Effect of aqueous and alcoholic extract of Cinnamon plant on some bacterial isolates

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Abstract
The inhibitory activity of hot, cold, and alcoholic extracts of cinnamon Phloem powder was studied1) Samples Used A- Plant Samples: B- Bacterial Samples Two types of bacteria were obtained: one Gram-negative rod-shaped bacterium, *E. coli*, and the other Gram-positive bacterium, *Staphylococcus aureus*. against several pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The hot aqueous extract of cinnamon showed the highest activity, with an inhibition diameter of 11 mm for *Staphylococcus aureus* and 8 mm for *E. coli*. The cold aqueous extract followed, with an inhibition diameter of 10 mm for Staphylococcus aureus and 7 mm for *E. coli*. The alcoholic extract showed an inhibition diameter of 8 mm for *Staphylococcus aureus* and 7 mm for *E. coli*. The constituents of the cinnamon extract were also identified. It contained saponins, resins, phenols, and alkaloids, but did not contain flavonoids, glycosides, or proteins. The hot aqueous extract of cinnamon Phloem showed better inhibitory activity against the studied bacteria than the other extracts, while the alcoholic extract was less effective than the other extracts tested against the two types of bacteria (*Escherichia coli* and *Staphylococcus aureus*).

Keywords: Cinnamon plant, alcoholic extract, bacterial isolates.
(It is not necessary that the keywords are taken from the title)

Introduction

Section One/General Introduction and the Importance of Medicinal Plants

Numerous studies have demonstrated the significant antimicrobial potential of plant-derived products , highlighting their remarkable efficacy against a broad spectrum of microbial pathogens while exhibiting fewer adverse effects than conventional antibiotics , Consequently , there has been growing scientific interest in medicinal plants as valuable sources of novel therapeutic agents plants produce a diverse array of bioactive secondary metabolites including alkaloids, flavonoids ,tannins ,lectins and peptides all of which possess various biological activities these phytochemicals are considered promising candidates for the development of new antimicrobial agent due to their ability to inhibit microbial growth through multiple mechanisms of action targeting microbial cells among medicinal plants cinnamon (*Cinnamomum spp*.) has gained considerable attention because of its extensive use as both a culinary spice and a medicinal herb its biological activities particularly its antibacterial properties are primarily attributed to cinnamaldehyde the major bioactive constituent responsible for many of its pharmacological effects.(Miroslava and Natalia , 2025)

• Medicinal Plants:

The scientist Dragendra recognized that anything of plant origin that can be used to treat a specific disease is a medicinal plant. A plant is called a medicinal plant if one or more of its parts contain one or more chemical substances in low or high concentrations that have the physiological ability to treat a specific disease or at least reduce the symptoms of that disease when given to the patient in its pure form, as a fresh herb, or in a reduced or partially extracted form. (Medicinal plants have the ability to produce one or more types of active

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compounds, and they can also produce inactive compounds that have no medicinal effect) (Evans and Evans[1] ,2009)

Active compounds:

The active chemical components of medicinal photosynthesis processes, such as glycosides, alkaloids, volatile oils, phenolic compounds, and others. These substances have a therapeutic effect on many diseases, accelerating their healing and alleviating their symptoms. Therefore, these products are called active compounds.

Active compounds in medicinal plants:

1. Glycosides: These are organic compounds that, when hydrated with acids or yeasts, split into a reducing sugar component and a sugar component called aglycone. Glycosides are linked to a sugar. This substance is soluble in water and alcohol, and its solutions are bitter. It is non-volatile. Glycosides are broken down in plants by specific enzymes. The enzyme and the glycoside it acts upon are found in the same plant, but in separate cells. When the plant is ground, the enzyme mixes with the glycoside in the presence of water, and this reaction results in the breakdown of the glycoside. Although the physiological effect in the body is due to the non-sugar part of its structure, glycosides are very active components. Some of them have highly toxic properties (cyanogenic glycosides), while others have important medicinal properties, such as heart tonics (squill and digitalis glycosides), laxatives (anthraconic glycosides), vermifuges (fern glycosides), antirheumatics (salicylic glycosides in Salix), or vitamin properties.

2. Alkaloids: These are organic compounds containing nitrogen. They are highly physiologically active, as the toxicity of most plants is due to their alkaloid content. They are characterized by their bitter taste, and their physiological effects vary depending on their type and proportions. They can be central nervous system analgesics (morphine, codeine), central nervous system stimulants (strychnine, caffeine), local anesthetics (cacanine), or antiparasitic (quinine, emetine).

3. Tannins: These are a group of compounds derived from carbohydrates and are abundant in the plant kingdom. Virtually every plant species contains at least one type of tannin. They are amorphous substances that dissolve in water, alcohol, and glycerol. When dissolved in water, they form an acidic emulsion with an astringent taste. These substances have the ability to precipitate proteins and alkaloids from their solutions. This process occurs during leather tanning and is a characteristic feature of this group of plant components. The proteins that make up the skin become insoluble and are beneficial in treating intestinal disorders due to their astringent and antiseptic properties. They also cause vasoconstriction and are used to stop bleeding and treat wounds and ulcers.

4. Saponins: These are compounds derived from carbohydrates, known for being water-soluble and having the property of forming a thick lather when mixed with water. They are used in the manufacture of toothpaste and cosmetic products such as shampoo. They also help in expectorating phlegm and reducing fever. They are found primarily in the roots of the licorice plant.

5. Resins: These are complex substances resulting from the oxidation of various essential oils. They are secreted by ducts or cavities within the plant and then flow onto the Phloem surface, where they solidify upon exposure to air. Trees are typically targeted for large quantities. They are insoluble in water but soluble in ether. They are often found combined with gums and essential oils and possess strong antiseptic and medicinal properties.

6. Volatile Oils: Also known as aromatic oils or essential oils, these are oils that evaporate or volatilize without decomposing. They have a strong aromatic scent and may have medicinal, antiseptic, antibacterial, or soothing value, in addition to their use in the perfume, soap, and cosmetic industries.

7. Fats and Lipids: These are a large group of glycerides. Unlike volatile oils, they do not evaporate or volatilize and cannot be distilled without decomposition. Some lipids are used

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as is in food (e.g., olive oil and pistachio oil). On the other hand, some fatty substances must be consumed with food because the body cannot synthesize them. A deficiency in these fatty acids can lead to skin lesions and hair loss. Additionally, corn oil and sunflower oil play a role in preventing atherosclerosis. Some oils also possess emollient and laxative properties, while others, like castor oil, have cholagogue or laxative properties.

Different Classifications of Medicinal Plants: Medicinal plants are classified into groups based on shared characteristics, features, or similar properties. This classification facilitates the identification of these groups and the study of all the characteristics that unite these plants. This classification can be summarized in several ways, including:

A. Morphological Classification: Medicinal and aromatic plants are classified according to the part used, which contains the active ingredient, into:

1. Plants Used Entirely: These are plants in which the active chemical substances are found in various plant parts without tending to concentrate or accumulate in one specific plant organ over another. Examples include black pine, Vinca, Artemisia cina, and Datura..

2. Plants whose leaves are used: These are plants whose active chemical compounds are found in their leaves. Examples include basil, mint, aloe vera, tea, and henna.

3. Plants whose inflorescences or flowers are used: These are plants whose active compounds are found either in the inflorescence, such as chamomile and chrysanthemum, or in the petals, as in roses, jasmine, and gardenia, or in the calyx, as in hibiscus, or in the stigmas, as in saffron.

4. Plants whose fruits are used: These are plants whose fruits contain the active chemical compounds, such as senna, ammi visnaga, and caraway.

5. Plants whose seeds are used: These are plants whose seeds contain the active chemical compounds, such as black cumin, mustard, cocoa, coffee, castor bean, sunflower, and fenugreek.

6. Plants whose Phloem is used: Such as cinnamon, willow, poplar, and chestnut.

7. Plants whose underground parts are used: These may be modified underground stems, taproots, or tuberous roots, and contain the active chemical compounds, such as (Mughat, Gypovial, Saponaria officinalis, Liquorice, and orchid tubers).

B. Physiological or Therapeutic Classification: This classifies plants according to the nature of the treatment or the benefit that can be gained from using these plants:

1. Laxative plants: Such as senna, castor bean, and Liquorice.

2. Analgesic plants And narcotic plants: such as Salix and poppy.

3. Plants that prevent capillary rupture: such as citrus fruits and buckwheat.

4. Plants that stimulate the heart: such as oleander, white squill, and foxglove.

5. Plants that cause localized redness: such as white and black mustard and Sudanese chili pepper.

C. Commercial Classification:

Classification is based on the nature of the commercial use of these plants, as follows:

1. Medicinal plants: These are plants traded commercially for use in the manufacture of medicines, including datura, mint, marjoram, and khella. Spices, seasonings, flavor enhancers, and natural ingredients are those used for culinary purposes, including black cumin, nutmeg, and cumin.

2. Aromatic plants: This group of plants contains volatile aromatic oils in a large or larger part of their plant organs, which can be used in the manufacture of perfumes and cosmetics. Examples include jasmine and rose. And basil.

3. Insect-resistant plants: These are plants used in their natural form or extracts to control and eliminate insects, such as pyrethrum and derris.

4. Plants used in making beverages: such as tea, coffee, cocoa, cola, fenugreek, salep, chamomile, tamarind, mint, and hibiscus.

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5. Natural products: These are organic compounds of natural origin that are important in metabolism and are isolated from plants and microorganisms.

Herbal medicine:

The interest in medicinal plants stems from their being among the first primary sources of medicine since the beginning of creation. Humans have used plants to alleviate pain and treat illnesses, either by using one or more parts of the plant after infusion, ingestion, or as a poultice, without verifying or researching the specific substance responsible for the therapeutic effect or which part of the plant had the therapeutic benefit. This was based solely on experience. With the advancement of science and technology, scientists have become able to isolate the active compounds from the plants they contain and prepare them in a way that suits their use and the specific ailment being treated. For her (Majrab, 2020).

Types of Medicinal Herbs:

Examples of medicinal herbs include turmeric, mint, rosemary, ginger, evening primrose oil, cinnamon, garlic, fenugreek, cayenne pepper, basil, sage, black cumin, green tea, licorice, chamomile, caraway, thyme, garden cress, wild oats, fennel, bitter lupin, and rosemary.

Cinnamon:

Cinnamon is a perennial, evergreen tree with aromatic leaves. Cinnamon exists in two main varieties: Ceylon cinnamon (Cinnamon ceylanicum) and Chinese cinnamon (Cinnamomum cassia). The original home of cinnamon is Sri Lanka, hence Ceylon cinnamon takes its name from its place of origin, while Chinese cinnamon originated in China (Fendy, 2022).Cinnamon contains antioxidants that are essential for protecting the body from many chronic diseases.

Table (1-1) Scientific Classification of Cinnamon

Scientific classification	
Plantae	Kingdom
Viridiplantae	Subkingdom
Magnoliophyta	Phylum
Magnoliopsida	Class
Magnolianae	Super order
Laurales	Order
Lauraceae	Family
Cinnamomum Schaeff	Genus
Zeylanicum Cinnamomum	Species

The stages of cinnamon tree growth and processing:

• After planting, the cinnamon tree takes about two years to grow to a height of 12-15 feet. Once it reaches this height, it is ready for harvest, as shown in Figure 1-1.

• Cinnamon is harvested manually using a sickle-like blade. Once cut, it is loaded onto a truck and transported to a peeling factory. The branches are then soaked in water to soften

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them, and the outer Phloem is removed to obtain the outer layer, as shown in Figure 2-1.
• The Phloem pieces cut from the peeled branches are then gathered and stuffed together to form the coiled cinnamon stick, known as cinnamon quills.
• The quills are then placed high up near the surface and left to dry.
• Finally, they are collected, weighed, and packed into containers for shipment to the factories.
• Upon arrival at the factory, the cinnamon sticks undergo quality testing in laboratories to determine the suitability of the final product. Once ground into a powder, the cinnamon is placed in an airtight container. It is then packaged using specialized machinery before being transported to stores for sale.

Nutritional Value of Cinnamon:
Cinnamon contains dietary fiber and several vitamins in small amounts, such as vitamin E, niacin, and vitamin B6, as well as minerals like magnesium, potassium, zinc, and copper (Al-Sayed, 2003).

Table (2-1) Nutritional Value of Cinnamon

Nutrient	Nutritional value
Calories	19 calories
Protein	0.3 mg
Carbohydrates	6.2 mg
Fats	0.1 mg
Dietary Fiber	4.1 mg
Manganese	1.4 mg
Calcium	77.7 mg
Iron	0.6 mg
Vitamin K	2.4 µg

The chemical components of cinnamon:
1. Cinnamaldehyde: An organic compound belonging to the aldehyde group, it is the active ingredient that gives cinnamon its distinctive flavor and aroma. Its medicinal benefits include strengthening the body's defenses (blood flow rate in the gastric mucosa), inhibiting the reduction of the gastric mucosa, and protecting the mucosa. It also promotes digestive function.

2. Cinnamic acid: A natural, protective plant compound found in cinnamon Phloem, ginseng, whole grains, honey, and many other foods. It improves gut health and has been shown to possess prebiotic-like properties, meaning it promotes the growth of beneficial bacteria in the gut while suppressing the growth of harmful bacteria. Furthermore, a 2020 study demonstrated that cinnamic acid inhibited the growth of Escherichia coli in the gut, a strain of bacteria associated with food poisoning.

3. Procyanidins: Possesses antioxidant activity.
4. Catechins: Possesses antioxidant activity.

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Table (3-1) Comparison of Cinnamon Types

Cassia 95% Cinnamaldehyde oil	Ceylon	**No.**
Origin: China	50%-63%	**1**
Widely distributed	Cinnamaldehyde oil	**2**
Available in most markets	Origin of India	**3**
Cheaper	Rarely widespread	**4**
Stronger aroma	Not available	**5**
Dark reddish-brown color	More expensive	**6**
Hard and difficult to break	Milder odor	**7**
High coumarin content	Light brown color	**8**

Section Two/ Literature Review

In recent years, interest in medicinal plants and herbs has increased, both as primary sources for producing pharmaceutical drugs and as sources of active compounds used in drug formulations. They are also used as raw materials for the production of certain chemical compounds and pharmaceutical substances. Many studies have examined the effect of plant extracts on the growth of microorganisms and, consequently, their potential use in treating various diseases resulting from microbial infections. Among these studies are the following:

In, Majid and Al-Shatti 2002conducted a study investigating the effect of different concentrations of aqueous extracts from Citrullus colocynthis, mint, Stachys, Cordyceps myxa, and pomegranate peel inhibit the growth of microorganisms such as bacteria, molds, and yeasts. The results showed that extracts of mint and pomegranate on the growth of bacteria, molds, and yeasts. The results showed that mint and Stachys extracts had no effect on the growth of the studied microorganisms, while galangal, colocynth, and pomegranate peel extracts exhibited varying effects.

Eucalyptus, or eucalyptus plant, is a fast-growing evergreen tree used as a natural remedy for coughs and colds. Research has shown that it widens the bronchi and airways and reduces phlegm secretion. It contains eucalyptus (also known as cineole), and eucalyptus extract has inhibitory activity against microorganisms that cause urinary tract infections, as noted by Hussein and Amir (2016).Mint is a perennial aromatic plant belonging to the peppermint family and the seed-bearing herb family. It is used for eating and drinking, or its leaves are added to dishes after being dried and ground. Peppermint oil is also considered medicinally important, as noted by Hussein and Fawzi Taha Qutb (1981). Peppermint is used to treat many diseases, especially stomach and intestinal ailments, and is beneficial in treating headaches. Peppermint oil has antiseptic properties, which is why it is used in toothpaste and soap making.The plant kingdom is rich in bitter byproducts that have been used to treat most intractable diseases affecting humans and animals. These byproducts have also been used to kill and inhibit the growth of many pathogenic microorganisms that infect humans through food (Al-Saadi, 2019 , Hassan, 2012).Cinnamon is found primarily in two

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species: Ceylon cinnamon (Cinnamon ceylanicum) and Chinese cinnamon (Cinnamomum cassia blume). (2) indicated that the essential oil of the cinnamon plant has inhibitory activity against Haemophilus bacteria.

A study conducted by (3) on Strep pneumoniae, Strep pyogenes, Staphylococcus aureus, and Staphylococcus influenzae showed that extracts of Chinese cinnamon Phloem have inhibitory activity against 13 bacterial species and isolates, including: Listeria, Enterococcus faecalis, B. cereus EU, Bacillus brens FMC3, K. pneumoniae, Micrococcus lotevus LA2971 monocytogenes SCOTTA, and Yersininia enterocolitica, with varying diameters of inhibition zones at a concentration of 20 µg.

In Ali 2007, tested the inhibitory activity of cold, hot, alcoholic, and oil extracts of cinnamon Phloem on various bacteria and yeasts using the burrow diffusion method. He observed that the inhibitory activity of the four extracts varied depending on the extraction solvent and the microorganisms. The 25% oil extract showed significantly greater activity than the other extracts, followed by the ethyl alcohol extract, while the 35% water extract exhibited the lowest inhibitory activity.

In Yassin and Hardan 2012, found that using cinnamon Phloem extract on aerobic and anaerobic bacterial isolates causing bad breath had a higher inhibitory effect than traditional antibiotics such as chloramphenicol and metronidazole, except for Staphylococcus bacteria, where the antibiotics gentamicin and rifampin showed higher inhibitory activity than the cinnamon Phloem extract.

Researchers Al-Sharif and Al-Malh (2017) studied the extraction of cinnamon oil from cinnamon sticks using simple hydrodistillation. They found that the extracted oil yield was 0.99%. High-performance liquid chromatography (HPLC) also revealed a high concentration of the main component, cinnamaldehyde, in the laboratory-extracted cinnamon oil.

In the same study, the biological effects of the extracted cinnamon oil were investigated, and its antibacterial activity was determined. This was compared to cinnamon oil available in herbal shops and pharmacies using the disc diffusion method. The study targeted three types of bacteria: Escherichia coli and Klebsiella pneumoniae (Gram-negative), and Staphylococcus aureus (Gram-positive). Cinnamon oil extracted in vitro demonstrated high inhibitory activity, with aura diameters ranging from 24 to 26 mm. In contrast, commercially available cinnamon oil from herbal shops and pharmacies showed moderate to weak activity, with aura diameters ranging from 13 to 17 mm. This effect was compared to that of certain antibiotics against these types of bacteria.

The effect of cinnamon oil on Candida fungi and on the vegetative growth and conidia of the pathogenic fungus Aspergillus niger was also studied. Cinnamon oil extracted in vitro showed high activity against Candida, with an aura diameter of 40 mm, compared to 31 mm for the commercially available oil from herbal shops and pharmacies. However, commercially available cinnamon oil showed no inhibitory activity against the vegetative growth of the pathogenic fungus. Aspergillus nige

The current study aims to chemically identify the active compounds of Aspergillus nige and to assess the inhibitory activity of cold, hot, and alcoholic aqueous extracts of cinnamon on certain bacterial isolates.

Section Three/Importance and aim of the Study

Importance of the Study:

Cinnamon, like other medicinal herbs with active compounds, may contribute to finding alternatives to antimicrobial agents, which have become resistant due to the widespread use of antibiotics in treating many diseases, including the following:

1. Benefits of Cinnamon for Cancer Patients:

Cinnamon extracts may help prevent cancer by reducing the growth of cancer cells and contributing to their elimination.

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2. Benefits of Cinnamon for Inflammation:
Cinnamon can contribute to treating inflammation. This benefit stems from its content of cinnamaldehyde, a compound with antibacterial and antifungal properties, which can help with conditions such as respiratory infections, tooth decay, and bad breath.
3. Benefits of Cinnamon as a Pain Reliever:
Cinnamon possesses analgesic and wound-healing properties, making it potentially helpful in relieving pain, such as perineal pain after childbirth for women, as well as for menstrual cramps. Study Objectives:

The current study aims to:
1. Investigate the effect of aqueous and alcoholic extracts of cinnamon on pathogenic bacteria.
2. Test the inhibitory activity of cold, hot, and alcoholic aqueous extracts of cinnamon Phloem extract on the bacteria under study.
3. Explore the use of cinnamon extract as a natural remedy in the medical field for treating diseases, as an alternative to pharmaceuticals and other chemical or synthetic substances, given its lower cost.

Materials, Equipment, and Procedures
Section One: Materials and Equipment
A- Materials Used:
1. Slide and slide cover.
2. Ethyl alcohol (70%).
3. Filter cloth, cotton, and gauze.
4. Filter paper of various sizes.
5. Sterile distilled water.
7. Glassware of various types and sizes.
8. Hydrochloric acid.
9. Sodium hydroxide.
10. Iodine solution.
11. Ferric chloride.
12. Hydrated copper sulfate.
13. Mercuric chloride.
14. Solid bacterial culture media (Mueller-Hinton) agar.
15. Cotton.
16. Antibiotics: Clavulanic acid, Amoxicillin, Ceffadroxil.
Memmert bacterial incubator.
Loop.

Heat source for sterilization.
B- Equipment used:

Table (1-2) Equipment used

Manufacturer's name	**Equipment Name**
Al MARWANI	**Electric Grinder (Model_100)**
Newal	**Electric Mixer**
Stuart	**Hot Plate**
OPTIKA	**Binocular Light Microscope**
Guohua Group Co., Ltd	**Centrifuge**
Kern	**Sensitive Balance**
Guohua Group Co., Ltd	**Water Bath**
	Oven
Vestel	**Vertical Refrigerator for Sample Storage**

Section Two: Methods of Work
1) Samples Used:
A- Plant Samples:

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Samples of Ceylon cinnamon were obtained from local markets in Basra Governorate. The Phloem was used, identified microscopically, and ground using an electric grinder to obtain a homogeneous powder. The powder was then stored in sterile bags in the refrigerator until use.

B- Bacterial Samples:

Two types of bacteria were obtained: one Gram-negative rod-shaped bacterium, E. coli, and the other Gram-positive bacterium, S. aureus. These were isolated and identified in the Microbiology Laboratory of the Nursing Techniques Department at the Basra Technical Institute. These species were chosen because they are common causes of certain human diseases and are also known contaminants that cause food spoilage and poisoning. The purpose of these samples was to investigate the inhibitory activity of Ceylon cinnamon extract.

2) Preparation of Plant Extracts:

a- Preparation of Aqueous Extracts:

Plant extracts are generally secondary metabolites and may be in the form of aqueous or alcoholic extracts, oils, powders, or any other form from which their active compounds can be utilized. Preparing the extract is the first and most important stage for obtaining high concentrations.

Plant extracts are antibacterial and antifungal. They are plant extracts that have antibacterial and antifungal effects, and in some cases, they repel agricultural pests. They are characterized by very low toxicity, rapid decomposition, and low resistance to substances.

Preparation of the cold aqueous extract of Ceylon cinnamon Phloem powder:

The extract was prepared by dissolving 10 grams of Ceylon cinnamon powder - after grinding it for 3 minutes - in 100 ml of cold water. The powder was sparingly soluble in cold water. The solution was shaken for 10 minutes, then filtered by centrifugation for 5 minutes, then filtered again through linseed paper. The extract was placed in an opaque bottle in the refrigerator for 11 days,

Preparation of the hot aqueous extract of Ceylon cinnamon Phloem powder:

The extract was prepared by dissolving 10 grams of Ceylon cinnamon powder (after grinding it for 3 minutes) in 100 ml of hot water. The powder dissolved rapidly in hot water. The solution was shaken for 3 minutes and placed in an opaque bottle in the refrigerator for one week. It was then filtered by centrifuge for 5 minutes, followed by a second filter using a filter paper. The extract was then placed in an opaque bottle in the refrigerator for 4 days,

b) Preparation of the Alcoholic Extract:

The alcoholic extract was prepared by dissolving 10 grams of Ceylon cinnamon powder (after grinding it for 3 minutes) in 100 ml of 70% ethyl alcohol solution. The powder dissolved rapidly in alcohol (30% faster than in hot water). The solution was shaken for 2 minutes, left to stand for 5 minutes, and then filtered by centrifuge for 5 minutes. Minutes, then it was filtered again through the squill paper and the extracted solution was placed in an opaque bottle in the refrigerator for 4 days,

3- Chemical Detection of Active Compounds:

Detection of Saponins:

a- Cinnamon was dissolved in hot water, and the solution was then placed on a hot plate for five minutes at a temperature of 280°C, as shown in Figures (11) and (12).

b- After the solution began to boil, the temperature was reduced to 70°C.

c- A layer of bubbles, representing saponins, formed on top of the solution, as shown in Figure (8-2).

Result: The amount of saponins in the extract was high

Resin Detection:

The reagent, composed of 70% ethyl alcohol and hydrochloric acid (HCl), is shown in Figure 9-2.

a. 5 ml of the aqueous cinnamon extract was placed in a glass container.

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b. 3 ml of the reagent was added to the extract container using a dropper, as shown in Figure 10-2.
c. The glass container was placed on a heat source (hot plate) at 245°C, as shown in Figure 10-2.
d. The extract boiled, steam rose, and turbidity appeared, as shown in Figure 11-2.
Result: The aqueous cinnamon extract contains a high percentage of resins.

Detection of Glycosides:
1- Iodine Solution: To detect polysaccharides.
a- 1 ml of cold aqueous cinnamon extract was placed in a test tube.
b- Then 1 ml of iodine solution was added.
We shook the solution briefly and observed that it did not turn blue. Conclusion: The cold aqueous extract of cinnamon does not contain polysaccharides, as shown in Figure (13-2).



Figure (12-2) Iodine Solution



Figure (13-2) Aqueous extract before and after iodine solution

2- Benedict's Reagent: To detect reducing sugars
a- 1 ml of hot aqueous cinnamon extract was placed in a test tube.
b- Then 1 ml of Benedict's reagent was added to the test test tube.
After shaking slightly, a green precipitate appeared.
Result: The extract contains polysaccharides, as shown in Figure (15-2).



Figure (15-2) Aqueous extract before and after Benedict's reagent



Figure (14-2) Benedict's reagent

Detection of phenolic compounds:
a) 1 ml of alcoholic cinnamon extract was placed in a test tube.

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b) 1 ml of phenolic reagent was added to the test tube.
After a brief shake, a green precipitate was observed. Conclusion: The presence of phenolic compounds in the alcoholic cinnamon extract is shown in Figure (17-2).





Figure (17-2) Aqueous extract before and after phenol detection Figure (16-2) Phenol Detection

Flavones Detection:
Reagent Preparation: Prepared by measuring 1 mg of NaOH and dissolving it in 100 ml of ethyl alcohol.
Flavones Detection in Aqueous Cinnamon Extract:
1. Place 1 ml of aqueous cinnamon extract in a test tube.
2. Add one drop of flavones reagent.
3. No yellow color appeared.
Result: Aqueous cinnamon extract does not contain flavones, as shown in Figure (19-2).
Flavones Detection in Alcoholic Cinnamon Extract:
1. Place 1 ml of alcoholic cinnamon extract in a test tube.
2. Add one drop of flavones reagent.
3. No yellow color appeared.
Result: Alcoholic cinnamon extract does not contain flavones, as shown in Figure (20-2).

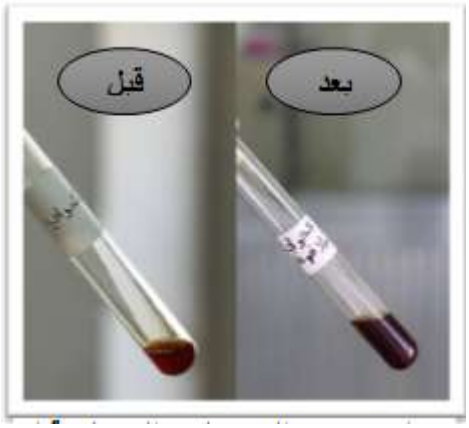




Figure (19-2) Alcoholic Extract Before and After Flavonoid Detection Figure (18-2) Flavonoid Detection

Protein Detection:
Pyruit's Reagent:
Reagent Preparation: Weigh 1 gram of copper sulfate and place it in a glass beaker. Add distilled water to the powder until the solution volume reached 100 ml. Then, add 1 gram of NaOH crystals to the solution and stir to dissolve the substances.

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Protein Detection in Aqueous Cinnamon Extract:
1. Place 1 ml of aqueous cinnamon extract in a test tube.
2. Add a drop of Pyruit's reagent.
3. Observe that no purple color appeared.
Result: Aqueous cinnamon extract does not contain proteins, as shown in Figure (24-2).

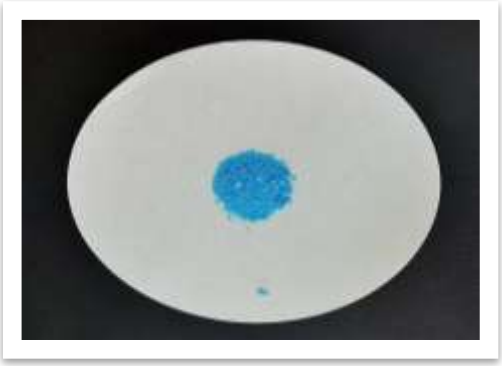


Figure (21-2) Copper sulfate



Figure (22-2) Protein reagent



Figure (23-2) Protein reagent

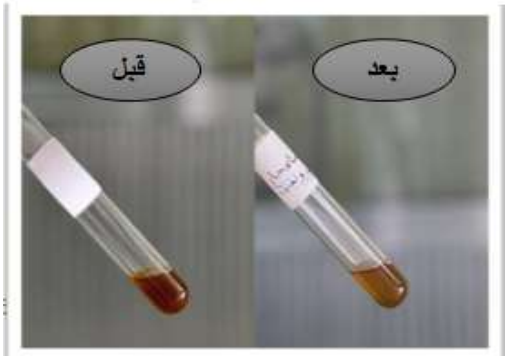


Figure (24-2) Aqueous extract before and after protein detection

Detection of Alkaloids:
Mayer's Reagent
Preparation of the reagent: It was prepared by measuring 1 mg of silver chloride (AgCl) and dissolving it in 50 ml of distilled water. Then, 5 g of potassium iodide (KI) was added, and the distilled water was brought up to 100 ml.
Detection of Alkaloids in Aqueous Cinnamon Extract:
1. One ml of aqueous cinnamon extract was placed in a test tube.
2. A drop of Mayer's reagent was added.
3. An orange color was observed.
Result: Aqueous cinnamon extract contains alkaloids, as shown in Figure (27-2).

Detection of Alkaloids in Alcoholic Cinnamon Extract:
1. One ml of alcoholic cinnamon extract was placed in a test tube.
2. A drop of alkaloid reagent was added.
3. A slight orange color was observed.
Conclusion: The alcoholic cinnamon extract contains alkaloids, but in smaller quantities than the aqueous cinnamon extract, as shown in Figure (28-2).

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Figure (26-2) Alkaloid Detection Alkaloid Detection



Figure (27-2) Aqueous Extract Before and After

Table (2-2) Results of chemical analysis of aqueous and alcoholic cinnamon plant extract

alcoholic extract	aqueous extract	Name of detector	Type of detection
–	–	Iodine test / Benedict's test	Glycosides detection
+	++	Ferrous chloride FeCl2%	Phenols detection
–	–	Ethyl alcohol NaOH+	Flavones detection
–	–	Bioritant 1D CuSO3+NaOH	Protein detection
+	+	Meyer's test HgCl2+K	Alkaloids detection
+	++	Shake and heat	Saponins detection
+	-	Ethyl alcohol HCl+70%	Resins detection

4- Drug Susceptibility Testing:

The Agar Disk Diffusion method (NCCLS, 2000) was used to test the drug susceptibility of bacteria.

1- A quantity of the hot aqueous extract, the cold aqueous extract, and the alcoholic extract were placed separately in three clean, sterile Petri dishes.

2- Using a piercing tool, filter papers were perforated to a diameter of 6 mm and dipped into the extract. They were then left to incubate for three weeks in a refrigerator at 4°C to allow for saturation.

3- Mueller-Hilton Agar medium and the streaking method were used to culture E. coli and S. aureus bacteria under strict sterile conditions (Turk et al., 2020).

4- Using sterile metal forceps, tablets containing the synthetic antibiotics amoxicillin, calvulanic acid, and cfadroxil were placed on the culture medium, followed by filter paper discs saturated with cinnamon extract.

5- The plates were kept in the incubator at a temperature of 37°C for 24 hours.

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Figure (29-2) Cold water extract



Figure (30-2) Alcoholic extract



Figure (31-2) Hot water extract



Figure (37-2) Müller-Hinton solid medium



Figure (38-2) Streaking method



Figure (39-2) Staphylococcus aureus

Results and Discussion

The biological activity of the active compounds in the aqueous (cold and hot) and alcoholic extracts of cinnamon was studied against pathogenic bacteria. The activity of each extract was investigated individually using two types of pathogenic bacteria: Staphylococcus aureus and Escherichia coli.

The aqueous (hot) extract of cinnamon showed the highest activity, with an inhibition plate diameter (the transparent, growth-free areas) of approximately 11 mm for Staphylococcus aureus and 8 mm for E. coli. This was followed by the cold aqueous extract, which had an inhibition plate diameter of 10 mm for E. coli. (S. aureus) and (7) mm for E. coli.

The diameter of the inhibition zone for the alcoholic extract was (8) mm for S. aureus and (7) mm for E. coli, as measured by a ruler, for the cinnamon extract, as shown in the table below:

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Table (1-3) Inhibition Results of Aqueous and Alcoholic Cinnamon Extract

Inhibition zone diameters (mm(for cinnamon extract			Bacteria	No.
Alcoholic	**Hot aquatic**	**Cold aquatic**		
7	**8**	**7**	***Escherichia coli***	1
8	**11**	**10**	***Staphylococcus aurus***	2

The inhibitory effect of cinnamon extract may be attributed to its tannin content, which includes phenolic compounds such as gallic acid and tannic acid. These compounds have a biological effect against many bacterial species due to the presence of hydroxyl (OH) groups. These hydroxyl groups can form hydrogen bonds between the hydroxyl group in these compounds and the water molecules in the bacterial cell, which constitute 90% of its weight. This leads to the disruption of vital functions within the bacterial cell, as observed by Ali et al. (2007).

These compounds also have the ability to coagulate bacterial cell proteins and break down enzymes involved in the synthesis of amino acids essential for cell division.

The aqueous extract showed higher inhibitory activity than the alcoholic extract. This is attributed to the varying proportions of active compounds in different extracts, such as saponins and phenolic compounds, compared to other components in cinnamon. Water is also one of the best solvents used in extraction (Shehab&Mahdi, 2012).

Shen et al. (2015) noted that cinnamon cinnamaldehyde destroys the sticky layer formed by Staphylococcus aureus and Escherichia coli bacteria, known as the biofilm. This biofilm helps bacteria adhere and permeate, and its integrity is compromised when exposed to even minimal inhibitory concentrations. Furthermore, the higher the cinnamaldehyde concentration, the more severe the damage to the bacterial biofilm.The current study observed that the alcoholic extract was less effective than the aqueous extract, and this may be due to:
The difference in the polarity of the solvent used leads to variations in the overall composition of the groups.
This aligns with what Giese (1994) stated: that the inhibitory activity of plant extracts against bacteria varies depending on the type of plant and microorganism.

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Figure (1-3) Inhibitory activity of hot and aqueous cinnamon extracts on S. aurus bacteria



Figure (2-3) Inhibitory activity of cinnamon alcoholic extract on S. auras bacteria



Figure (4-3) Inhibitory activity of cold and hot aqueous cinnamon extracts on E. coli bacteria



Figure (3-3) Inhibitory activity of alcoholic cinnamon extract on E. coli bacteria

Conclusions and Recommendations

Conclusions:

We conclude from the study that the extract of Ceylon cinnamon Phloem contains several active groups, including phenols, resins, alkaloids, and saponins. This is evident from the chemical analysis of the extract. The hot aqueous extract of cinnamon Phloem showed better inhibitory activity against the studied bacteria than the other extracts, while the alcoholic extract was less effective than the other extracts tested against the two types of bacteria (*Escherichia coli* and *Staphylococcus aurus*).

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