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Silencing Bacterial Communication: Quorum Sensing Inhibition by Cinnamon Extract

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KEYWORDS

Quorum sensing, Cinnamon, LasR, anti-bacterial, molecular interaction.

ABSTRACT:

Quorum sensing (QS) is a cell–cell communication system employed by many bacteria to coordinate population density–dependent physiological and pathogenic behaviors. Targeting QS has emerged as a promising anti-virulence strategy that does not exert direct selective pressure for resistance. The present study aimed to identify and evaluate quorum sensing inhibitory activity using *Cinnamomum verum* (cinnamon) extract. QS-interfering bacteria were isolated from environmental water samples using nutrient agar medium, followed by sub-culturing to obtain pure colonies. Molecular sequencing was performed to determine the taxonomic identity of the isolates. Cinnamon extract was prepared using 75% (v/v) methanol and assessed for antibacterial activity against the isolates by the agar well diffusion method on Mueller–Hinton agar. In addition, molecular interaction analysis was conducted to examine the binding relationship between the QS regulatory protein LasR and the bioactive compound cinnamaldehyde. The findings demonstrate that cinnamon extract exhibits notable quorum sensing inhibitory potential, supported by its interaction with LasR. These results suggest that cinnamon, a widely consumed spice, may serve as a natural quorum sensing inhibitor capable of attenuating bacterial virulence and pathogenesis, highlighting its potential application in antimicrobial and therapeutic strategies.

Introduction

Many bacteria use a cell-cell communication system called quorum sensing to coordinate population density-dependent changes in behaviour (1). Quorum sensing (or quorum signalling) is the ability of gene regulation to detect and respond to cell population density. Quorum sensing (QS), for example, allows bacteria to limit the expression of specific genes to high cell densities where the phenotypes will be most

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beneficial. Many bacterial species use quorum sensing to coordinate gene expression based on the density of their local population (2). Quorum sensing has many applications in computing and robotics, in addition to its role in biological systems. In general, quorum sensing can be used as a decision-making process in any decentralised system where components can determine the number of other components with which they interact and a standard response once a threshold number is detected (3).

Cinnamon (*Cinnamomum verum*) is an indigenous spice from the *Lauraceae* family that can be found in almost every home. It has long been a significant component of our food, primarily as a flavouring agent. Our forefathers used it as a cure for respiratory and digestive illnesses for a long time. However, little is known about its antioxidative, anti-inflammatory, antilipemic, antidiabetic, antimicrobial, and anticancer properties (4).

Bacteria need to possess three qualities in order to use quorum sensing constitutively: They have to release a signalling molecule, an autoinducer, detect variations in the quantities of signalling molecules, and respond by regulating gene transcription. The diffusion mechanism of signalling molecules is crucial to this process. Individual bacteria generally secrete only a modest number of QS signalling molecules. The chemicals may just diffuse away at low cell density. At high cell density, the local concentration of signalling molecules may surpass their threshold level, altering gene expression (5).

Materials and Methods

Extraction of cinnamon extracts

Readily available Cinnamon sticks were outsourced from the local store in Arumbakkam, Chennai. These sticks were ground into fine powder and sieved using a sieve. This powder was stored in air tight container for further use. The 50g powdered sample was extracted for 3-4 days with 300ml of 75 percent (v/v) aqueous methanol and then repeated if necessary. The extract is now filtered in a beaker and allowed to fully evaporate. The excess methanol was entirely removed using a rotary evaporator. The extract is then powdered after the base of the beakers is scraped out. For further experimental assays, the extract was weighed (3.54g) and redissolved in suitable concentrations of dimethyl sulfoxide (DMSO). This extract is stored in a screw cap bottle (6).

Isolation of bacterial source

A bottle of water was incubated for a few days at room temperature, untouched and undisturbed, to form a biofilm layer on the bottle's side. A swab was taken from the bottle's sides and inoculated in nutrient agar medium after a few days. For 24 hours, this was kept at room temperature.To isolate pure culture colonies, the colonies were streaked on MacConkey agar media and incubated at 37 °C for 24 hours. Two distinct colonies were discovered in a well-separated area. The pure colonies were cultured aerobically in Luria-Bertani broth (LB) at 27°C with 150 rev min $^{-1}$ agitation in a shaking incubator (7).

Table 1. Bacterial colonies isolated from the water sample

Plate No	Colony	Isolated
1	Colony 1	PSW
2	Colony 4	PSW1

Quantification of Quorum Sensing

The quantification of quorum sensing was carried out by the tube method. Firstly, 0.5g of glucose was added to 10ml of Luria-Bertani broth (LB). This broth was autoclaved and then chilled. This sterile broth was inoculated with a loop full of colonies and incubated for 24 h at 35 ° C. The tubes were discarded after the incubation period and

Journal of Chemical Health Risks
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JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727

washed with Phosphate saline buffer (pH=7.3). The tubes were then stained with crystal violet stain and washed with distilled water to remove any excess stains (8). Tubes were inverted and allowed to dry completely. Biofilm formation was graded as 1-weak/none, 2-moderate, or 3-high/strong.

Anti- Bacterial Activity

The Well-diffusion technique was used to test antibacterial activity. The culture was inoculated into sterile peptone water and left for 5 hours to incubate. Petri plates were sterilised, and 20ml of Muller-Hinton Agar was prepared for each plate. The medium was preserved for sterilisation and then poured onto the plate after sterilisation. The test culture was swabbed on top of the solidified medium and allowed to dry. Three wells were made on the media using sterile well puncture. Cinnamon extract was applied to the well in various concentrations. The plates were incubated at 30°C for 22-24 hours, and growth-inhibiting zones were observed (9).

Interaction of molecular analysis

Retrieval of structures

The three-dimensional (3D) structure of the LasR protein of *Pseudomonas aeruginosa* (PDB ID:2D5L) was retrieved from the RCSB protein data bank (http://www.rcsb.org/pdb). All water molecules were removed and Kollman charges were assigned to the protein structure.

Molecular Docking

Molecular docking was carried out by using the AutoDock Tools 4.2 graphical user interface (10). The LasR protein of *Pseudomonas aeruginosa* and the cinnamaldehyde compound from cinnamon were taken as receptor and ligand, respectively, followed by docking analysis. Kollman united atom charges, polar hydrogen and solvation parameters were added into the receptor PDB file for protein preparation in docking simulation. The grid box size was set to the maximum of 120 A°, 120 A° and 120 A° (x, y, and z) to encompass all the amino acid residues that exist in rigid macromolecules. Auto Grid 4.2 Program, furnished with Auto Dock 4.2, was used to produce grid maps. 0.375 angstroms is the spacing between grid factors. The Lamarckian Genetic Algorithm (LGA) forty-eight was chosen search for the best conformers. During the docking process, most of the 10 conformers were considered. The population measurement was set to a hundred and fifty, and the individuals were initialised randomly.

Visualisation

Auto dock results were analysed for interaction studies and the binding energy of the docked structure. The docking results were visualised using the Biovia Discovery Studio visualizer tool for molecular visualisation. The overlapping of atomic orbitals forms a bond between two atoms. Only at certain distances between the atoms do atomic orbitals overlap to form molecular orbitals.

Results and Discussion

Extraction of Cinnamon Extract

The solvent extraction of 50g of Cinnamon powder against 300ml of Methanol, solvents by maceration method for 3 days in a shaker. The collected extracts were washed and dried before being dissolved in a suitable concentration of dimethyl sulfoxide (DMSO). Methanol was selected due to its high polarity, which enables efficient solubilization of key cinnamon phytochemicals such as cinnamaldehyde, eugenol, and other phenolic derivatives that are known to exhibit antimicrobial and quorum-sensing inhibitory properties (11) . The solvent-to-sample ratio (50 g of cinnamon powder in 300 mL of methanol) ensured adequate solvent penetration and mass transfer, thereby enhancing extraction efficiency.

Maceration over a period of three days with continuous agitation in a shaker facilitated

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JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727

prolonged contact between the solvent and plant matrix, promoting the diffusion of intracellular bioactive compounds into the solvent phase. The dried extract was reconstituted in dimethyl sulfoxide (DMSO), a solvent commonly used in antimicrobial and quorum-sensing studies due to its ability to dissolve both polar and non-polar compounds. At appropriate concentrations, DMSO exhibits minimal interference with bacterial growth and cellular signalling, making it suitable for bioactivity evaluation. This step ensured uniform dispersion of the extract and accurate dosing during antibacterial and quorum-sensing inhibition assays (12).

Fig.1 Powdered Cinnamon sticks and methanolic extract of Cinnamon

Isolation of Bacteria

A swab was taken from the sides of the bottle and inoculated in nutrient agar medium. This was kept at room temperature for 24 hours. And were streaked in MacConkey agar media . In a well-separated region, two distinct colonies were discovered. The pure colonies were cultured aerobically in Luria-Bertani broth, in a shaking incubator, at 27°C with 150 rev min^{-1} agitation. Initial inoculation of swab samples onto nutrient agar and incubation at room temperature for 24 hours allowed for the recovery of heterotrophic bacteria associated with surface-adhered biofilms. Subsequent streaking onto MacConkey agar facilitated the isolation of pure cultures and provided preliminary differentiation based on lactose fermentation characteristics, enabling selective enrichment of Gram-negative bacteria commonly implicated in quorum sensing and biofilm formation(13).

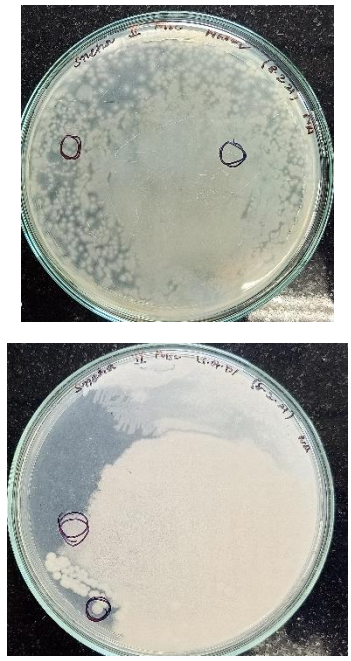

Fig.2 Lawn culture and distinct bacterial colonies isolated from water bottle biofilm

Molecular Identification of Bacterial Isolates

Pure cultures obtained from the biofilm-associated water sample were designated as PSW and PSW1 and subjected to morphological and molecular characterisation to establish their identity and phylogenetic relationships. Morphological analysis of both isolates, including colony characteristics and growth patterns, provided preliminary differentiation between PSW and PSW1, indicating phenotypic diversity within the biofilm microbial community. Such diversity is commonly observed in surface-associated bacterial populations and is often linked to adaptive strategies such as quorum–sensing–mediated coordination and biofilm formation (14).

Journal of Chemical Health Risks
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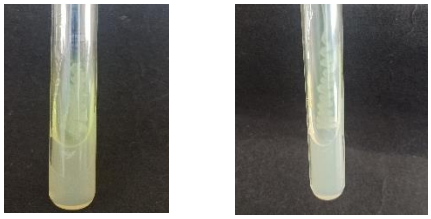
JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727



(a) (b)

Fig.3 Pure culture of (a) PSW and (b) PSW1

Table 2. Colony morphology and characterisation of the isolates

S. No	Name of the Test	PSW	PSW1
1	**Colour**	Green	Cream
2	**Appearance**	Smooth	Mucoid
3	**Form**	Circular	Circular
4	**Margin**	Undulate	Entire
5	**Elevation**	Flat	Convex
6	**Morphology**	Rods	Rods
7	**Motility**	Motile	Motile

To achieve accurate taxonomic identification, the 16S rRNA gene sequence of isolate PSW was analysed, as this gene is highly conserved among prokaryotes and serves as a reliable molecular marker for bacterial identification. Sequencing and subsequent submission of the 16S rRNA gene to the NCBI database enabled standardised documentation and ensured reproducibility and accessibility of the data for future comparative studies. The molecular identification complemented the morphological observations, overcoming the limitations of phenotype-based classification, which can be influenced by environmental conditions and growth media (15).

>PSW_contig_1

CTCAGATTGAACGCTGGCGGCAGGCCT
AACACATGCAAGTCGAGCGGATGAAGGGAGCT
TGCTCCTGGATTCAGCGGCGGACGGGTGAGTA
ATGCCTAGGAATCTGCCTGGTAGTGGGGGATA
ACGTCCGGAAACGGGCGCTAATACCGCATACG
TCCTGAGGGAGAAAGTGGGGGATCTTCGGACC
TCACGCTATCAGATGAGCCTAGGTCGGATTAGC
TAGTTGGTGGGGTAAAGGCCTACCAAGGCGAC
GATCCGTAACTGGTCTGAGAGGATGATCAGTC
ACACTGGAACTGAGACACGGTCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGGACAATG
GGCGAAAGCCTGATCCAGCCATGCCGCGTGTG
TGAAGAAGGTCTTCGGATTGTAAAGCACTTTAA
GTTGGGAGGAAGGGCAGTAAGTTAATACCTTG
CTGTTTTGACGTTACCAACAGAATAAGCACCGG
CTAACTTCGTGCCAGCAGCCGCGGTAATACGA
AGGGTGCAAGCGTTAATCGGAATTACTGGGCG
TAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA
TGTGAAATCCCCGGGCTCAACCTGGGAACTGC
ATCCAAAACTACTGAGCTAGAGTACGGTAGAG
GGTGGTGGAATTTCCTGTGTAGCGGTGAAATGC
GTAGATATAGGAAGGAACACCAGTGGCGAAGG
CGACCACCTGGACTGATACTGACACTGAGGTG
CGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTA
GCCGTTGGGATCCTTGAGATCTTAGTGGCGCAG
CTAACGCGATAAGTCGACCGCCTGGGGAGTAC
GGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGAAGAACCTTACCTGGC
CTTGACATGCTGAGAACTTTCCAGAGATGGATT
GGTGCCTTCGGGAACTCAGACACAGGTGCTGC
ATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGTAACGAGCGCAACCCTTGTCC
TTAGTTACCAGCACCTCGGGTGGGCACTCTAAG
GAGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAGTCATCATGGCCCTTACGGCC
AGGGCTACACACGTGCTACAATGGTCGGTACA
AAGGGTTGCCAAGCCGCGAGGTGGAGCTAATC
CCATAAAACCGATCGTAGTCCGGATCGCAGTCT
GCAACTCGACTGCGTGAAGTCGGAATCGCTAG
TAATCGTGAATCAGAATGTCACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCA
TGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTA
ACCGCAAGGGGGACGGTTACCACGGAGTGATT
CATGACTGGGGTGA

Fig 4. 16s RNA sequence of the isolate PSW

Phylogenetic analysis based on the 16S rRNA gene sequence further elucidated the evolutionary placement of isolate PSW among closely related bacterial taxa. The constructed phylogenetic tree demonstrated the genetic

Journal of Chemical Health Risks
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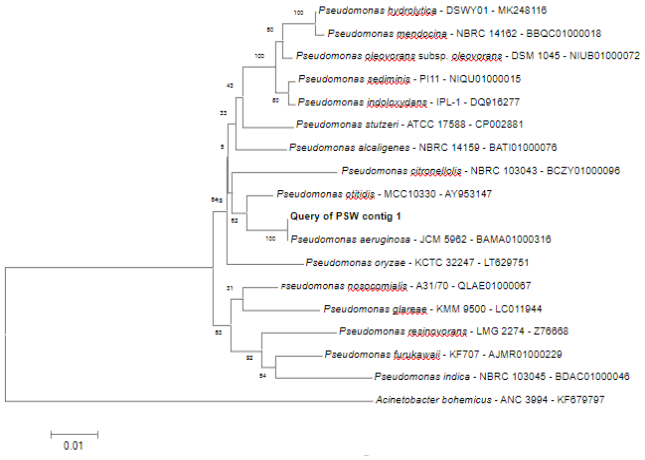
JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727

relationship of PSW with reference strains, confirming its taxonomic affiliation and highlighting its evolutionary proximity to quorum–sensing–competent and biofilm-forming bacteria (16). Such phylogenetic positioning is particularly relevant in the context of this study, as it supports the selection of PSW for subsequent quorum-sensing inhibition and molecular interaction analyses.



S.No	Strain	Isolate name	Accession Number
1	PSW	*Pseudomonas aeruginosa*	MW757180

Fig 5. Phylogenetic tree of isolate PSW

Quorum Sensing:

Biofilm formation by the selected bacterial isolates was quantitatively evaluated using a static culture method in Luria–Bertani (LB) broth supplemented with glucose. The addition of glucose (0.5 g) served as an external carbon source to enhance bacterial adhesion and extracellular polymeric substance (EPS) production, both of which are critical determinants of biofilm development. Glucose-mediated stimulation of biofilm formation has been widely reported, as increased carbohydrate availability promotes quorum-sensing activity and facilitates the transition from planktonic to sessile growth (17).

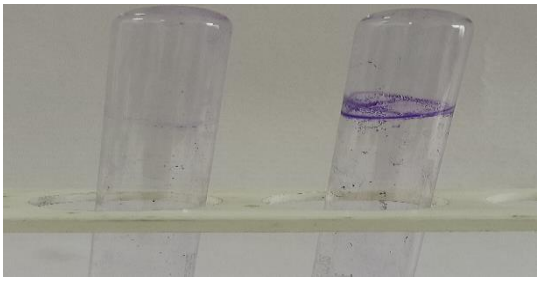


Fig 6. Biofilm formation of isolates PSW and PSW1, respectively

Biofilm formation was subsequently measured and categorised based on the criteria outlined in Table 3, which enabled comparative evaluation of the isolates according to their biofilm-forming capacity. Grading the biofilm intensity provided a semi-quantitative framework to distinguish weak, moderate, and strong biofilm producers. Such classification is particularly valuable in quorum-sensing studies, as strong biofilm-forming isolates are often associated with enhanced cell–cell communication and increased resistance to antimicrobial agents (18).

The observed differences in biofilm formation among the isolates underscore the inherent variability in surface adhesion and biofilm regulatory mechanisms. These findings support the selection of robust biofilm-forming strains for downstream analyses, including quorum-sensing inhibition assays and molecular interaction studies.

Table 4. Scoring of the isolates

S. No	Isolates	Score
1	PSW1	1-Weak
2	PSW	3-Strong

Anti-bacterial Activity

The antibacterial activity of the cinnamon methanolic extract was evaluated against two bacterial isolates, PSW and PSW1, using the Kirby–Bauer agar well diffusion method. The extract, prepared by dissolving 5.3 g of crude extract in 6

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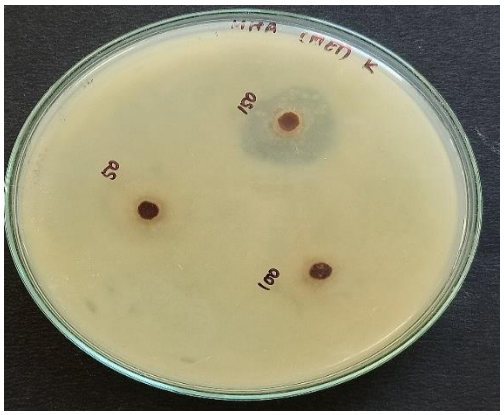
mL of DMSO, was tested at increasing concentrations (50 µL, 100 µL, and 150 µL). Clear zones of inhibition were observed around the wells in both plates, indicating that the cinnamon extract possesses antibacterial activity against both isolates. The diameter of the inhibition zones increased with increasing extract concentration, demonstrating a dose-dependent antibacterial effect (19). Among the two isolates, PSW exhibited a larger zone of inhibition compared to PSW1 at corresponding concentrations, suggesting that PSW is more susceptible to the cinnamon extract.

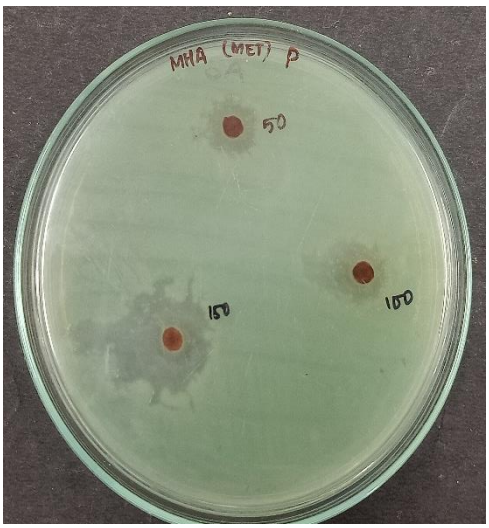
Table 5. Zones of Inhibition produced by methanolic extracts of Cinnamon

Isolate	Concentration (µL)	Zone of Inhibition (mm)
PSW1	50	6mm
	100	9mm
	150	12mm
PSW	50	8mm
	100	12mm
	150	15mm

The observed zones of inhibition confirm that the cinnamon extract exhibits significant antibacterial activity against both isolates. The effectiveness of the extract increased with concentration, indicating a clear dose-dependent response, which is a characteristic feature of bioactive phytochemical-mediated antimicrobial activity (20). Isolate PSW showed comparatively larger inhibition zones than PSW1 across all concentrations tested, suggesting differential susceptibility between the two isolates. Such variation may be attributed to differences in cell wall structure, membrane permeability, metabolic activity, or quorum–sensing–regulated resistance mechanisms. Environmental isolates often exhibit heterogeneous responses to phytochemicals, particularly those involved in biofilm formation (21). The antibacterial effect observed can be primarily attributed to bioactive constituents of cinnamon, such as cinnamaldehyde and other phenolic compounds, which are known to disrupt bacterial cell membranes, alter enzyme activity, and interfere with quorum-sensing pathways. Dissolution of the extract in DMSO ensured effective diffusion of these compounds into the agar medium, enabling consistent antimicrobial action (22).



(a)



(b)

Fig 6. Anti-Bacterial activity of (a) PSW and (b) PSW1, respectively

Journal of Chemical Health Risks
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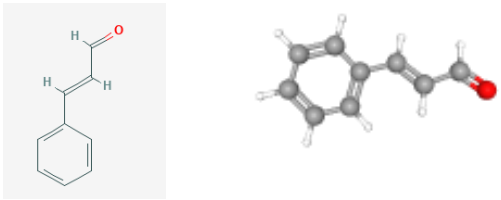
JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727

Computational analysis

Molecular docking analysis was performed to investigate the interaction between cinnamaldehyde, the major bioactive compound of Cinnamomum verum, and the quorum-sensing regulatory protein LasR of *Pseudomonas aeruginosa*. Prior to that, the ligand was optimised using ACD ChemSketch and saved in a compatible format using the Open Babel Molecular converter tool (23). The ligand structures that were discovered are depicted in the figure 7.



(a) (b)

Fig 7. (a) 2D and (b) 3D structure of cinnamaldehyde compound of *Cinnamomum verum*

The best conformers were selected using the Lamarckian Genetic Algorithm (LGA) based on the most favorable ligand–receptor orientation, characterised by the lowest binding energy and minimal solvent accessibility (24). This selection criterion ensured identification of a stable and biologically relevant binding pose.

Visualization of the docked complex using BIOVIA Discovery Studio revealed a stable interaction between cinnamaldehyde and the LasR protein, represented in a stick model. The docking results indicated that cinnamaldehyde binds within the active site of LasR, forming a hydrogen bond interaction with key amino acid residues involved in quorum sensing regulation. The predicted free binding energy for this interaction was −5.1 kcal/mol, suggesting a thermodynamically favorable and stable ligand–protein complex. The presence of a hydrogen bond further supports the specificity and strength of the interaction, which is critical for effective inhibition of transcriptional regulators such as LasR (25).

By occupying the ligand-binding domain of LasR, cinnamaldehyde is likely to disrupt the binding of native autoinducers, thereby inhibiting downstream quorum–sensing–regulated gene expression. However, exposure to cinnamon extract resulted in a significant reduction in antibacterial growth, biofilm formation, and quorum-sensing responsiveness (26). This phenotypic inhibition supports the molecular docking results, suggesting that cinnamaldehyde-mediated interaction with LasR plays a pivotal role in disrupting quorum-sensing pathways (27).

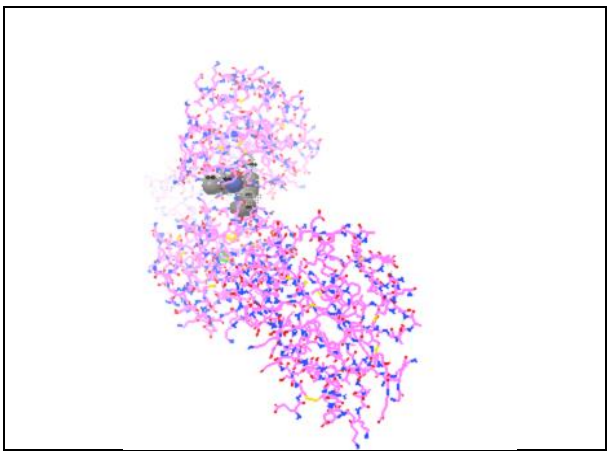


Fig 8. Docked conformation of cinnamaldehyde interacting with the LasR protein of Pseudomonas aeruginosa, highlighting the stable ligand–protein complex within the quorum-sensing regulatory domain.

Conclusion

This study was designed to identify novel quorum-sensing inhibitory activity and elucidate its underlying mechanisms using *Cinnamomum verum* methanolic extract. Through systematic extraction of cinnamon phytochemicals, isolation of biofilm-forming bacteria from water samples, quantification of quorum-sensing activity, and evaluation of

Journal of Chemical Health Risks

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JCHR (2026) 16(1), 2198-2207 | ISSN:2251-6727

antibacterial efficacy, the work establishes a comprehensive experimental framework. Furthermore, molecular interaction analysis between cinnamaldehyde and the *Pseudomonas aeruginosa* LasR protein provides mechanistic insight into quorum-sensing disruption. Collectively, these approaches strengthen the potential of cinnamon-derived compounds as effective quorum-sensing inhibitors and alternative strategies for controlling biofilm-associated bacterial infections. From the present study it can been concluded that the strain PSW *Pseudomonas eruginosa* showed sensitivity against cinnamon. The results indicate that a small amount of cinnamon ingested in any form is beneficial to human health by inhibiting quorum sensing and preventing bacterial pathogenesis.

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