive index

The refractive index of the extracted cinnamon oil was found to be(1.50), while for standard cinnamon oil is(1.54) -[approximately similar]

Color:

The color of extracted cinnamon oil was found to be reddish yellow ,whitch appeared at 30^{0} (yellow)

And 1.7° (red) ,null blue-while for standard cinnamon oil the color is yellow[then the extracted oil –null for blue-is of high quality]

Density:

The density of extracted cinnamon oil was found to be (0.90392glml), while for standard is(1.047glml)

[it is less dense than water]

Viscosity:

The stander oil viscosity is medium to watery (the viscosity of water = 0.890 cP) , the extracted oil viscosity was found to be 4.133 cp[medium to watery]

3.4.2 Chemical properties:

Acid value:

Acid value was found to be 0.84 and F.F.A = 0.419 this value is indicate there is no dissociation tri glyceride due to enzyme, air and bacteria

Peroxide value

Peroxide value of extracted oil was found to be 1.5 (far less than 10) which indicate no sign of auto oxidation or rancidity

3.5 Anti oxidant:

The cinnamon oil is strong anti oxidant and that refer to the value of peroxide and acid value of sun flower oil is lower than blank special and that present when added (0.3 - 0.5) of cinnamon oil

3.6 Antimicrobial:

The rang of activity:

9-13: weak

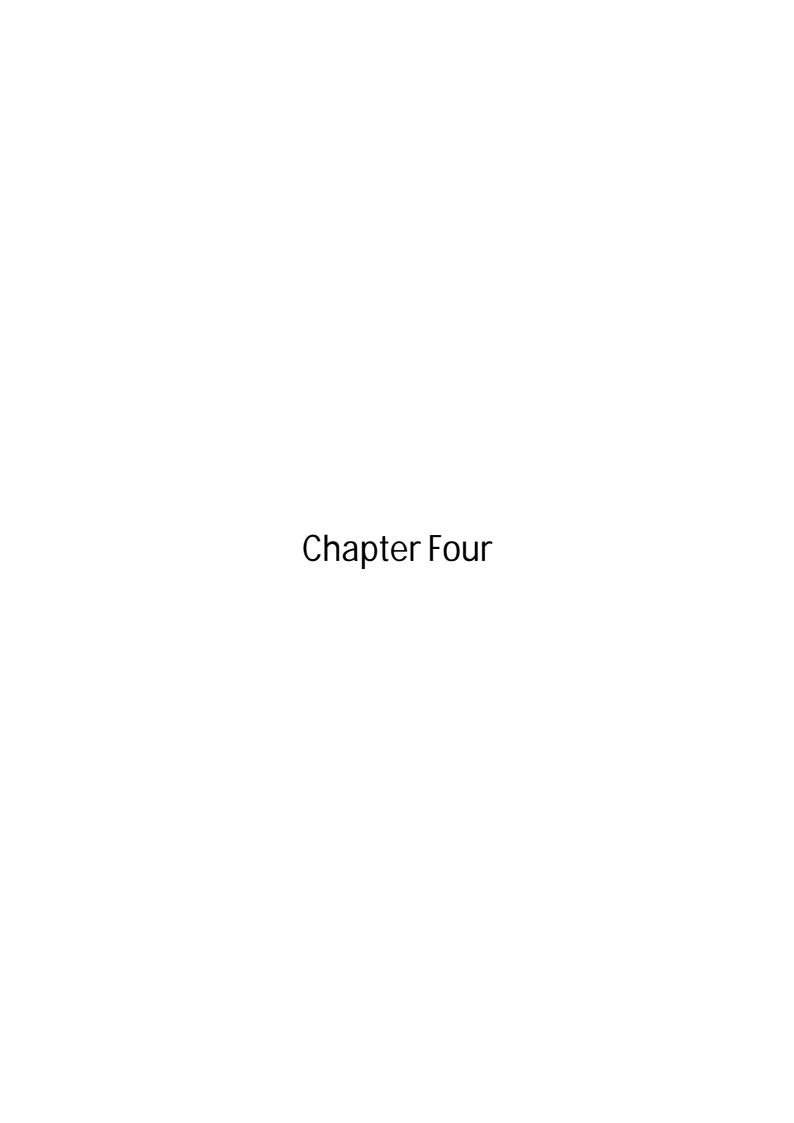
13-18: active

Above -18; high activity

From the result cinnamon oil is classified strong antimicrobial

. Conclusion

- . THE cinnamon oil was extracted and the physical and chemical properties of it were determined.
- . Cinnamon oil was analyzed and it was found to be has anti bacterial and anti oxidant effects



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Recommendation

- -Extraction of oil always should be carried out by distillation (hydro distillation) due to its volatile oil.
- it recommended to save extracted cinnamon oil under appropriate condition (reach of light , at temperature 5^0c) to avoid damage of oil.