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Research Article

Study of the Effectiveness of *Cinnamomum camphora* Leaf Extracts against Bacteria Causing Respiratory Infections

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Abstract

This study aimed to investigate the inhibitory activity of alcoholic and hot aqueous extracts of *Cinnamomum camphora* leaves against bacteria isolated from respiratory infections, including *Streptococcus pyogenes*, *Streptococcus pneumoniae, and Klebsiella pneumoniae,* collected from Al-Hakim General Hospital in Najaf Al-Ashraf from December to April 2021. These bacteria are clinically significant as they cause respiratory tract infections. A total of 133 samples was collected from patients exhibiting symptoms of respiratory tract infections, yielding 92 positive samples (69.17%) and 41 negative samples (30.83%). The bacterial strains identified included *Streptococcus pyogenes*, *Streptococcus pneumoniae, and Klebsiella pneumoniae*, with prevalence rates of 46.7%, 17.3%, and 2.17%, respectively. The study results demonstrated that these extracts effectively inhibited bacterial growth in vitro.The antimicrobial activity of the plant extracts, both alcoholic and hot aqueous, was assessed using the agar well diffusion method at a concentration of 200 mg/mL. The highest inhibition zone was observed for *Streptococcus pyogenes*, measuring 20 mm at 200 mg/mL, while the lowest inhibition zone was recorded for *Klebsiella pneumoniae*, measuring 10 mm at concentrations of 100 mg/mL and 200 mg/mL For both the alcoholic and hot aqueous extracts of *Cinnamomum camphora* leaves. Preliminary phytochemical screening of the hot alcoholic extract of *Cinnamomum camphora* leaves revealed the presence of active compounds, including flavonoids, amino acids, saponins, glycosides, alkaloids, tannins, and terpenoids. In contrast, the hot aqueous extract contained the same active compounds except for amino acids and terpenoid glycosides.

1. Introduction

Bacterial respiratory tract infections are among the most common diseases in children, ranking first in prevalence before urinary tract infections [1]. Respiratory tract infections typically result from pathogenic microorganisms attacking the respiratory system, with most infections caused by *Streptococcus pneumoniae* (pneumococcus) [2]. This pathogen is a major cause of bacterial pneumonia. Additionally, other pathogens such as Streptococcus pyogenes and *Klebsiella pneumoniae* contribute to laryngitis and lung infections. *Klebsiella pneumoniae* is a medically significant enterobacterium that causes pneumonia. The virulence of these bacteria and their ability to infect the host play a critical role in the onset and progression of infections, which depend on a series of interactions between the pathogen and the host [3]. Medicinal plants and herbs have been used as new sources of antimicrobial agents [4]. Plants produce secondary metabolites that contribute to various biological activities, including defense mechanisms and antimicrobial properties [5]. Among these plants, *Eucalyptus camaldulensis* has been used to treat urinary and respiratory tract diseases. Similarly, *Cinnamomum camphora* is a medicinal plant belonging to the *Cinnamomum* genus and has significant therapeutic applications. It has been used in various treatments due to its bioactive compounds,

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including steroids, tannins, glycosides, and flavonoids, which help regulate and reduce microbial populations in the body. This plant is known for its broad-spectrum antimicrobial properties, inhibiting the growth of bacteria, fungi, and viruses [6]. The essential oil extracted from *Cinnamomum camphora* is beneficial in disinfecting the respiratory tract, treating skin infections, whooping cough, asthma, influenza, and colds when used as a massage oil or inhaled as steam. It also helps relieve pulmonary congestion and acts as an expectorant due to its active compounds [7]. Additionally, its bark is used in Asian countries to treat gastrointestinal disorders and infections [8]. The plant is also effective in treating respiratory mucosal inflammation associated with excessive secretions, stimulating circulation in diabetic patients, and serving as a bactericidal, insecticidal, and antimicrobial agent. It is also known to regulate heartbeats. This study was conducted to explore the potential medical applications of *Cinnamomum camphora* extracts and their possible use in treating infections caused by these microorganisms.

2. Materials and Methods

Collection of Plant Samples

Cinnamomum camphora leaves were collected from local markets and classified in the herbarium of the College of Education for Women, University of Kufa. After being cleaned from dust using tap water, the leaves were air-dried in the shade. Once dried, they were ground using an electric grinder to obtain a fine leaf powder. The powder was then stored in clean, sterile, dry nylon bags and kept in a refrigerator until use in microbiological studies.

Preparation of the Extract

Preparation of Hot Aqueous and Alcoholic Extracts of *Cinnamomum camphora* Leaves

The method described in [9] was used to prepare the hot aqueous extract of *Cinnamomum camphora* leaves. This process involved taking 40 g of dried leaf powder and placing it in a glass flask containing 350 mL of hot distilled water. The flask was then placed on a magnetic stirrer (Hot Plate Stirrer) for continuous mixing for 1.5 hours. *Cinnamomum camphora* is widely used for its medicinal properties, including pain relief, treatment of stomach aches, diarrhea, and severe headaches during high fevers. It is also an effective treatment for tuberculosis [10]. Additionally, camphor oil is used for treating scabies and itching. *Cinnamomum camphora* exhibits significant antifungal and antibacterial activity, with its alcoholic extract showing the strong antibacterial effects against both Gram-positive and Gram-negative bacteria [11]. The bioactivity of compounds isolated from *Cinnamomum camphora* is attributed to the presence of flavonoids [12]. After covering, the solution was left to stand for 24 hours to allow the plant components to settle. The solution was then filtered through a clean cloth, followed by filtration using Whatman No.1 filter paper. The filtrate was then centrifuged at 3000 rpm for 10 minutes to separate the precipitate and obtain the extract. The extract was then concentrated using a rotary evaporator at 40–45°C, collected in a closed container, and stored in the refrigerator until use.

Preparation of the Stock Solution of *Cinnamomum camphora* Leaf Extract

A stock solution of the extract was prepared at a concentration of 200 mg/mL. The solutions were sterilized using membrane filters with a pore size of 0.45 µm and stored in a refrigerator at 4°C. Different concentrations of each extract were prepared from the stock solution using the dilution formula: N1V1 = N2xV2

Phytochemical Screening of Bioactive Compounds

Alkaloid Reagents

Mayer's Reagent: Prepared by dissolving 12.5 g mercuric chloride and 5 g of potassium iodide in 1 liter of distilled water. It was used to detect alkaloids by adding 1–2 mL of the aqueous or alcoholic extract, resulting in a white to brown precipitate [13].
Tannic Acid Reagent: Prepared at a 1% concentration of tannic acid and used for alkaloid precipitation by adding 1–2 mL to 5 mL of the aqueous or alcoholic extract, leading to a white, cloudy precipitate [14].

Phenol Reagents

1% Lead Acetate Reagent: A 1% aqueous solution of lead acetate was used to detect tannins by adding an equal volume of reagent to the aqueous or alcoholic extract, producing a greenish-blue precipitate [15].
1% Potassium Hydroxide (KOH) Reagent: Used to detect coumarins and flavonoids by adding 10% potassium hydroxide solution to an equal volume of aqueous or alcohol eExtract resulting in a yellow or greenish-yellow color [16].
Terpenoid Reagents: Foam Test (Saponin Test): To detect the presence of saponins, a tightly closed bottle containing the aqueous extract was shaken vigorously. The presence of dense foam that remained for a long time indicated the presence of terpenoids [15].
Mercuric Chloride ($HgCl_2$) Reagent: To detect the presence of saponins among the terpenoids, 1–2 mL of mercuric chloride was added to 5 mL of the aqueous or alcoholic extract. The formation of a white precipitate indicated a positive result [17].

Concentrations of Plant Extracts

To prepare the **stock solution** of the aqueous extract, 2 g of plant extract powder was dissolved in 10 mL of sterile distilled water, resulting in a stock solution with a concentration of 0.2 g/mL. The solution was sterilized by filtration using a membrane filter and Whatman No.10 filter

paper to remove microbial contaminants, ensuring a sterile stock solution. This solution was then used to prepare concentrations of 100 mg/mL and 200 mg/mL.

For the alcoholic extract, 2 g of the plant extract were dissolved in 3 mL of ethyl alcohol, and the volume was adjusted to 10 mL with distilled water, obtaining a concentration of 200 mg/mL, from which further dilutions (100 mg/mL and 200 mg/mL) were prepared.

Antibacterial Activity of the Extracts

A total of 133 bacterial isolates was collected and identified from respiratory infections at the Chest Diseases Center of Al-Hakim General Hospital in Najaf between December and April 2021. The samples were collected from both male and female patients in sterile tubes, then immediately cultured on nutrient agar to prevent contamination. Bacterial identification was based on colony morphology and biochemical tests, and the isolates were preserved using two methods: hort-term preservation: The bacteria were inoculated into slant tubes containing solid nutrient medium and incubated at 37°C for 24 hours, then stored at 4°C in the refrigerator until use.
Long-term preservation: The bacteria were inoculated into liquid nutrient medium supplemented with 15% glycerol, incubated for 24 hours, and then stored at -20°C [18].

Three bacterial isolates were Selected, calibrated using a McFarland standard, and spread on Mueller-Hinton agar to study the effects of the extract.

Bacterial Susceptibility Testing Against the Plant Extract

Extract concentrations of 100 mg/mL and 200 mg/mL were prepared using distilled water for aqueous extracts and 10% DMSO (dimethyl sulfoxide) for alcoholic extracts. The bacterial susceptibility was tested using the well diffusion method (Agar Well Diffusion Assay) [19].
• 0.1 mL of each bacterial suspension was spread on solid Mueller-Hinton agar using a sterile cotton swab.
• A cork borer was used to create three wells (6 mm in diameter) in each plate.
• 0.1 mL of the plant extract at different concentrations was added to the wells.
• A negative control well containing distilled water was included.
• Plates were left at room temperature for 15 minutes, then Incubated at 37°C for 24 hours.
• The inhibition zone diameter (zone of bacterial growth inhibition) was measured using a ruler [20].

Detailed Diagnostic Tests

Morphological and Cultural Characteristics

Bacterial colonies grown on MacConkey agar were identified based on:
• Colony size, shape, and color
• Viscosity
• Ability to ferment lactose
• Cell shape, size, arrangement, and Gram reaction

Biochemical Tests

Several biochemical tests were performed to confirm bacterial identification, including:
• Catalase test
• Oxidase test
• Ureas test
• Growth in Kligler Iron Agar (KIA) and gas production
• Indole test
• Methyl Red (MR) test
• Voges-Proskauer (VP) test
• Citrate utilization test
• Motility test
The entire 18 System [21] was used for confirmatory testing, with results Interpreted as follows:
• Positive fermentation of lactose, mannitol, inositol, sorbitol, sucrose, arabinose, and raffinose resulted in a yellow color, while a negative result appeared greenish-blue.
• Arginine, ornithine, and lysine decarboxylation tests:
  ◇ Negative results appeared orange-yellow
  ◇ Positive results appeared red
• β-Galactosidase enzyme activity:
  ◇ Negative result was colorless
  ◇ Positive result was yellow
• Malonate utilization, acetoin production, H_2S production, and indole tests:
  ◇ Negative results appeared yellow
  ◇ Positive results varied:
• H_2S production $\rightarrow$ black
• Malonate utilization $\rightarrow$ greenish-blue
• Indole production $\rightarrow$ red

• Acetoin production → pink-red
• Phenylalanine deaminase (PD) test:
  ◇ Positive result → black-brown
  ◇ Negative result → pale green
• Ureas test:
  ◇ Positive result → red-fuchsia
  ◇ Negative result → orange-yellow
• Final biochemical test:
  ◇ Positive result → black-brown
  ◇ Negative result → yellow

3. Results and Discussion

The qualitative screening results for some active compounds in the plant extracts under study revealed that the leaves of *Cinnamomum camphora* contain various bioactive compounds. Several detection methods, as outlined in Table 1, were employed to identify these compounds. The chemical screening results of the *Cinnamomum camphora* leaves indicated that the hot aqueous extract contained several active compounds, including saponins, tannins, and flavonoids only. In contrast, the hot alcoholic extract contained all these compounds found in the aqueous extract in addition to glycosides, alkaloids, and amino acids. Furthermore, it was found to contain essential oils, which are responsible for its antimicrobial activity, aligning with the findings of [22].

Additionally, the extract was found to contain oleic acid and fatty acids, fatty acid methyl esters, which is consistent with the findings of [23]. Phenolic compounds were identified as a rich source of antioxidants [24].

Regarding the nature of the extracts, they were characterized by a viscous texture and a distinctive aromatic scent. The characteristic aroma of *Cinnamomum camphora* is attributed to the presence of volatile oils, which contain essential oils in different plant parts. Key compounds identified in these oils include sitosterol, oleic acid, γ-linolenic acid, and linoleic acid [25]. The results showed that all bacterial isolates were sensitive to both the hot aqueous and alcoholic extracts of *Cinnamomum camphora* leaves. The application of concentrations (100 and 200 mg/mL) of the hot aqueous and alcoholic extracts demonstrated antimicrobial activity, as measured by the inhibition zone diameter. The findings indicated a direct proportionality between the concentration used and the observed antimicrobial effect. Table 2 illustrates the effect of the 200 mg/mL concentration of the extracts on bacterial isolates obtained from respiratory infections.

Table 1: The chemical composition of the aqueous and alcoholic extracts of *Cinnamomum camphora* leaves.

Active compounds	Hot Ethanol Extract	Hot Water Extract
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Glycoside	+	-
Alkaloids	+	+
Aminoacid	+	-
Terpenoid	+	-

(+) = The substance is present in the extract (-) = Substance not present in the extract

Table 2: Shows the concentration effect (200) mg/ml of extracts on isolated bacteria.

Bacterial Species	Diameter of inhibition area (milleter) for extracts	
	Hot Alcohol Extract	Hot Water Extract
Streptococcus pyogens	**14**	**12**
Streptococcus pneumoniae	**20**	**18**
Klebsiella pneumoniae	**14**	**10**

The Table illustrates the inhibition zone diameter, indicating that the highest antimicrobial activity was observed with the hot alcoholic extract, followed by the hot aqueous extract, which exhibited a lower effect.

Table 3: Effect of alcohol and water extracts against bacteria.

Bacteria	Alcohol extract	Water extract
Streptococcus pneumonia	60%	40%
Streptococcus peyogenes	60%	40%
Klebsiella pneumonia	40%	20%

From the Tables 1, 2, 3, a clear variation in bacterial growth inhibition was observed based on the concentration used. The maximum inhibitory effect was recorded at a 200 mg/mL concentration of the extract. Regarding the aqueous extract, the inhibition zone diameter at 100 mg/mL for S*treptococcus pneumoniae* and *Streptococcus pyogenes* was 25.33 mm and 24.60 mm, respectively, representing the highest inhibition levels.

Conversely, *Klebsiella pneumoniae* exhibited the lowest inhibition rate, with an inhibition zone of 5 mm at both 100 and 200 mg/mL concentrations of the hot aqueous extract. For *Streptococcus pyogenes*, the inhibition zone reached 8 mm. The increased antimicrobial

activity of the hot aqueous and alcoholic extracts of *Cinnamomum camphora* leaves may be attributed to the presence of active compounds listed in Table 1 and potentially additional bioactive components since these are crude extracts . Moreover, the alcoholic extracts' ability to penetrate bacterial cell membranes could be due to the affinity of bacterial membranes for the lipids present in the extract [26].

Additionally, ethyl alcohol has a high capacity to extract the active compounds from plant samples due to its strong polarity [27].

The findings also revealed that Gram-negative bacteria were more affected than Gram-positive bacteria. This difference is due to Gram-negative bacteria having an outer membrane composed of lipoproteins and phospholipids, whereas Gram-positive bacteria have a lower lipid content and a higher percentage of peptidoglycan in their cell walls [28].

Minimum Inhibitory Concentration (MIC) Determination for the Plant Extracts Nutritional scientists have also found that flavonoids, which are compounds soluble in water and organic solvents, are present in the roots of *Cinnamomum camphora* and exhibit antiviral activity [18]. Additionally, the plant contains pectin, which is used as a potent antioxidant against free radicals [29]. The current study included 133 bacterial isolates identified from respiratory infections at the Chest Diseases Center of Al-Hakim General Hospital in Najaf during the period from December to April 2014, involving both genders. Among the collected samples, 92 were positive (69.17%), while 41 samples (30.83%) tested negative.

The identified bacterial isolates included ***Streptococcus pyogenes*** (17.3%), ***Streptococcus pneumonia*** (46.7%), and ***Klebsiella pneumonia*** (2.17%) of the total collected samples. The lower isolation rate of certain bacterial strains may be attributed to prior antibiotic use by patients before undergoing testing [30].

Table 4: The inhibitory activity of the alcoholic extract of eucalyptus leaves.

Bacteria	***Klebsiella pneumonia***	***Streptococcus pyogenes***	***Streptococcus pneumonia***
St.	10±0.29	29.77±0.99	31.33±1.15
0.8	8±0.61	27.11±0.81	18.33±0.69
0.6	6.44±0.21	25.66±1.01	18±0.52
0.4	6±0.33	23±0.95	16.66±0.45

Table 5: The inhibitory activity of the aqueous extract of camphor leaves.

Bacteria	***Klebsiella pneumonia***	***Streptococcus pyogenes***	***Streptococcus pneumonia***
St.	8±0.25	25.33±0.61	24.00±1.11
0.8	-±0.0	23±0.63	23.33±0.88
0.6	-±0.0	22.66±0.85	17±0.87
0.4	-±0.0	21±0.51	15.66±0.09

Aqueous extract (ml), *(-) = no inhibition, 0.01 $<$ P = standard error value St. = stock mg/ml

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