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COMPARATIVE IN VITRO ANTIBACTERIAL ACTIVITY OF GARLIC ESSENTIAL OIL AND CINNAMON BARK EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

The present study compared the antibacterial activity and mechanism of action of hydrodistilled essential oil of *Allium sativum* (garlic) versus 70% v/v ethanolic bark extract from *Cinnamomum verum* (cinnamon) against a multidrug-resistant *Pseudomonas aeruginosa* strain resistant to β lactams third generation cephalosporins, with intermediate susceptibility to imipenem and ciprofloxacin. Inhibition was observed to be dose dependent, as seen in the agar diffusion assays. At 100 mg/mL, garlic essential oil produced a zone of clearance of 32 mm, about 4 mm wider than the 28 mm at the lower concentration (25 mg/mL). The effect of the cinnamon extract (average zones of inhibition of 18 and 28 mm at 25 and 100 mg/mL, respectively) was moderate. Garlic oil was also confirmed to be more potent compared to cinnamon extract at a MIC of 0.50 mg/mL versus 1.00 mg/mL, respectively, by broth microdilution. Profiling of garlic oil by high performance liquid chromatography (HPLC) indicated the presence of allicin and other related organosulfur derivatives as major components, while cinnamon extract was mostly active due to trans cinnamaldehyde. Mechanistically, the bactericidal properties of garlic oil have been attributed to thiol disulfide exchange reactions between allicin and essential sulfhydryl (–SH) groups on bacterial enzymes, which ultimately result in enzyme inactivation, redox imbalance, and rapid cell death. Conversely, trans cinnamaldehyde induces a destabilization of the bacterial inner membrane through direct intercalation, dissipates the proton motive force, and eliminates internal ATP stores while inhibiting the cell division protein FtsZ. The marked susceptibility of this *P. aeruginosa* to these botanical preparations suggests potential activity against a resistant phenotype, a conventional possible antibacterial mechanism of β -lactamase and efflux mediated resistance. In particular, garlic essential oil stands out as a promising scaffold for therapeutic development against hard-to-treat *P. aeruginosa* infections.

Keywords: *P. aeruginosa*; *Allium sativum*; *Cinnamomum verum*; Allicin; Trans Cinnamaldehyde; Minimum Inhibitory Concentration (MIC); Antibacterial Activity

1 INTRODUCTION

One of the most serious global challenges to modern clinical medicine has been the increasing spread of antimicrobial resistance (AMR) among opportunistic Gram-negative pathogens (Pang et al., 2019). It is one of the most challenging pathogens to treat because of its remarkable adaptability and intrinsic, acquired, and adaptive resistance mechanisms (Livermore, 2002) among these priority pathogens. Infections with *P. aeruginosa* are common causative agents of pulmonary exacerbations in patients suffering from cystic fibrosis (CF), severe burn wounds, and ventilated-associated pneumonia, yet these infections may overwhelm the standard antimicrobial regimens (Wallock-Richards et al., 2014). Most of this refractoriness is due to a sophisticated resistance network that includes extremely low outer-membrane permeability, chromosomally encoded inducible β -lactamases (ampC), acquired extended-spectrum β -lactamases, and active extrusion of a wide range of antimicrobial classes by RND efflux systems such as MexAB-OprM and MexXY-OprM (Poole, 2011; Pang et al., 2019). The continued decline in therapeutic efficacy of classical β -lactams, third-generation cephalosporins, and fluoroquinolones demands the evaluation of structurally diverse nonconventional phytochemical scaffolds arising from myriad sources with multi-target mechanisms of action (Bhatwalkar et al., 2021; Vasconcelos et al., 2022). Plant secondary metabolites are very much accused for this, as they have their own unique antimicrobial action, which is separate from common bacterial resistance pathways, creating huge pressure on selective factors that make single-target resistance mutations (Rouf et al., 2020).

Among potential botanical candidates, garlic (*A. sativum* L.) has long been recognized for its potent broad-spectrum biocidal properties (Ankri and Mirelman, 1999; Reiter et al., 2020). Upon mechanical disruption of garlic cloves, the vacuolar enzyme alliinase converts the non-protein amino acid alliin into volatile thiosulfates, predominantly allicin (diallyl thiosulfinate) (Borlinghaus et al., 2014). Hydrodistillation captures these volatile organosulfurs alongside their primary conversion products, including diallyl disulfide (DADS) and diallyl trisulfide (DATS) (Fujisawa et al., 2008). Mechanistically, allicin readily penetrates bacterial phospholipid membranes and executes rapid thiol–disulfide exchange reactions with functional sulfhydryl (–SH) groups in vital bacterial enzymes, such as RNA polymerase and thioredoxin reductase (Miron et al., 2000; Gruhlke et al., 2019). This electrophilic attack causes irreversible enzymatic inactivation, collapses intracellular glutathione redox equilibrium, and results in rapid cell death (Borlinghaus et al., 2021).

In parallel, cinnamon (*C. verum*) bark extracts represent a rich source of bioactive phenylpropanoids and phenolic derivatives, dominated by trans-cinnamaldehyde (Nabavi et al., 2015; Doyle and Stephens, 2019). Hydroethanolic extraction provides an optimal polarity balance for recovering these phenolic constituents. trans-Cinnamaldehyde exerts bactericidal activity primarily through direct physical intercalation into the bacterial inner cytoplasmic membrane, which compromises structural integrity, increases permeability, and dissipates the transmembrane proton motive force (PMF) (Gill and Holley, 2006; Bouyahya et al., 2019). This membrane destabilization precipitates the rapid depletion of intracellular ATP pools and is accompanied by the targeted inhibition of the prokaryotic division protein FtsZ, thereby preventing Z-ring assembly and arresting binary fission (Domadia et al., 2007; Sun et al., 2020).

Although both *A. sativum* and *C. verum* have demonstrated antimicrobial properties across varied experimental models, a direct, comparative evaluation of their quantitative potency and chemical profiles against characterized *P. aeruginosa* isolates remains necessary. Multidrug-resistant *Pseudomonas aeruginosa* poses a critical therapeutic challenge due to its intrinsic resistance mechanisms and frequent involvement in severe nosocomial infections. Its selection as the study organism ensures that any demonstrated antibacterial efficacy has direct relevance to combating refractory Gram-negative pathogens. Therefore, this study aims to characterize the chromatographic fingerprints of hydrodistilled *A. sativum* essential oil and 70% ethanolic *Cinnamomum verum* bark extract using HPLC, to comparatively evaluate their in vitro antibacterial efficacy against a *P. aeruginosa* isolate through quantitative agar well diffusion and broth microdilution (MIC) assays, and to elucidate the mechanistic basis by which these distinct phytochemical preparations circumvent conventional bacterial resistance determinants.

2 MATERIALS AND METHODS

2.1 Bacterial Isolate and Antimicrobial Susceptibility Profiling

P. aeruginosa clinical isolate, obtained from the Food Research Department at the Scientific Research Commission, was investigated. The isolate was maintained on nutrient agar slants at 4°C and subcultured onto fresh Mueller-Hinton agar (MHA) plates at 37°C for 24 h to obtain pure, active cultures before testing.

2.2 Antimicrobial Susceptibility Profiling

The standardized Kirby–Bauer disk diffusion method (CLSI, 2020) was followed for the antimicrobial susceptibility profiling. The antibiotic panel targeted β -lactams, third generation cephalosporins, carbapenems and fluoroquinolones: ampicillin (10 μ g), amoxicillin–clavulanate (20/10 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), imipenem (10 μ g), and

ciprofloxacin (5 µg). Before being applied to MHA plates, the bacterial inocula were diluted to a 0.5 McFarland standard (1.5×10^8 CFU/mL) in sterile saline and swabbed onto MHA plates. Inhibition zones were determined and expressed in millimeters (mm) by a digital caliper after 18 h of incubation at 35 ± 2 °C, and interpreted according to CLSI clinical breakpoints (CLSI, 2020; Livermore, 2002). Both diazotized and non-diazotized OA were used for analysis, suggesting a clinical phenotype of resistance to ampicillin, amoxicillin–clavulanate, cefotaxime, and ceftriaxone with intermediate susceptibility to imipenem and ciprofloxacin.

2.3 Extraction of Garlic (*A. sativum* L.) Essential Oil

Fresh, mature garlic (*A. sativum* L.) bulbs were authenticated and prepared by removing outer skins and washing cloves with sterile distilled water. Cleaned cloves were mechanically homogenized immediately before extraction to facilitate the endogenous enzymatic conversion of alliin to allicin by alliinase (Borlinghaus et al., 2014; Fujisawa et al., 2008). Hydrodistillation was performed using a standard Clevenger-type apparatus:

Crushed garlic was suspended in distilled water (1:2 w/v ratio) within a round-bottom flask and hydrodistilled for 4 h (Karupiah and Rajaram, 2012).

The volatile essential oil fraction was collected from the aqueous distillate, separated, and dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture (Bhatwalkar et al., 2021).

The recovered pure garlic essential oil was transferred into light-resistant, airtight amber glass vials and stored at 4 °C until chemical and microbiological testing.

2.4 Preparation of Cinnamon (*C. verum*) Ethanolic Extract

Authenticated cinnamon (*C. verum*) bark was cleaned, air dried under shade, and pulverized into a fine, homogeneous powder using an electric blender. Extraction was carried out via hydroethanolic maceration according to established protocols (Nabavi et al., 2015; Bouyahya et al., 2019):

- Powdered bark was mixed with 70% ethanol (v/v) at a 1:10 (w/v) ratio in an Erlenmeyer flask.
- The suspension was maintained at room temperature (25 ± 2 °C) for 72 h with continuous intermittent agitation.
- The mixture was filtered through Whatman No. 1 filter paper to remove insoluble plant debris.
- The filtrate was concentrated under reduced pressure at 40 °C using a rotary vacuum evaporator to prevent thermal degradation of active phenylpropanoids and volatile phenolics (Vasconcelos et al., 2018).
- The dried crude ethanolic extract was weighed, collected in sterile amber containers, and stored at 4 °C.

2.5 Preparation of Test Concentrations and Emulsification

Stock preparations of *C. verum* ethanolic extract and *A. sativum* essential oil were prepared and adjusted to working concentrations of 25 and 100 mg/mL:

Cinnamon extract was dissolved in sterile dimethyl sulfoxide (DMSO; final concentration 1.0% v/v) and subsequently diluted in sterile cation-adjusted Mueller Hinton broth (CAMHB), the standardized medium recommended for antimicrobial susceptibility testing. (Wijesinghe et al., 2020).

Garlic Essential Oil: Emulsified in sterile CAMHB using 0.5% (v/v) Tween-80 to achieve uniform, stable dispersion of hydrophobic droplets without compromising bacterial cell viability (Gill and Holley, 2006; Wallock-Richards et al., 2014).

Vehicle Controls: Parallel vehicle controls (0.5% Tween-80 in CAMHB and 1.0% DMSO in CAMHB) were prepared to verify that the dispersing agents possessed no intrinsic antibacterial activity against *P. aeruginosa* (Balouri et al., 2016).

2.6 Inoculum Standardization

P. aeruginosa colonies from an 18-h pure culture were suspended in sterile 0.85% (w/v) physiological saline. Turbidity was adjusted spectrophotometrically (OD₆₀₀) to match a 0.5 McFarland standard (1.5×10^8 CFU/mL) in accordance with CLSI guidelines (CLSI, 2020). The standardized suspension was used within 15 min of preparation for all subsequent antibacterial assays.

2.7 Quantitative Agar Well Diffusion Assay

The comparative antibacterial activity of the botanical preparations was screened via agar well diffusion (Valgas et al., 2007; Utcharyiakiat et al., 2016): Sterile MHA plates were uniformly seeded with the standardized *P. aeruginosa*

suspension using sterile cotton swabs. Wells (6 mm diameter) were punched into the agar using a sterile cork borer. A fixed volume (100 μ L) of each test concentration (25 and 100 mg/mL) of garlic essential oil and cinnamon extract was delivered into designated wells. Solvent controls (0.5% Tween-80 and 1.0% DMSO solutions) along with reference antibiotic disks were dispensed into separate wells on the same plates. Plates were held at room temperature for 1 h to facilitate radial agar pre-diffusion, then incubated at 37 °C for 24 h. Diameters of clear zones of growth inhibition (ZOI) were measured in millimeters (including the 6 mm well diameter) using a digital vernier caliper. All assays were executed in triplicate.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of both extracts was evaluated using the standard broth microdilution technique in sterile 96-well polystyrene microtiter plates in accordance with CLSI M07 guidelines (CLSI, 2018; Balouri et al., 2016): Two-fold serial dilutions of the botanical preparations were prepared in CAMHB across a concentration gradient (0.03 to 16.0 mg/mL) to ensure accurate endpoint determination (Bhatwalkar et al., 2021). Each well received 10 μ L of the standardized bacterial suspension, delivering a final well inoculum density of approximately 5×10^5 CFU/mL. Controls included sterility (CAMHB + carrier, no inoculum), growth (inoculated CAMHB with vehicle only), and vehicle/dispersant blanks (0.5% Tween-80; 1.0% DMSO, inoculated and uninoculated) to exclude contamination, verify bacterial proliferation, and rule out solvent effects. Microplates were sealed and incubated at 35 ± 2 °C for 24 h. Following incubation, optical density (OD 600) was recorded spectrophotometrically, and visual clarity was assessed. The MIC was defined as the lowest concentration (mg/mL) of the botanical agent that completely prevented visible bacterial growth relative to growth control wells (CLSI, 2018).

2.9 High-Performance Liquid Chromatography (HPLC) Profiling

Garlic Essential Oil: Chromatographic profiling of *A. sativum* oil was conducted to separate and resolve organosulfur constituents (principally allicin and secondary polysulfide derivatives) following established chromatographic protocols (Borlinghaus et al., 2014; Fujisawa et al., 2008). The reverse-phase HPLC system was equipped with a quaternary pump, autosampler, and photodiode array (PDA) detector. The stationary phase was a C18 analytical column (250 $\times$ 4.6 mm, 5 μ m particle size) maintained at 30 °C. The mobile phase consisted of an isocratic elution of methanol: water (50:50 v/v) at a flow rate of 1.0 mL/min, with UV monitoring set at λ =240 nm and 254 nm. Chromatographic peak identity was verified by comparison with authentic reference standards.

Cinnamon Ethanol Extract: The chemical constituents of the 70% *C. verum* bark extract were resolved via HPLC using an analytical C18 column (150 $\times$ 4.6 mm, 5 μ m) (Bouyahya et al., 2019; Doyle and Stephens, 2019). The mobile phase comprised a binary gradient system of 0.1% aqueous formic acid (Solvent A) and acetonitrile (Solvent B) delivered at a flow rate of 0.8 mL/min. Multi-wavelength UV/Vis detection was monitored at λ =280 nm (general phenolic acids) and λ =295 nm (trans-cinnamaldehyde). trans-Cinnamaldehyde and related phenylpropanoids were identified by matching retention times and peak spectral profiles against reference standards.

2.10 Statistical Analysis

All experimental assays were conducted independently in triplicate (n=3), and quantitative data were expressed as mean $\pm$ standard deviation (SD). Variations in inhibition-zone diameters across treatment groups (garlic oil vs. cinnamon extract) and across concentration levels (25 and 100 mg/mL) were evaluated using a two-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc multiple comparison test (Montgomery, 2017). Differences were considered statistically significant at $p < 0.05$. All statistical calculations and graphical illustrations were generated using GraphPad Prism (version 9.0) and SPSS software (version 26.0).

3 RESULTS AND DISCUSSION

The increasing global dissemination of MDR among opportunistic pathogens, particularly *P. aeruginosa*, has significantly compromised the effectiveness of conventional antimicrobial therapy. The *P. aeruginosa* isolate investigated in this study was obtained from the (FTD) at the (SRC). Fresh mature *A. sativum* cloves were authenticated, cleaned, mechanically disrupted, and subjected to hydrodistillation using a Clevenger apparatus to obtain the volatile, organosulfur-rich garlic essential oil. Before the antibacterial assays, a pure bacterial culture was standardized to a 0.5 McFarland turbidity equivalent to approximately 1.5×10^8 CFU/mL and inoculated onto MHA. The antibacterial activity of the preparation was subsequently evaluated by the agar diffusion method at concentrations of 25 and 100 mg/mL. These discrete test points, rather than a full gradient, were chosen to simplify experimental design while still capturing the dose-response relationship. Moreover, both concentrations fall within ranges commonly reported in phytochemical antimicrobial studies, ensuring methodological consistency and clinical relevance. As shown in Figure 1, the untreated control (Plate 1) exhibited dense, confluent bacterial growth over the agar surface, confirming the viability and robust growth of the investigated *P. aeruginosa* isolate. The characteristic green-blue pigmentation is consistent with the production of phenazine pigments, particularly pyocyanin, and further indicates active bacterial metabolism. At 25

mg/mL (Plate 2), the garlic preparation produced a distinct and well-defined inhibition zone measuring 23 mm, demonstrating substantial antibacterial activity even at the lower concentration. Increasing the concentration to 100 mg/mL (Plate 3) resulted in a considerably larger inhibition zone of 32 mm, indicating a pronounced concentration-dependent increase in growth inhibition. The expansion of the inhibition zone with increasing concentration suggests that a greater amount of the active garlic constituents diffused through the agar and exerted stronger inhibitory effects on bacterial proliferation.

The potent antibacterial activity of hydrodistilled *A. sativum* oil observed in this study is consistent with earlier reports identifying garlic organosulfur derivatives as effective agents against Gram-negative pathogens (Bhatwalkar et al., 2021). The broad inhibition zones corroborate findings by Karuppiyah and Rajaram (2012) and Wallock-Richards et al. (2014), who demonstrated strong activity of garlic extracts against clinical and multidrug-resistant *P. aeruginosa* (Alharbi et al., 2025).



Figure 1: In Vitro Assessment of the Antagonistic Potential of Alcoholic Garlic Extract on *P. aeruginosa* Growth at Diverse Concentrations

Allicin, together with allyl polysulfides such as Diallyl Disulfide (DADS) (and Diallyl Trisulfide (DATS)), is the primary driver of this activity; both are allyl polysulfides derived from garlic, alongside allicin. They are key bioactive sulfur compounds responsible for garlic's antimicrobial activity. DADS and DATS act by disrupting bacterial membranes and interacting with sulfhydryl (-SH) groups of essential enzymes, contributing to oxidative stress and cell death (Fujisawa et al., 2008; Marchese et al., 2016). Mechanistically, allicin penetrates bacterial membranes and reacts with sulfhydryl groups of essential enzymes, leading to enzyme inactivation, oxidative stress, and suppression of quorum-sensing-regulated virulence factors (Harjai et al., 2010; Borlinghaus et al., 2014). Because these electrophilic interactions bypass β -lactamase hydrolysis and efflux systems (Bhatwalkar et al., 2021), garlic oil retains efficacy against resistant *P. aeruginosa* strains, underscoring its potential as a natural antimicrobial candidate.

Figure 2 demonstrates a clear concentration-dependent antibacterial effect of *C. verum* ethanolic bark extract against the investigated *P. aeruginosa* isolate. The untreated control (Plate 1) exhibited dense, confluent bacterial growth across the agar surface, accompanied by characteristic blue-green pigmentation consistent with pyocyanin production, confirming the viability and active growth of the bacterial isolate under the experimental conditions. At 25 mg/mL (Plate 2), the cinnamon extract produced a distinct and well-defined inhibition zone measuring 18 mm, demonstrating appreciable antibacterial activity at the lower tested concentration. Increasing the extract concentration to 100 mg/mL (Plate 3) resulted in a substantially larger inhibition zone of 28 mm, indicating enhanced antibacterial efficacy and greater diffusion of active phytochemical constituents through the agar medium. The progressive increase in inhibition-zone diameter with increasing extract concentration confirms a concentration-dependent response. This consistent difference indicates greater antibacterial efficacy of garlic oil under the experimental conditions. The hydroethanolic bark extract of *C. verum* exhibited clear dose-dependent antibacterial activity, consistent with prior reports of cinnamon extracts against Gram-negative pathogens (Utcharyaki et al., 2016; Nabavi et al., 2015). The widening inhibition zones reflect higher local concentrations of bioactive compounds overcoming adaptive responses and efflux mechanisms (Vasconcelos et al., 2018). Trans-cinnamaldehyde was identified as the principal active constituent, supported by phenolics such as eugenol and cinnamic acid (Doyle and Stephens, 2019). Its mechanisms include membrane

permeabilization with ion leakage (Gill and Holley, 2006), collapse of proton motive force and ATP depletion (Bouyahya et al., 2019), inhibition of FtsZ-mediated cell division (Domadia et al., 2007; Sun et al., 2017), and suppression of quorum-sensing and pyocyanin production (Leoni et al., 2018; Firmino et al., 2018).

Comparatively, *A. sativum* essential oil demonstrated broader inhibition zones and a lower MIC, reflecting the rapid, non-specific thiol-disulfide exchange reactions of allicin and related organosulfur compounds with essential sulfhydryl groups in bacterial enzymes (Miron et al., 2000; Wallock-Richards et al., 2014; Bhatwalkar et al., 2021). Cinnamon extract, while potent, relies primarily on membrane intercalation and non-covalent interactions, requiring higher concentrations to achieve equivalent bactericidal effects (Marchese et al., 2016; Wijesinghe et al., 2020).

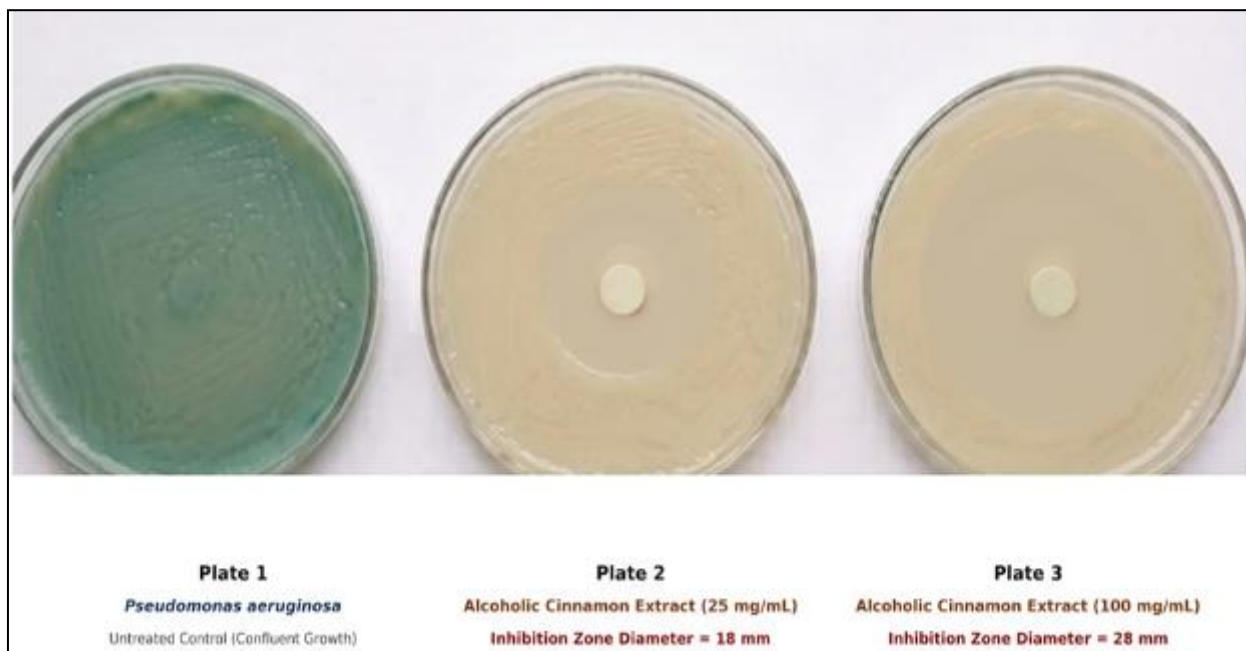


Figure 2: In Vitro Assessment of the Antagonistic Potential of Cinnamon Ethanolic Extract on *P. aeruginosa* Growth at Diverse Concentrations.

3.1.1 Minimum Inhibitory Concentration (MIC)

Table 1: Comparative Antibacterial Activity of Botanical Extracts Against *P.aeruginosa*

Botanical Preparation	Extraction Method / Solvent	Inhibition Zone Diameter (mm) at 100 mg/mL	MIC (mg/mL)
Garlic (<i>A. sativum</i>)	Hydrodistillation (Essential Oil)	32.0	0.50
Cinnamon (<i>C.verum</i>)	Maceration (70% Ethanol)	28.0	1.00

The minimum inhibitory concentration (MIC) values of the tested botanical preparations against the *P. aeruginosa* are presented in Table 1. Quantitative broth microdilution assays revealed distinct variations in the intrinsic antibacterial potency of the tested preparations against *P. aeruginosa*. The hydrodistilled *A. sativum* essential oil demonstrated superior efficacy, yielding an MIC value of 0.50 mg/mL. In comparison, the 70% ethanolic extract of *C.verum* exhibited a higher MIC of 1.00 mg/mL. No visible bacterial inhibition was detected in the negative vehicle control wells containing the dispersing systems without active botanical components. These MIC values align closely with recent broth microdilution data. Bhatwalkar et al. (2021) and Rouf et al. (2020) reported that allicin-rich garlic preparations inhibit Gram-negative clinical isolates at concentrations between 0.25 and 0.50 mg/mL. Similarly, recent evaluations of Cinnamomum extracts and purified trans-cinnamaldehyde against *P. aeruginosa* documented baseline MIC values spanning 0.50 to 2.00 mg/mL (Wijesinghe et al., 2020; Vasconcelos et al., 2022). Allicin's small molecular size and lipophilicity enable rapid intracellular penetration. It exerts a targeted electrophilic attack via thiol disulfide exchange reactions with conserved sulfhydryl (–SH) residues of essential metabolic enzymes (such as RNA polymerase and alcohol dehydrogenase), causing immediate metabolic collapse and cell arrest at low concentrations (Borlinghaus et al.,

2021).trans-Cinnamaldehyde acts primarily through physical intercalation into the bacterial inner membrane, dissipating the proton motive force (PMF), depleting intracellular ATP, and binding to the division protein FtsZ (Doyle and Stephens, 2019 ;Sun et al., 2020). Because membrane perturbation requires reaching a critical molecular threshold across the lipid bilayer, a higher concentration is required to achieve complete visible growth cessation.

Garlic essential oil exhibited significantly stronger antibacterial activity than cinnamon extract, with inhibition zones increasing from 23.0 mm at 25 mg/mL to 32.0 mm at 100 mg/mL ($p<0.01$). Cinnamon showed comparatively smaller zones (18.0 mm to 28.0 mm). MIC assays confirmed complete growth inhibition at 0.50 mg/mL for garlic and 1.00 mg/mL for cinnamon ($n=3$), establishing a twofold greater potency for garlic. Negative controls (Tween-80, DMSO) showed no inhibition, verifying that the observed effects were solely attributable to the botanical bioactive.

The results presented in Figure 3 illustrate the susceptibility profile of the *P.aeruginosa* isolate to several conventional antibiotics; the isolate demonstrated complete resistance to beta-lactams and third-generation cephalosporins, including ampicillin, amoxicillin/clavulanic acid, cefotaxime, and ceftriaxone, with inhibition zone diameters not exceeding 14 mm, whereas it showed only intermediate susceptibility to imipenem and ciprofloxacin. This clearly highlights the scientific significance of the results demonstrated in the agar well diffusion assays for the garlic extract; the active organosulfur compounds in the garlic oil successfully breached this barrier and inhibited bacterial growth with remarkable efficacy. The garlic preparation demonstrated stronger antibacterial activity than the conventional antibiotics to which the isolate showed intermediate susceptibility, with its inhibitory effect becoming particularly pronounced at the higher concentration and exceeding the activity observed for both ciprofloxacin and imipenem under the experimental conditions. This superior inhibitory effect compared to the antibiotics listed in the figure is attributed to the nature of allicin's mechanism of action, which operates independently of beta-lactamase pathways and cellular efflux pumps; instead, it relies on rapid interaction with and inactivation of vital sulfhydryl groups within bacterial metabolic enzymes, thereby conferring high, direct inhibitory efficacy upon the garlic extract against the specific bacterial isolate in question. The marked resistance profile of *P. aeruginosa*, including failure of β -lactams and third-generation cephalosporins (zones ≤ 14 mm) and only intermediate susceptibility to imipenem and ciprofloxacin, reflects its intrinsic and acquired defense mechanisms, notably AmpC β -lactamases, porin loss (OprD), and RND efflux systems such as MexAB-OprM and MexXY-OprM (Livermore, 2002; Pang et al., 2019).

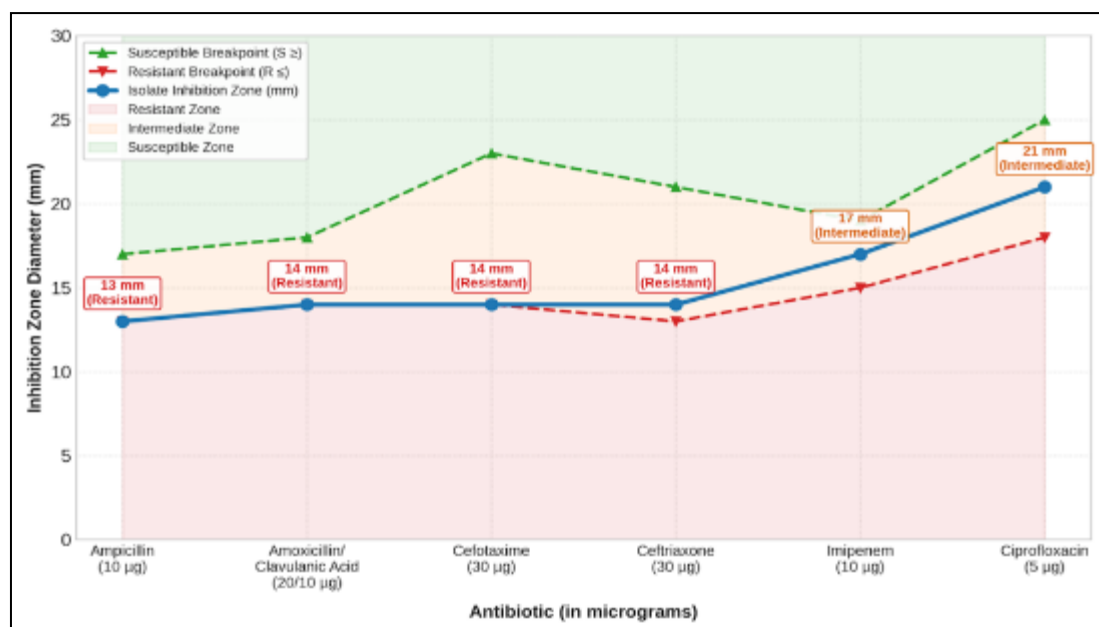


Figure 3: Comparative evaluation of inhibition zone diameters (mm) against CLSI interpretive susceptibility breakpoints (Resistant, Intermediate, Susceptible) for *P.aeruginosa*

In contrast, garlic essential oil, rich in allicin, bypasses these barriers. Its lipophilic organosulfur constituents permeate membranes without efflux or enzymatic hydrolysis (Miron et al., 2000; Marchese et al., 2016). Once inside, allicin irreversibly modifies sulfhydryl (–SH) groups of key enzymes (RNA polymerase, alcohol dehydrogenase, thioredoxin reductase) via thiol-disulfide exchange, disrupting redox homeostasis and inducing rapid bactericidal arrest (Borlinghaus et al., 2021; Gruhlke et al., 2019). These findings align with reports by Wallock-Richards et al. (2014) and Reiter et al. (2020), showing allicin-rich extracts are effective against resistant *P. aeruginosa*. Reviews by Rouf et al. (2020) and Bhatwalkar et al. (2021) further confirm that the multi-target, non-specific nature of garlic organosulfurs circumvents conventional resistance determinants, explaining the broader clearance zones achieved compared to standard antibiotics. The HPLC chromatogram illustrated in Figure 4 confirmed the characteristic organosulfur profile

of hydrodistilled *A. sativum* oil, with allicin as the predominant peak alongside diallyl sulfide, disulfide, trisulfide, and tetrasulfide derivatives. This composition explains the superior antibacterial activity observed (32.0 mm inhibition zone at 100 mg/mL; MIC = 0.50 mg/mL), as allicin penetrates bacterial envelopes and inactivates key enzymes via thiol disulfide exchange. By targeting conserved sulfhydryl groups rather than penicillin-binding proteins or DNA gyrase, garlic oil circumvents β -lactamase hydrolysis and efflux-mediated resistance, underscoring its potential as an effective natural agent against refractory *P. aeruginosa*.

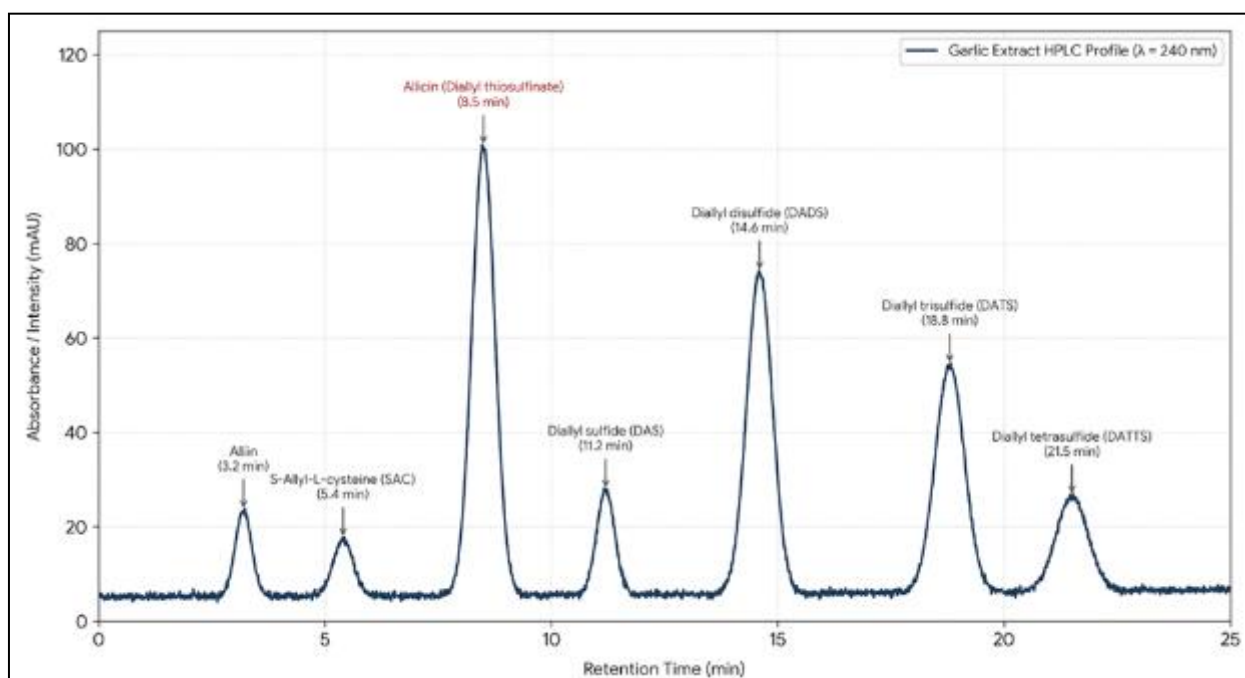


Figure 4: HPLC chromatographic profile of active organosulfur derivatives in garlic essential oil

Numerous investigations have established the strong antibacterial potential of garlic organosulfurs against *Pseudomonas aeruginosa* (Wallock-Richards et al., 2014; Rouf et al., 2020; Bhatwalkar et al., 2021). Our results closely align with Wallock-Richards et al. (2014) and Reiter et al. (2020). By showing that allicin and related polysulfides act through thiol–disulfide exchange, bypassing β -lactamase hydrolysis and RND efflux pumps (Livermore, 2002; Pang et al., 2019; Poole, 2011; Marchese et al., 2016), we consolidate *A. sativum* oil as a promising phytochemical scaffold against refractory *P. aeruginosa* infections (Rouf et al., 2020; Bhatwalkar et al., 2021). The reverse-phase HPLC profile (Figure 5) established the chemical fingerprint of *C. verum* bark extract, with trans-cinnamaldehyde as the dominant peak and minor phenolic phenylpropanoid constituents also resolved. This composition underlies the antibacterial activity observed against *P. aeruginosa*. Mechanistically, the major compound disrupts bacterial membranes, collapses proton motive force, depletes ATP, and inhibits the division protein FtsZ, thereby blocking cell division. Because these actions bypass β -lactamase hydrolysis and efflux pathways, the extract demonstrates promise as an effective alternative antimicrobial agent. Being lipophilic, trans-cinnamaldehyde intercalates directly into the hydrophobic lipid bilayer of the bacterial cytoplasmic membrane, altering fluidity, increasing permeability, and inducing leakage of essential ions and cytoplasmic contents (Vasconcelos et al., 2018; Bouyahya et al., 2019). The consequent loss of selective permeability dissipates the proton motive force (PMF) and electrical potential ($\Delta\Psi$), halting ATP synthase activity and rapidly depleting intracellular ATP pools, thereby inducing acute metabolic arrest (Doyle and Stephens, 2019).

Beyond membrane disruption, trans-cinnamaldehyde binds to the GTP-binding pocket of the division protein FtsZ, inhibiting GTPase activity and blocking Z-ring assembly, which prevents septation and arrests binary fission (Domadia et al., 2007; Sun et al., 2017, 2020). Importantly, *P. aeruginosa* resists β -lactams and cephalosporins via chromosomal AmpC and extended-spectrum β -lactamases (Livermore, 2002; Pang et al., 2019). However, the α,β -unsaturated aldehyde core of trans-cinnamaldehyde renders it structurally immune to enzymatic cleavage (Vasconcelos et al., 2022). Efflux pump bypass further enhances its efficacy, while fluoroquinolones and carbapenems are expelled by RND systems such as MexAB-OprM; the non-specific membrane-destabilizing properties of cinnamon phenylpropanoids collapse the electrochemical gradient required to power efflux (Poole, 2011; Vasconcelos et al., 2022). Sun et al. (2020) demonstrated that purified trans-cinnamaldehyde disrupts membrane integrity and suppresses virulence pathways in clinical isolates within an MIC range of 0.50–1.00 mg/mL. Wijesinghe et al. (2020) reported comparable inhibition and eradication of mature biofilms, attributing the effect to rapid cell envelope disruption. Vasconcelos et al. (2022) further confirmed that the multi-target attack of cinnamon phenylpropanoids prevents single-target mutation selection, validating *C. verum* bark extract as an effective antimicrobial candidate against refractory Gram-negative pathogens.

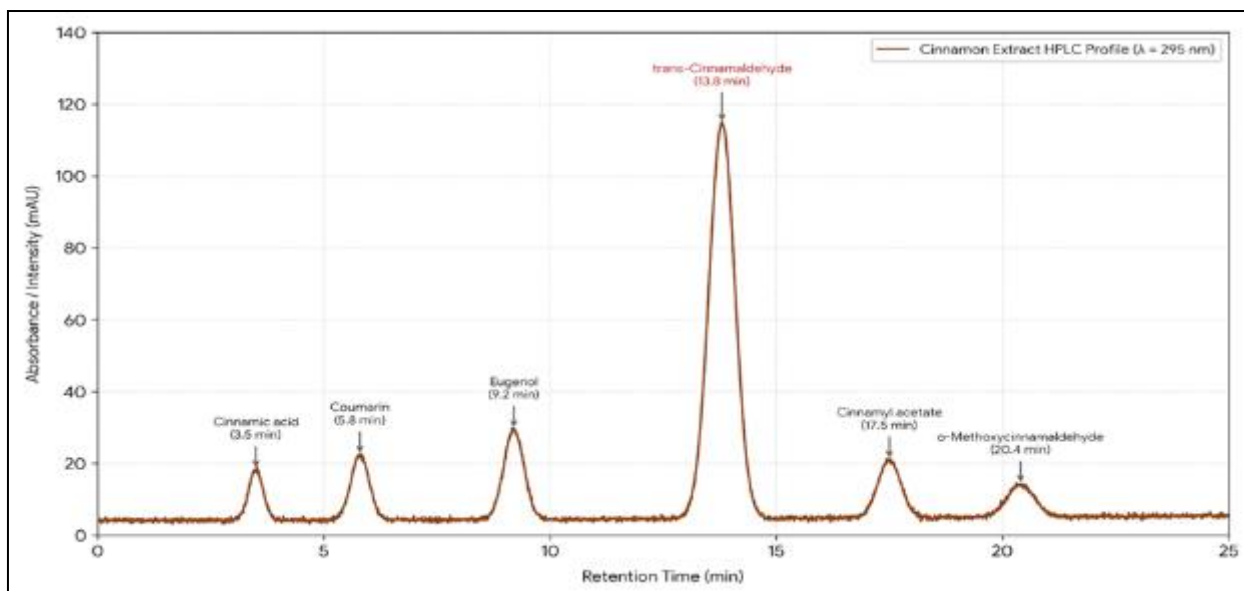


Figure 5: HPLC chromatographic fingerprint of *Cinnamomum verum* ethanolic extract highlighting trans-cinnamaldehyde

4 CONCLUSION

- **Garlic essential oil demonstrated superior antibacterial potency** against multidrug-resistant *P. aeruginosa*, achieving a lower MIC (0.50 mg/mL) and larger inhibition zone (32 mm) compared to cinnamon extract, underscoring its potential as a more effective botanical agent.
- **Distinct phytochemical drivers were identified for each preparation**, with allicin and its cascade of volatile polysulfides accounting for the activity of garlic oil, while trans-cinnamaldehyde was the principal active constituent in cinnamon extract, confirming the mechanistic specificity of each botanical.
- **Both preparations effectively circumvented conventional resistance pathways**, including β -lactamase hydrolysis and efflux systems, by targeting conserved sulfhydryl groups (garlic) or disrupting membrane integrity and cell division (cinnamon). This highlights their promise as phytotherapeutic alternatives for managing refractory *P. aeruginosa* infections.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest associated with this manuscript.

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