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Research Article

Antimicrobial Activity of *Cinnamomum Camphora* against Pathogens Isolated from Burn Patient

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Abstract: *Cinnamomum camphora*, a medicinal herb belonging to the family Lauraceae, is known for its potential therapeutic applications. This study aimed to evaluate the antibacterial activity of *C. camphora* against pathogens isolated from burn patients. The prepared extracts were tested using the agar well diffusion method, employing solvents such as acetone, chloroform, distilled water, ethanol, hexane, and methanol. The antibacterial activity was assessed against *Staphylococcus aureus* and *Escherichia coli*, with zones of inhibition measured in millimeters. The ethanolic extract exhibited significant antibacterial activity, with inhibition zones of 14 mm for *E. coli* and 15 mm for *S. aureus*. Similarly, acetonic, ethanolic, methanolic, and aqueous extracts demonstrated larger zones of inhibition, indicating their potential to resist microbial growth. In contrast, chloroform and hexane extracts did not exhibit antibacterial activity against either *E. coli* or *S. aureus*.

Keywords: *Cinnamomum camphora* (kafor), Burn Patient, Pathogens

Introduction

In recent decades, the search for natural products with antimicrobial properties has intensified to counteract the adverse effects and microbial resistance associated with synthetic antibiotics. Synthetic antimicrobials often lead to microbial resistance and can pose significant health risks. In contrast, aromatic plants have long been used in phytotherapy and food preservation due to their ability to synthesize active secondary metabolites with therapeutic potential (Sahraei et al., 2014). Consequently, the interest in herbal medicine has grown rapidly, fueled by concerns over the toxicity and side effects of allopathic treatments. This has led to an increased focus on medicinal plants as a foundation for the development of new, safer drugs (Li et al., 2020). These natural sources provide an extensive range of structurally diverse and biologically active compounds (Agarwal, 2005).

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Cinnamomum camphora, belonging to the family Lauraceae, is a medicinal plant widely recognized for its chemical constituents and aromatic properties. It is a significant source of safrole, used in manufacturing heliotropin, a flavor and fragrance compound (Chelliah, 2008). Previous studies have demonstrated that oils derived from camphor bark, leaves, and roots vary significantly in chemical composition, with cinnamaldehyde, eugenol, and camphor being the primary constituents, respectively. These distinct properties have garnered interest in the biosynthetic pathways of *C. camphora* and its potential applications in the flavor and pharmaceutical industries (Paranagama et al., 2001).

Burn wounds are complex injuries caused by thermal, chemical, electrical, or radiation-induced energy, leading to disruptions in the skin's anatomical stability and functional integrity. Such wounds range from minor superficial burns to severe injuries involving extensive tissue damage. The healing of burn wounds is critical for restoring the skin's normal structure and function. Without appropriate care, these injuries may result in severe complications, including infections caused by opportunistic pathogens (Ayyanar and Ignacimuthu, 2009).

Despite the widespread use of medicinal drugs, there is often limited understanding of their active ingredients and compositions. This underscores the need to explore the full therapeutic potential of medicinal plants like *C. camphora*, which are extensively utilized but remain under- researched. By isolating their active compounds, it is possible to formulate more effective remedies to improve healthcare outcomes (Xu et al., 2022).

This study aimed to investigate the antimicrobial activity of *Cinnamomum camphora* extracts against bacterial pathogens isolated from burn patients, highlighting its potential as a natural therapeutic agent in wound care.

Materials And Methods

Collection of Microbial Samples

Microbial flora was collected from burn patients using sterilized cotton swabs. Samples were taken from seven hospitalized patients at Mayo Hospital, Lahore, who had been admitted for second-degree burns caused by fire for a period of three weeks or more over seven months.

Preparation of Culture Media

Nutrient agar (7.7 g in 250 mL distilled water) and MacConkey agar (13.7 g in 250 mL distilled water) were prepared according to manufacturer instructions. Additionally, nutrient broth was prepared by dissolving 0.65 g in 50 mL distilled water (Wang et al., 2021). All media were sterilized and stored for use in bacterial culture experiments.

Isolation and Cultivation of Bacteria

Pathogenic and non-pathogenic bacterial cultures were isolated from the collected samples. Each culture was streaked onto sterile nutrient agar and MacConkey agar plates, labeled accordingly, and

incubated at 37°C for 24 hours. Following incubation, the plates were stored in a refrigerator until further use.

Collection and Extraction of *Cinnamomum camphora* Leaves

Leaves of *Cinnamomum camphora* were collected from Bagh-e-Jinnah, Lahore, and identified by a certified plant anatomist. The leaves were shed-dried, ground into a fine powder using a mortar and pestle, and soaked in various solvents—acetone, chloroform, ethanol, hexane, methanol, and water—in a 1:2 ratio for 1–2 days. The resulting extracts were filtered, and the filtrates were concentrated using a rotary evaporator. The concentrated extracts were dissolved in distilled water and stored for further analysis.

Antibacterial Assay

Fresh bacterial cultures were prepared by inoculating a loopful of stock culture into 5 mL sterile nutrient broth and incubating it at 37°C for 24 hours. The antibacterial activity of *C. camphora* leaf extracts were assessed using the agar well diffusion method.

Sterile nutrient agar was poured into petri dishes and incubated at 37°C for 24 hours. The plates were labeled with the names of the respective bacterial cultures. Fresh cultures were streaked onto the nutrient agar plates using sterile cotton swabs. The plates were dried at 37°C for 30 minutes with lids partially opened. Wells were cut in the agar using a sterile cork borer, and each well was filled with the respective *C. camphora* leaf extract. The plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters. All experiments were performed in triplicate, with each test run in duplicate to ensure reproducibility. The data were statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test (SPSS software). Differences between treatments were considered statistically significant at $P \leq 0.05$.

Results and Discussion

This study aimed to evaluate the antimicrobial activity of *Cinnamomum camphora* against pathogens isolated from burn patients and assess the effects of different solvent extracts on various bacterial strains. The agar well diffusion method was employed to measure t antimicrobial activity of camphor leaf extracts. The results, summarized in Tables 1 and 2, present the inhibition zones (measured in millimeters) of extracts prepared using distilled water, methanol, ethanol, acetone, chloroform, and hexane. A standard metric ruler was used for these measurements.

The antimicrobial activity of *C. camphora* extracts was evaluated against two bacterial strains: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Significant variations were observed in the inhibitory effects depending on the solvent used for extraction. Ethanol, methanol, and acetone extracts exhibited the highest zones of inhibition against *E. coli* at 14±0.4 mm, 9±0.4 mm, and 11±0.4 mm, respectively. In contrast, hexane and chloroform extracts, as well as their controls, showed no inhibition (0.00±0.0 mm). These findings underscore the potential of polar

solvents in extracting bioactive antimicrobial compounds from *C. camphora*.

Table 1: Antibacterial activity of camphor leaf extracts against *Escherichia coli*

	Zone of inhibition in mm Mean ± S. D					
Concentrate	**Ethanol**	**Methanol**	**Acetone**	**Water**	**Chloroform**	**Hexane**
Control	6 ± 0.4^{d}	4 ± 0.4^{c}	2 ± 0.4^{b}	1 ± 0.4^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Extract	14 ± 0.4^{f}	9 ± 0.4^{e}	12 ± 0.4^{f}	7 ± 0.4^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

All values presented are the means of three replicate measurements. The symbol ± represents the standard error of the mean (± SEM) across these replicates. Statistical analysis revealed that the mean differences were significant at $p \leq 0.05$, determined using One-Way ANOVA. Duncan's New Multiple Range Test was used to group means with different letters denoting significant differences.

The results for the zone of inhibition (shown in the table) indicated that the control groups of ethanol, methanol, acetone, and water extracts exhibited lower inhibition zones of 8±0.5 mm, 3±0.5 mm, 5±0.5 mm, and 2±0.5 mm, respectively, against *S. aureus*. These control groups demonstrated significantly smaller inhibition zones compared to the extracts. For the control treatments, ethanol, methanol, acetone, and water showed inhibition zones of 6±0.4 mm, 4±0.4 mm, 2±0.4 mm, and 1±0.4 mm, respectively. Both hexane and chloroform extracts, along with their controls, showed no inhibition against *S. aureus* (0.00±0.0 mm).

Table 2: Antibacterial activity of camphor leaf extracts against *Staphylococcus aureus*

	Zone of inhibition in mm Mean± S. D					
Concentrates	**Ethanol**	**Methanol**	**Acetone**	**Water**	**Chloroform**	**Hexane**
Control	8.1 ± 0.5^{d}	3 ± 0.5^{b}	5 ± 0.5^{c}	2 ± 0.5^{b}	0.0 ± 0.5^{a}	0.0 ± 0.0^{a}
Extract	18 ± 0.5^{f}	10 ± 0.5^{e}	11 ± 0.5^{f}	9 ± 0.5^{d}	0.0 ± 0.5^{a}	0.0 ± 0.0^{a}

All values represent the means of three replicate measurements. The ± symbol indicates the standard error of the mean (± SEM) across these replicates. Statistical analysis confirmed that the differences in means were significant at $p \leq 0.05$, as determined by One-Way ANOVA. Duncan's New Multiple Range Test was used to assign different letters to means showing significant differences.

Investigating the antimicrobial properties of plants offers promising alternatives to conventional antibiotics, particularly for pathogenic burn-associated organisms where antibiotics are often ineffective. Such alternative antimicrobial agents are essential for managing these challenging infections (Sun et al., 2023).

Our findings revealed that *Staphylococcus aureus* was the most prevalent isolate, consistent with previous research conducted at Mayo Hospital, Lahore. This prevalence may be attributed to *S. aureus* being a common component of the skin's normal flora, which can become opportunistic under certain conditions and cause severe infections (Gill et al., 2005).

The antimicrobial activity of *Cinnamomum camphora* ethanolic extract has been extensively documented, with studies consistently demonstrating superior inhibition zones compared to other solvent extracts. For instance, Waty et al. (2018) observed that ethanolic extracts of *C. camphora* at concentrations of 6.25%, 12.5%, and 25% produced inhibition zones of 6.78 mm, 9 mm, and 11.68 mm, respectively, against *Streptococcus aureus*. In the present study, the ethanolic extract of camphor leaves exhibited significantly larger inhibition zones of 14±0.04 mm and 18±0.04 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively, confirming its potent antimicrobial activity.

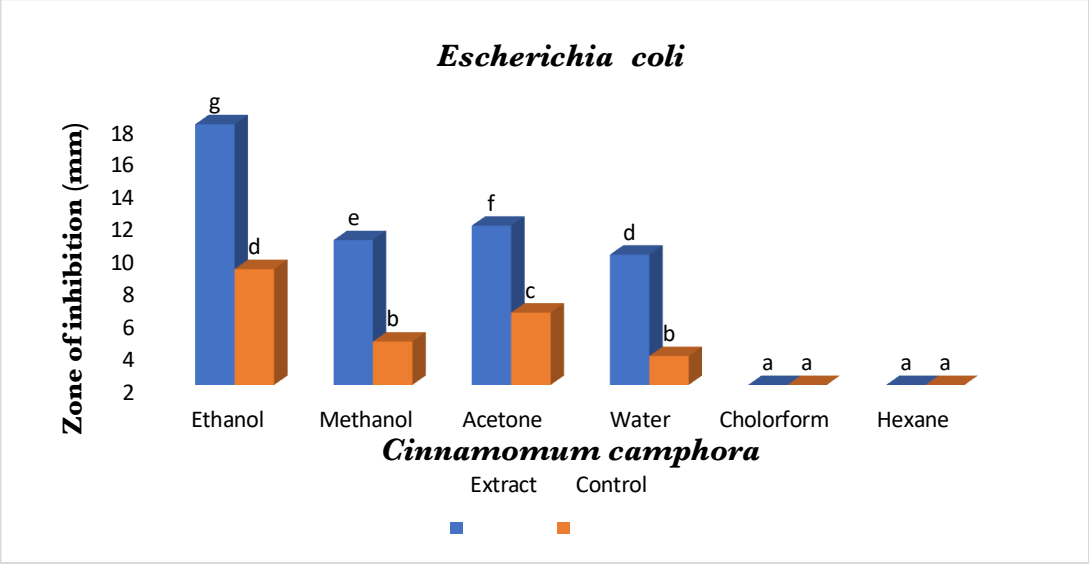

Figure 1: Antibacterial activity of camphor leaf extracts against *E. coli.* Values with different letters at the top of the bar are determined by Duncan's new multiple-range test

The results of this investigation demonstrated significant differences in the bacterial inhibition zones produced by various solvent extracts. Among these, acetonic, distilled water, ethanolic, and methanolic extracts of *Cinnamomum camphora* exhibited substantial antimicrobial activity against the sensitive pathogens isolated from burn patients. In contrast, hexane and chloroform extracts showed no inhibitory effect on the tested pathogens. A comparative analysis of the antimicrobial efficacy of different solvent extracts is presented in Figures 1 and 2. The ethanolic extract displayed

remarkably superior results compared to hexane and chloroform extracts. While water, methanol, and acetone extract also inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*, their zones of inhibition were smaller than those observed with the ethanolic extract. Notably, the acetonic extract exhibited a significant inhibition zone against *Staphylococcus aureus*, aligning with findings by Laal and Maeyer (2000), who reported the potent antimicrobial effects of acetone against *S. aureus*. Additionally, Gram-positive bacteria appeared to be more susceptible to the inhibitory action of the acetonic extract compared to Gram-negative bacteria, highlighting a potential specificity in the extract's antimicrobial activity.

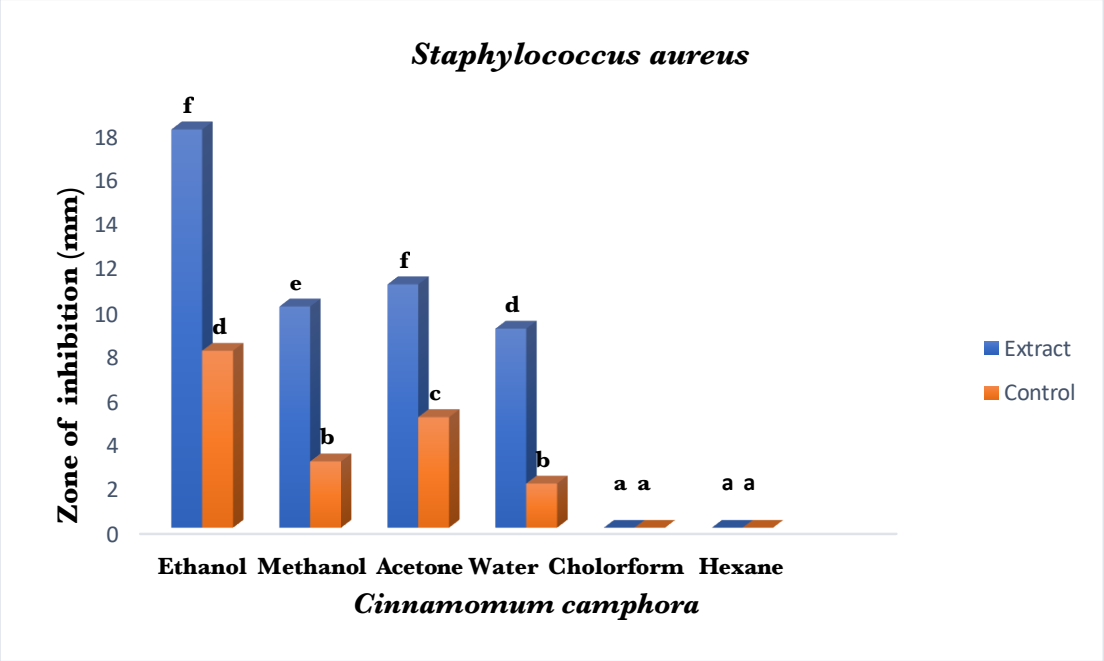

Figure 2: Antibacterial activity of camphor leaf extracts against *Staphylococcus aureus*. Values with different letters at the top of the bar are determined by Duncan's new multiple range

Identifying the specific antimicrobial compounds present in the camphor leaf extract would be a significant advancement. Once the primary active compound is isolated, it could undergo further testing in vitro using mammalian cell lines and in vivo experiments with animal models to evaluate its efficacy and safety.

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Conflict of Interest: No potential conflict of interest is declared by any author.

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