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Cinnamaldehyde Extraction from Cinnamon by Solid Phase Extraction and Evaluate the Antimicrobial Activity

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Abstract: Cinnamaldehyde is an essential component of cinnamon has antibacterial property, and thus it provides a potential application to the global problem of antibiotic resistance. The goal of this paper is to improve the extraction method for cinnamaldehyde from cinnamon and evaluate it antibacterial efficiency against pathogenic bacteria. The cinnamaldehyde extracted from cinnamon powder with 95% ethanol, then the cinnamonaldehyd separated by solid phase extraction (SPE) with a carbon based sorbent (CBS) resin. To obtain better extraction yield, SPE parameters were optimized. The concentration of cinnamonaldehyde was evaluated by high performance liquid chromatography (HPLC). The extraction results indicate that the optimum parameters for SPE are pH 9, 10 min contact time, and using isopropanol as elution solvent. The cinnamaldehyde yield was 0.72%. To evaluate the antimicrobial activity, clinical specimens were collected and bacterial isolates were identified, then susceptibility to antibiotics was tested, and minimum inhibitory concentration (MIC) against bacterial strains was estimated using a microdilution technique. Cinnamaldehyde demonstrated bactericidal activity against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* with a MIC ranging from 128 to 2048 µg/ml.

Keyword: Cinnamaldehyde, *Cinnamomum verum*, Solid phase extraction, antibacterial activity, Antibiotic Sensitivity.

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Introduction

Cinnamaldehyde, a natural ingredient presented in cinnamon, has many applications in food, medicine, and environmental fields. Cinnamon, derived from the outermost layer of Cinnamomum trees, is known by its unique taste and smell. Cinnamaldehyde has antibacterial, antioxidant, and anti-inflammatory property. The Cinnamaldehyde has a capacity to inhibit microorganisms which give a potential to prevent infections caused by microorganisms (1).

Solid phase extraction (SPE) is used to separate natural product compounds. SPE has properties including increased selectivity, less solvent usage, and provide higher purity compound, making it a suitable method for extracting bioactive compounds from natural products (2).

Othman and others studied the optimization of cinnamaldehyde extraction using 95% (v/v) ethanol. The

optimal conditions for extract the cinnamaldehyde were 37°C and 5 h extraction time. The yield was 3.05 mg/g(3). Wardatun *et al.* (4) explained the extraction of cinnamaldehyde from cinnamon using 96% ethanol. The cinnamaldehyde content was 124.14 ± 1.17 mg/g of dry extract and trans-cinnamic acid content was 151.35 ± 1.24 mg/g of dry extract. 96% ethanol and 70% ethanol solvents had a significant effect on cinnamaldehyde and trans-cinnamic acid extraction (4). supercritical fluid extraction (SFE) method was used to extract cinnamaldehyde from cinnamon. The extraction yield was 7.8%. The highest amount of cinnamaldehyde in the SFE extraction was at 70 °C and 160 bar. The extraction of cinnamaldehyde influenced by the pH in hydrodistillation extraction, the optimum pH was 4.1 (5). Satya *et al*. purified the cinnamaldehyde utilizing column chromatography method. By using the column chromatography the cinnamaldehyde yield increased from 44.6 mg/L to 52.0 mg/L at the optimum parameters (6).

Furthermore, the research extends its focus to explore the antibacterial activity of cinnamaldehyde. In light of the growing concern over antibiotic resistance, natural compounds with antimicrobial properties, such as cinnamaldehyde, present promising alternatives for combating bacterial infections.

Cinnamaldehyde used to inhibit the growth of *Salmonella enterica*, *E. coli*, *Staphylococcus aureus*, and *Clostridium botulinum* (7). *trans*-Cinnamaldehyde has been reported as the component with antimicrobial properties. Extracted cinnamaldehyde has been proved to inhibit growth of a variety of microorganisms such as bacteria, molds, and yeasts. It has also been shown to reduce producing of bacterial toxin (8–10).

Cinnamon aldehyde's bioactivities include a remarkable range of antimicrobial properties. It has been demonstrated to be highly effective *against S. aureus, E. coli, Salmonella spp., and Bacillus spp.* (11).

The cinnamaldehyde has ability to interact and destroy the bacterial cell wall making it highly antibacterial compound. In reality, the hydrophilic compound on the surfaces of bacteria quickly attracts with aldehydic group of cinnamaldehyde, which may then pass inside the cell and initiate of inhibition mechanism by rupturing the polysaccharide structure, making pores in cell wall that allowing ions, proteins, and nucleic acids to pass out from a bacterial cell. Furthermore the cinnamaldehyde have ability to inhibition of Adenosine Triphosphate (ATP) generation and biofilm formation, as well as interference with quorum sensing systems (12). Cinnamaldehyde's antibiotic-potentiating properties have been extensively studied in recent years, notably in superbug strains (13). The current study examined the existing literature to investigate cinnamaldehyde as anti-bacterial agent and to highlight the possibility for future interest in this natural molecule as an exciting method for managing resistant bacteria. This investigation aims to evaluate the antibacterial efficacy of cinnamaldehyde against a spectrum of bacterial strains, shedding light on its potential as a therapeutic agent.

Materials and methods

Cinnamon Sample preparation and digestion

The cinnamon acquired locally and grounded to a fine powder. A total of 10 grams of the plant powder underwent extraction with 100 ml 50% ethanol(14,15) through agitation in a dimly light area at room temperature for 48 hours. The solvent was evaporated in a vacuum at 30°C. The extracted cinnamaldehyde was dried via vacuum oven at 30°C for 48 hours then kept in a dark place for further evaluation (16).

Carbon-based sorbent preparation

The CBS material was cleaned by methanol and ethanol solvents (17). The cleaning solvents were tested by UV spectrophotometer to ensure the cleaning efficiency and assure there are no longer any residual pollutant.

Optimization of Parameter solid phase extraction

To enhance the solid phase extraction (SPE) separation, cinnamon aldehyde standard (95% from Merck) has been used. The extraction parameters including pH, contact time, CBS amount, and eluting solvent were investigated. The SPE parameters optimization was tested by extraction 25 mL of standard solution contains 10 PPM. The pH level was systematically adjusted using phosphoric acid and sodium acetate to create an experimental environment with pH values of 3, 5, 7, and 9. By employing these specific pH conditions, it has intended to explore the impact of varying acidity on the extraction of cinnamaldehyde. Additionally, the influence of sorbent mass on extraction performance was examined. The choice of washing solvent and elution solvent was carefully considered, with different solvents tested to identify those that yield the highest selectivity and recovery (18).

Determination of cinnamon aldehyde

HPLC analysis was conducted at the National Health Factory, Baghdad, employing a Shimadzu LC 2030 HPLC system manufactured in Japan. The utilized column was a GL Sciences C18 column with dimensions of 4.6 x 250 mm and a particle size of 5µm. The determination of cinnamaldehyde was achieved using a mobile phase composed of water and MeOH (Methanol) in a 40:60 v/v ratio, with a flow rate of 1.0 mL/min and a column temperature set at 35°C. The injection volume for the samples was 20 µl, and the detection of cinnamaldehyde was performed at 286 nm (19).

Isolation of bacteria from wound and burn infection

In the timeframe spanning from October 2021 to December 2021, a total of 100 samples were collected from several Baghdad hospitals, namely Baghdad Teaching Hospital, AL-Shahid Ghazi Al-Hariri for specialized surgery, Al-Shahid Dhar Al-Faiad hospital, and Burns Specialized Hospital. The sample collection adhered to the protocol outlined (20). All samples were transported to the laboratory without delay. The samples were inoculated on Mannitol salt agar, blood agar and MacConkey agar for overnight at 37 ºC under aerobic conditions.

Identification of bacterial isolates

The identification of bacterial isolates involved a comprehensive approach. Microscopic, Macroscopic examination and Biochemical tests. Then confirm the type of isolate by VITEK 2 Compact system (21).

Antibiotic Sensitivity Test of Wound and Burn Infection

The antibiotic sensitivity test for wound and burn infections was conducted.

Determination of minimum inhibitory concentration (MIC) of extracted cinnamaldehyde

A cinnamaldehyde stock solution was meticulously prepared by dissolving 0.04 g of cinnamaldehyde in 10 ml of dimethyl sulfoxide (DMSO), yielding a concentration of 4 mg/ml Overnight cultures of *S. aureus, P. aeruginosa, E. coli, A. baumannii* and *K. pneumoniae* strains were diluted (1:100) using Mueller Hinton broth (MHb) then cultivated to achieve an optical density of 0.1 at 600 nm. Then 10 µL of the suspension was added to well which each well included various concentrations of cinnamaldehyde (2048 µg/mL to 64 µg/mL). The MIC was determined after 24 hours of incubation at 37 °C using resazurin 0.03%. Aliquots of the wells without turbidity were cultivated on nutrient agar plates to measure the minimum bactericidal concentration (MBC). All tests were carried out in three replicate(22).

Statistical analysis

The equation of the calibration curve for the standard cinnamaldehyde were measured using Origin lab software. Statistical analysis was performed using Excel from Microsoft office. All values are presented as the mean ± SD of at least three independent experiments.

Result and discussion

Optimizing the solid phase extraction parameters

pH number values

The result showed the extraction of cinnamaldehyde is highly influenced by the pH media. At lower pH values, where the surrounding is more acidic, the separation of cinnamaldehyde detected to be less efficient, due to the protonation of cinnamaldehyde molecules, as a result, it prefers the aqueous phase. However, as the pH raises to 9, a significant enhancement in cinnamaldehyde extraction is noted, and the extraction is 99%. This improvement referred to the reduction of cinnamaldehyde polarity at higher pH levels, leading to increase the affinity for the CBS resin (non-polar sorbent) (23).

Contact time

The contact time of the solution with CBS in SPE process plays an important role in the separation of cinnamaldehyde (24).

The results indicate the separation of cinnamaldehyde influenced by contact time with CBS. At a contact time of 2 min, a significant extraction efficiency of 70% is achieved. As the contact time is enlarged to 5 minutes, the extraction efficiency increasing to 85%. Further time extension to 10 min contact time results in a slightly extraction enhancement, reaching an extraction efficiency to 90%. These results indicated that the adsorption of cinnamaldehyde onto the CBS is relatively rapid, with significant extraction arise within the initial 2 to 5 min. Over 10 min, the rate of increase becomes less obvious.

Carbon-based sorbent amount

The CBS mass, representing the amount of solid-phase material used in the extraction, which is impact on the adsorption capacity and selectivity for the target compound (25). By using various amount of CBS, the study objects to investigate the impact of resin amount on the extraction performance. Higher CBS amount may increase the separation capacity but also could lead

to fading returns or even saturation. Equally, lower sorbent masses might result lower separation. The optimal resin mass that achieves best extraction efficiency was 20 mg CBS for 0.25 mg cinnamaldehyde.

Elution Solvent

The elution efficiency of cinnamaldehyde from the CBS was investigated using three different solvents: methanol, ethanol, and isopropanol. The results indicate the elution of cinnamaldehyde from CBS resin is influenced by the solvent. The lowest recovery of cinnamaldehyde from CBS was obtained by methanol solvent (65%), and the most effective elution was obtained by (96%) isopropanol about 1.5times of that obtained by methanol. Ethanol lies in the middle with a recovery of 84%. The result indicates the recovery of cinnamaldehyde from the resin following the order of isopropanol > ethanol> methanol, which is the opposite sequence for relative polarity 0.546, 0.654, and 0.762 respectively(26).

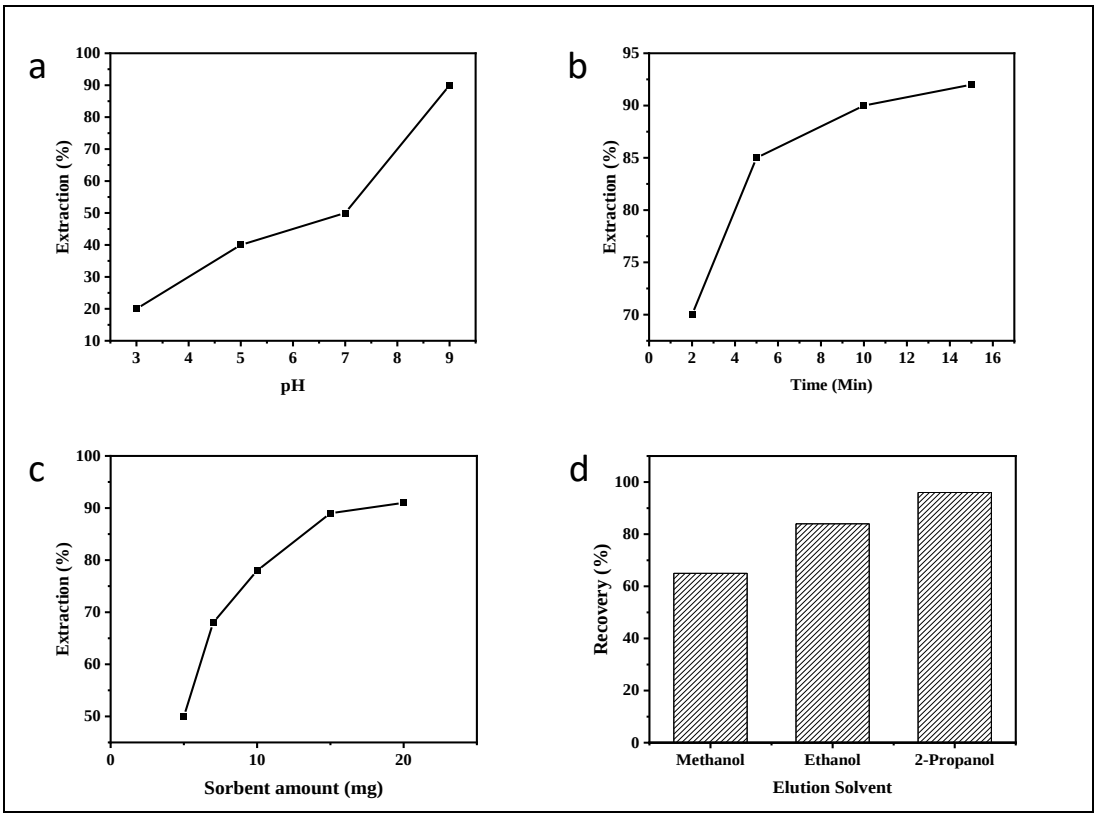

Figure (1): Effect of pH (a), effect of time (b), effect of sorbent amount (c), effect of elution solvent type (d).

The extract yield

The samples were extracted under optimized conditions, with a pH of 9, a contact time of 10 min, and 25 grams of CBS. Isopropanol was employed as the elution solvent. Under these parameters, the extraction yield of cinnamaldehyde recovery of 0.72 % ± 0.04. as a comparative study the cinnamaldehyde yield in cinnamon was 0.376, 1.227 and 5.914% by Hydro distillation, supercritical fluid extraction and reflux extraction, respectively (27).

Collection and Isolation of bacteria

The study conducted in several Baghdad hospitals involving 100 wound and burn swabs the result indicate a 38% positivity rate of bacteria, with *S. aureus* being the predominant isolate (36.84%), followed by *P. aeruginosa* (28.95%), *E. coli* (13.16%), *A. baumannii* (10.53%), and *K. pneumoniae* (10.53%) (Table 1). The identification of bacterial isolates through biochemical tests and confirmation via VITEK 2 Compact system. The results indicate that significant impact of bacterial infections, which might have therapeutic consequences for picking an antibiotic in wound and burn treatment.

Table (1): Distribution of bacteria species among patient.

Type of isolates	No. of isolates	Percentages of isolates
S. aureus	14	36.84%
P. aeruginosa	11	28.95%
E. coli	5	13.16%
A. baumannii	4	10.53%
K. pneumoniae	4	10.53%
Total	38	100.00%

Antibiotic susceptibility test

The antibiotic resistance profiles of *P. aeruginosa*, *E. coli*, *A. baumannii* and *K. pneumoniae* were tested in this study. The resistance percent against different antibiotics have been examined to better understand the frequency and patterns of antibiotic resistance among these bacterial species (figures 1and2).

P. aeruginosa showed significant resistance to a variety of antibiotics, specifically significant resistance against AMC (100.00%), TE (90.91%), and CRO (90.91%). Resistance to these essential antibiotics raises concerns since they are widely utilized in therapeutic settings. In contrast, IMP (9.09%) and TGC (0.00%) showed lower resistance rates.

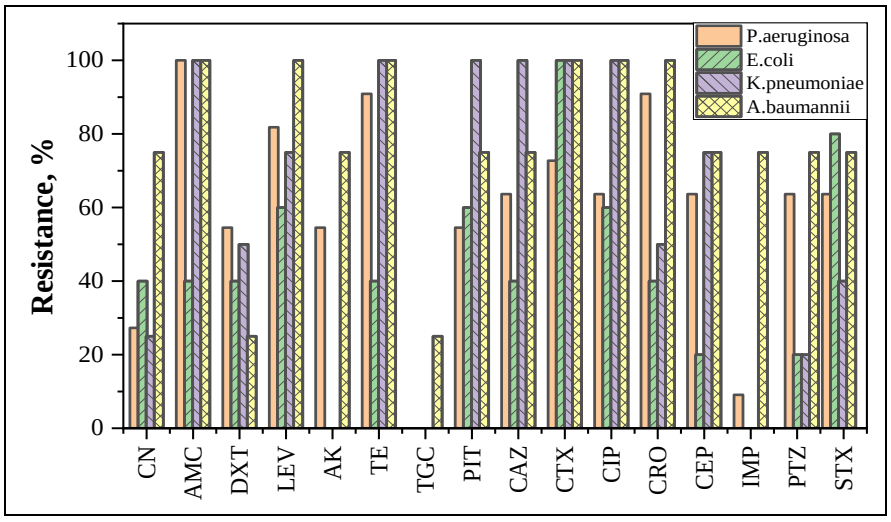

Figure (1): Antibiotic resistance of gram negative bacteria.

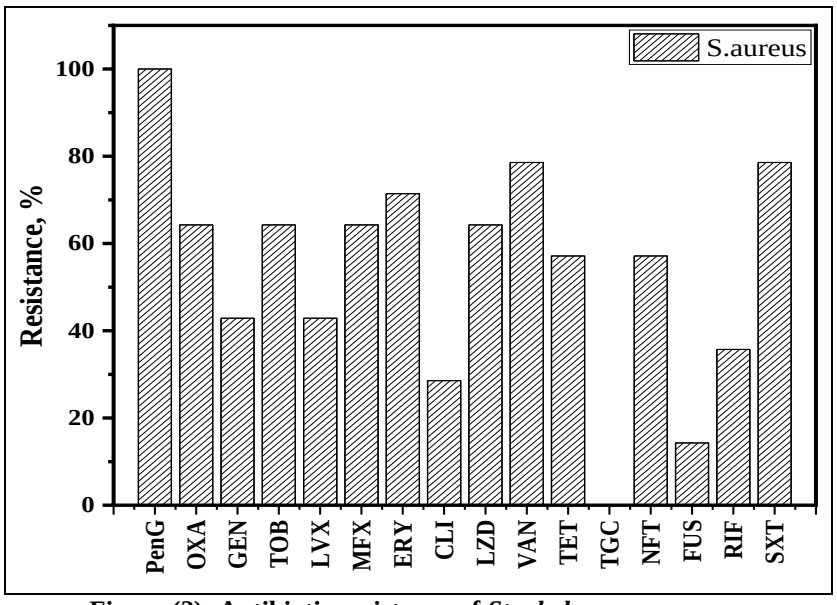

Figure (2): Antibiotic resistance of *Staphylococcus aureus*.

E.coli showed a wide range of resistance patterns, including crucial resistance to CTX (100.00%), CIP (60.00%), and STX (80.00%). Particularly, both AK (0.00%) and TGC (0.00%) showed total susceptibility.

K. pneumoniae was highly resistant to various antibiotics, including AMC (100.00%), CTX (100.00%), and CIP (100.00%). and showed little susceptibility against CN (25.00%) and DXT (50.00%)

A. baumannii showed significant resistance to multiple antibiotics, including AMC (100.00%), LEV (100.00%), and CIP (100.00%). The resistance observed over numerous antibiotic classes indicate the enormous difficulty of controlling infections caused by this *A. baumannii*. In particular, lower resistance rates were found for IMP (75.00%) and PTZ (75.00%).

The antibiotic resistance profiles of 14 *S. aureus* isolates the result indicate all isolates are resistant to Penicillin G (100%). The majority are resistant to Oxacillin (64.29%), Gentamicin (42.86%), Tobramycin (64.29%), Levofloxacin (42.86%), Moxifloxacin (64.29%), Erythromycin (71.43%), Linezolid (64.29%), Vancomycin (78.57%), Tetracycline (57.14%), Nitrofurantoin (57.14%), Rifampin (35.71%), and Trimethoprim/ Sulfamethoxazole (78.57%). Particularly, none isolates are resistant to tigecycline. These results underline the critical information for cautious antibiotic usage, surveillance, and the development of alternate treatment methods for multidrug-resistant *S. aureus* infections.

The antibacterial activity of cinnamaldehyde

The antibacterial activity of cinnamaldehyde, in term of MIC, against bacterial strains in Table 2 and Figure 3. All of the examined bacterial strains showed sensitivities for cinnamaldehyde with different concentration MIC 1024 - 128 µg/ml.

Table (2): MIC and MBC OF Cinnamaldehyde.

Microorganism	Cinnamaldehyde	
	MIC (µg/ml)	MBC (µg/ml)
S. aureus	1024	2048
P. aeruginosa	1024	1024
E. coli	512	1024
K. pneumoniae	128	128
A. baumannii	512	1024

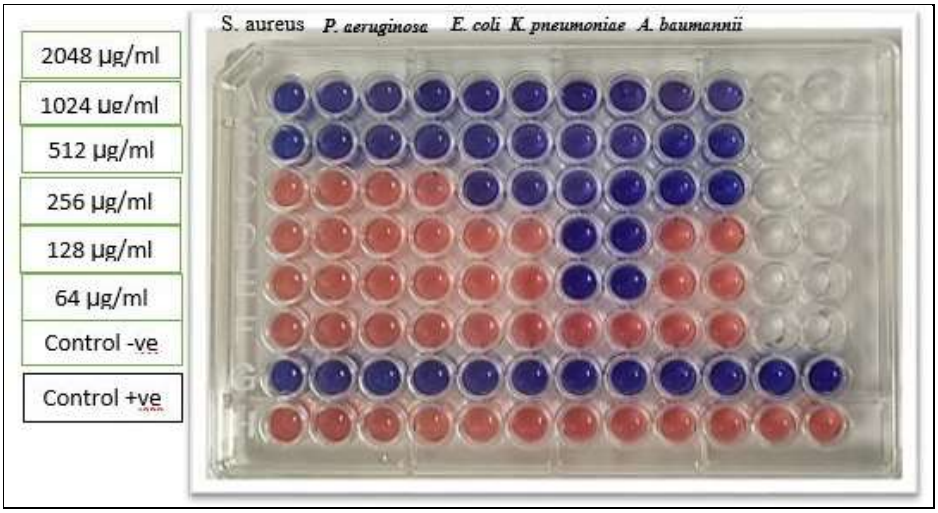

Figure (3): The MIC via microplate.

Cinnamaldehyde demonstrates varying degrees of antimicrobial effectiveness against different bacterial strains. In the case of *Staphylococcus aureus*, the minimum inhibitory concentration (MIC) is 1024 µg/ml, with a slightly higher minimum bactericidal concentration (MBC) at 2048 µg/ml, indicating effective growth inhibition and bactericidal activity at a slightly elevated concentration. Conversely, *Pseudomonas aeruginosa* exhibits equal MIC and MBC values of 1024 µg/ml, highlighting cinnamaldehyde's uniform efficacy in inhibiting and killing this Gram-negative bacterium. *E. coli* has a MIC and MBC 512 and 1024 µg/ml respectively, indicating efficient growth inhibition and requiring a higher concentration from Cinnamaldehyde for bactericidal action. Cinnamaldehyde effectively inhibits growth and induces bacterial mortality at the same concentration, as evidenced by *Klebsiella pneumoniae* reduced MIC and MBC of 128 µg/ml. the cinnamaldehyde showed antibacterial activity against *Acinetobacter baumannii*, cinnamaldehyde reveals a MIC and MBC 512 and 1024 µg/ml respectively, indicating its effectiveness in inhibiting growth, but a higher concentration is necessary for full bactericidal activity.

In a study by Ooi *et al.* (28), indicated that the essential oil extracted from *Cinnamomum cassia* contained around 85.06% of (E)-cinnamaldehyde. Furthermore, antibacterial properties of (E)-cinnamaldehyde has been shown towards strains of *S. aureus, E. coli*, and *P. aeruginosa*. The results showed a significant inhibitory activity, with the MICs of (E)-cinnamaldehyde reaching

0.3 mg/ml for *E. coli*, and *P. aeruginosa* and 0.25 mg/ml for *S. aureus*.

In a study Sanla-Ead *et al.* (29), three fungus species and some bacterial strains were tested against (E)-cinnamaldehyde. Based on the categorization system provided by (30), the results indicated an inhibitory activity of cinnamaldehyde. The MIC values ranging from 0.78 to 12.5 µl/ml, with specific values of 1.56 and 12.5 µl/ml against fungus.

Zhang *et al*. (31) indicate the inhibitory action of cinnamaldehyde toward *S. aureus* and *E. coli*. In them investigate, cinnamaldehyde had MIC concentration of 0.25 μl/ml and MBC concentration of 0.5 μl/ml for both *S. aureus* and *E. coli*. Furthermore, the scanning electron microscopy indicated morphological modifications, which were confirmed by an raise in protein concentration and nucleic acid in suspension, suggesting that cell membrane was destroyed. As a result, the authors believe that cinnamaldehyde has activity in disrupting the bacterial cell membrane (31).

Cinnamaldehyde's minimum inhibitory concentration (MIC) against *S. aureus* and *Uropathogenic E.coli* was determined under static conditions. Most Cinnamaldehyde's exhibited MICs of 400 µg/mL, whereas 4-nitroCinnamaldehyde only had a MIC of 100 µg/mL against *S. aureus* and Uropathogenic *E.coli* (32).

The study by Shen *et al.* (33) was indicate that *trans*- Cinnamaldehyde at (31 mg/mL) concentration caused membrane lysis in *S. aureus* and *E. coli*. Cinnamon oil has the ability to inhibit all strains tested by Pereira *et al*. (22) including *S. aureus*, *P. aeruginosa* and *E. coli*., with the MIC of 4.88, 4.88, and 19.53 µg/ml (34).

Conclusion

The research provides critical insights into the extraction of cinnamaldehyde from cinnamon and its potential as an antibacterial agent. Optimization of SPE parameters highlighted the importance of pH, contact time, sorbent amount, and elution solvent in achieving efficient cinnamaldehyde separation. The antibacterial activity of cinnamaldehyde, evidenced by MIC values, suggests its potential application in treating bacterial infections. The study's comprehensive approach, from extraction to antibacterial testing, contributes valuable information for future applications in wound and burn care.

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