

The Assessment of Cinnamon (*Cinnamomum* spp.) Aqueous and Ethanolic Extracts' Antibacterial Properties against Antibiotic-Resistant *Staphylococcus* Species

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ABSTRACT

The increasing prevalence of antibiotic-resistant bacteria has encouraged research into natural antimicrobial agents derived from medicinal plants. This study evaluated the antibacterial activity of ethanolic and aqueous extracts of cinnamon (*Cinnamomum* spp.) against three *Staphylococcus* species: *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. The antibacterial activity was assessed using the disc diffusion method at concentrations of 50, 100, 200, and 400 mg/mL. The sensitivity of the bacterial isolates to several commonly used antibiotics was also investigated. The results showed that the ethanolic extract had a stronger antibacterial effect than the aqueous extract, with activity increasing as the concentration increased. At 400 mg/mL, the ethanolic extract produced inhibition zones of 12.00 ± 2.65 mm against *S. saprophyticus*, 12.67 ± 2.08 mm against *S. epidermidis*, and 12.33 ± 1.53 mm against *S. aureus*. In comparison, the aqueous extract showed lower activity, producing inhibition zones of 6.67 ± 0.58 mm against *S. saprophyticus* and 7.33 ± 0.58 mm against *S. epidermidis*. The ethanolic extract showed no inhibitory activity against *S. saprophyticus* at 50 mg/mL, while only limited activity was observed against *S. epidermidis* and *S. aureus*. The aqueous extract showed no inhibitory effect against any of the tested *Staphylococcus* strains at 50 and 100 mg/mL. The greater effectiveness of the ethanolic extract may be attributed to ethanol's ability to extract lipophilic and phenolic compounds, particularly cinnamaldehyde and eugenol. Overall, cinnamon extract may serve as a potential natural antibacterial agent against antibiotic-resistant *Staphylococcus* species.

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INTRODUCTION

Antimicrobial resistance constitutes one of the major challenges of modern public health care. Misuse and excessive use of antimicrobial agents have resulted in the appearance and spread of resistant strains of bacteria, limiting the efficiency of existing treatment regimens and contributing to the rising threat from infectious diseases (WHO, 2023; Vasconcelos *et al.*, 2018). Such a situation has led to an increased interest among researchers to search for novel antimicrobials and sources thereof (Doyle and Stephens, 2019). Cinnamon is a common spice and medicinal herb that has long been used in traditional medicine. It is derived from a variety of plants in the genus *Cinnamomum* of the family *Lauraceae* (Nabavi *et al.*, 2015). The plant's many parts, particularly its bark, have been used for medicinal and culinary purposes. Numerous biological properties, including antibacterial, antioxidant, and anti-inflammatory properties, have been associated with cinnamon (Rao and Gan, 2014). The biological effects of cinnamon can be attributed to its phytochemical makeup. One of the active ingredients in cinnamon bark is cinnamaldehyde, which is said to play a major role in its antimicrobial effects. Some other ingredients that might contribute to the biological activity of cinnamon include eugenol, cinnamic acid, cinnamyl acetate, and phenolic substances. The proportion of such ingredients may differ with regard to different *Cinnamomum* species, geographic location, plant source, and extraction technique (Burt, 2004).

There have been various explanations for the antimicrobial effects of cinnamon. The bioactive components may react

with the bacterial membrane, change its permeability, disrupt necessary biochemical functions, and hinder bacterial proliferation (Ooi *et al.*, 2006; Shan *et al.*, 2007). However, the success of extraction of the bioactive substances depends largely on the type of solvent used, which in this case could be better in ethanol than water (Otto, 2009). *Staph. aureus* is one of the clinically significant bacteria that causes infection not only in the community but also in health-care settings. The situation is becoming more complicated by the occurrence of methicillin-resistant *Staph. aureus* (MRSA), which shows resistance not only to β -lactams but also to other antimicrobial agents (Tong *et al.*, 2010). Another group of clinically significant bacteria is coagulase-negative staphylococci (CoNS), including *Staph. epidermidis* and *Staph. saprophyticus*. The former bacterium often causes health-care associated and device-related infections, while the latter is an etiologic agent of urinary tract infections (Shu *et al.*, 2024). Due to growing antibiotic resistance among different *Staphylococcus* bacteria, there is an ongoing need to research other antibacterial drugs. One possible source of such substances can be plant extracts (Nematollahi *et al.*, 2020). However, the choice of solvent, extract concentration, and species of bacteria all have a significant impact on the biological activity of such extracts.

In light of this, the current study sought to ascertain the antibacterial effects on *Staph. aureus*, *Staph. epidermidis*, and *Staph. saprophyticus* using aqueous and ethanolic extracts of cinnamon (*Cinnamomum* spp.) at different doses. In order to make comparisons, the study also attempted to ascertain the bacteria's patterns of antibiotic susceptibility.

MATERIALS AND METHODS

Plant Material

Cinnamon bark (*Cinnamomum* spp.) was collected from local markets at Samarra City, Salah Al-Din Governorate, Iraq. This was subjected to cleaning, drying if necessary, and grinding using an electrical grinder to obtain a fine powder, which was then kept in a clean dry airtight box before extraction.

Bacterial Isolates

The bacterial strains employed for the investigation include *Staph. aureus*, *Staph. epidermidis*, and *Staph. saprophyticus*.

The strains were collected from the Department of Biotechnology, College of Science, University of Samarra. The identification was done using standard techniques of culture and biochemistry (Mohammad *et al.*, 2022).

Antibiotics

Antibiotic discs were utilized for the determination of antimicrobial susceptibility patterns of the bacteria. The antibiotic discs were supplied by Bioanalyse Ltd. Turkey. The antibiotics along with their corresponding disc contents have been listed in Table 1.

Table 1: Antibiotic Discs used in the Study

Antibiotic	Disc content (µg/disc)
Methicillin	5
Cefotaxime	30
Clindamycin	2
Vancomycin	30
Ampicillin	10
Tetracycline	30
Ciprofloxacin	5

Preparation of Cinnamon Extracts

Aqueous Extract

This aqueous extract was obtained using a process called maceration which is normally used in the extraction of medicines from plants (Dai *et al.*, 2024). Essentially, this process involves combining 40 g of pulverized cinnamon bark in 160 mL of distilled water and stirring the mixture for 60 minutes using a magnetic stirrer. After the mixture has been homogenized and allowed to stand at 4 °C for 24 hours to improve extraction, it was later filtered through multiple layers of sterile gauze before being centrifuged to eliminate suspended matter.

Ethanollic Extract

A traditional solvent extraction approach was used to create the ethanollic extract (Jaramillo Jimenez *et al.*, 2024). In short, 250 mL of 90% ethanol and 50 g of powdered cinnamon bark were combined. After allowing the mixture to extract under the proper circumstances, the insoluble material was filtered out and centrifuged. A rotary evaporator operating at lower pressure was used to extract the ethanol. Before being used again, the resulting crude extract was gathered and kept in a tightly sealed container.

Preparation of Extract Concentrations

Stock solutions for both aqueous and ethanollic extracts of cinnamon were made and then diluted in order to have their final concentration at 50, 100, 200, and 400 mg/mL. While aqueous extract of cinnamon was made in sterile distilled water, the ethanollic crude extract of cinnamon was made in an appropriate solvent that can dissolve it completely. The solvent itself must be used without adding extract to make a negative control for the possible effect of the solvent.

Preparation of Bacterial Inoculant

New cultures of bacteria were suspended in sterile saline or broth and made into inoculums of a standard turbidity before performing the antimicrobial test. In the disc diffusion susceptibility test using the conventional technique, the suspension of bacteria needs to have a turbidity that represents the 0.5 McFarland standard, equaling roughly 1.5×10^8 CFU/mL (Li *et al.*, 2024).

Antibacterial Activity of Cinnamon Extracts

The agar well diffusion method was used to assess the antibacterial activity of cinnamon aqueous and ethanollic extracts. Mueller-Hinton agar plates were inoculated with standardized bacterial solutions using sterilized cotton swabs. Different amounts of cinnamon extracts (50, 100, 200, and 400 mg/mL) were added to sterile filter paper disks (Whatman No. 1; 6 mm in diameter). The plates were incubated for twenty-four hours at 37 °C. The size of the zone of inhibition surrounding each disk was measured in millimeters to determine the antibacterial action following the incubation period. Every treatment was done in triplicate, and the results were displayed as means $\pm$ SD (Musa *et al.*, 2019).

Antibiotic Susceptibility Testing

The Kirby-Bauer disk diffusion method on Mueller-Hinton agar was used to determine the bacterial isolates' susceptibility to the selected antibiotics using standardized antimicrobial susceptibility test recommendations (Li *et al.*, 2024). The antibiotic disks were aseptically positioned on the agar plate after it had been inoculated with a standardized bacterial suspension. The diameter of the zone of inhibition was measured in millimeters after the plate was incubated at $35 \pm 2^\circ\text{C}$ for the necessary amount of time.

Statistical Analysis

The Kirby-Bauer disk diffusion method on Mueller-Hinton agar was used to determine the bacterial isolates' susceptibility to the selected antibiotics using standardized antimicrobial susceptibility test recommendations (Li *et al.*, 2024).

RESULTS AND DISCUSSION

Antibiotic Susceptibility of the Bacterial Isolates

Antibiotic susceptibility patterns for *Staph. saprophyticus*, *Staph. epidermidis*, and *Staph. aureus* to tested drugs are provided in Table 2 and Figure 1. The diameter of the inhibition zones was not uniform for different bacteria and drugs. Tetracycline was rather active against all tested bacteria, forming inhibition zones at a distance of 18.00 ± 1.00 mm, 21.67 ± 0.58 mm, and 19.00 ± 1.00 mm from the test drug, respectively, for *Staph. saprophyticus*, *Staph. epidermidis*, and *Staph. aureus*.

Table 2: Antibacterial Activity of Antibiotics against *Staphylococcus* Isolates

Antibiotic	<i>Staph. saprophyticus</i>	<i>Staph. epidermidis</i>	<i>Staph. aureus</i>
Vancomycin	16.33 ± 0.58 ^b	17.67 ± 0.58 ^b	21.00 ± 1.00 ^a
Ampicillin	6.33 ± 0.58 ^c	7.33 ± 0.58 ^c	7.33 ± 0.58 ^c
Tetracycline	18.00 ± 1.00 ^a	21.67 ± 0.58 ^a	19.00 ± 1.00 ^a
Ciprofloxacin	16.00 ± 0.00 ^b	20.33 ± 0.58 ^a	18.33 ± 0.58 ^a
Methicillin	6.00 ± 0.00 ^c	11.33 ± 0.58 ^b	8.67 ± 0.58 ^b
Cefotaxime	6.33 ± 0.58 ^c	6.67 ± 0.58 ^c	7.33 ± 0.58 ^c
Clindamycin	14.67 ± 0.58 ^b	18.67 ± 0.58 ^a	17.33 ± 0.58 ^a

The values are shown as mean ± SD. Significant differences ($P < 0.05$) are shown by different superscript characters.

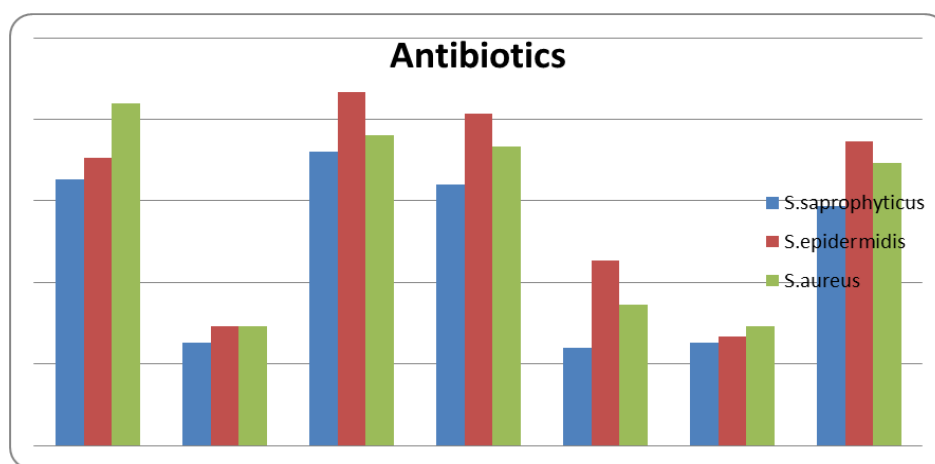


Figure 1: Antibacterial Activity of Standard Antibiotics against the Tested *Staphylococcus* Isolates

Variability was observed amongst the antibiotic susceptibilities. Vancomycin, tetracycline, ciprofloxacin, and clindamycin displayed the strongest antibacterial properties, while ampicillin and cefotaxime had weak antibacterial properties. Methicillin too showed poor activity against *Staph. aureus*, which proved the methicillin-resistant nature of MRSA.

Present observations correlate well with the findings presented by Tong *et al.* (2015), where MRSA has been described to be among the important multidrug-resistant pathogens, owing to the presence of resistance against β -lactam antibiotics. Likewise, CLSI guidelines (CLSI, 2023)

state that susceptibility testing needs to be performed for identifying drug-resistant strains of *Staphylococcus*.

Antibacterial Activity of the Aqueous Cinnamon Extract

Antibacterial activities of the cinnamon aqueous extract are shown in Table 3 and Figure 2. Antibacterial activities of the cinnamon aqueous extract against the test *Staphylococcus* bacteria were weak. Inhibition zones measured were 6.67 ± 0.58 mm, 7.33 ± 0.58 mm, and 6.33 ± 0.58 mm for *Staph. saprophyticus*, *Staph. epidermidis*, and *Staph. aureus* at 400 mg/mL concentration, respectively. Minimal inhibition was recorded at 200 mg/mL, while no inhibitory action was seen at 50 and 100 mg/mL concentrations.

Table 3: Antibacterial Activity of the Aqueous Cinnamon Extract against *Staphylococcus* Isolates

Concentration (mg/mL)	<i>Staph. saprophyticus</i>	<i>Staph. epidermidis</i>	<i>Staph. aureus</i>
400	7.67 ± 0.58 ^a	8.33 ± 0.58 ^a	7.33 ± 0.58 ^a
200	7.00 ± 0.00 ^a	7.00 ± 0.00 ^a	7.67 ± 0.58 ^a
100	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
50	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b

The values are shown as mean ± SD. Significant differences ($P < 0.05$) are shown by different superscript characters

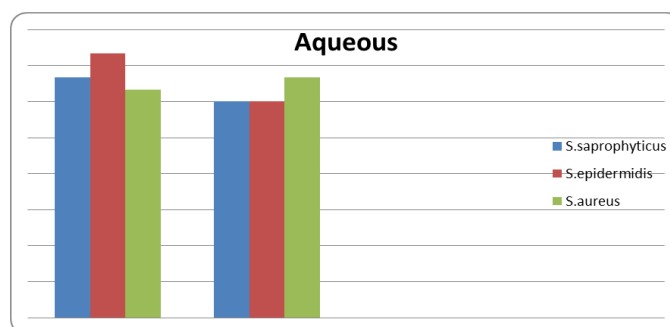


Figure 2: shows the Aqueous Cinnamon Extract's Concentration Dependent Antibacterial Activity against the *Staphylococcus* Isolates that were Tested

This may be due to the fact that the hydrophobic antimicrobial compounds, including cinnamaldehyde and eugenol, have low solubility in water.

This research is supported by the results of Rao and Gan (2014), Burt (2004), and Doyle and Stephens (2019), who found that aqueous extraction usually produces lesser quantities of antimicrobial chemicals compared to organic solvent. Shu *et al.* (2024) also observed that the quantity of cinnamaldehyde was much less in aqueous extracts.

Antibacterial Activity of the Ethanolic Cinnamon Extract

Antibacterial activity was higher for ethanolic cinnamon extract than for aqueous extract on all tested bacterial isolates (Table 4 and Figure 3). The level of antibacterial activity increased with increasing extract concentration.

The ethanolic extract reduced the growth of *Staph. saprophyticus* by 12.00 ± 2.65 mm and *Staph. epidermidis* by 12.67 ± 2.08 mm at a concentration of 400 mg/mL, and *Staph. aureus* by 12.33 ± 1.53 mm. When tested at a concentration of 200 mg/mL, the inhibitions were 10.33 ± 1.53 , 10.33 ± 2.08 , and 10.00 ± 2.00 mm, respectively. Further decrease in activity was seen at 100 mg/mL. The lowest concentration of 50 mg/mL showed no inhibition against *Staph. Saprophyticus*.

At all doses, the ethanolic extract had considerably more antibacterial activity than the aqueous extract ($P < 0.05$), indicating that ethanol is a superior solvent for the extraction of the antimicrobial chemicals from the cinnamon.

Table 4: Antibacterial Activity of the Ethanolic Cinnamon Extract against Staphylococcus Isolates

Concentration (mg/mL)	<i>Staph. saprophyticus</i>	<i>Staph. epidermidis</i>	<i>Staph. aureus</i>
400	13.00 ± 2.65^a	13.67 ± 2.08^a	13.33 ± 1.53^a
200	11.33 ± 1.53^b	11.33 ± 2.08^b	11.00 ± 2.00^b
100	7.67 ± 2.08^c	8.00 ± 0.00^c	7.67 ± 0.58^c
50	0.00 ± 0.00^d	3.33 ± 0.58^d	3.67 ± 1.53^d

The values are shown as mean $\pm$ SD. Significant differences ($P < 0.05$) are shown by different superscript characters

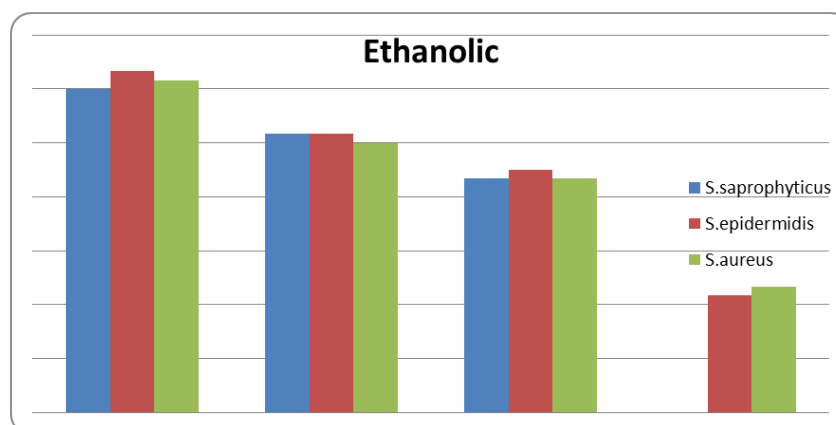


Figure 3: Antibacterial Effect of Ethanolic Cinnamon Extract Depending on its Concentration against Tested *Staphylococcus* isolates

The current results agree with those of Vasconcelos *et al.* (2018), Doyle and Stephens (2019), Sangavi and Gopinath (2019) and Nabavi *et al.* (2015), who indicated that the ethanolic cinnamon extracts have greater antimicrobial efficacy in contrast to aqueous extracts due to effective extraction of lipophilic bioactive substances, especially trans-cinnamaldehyde, eugenol, cinnamic acid, and phenols.

The high antibacterial properties of ethanolic extract could be explained by the presence of cinnamaldehyde at high concentrations in the cinnamon extract. Cinnamaldehyde is the main component that acts on bacterial cell walls by increasing their permeability, leaking of the intracellular materials, inhibition of ATP production, interference with quorum sensing, and preventing biofilm formation.

The results align with those found by Shu *et al.* (2024) and Jaramillo Jimenez *et al.* (2024), who showed that cinnamaldehyde is the compound behind the strong antibacterial effects exhibited by cinnamon due to various means.

Comparison between Aqueous and Ethanolic Cinnamon Extracts

Comparing the results of the two types of extractions, it was found that the ethanolic extract always formed significantly bigger inhibition zones than the aqueous extract at equivalent

concentrations. Thus, we can conclude that solvent is a very important factor which determines the antibacterial activity of cinnamon.

The low solubility of hydrophobic substances like eugenol and cinnamaldehyde in water may be the cause of the aqueous extract's low activity, but these compounds can easily dissolve in ethanol leading to better extraction efficiency and better antibacterial activity.

The current results agree with Burt (2004), Rao and Gan (2024), and Doyle and Stephens (2019) who have found that organic solvents, especially ethanol, give higher amounts of phenolic components and essential oils which determine the antibacterial effect of cinnamon, that extracts containing cinnamaldehyde had a superior ability to inhibit the growth of Gram-positive bacteria compared to aqueous extracts owing to their phenolic content.

Comparison with Conventional Antibiotics

Comparison between the ethanolic cinnamon extract and the other antibiotics indicated that while the tested antibiotics showed more inhibition zones, the ethanolic cinnamon extract was capable of inhibiting bacteria resistant to ampicillin, methicillin, and cefotaxime. This is significant since MRSA continues to be among the main organisms responsible for nosocomial infections around the world. Since the cinnamon

extract has the potential to inhibit resistant isolates, it is clear that it works through a different mode compared to other β -lactam antibiotics. These results support the study by Tong *et al.* (2015), where the authors stressed the urgency of developing alternative antimicrobial compounds to overcome the problem of antibiotic resistance of *Staph. aureus*. Similarly, Li *et al.* (2024) showed that the use of cinnamaldehyde increases the sensitivity of MRSA to β -lactam antibiotics due to SarA inhibition. In addition, the antibacterial effect on *Staph. epidermidis* and *Staphylococcus saprophyticus* is highly relevant since coagulase-negative *staphylococci* have recently become more prevalent as causative agents of catheter infection, urinary tract infection, septicemia, and other infections caused by foreign bodies. This work is consistent with Otto (2009), who states that coagulase-negative *Staphylococci* have an extraordinary ability to form biofilms and acquire resistance to multiple antibiotics, making the discovery of natural antibacterials more crucial than ever.

In summary, the results of this study have clearly shown that the antibacterial properties of cinnamon depend on the type of solvent and its concentration. Ethanol extracts showed higher antibacterial activity in comparison to aqueous extracts, confirming the assumption about higher extraction of biologically active phytochemicals by ethanol.

These results correspond with previous research done by Vasconcelos *et al.* (2018), Doyle and Stephens (2019), Nabavi *et al.* (2015), Shu *et al.* (2024), and Jaramillo Jimenez *et al.* (2024), who demonstrated that ethanol extracts of cinnamon rich in cinnamaldehyde possess high antibacterial activity towards *Staphylococcus* species including resistant ones.

Lastly, despite being less effective in terms of inhibition zones in comparison with the most effective antibiotics, the antibacterial effect of ethanolic extracts against resistant bacteria can be considered promising for further use as an alternative antimicrobial product. Further research should include determination of MIC, MBC, studying synergistic effects with antibiotics, identification of active components using chromatography (HPLC, GC-MS), and efficacy and safety in vivo models.

CONCLUSION

According to this investigation, *Staph. aureus*, *Staph. epidermidis*, and *Staph. saprophyticus* were all susceptible to the antibacterial activity of cinnamon (*Cinnamomum* spp.) extracts. According to the findings, the ethanolic extract exhibited more potent antibacterial activity than the aqueous extract, and the antibacterial activity rose as the extract concentration increased. In general, out of all the examined bacterial isolates, the ethanolic extract exhibited the strongest antibacterial activity. The strong antibacterial activity displayed by the ethanolic extract could be due to the higher ability of ethanol in extracting the key bioactive constituents of the cinnamon such as cinnamaldehyde, eugenol, and other phenolic compounds known to have antimicrobial activities. On the other hand, the aqueous extract showed low antibacterial activity especially at low concentrations because water is less efficient in extracting the hydrophobic antimicrobial compounds of cinnamon. The aforementioned results indicate that cinnamon is a potentially valuable natural source of antibacterial agents against *Staph. aureus* strains, including those resistant to conventional antibiotics. However, any direct comparison of raw plant extracts and conventional antibiotics requires cautious interpretation in light of variations in chemical composition, concentrations, diffusive properties, and modes of action of these agents.

Additional research is needed to establish MIC and MBC levels, to isolate and characterize the phytochemicals, and to study their modes of action, potential synergy with conventional antibiotics, and efficacy and safety in vivo.

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