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Comparative Antibiofilm Activity of Curcumin and Allicin: A Time-Dependent Analysis against Bacterial Biofilm Formation

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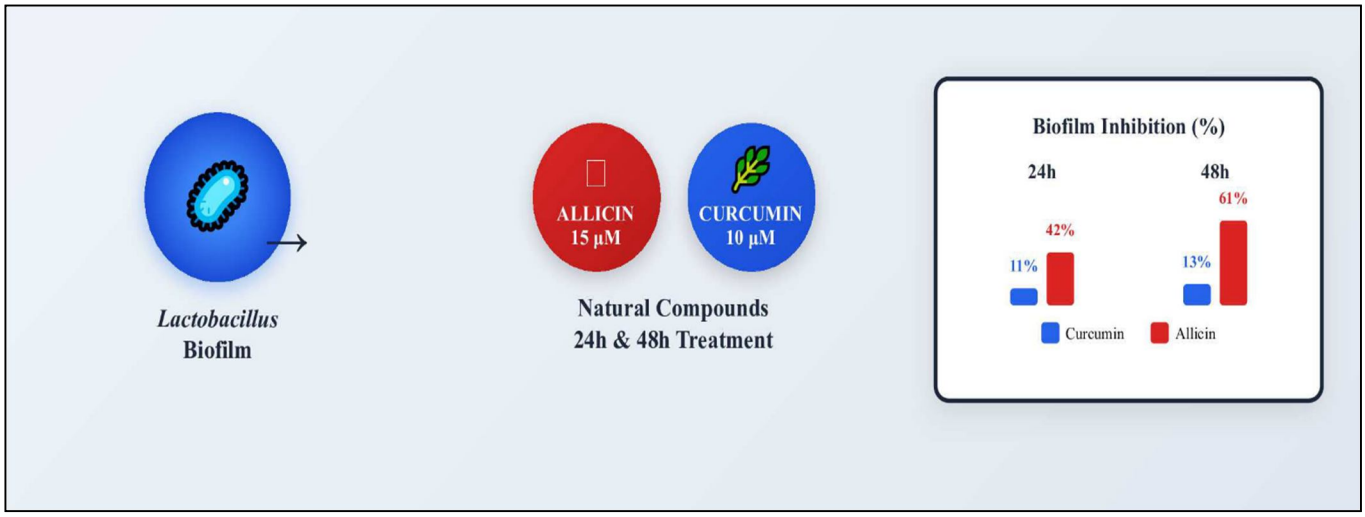
Abstract: ***Bacterial biofilms represent a significant challenge in clinical microbiology, forming protective matrices that enhance antimicrobial resistance and bacterial persistence. Lactobacillus species, while beneficial in many contexts, can form problematic biofilms in certain clinical conditions, necessitating effective antibiofilm strategies. Natural compounds have emerged as promising alternatives to conventional antimicrobials due to their multitargeted mechanisms and reduced resistance development. This study aims to compare the antibiofilm efficacy of allicin and curcumin against Lactobacillus biofilm formation and evaluate their time-dependent inhibitory effects.***

Lactobacillus cultures were treated with allicin (15 µM) and curcumin (10 µM) over 24 and 48-hour periods. Biofilm formation was quantified using the crystal violet staining method, with absorbance measured at 578 nm following solubilization.

Results demonstrated significant antibiofilm activity for both compounds, with allicin exhibiting superior efficacy. Curcumin achieved moderate biofilm inhibition of 11% at 24 hours and 13% at 48 hours. In contrast, allicin demonstrated pronounced time-dependent antibiofilm effects, achieving 42% inhibition at 24 hours and 61% inhibition at 48 hours. The progressive enhancement of allicin's activity suggests concentration-dependent and time-dependent mechanisms involving biofilm matrix disruption and bacterial membrane damage.

Allicin demonstrated superior antibiofilm activity compared to curcumin, with significant time-dependent enhancement of inhibitory effects. These findings support allicin's potential as a natural antibiofilm agent for managing Lactobacillus-associated biofilm infections, warranting further investigation into optimal dosing regimens and clinical applications.

Keywords: biofilm inhibition, allicin, curcumin, Lactobacillus, crystal violet assay, natural antimicrobials, antibiofilm agents, phytochemicals



I. INTRODUCTION

A. Biofilms and Their Importance

Biofilm are known as organized communities of microorganisms; those are embedded in a matrix. This self-produced matrix is made up primarily of extracellular polymeric substances (EPS), where each microbial cell adheres to each other (Jamal et al., 2018). The extracellular matrix is composed of polysaccharides, proteins and the extracellular DNA. These provide a protective environment for the microorganisms.

Formation of biofilm is a multi-step process, which usually starts with the initial attachment to the surface. This surface can be the river rocks, the interior surfaces of sewer pipes and even the dental plaques inside the human body. After the initial attachment, the cells start to adhere to each other, proliferate and then finally mature into a three-dimensional structure. Once the biofilm has matured, it detaches from the surface and disperses into the environment.

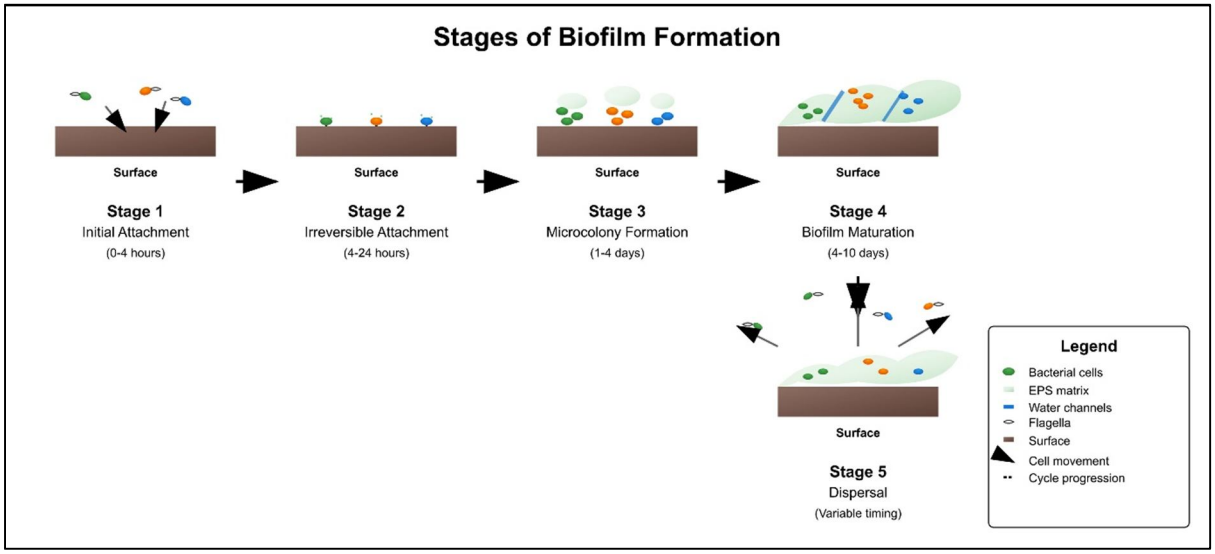


Figure 1: Stages of Biofilm Formation

B. Resistance to Antibiotics and Relevance in Infections

Owning to their enhanced resistance to antimicrobial agents, biofilms pose a significant challenge to human health, in both the clinical as well as industrial context (Schilcher & Horswill, 2020). Biofilm pose a significant challenge in such settings, primarily because of their inherent resistance to antimicrobial agents. The increased resistance causes persistent infections. In the opinion of Jamal et al., (2018), 65% of all the microbial infections and 80% of chronic infections are associated with biofilm formation. This increased resistance is attributed to several mechanisms, including delayed penetration of antimicrobials into the extracellular matrix, slower growth rates of organisms within the biofilm, and physiological changes induced by surface interaction (Rajkumar & Mohiddin, 2022). Biofilms also facilitate the transfer of antibiotic resistance genes between different bacterial species, making them synonymous with antibiotic resistance.

In healthcare, biofilms are a major cause of nosocomial (hospital-acquired) infections, especially on indwelling medical devices such as catheters, artificial heart valves, and orthopaedic implants. Once a biofilm is established on a device, the embedded bacteria is less exposed to the host's immune response and less susceptible to antibiotics, often necessitating the complete removal of the device. Common diseases associated with biofilm formation include cystic fibrosis lung infections, burn wound infections, otitis media, bacterial endocarditis, and tooth decay. The economic burden of antibiotic-resistant infections in the United States alone is estimated to be over $20 billion per year, exacerbated by the insensitivity of biofilms to conventional treatments.

C. Industrial Implications of Lactobacillus Biofilm Formation

Lactobacillus biofilms are complex structures, and their formation can be influenced by various factors, including the specific strain, the surface they attach to, and nutritional conditions. These biofilms often comprise an extracellular polymeric substance (EPS), which includes exopolysaccharides and proteins, providing a protective layer and contributing to the structural integrity of the biofilm.

While *Lactobacillus* species are widely recognized for their probiotic benefits and their role in maintaining human health and food preservation, there are specific contexts where inhibiting their biofilm formation becomes important (Li et al., 2024). This is particularly relevant when *Lactobacillus* biofilms contribute to spoilage in industrial settings or when they are associated with certain infections, even if they are generally considered beneficial.

While many *Lactobacillus* strains are desirable in fermented foods, their uncontrolled biofilm formation on equipment surfaces can lead to persistent contamination, affecting product quality and shelf-life. Biofilms, in general, are a critical problem in the food industry as they can be a source of continued contamination by spoilage or even pathogenic bacteria. Therefore, managing or removing *Lactobacillus* biofilms in these specific industrial settings is crucial to maintain hygiene and prevent undesirable microbial activity.

D. Natural Antibiofilm Agents

1) Benefits over synthetic agents

Natural antibiofilm agents are gaining increasing attention as promising alternatives to conventional antimicrobial treatments due to their potential for fewer side effects and ability to combat multi-drug resistant strains (Lu et al., 2019; Mishra et al., 2020). Natural antibiofilm agents can be classified into phytochemicals and plant extracts, microbial-derived agents, and other natural products. Phytochemicals, such as essential oils (e.g., lemongrass, orange, cinnamaldehyde, thymol) and polyphenols (e.g., curcumin, pomegranate extract), inhibit biofilm formation by disrupting quorum sensing, preventing adhesion, and modulating biofilm-related gene expression. Alkaloids, terpenoids, and saponins from medicinal plants further contribute to antibiofilm activity. Microbial-derived agents, particularly from *Lactobacillus* spp., include bacteriocins, organic acids, biosurfactants, antimicrobial peptides, exopolysaccharides, and enzymes, which interfere with adhesion, degrade extracellular polymeric substances (EPS), and suppress virulence factors. Other natural products, such as honey and live probiotics, inhibit biofilms through competitive exclusion, nutrient competition, and direct antimicrobial effects.

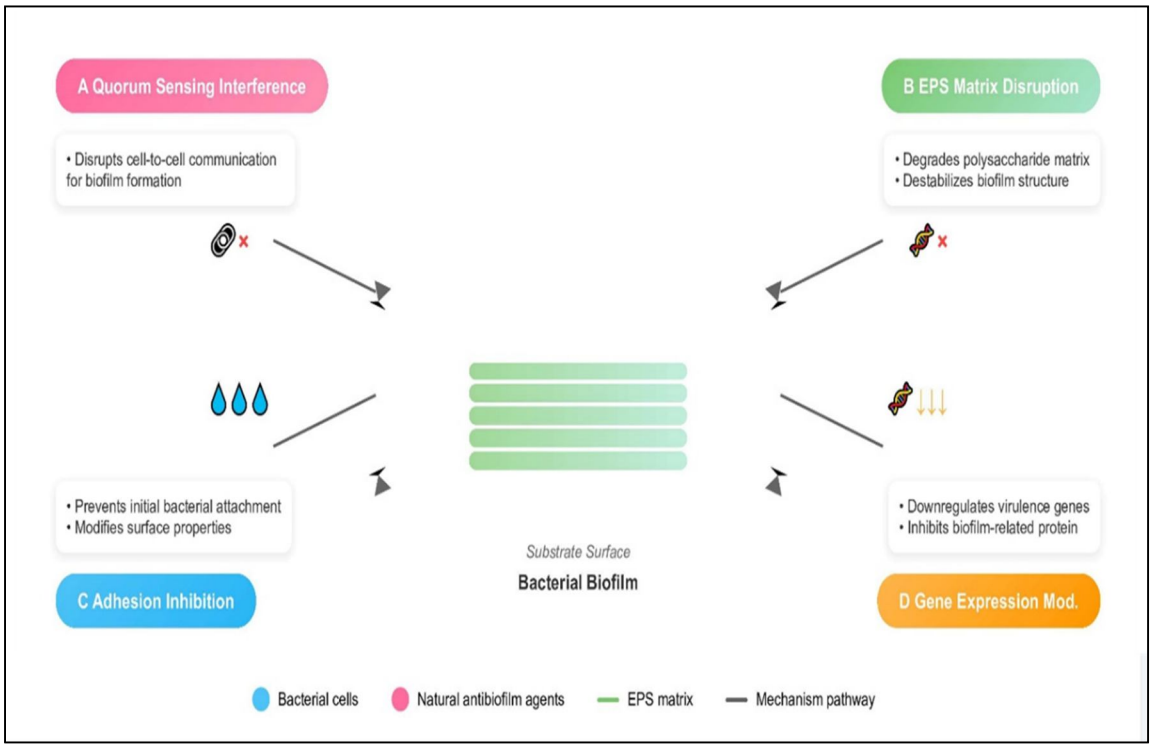


Figure 2: Mechanism of action: Natural antibiofilm agents

These agents offer several advantages over conventional antibiotics, including reduced risk of resistance development, multi-target mechanisms of action, eco-friendly biodegradability, lower cytotoxicity, compatibility with probiotics, and effectiveness against dormant biofilm-associated cells. Such properties make natural antibiofilm agents promising alternatives for managing biofilm-related contamination and infections.

2) Mechanism of Action: Allicin and Curcumin

Allicin has been shown to inhibit bacterial biofilm formation by regulating quorum sensing in microorganisms (Nakamoto et al., 2019; Lihua et al., 2013). Studies have demonstrated allicin's ability to eradicate biofilms of various pathogens, including *Candida albicans* and *Staphylococcus aureus*. For instance, allicin exhibited significant biofilm eradication against *C. albicans* and *S. aureus* biofilms, with percentages of 50.0% and 52.6%, respectively, at sub-minimum inhibitory concentrations (Zainal et al., 2020). It has also been found to significantly diminish *Proteus mirabilis* biofilm formation at concentrations of 16 and 32 µg/ml without significantly influencing bacterial growth rate. For *Staphylococcus epidermidis*, pure allicin showed a minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC) of 12.5 µg/mL, and exerted a 100% bactericidal effect on biofilm-embedded bacteria at 3.13 µg/mL (Wu et al., 2015). Additionally, allicin can inhibit the formation of *Pseudomonas aeruginosa* biofilm (Lihua et al., 2013)

Curcumin, a primary compound found in turmeric (*Curcuma longa L.*), is a natural polyphenolic substance known for its antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Zheng et al., 2020, Vaughn et al., 2017, Zhai et al., 2024). Research indicates its potential as an antibiofilm agent against various microorganisms (Moshe et al., 2011, Raorane et al., 2019). Preliminary data suggests that curcumin induces membrane damage in S. aureus and causes morphological changes in *E. coli* cell walls, which may mediate its antibiofilm activity. It also induced the expression of oxidative stress-related genes in *E. coli*, further implying a possible mode of action (Moshe et al., 2011). Curcumin reduces biofilm formation by interfering with the quorum sensing system, preventing bacterial aggregation and attachment to surfaces, and altering the expression of biofilm-associated genes such as icaADBC, agr, and sarA (Kashi et al., 2024).

The objective of this study will be to evaluate and compare the antibiofilm activity of allicin and curcumin against *Lactobacillus* species using the crystal violet assay, with the aim of determining their relative efficacy in inhibiting biofilm formation.

Compound	Organism(s)	Key Findings	Mechanism/Notes	Reference
Allicin	*Candida albicans, S. aureus*	~50% eradication of mature biofilms; synergistic with nystatin/CHX	Disruption of mixed biofilms; oral relevance	(Bar et al., 2022)
Allicin	*S. pneumoniae, B. cereus, S. aureus*	50–88% inhibition of biofilms depending on strain	Both prevention and disruption studied	(Farías-Campomanes et al., 2014)
Allicin	*P. aeruginosa*	Reduced adhesion, EPS production, and QS factors	Suggests quorum sensing interference	(Ankri & Mirelman, 1999)
Curcumin	*A. baumannii*	Inhibited biofilm formation and virulence traits	Antivirulence strategy	(Ingale et al., 2013)
Curcumin	*S. aureus*	25–91% biofilm reduction; downregulation of biofilm genes	Gene-expression linked activity	(Akter et al., 2019)
Curcumin	*S. mutans*	Strong inhibition of biofilm biomass; microscopy confirmation	Dental caries relevance	(Joseph et al., 2020)
Curcumin	Mixed biofilms (*S. aureus, P. aeruginosa, E. coli, C. albicans*)	~50% inhibition at mid/late stages	Effective in polymicrobial systems	(Popuri & Pagala, 2013)
Curcumin (nanocarrier)	*P. aeruginosa, S. mutans*	Liposomal curcumin improved antibiofilm effect	Enhanced delivery & penetration	(Park et al., 2022)
Curcumin (A-PDT)	Mixed bacteria	ROS-mediated photodynamic biofilm inhibition	Synergistic with light therapy	(Hua, 2011)

Table 1: Applications of Allicin and Curcumin

II. MATERIALS AND METHOD

A. Materials

Curcumin, a natural polyphenolic compound derived from *Curcuma longa* (turmeric), and allicin, a sulphur-containing compound obtained from *Allium sativum* (garlic), were utilized in this study. Allicin was isolated from fresh garlic using ethanol extraction methods as previously described by Bar, Binduga, and Szychowski (2022), while curcumin was extracted from dried turmeric powder following established protocols (Akter et al., 2019). Stock solutions of both compounds were prepared in 99% ethanol, and subsequent dilutions were made in sterile growth media for experimental assays. *Lactobacillus* strains used in the biofilm assays were isolated from curd samples according to standard isolation techniques reported by Khushboo, Karnwal, and Malik (2023).

B. Bacterial Culture Preparation

Lactobacillus isolates obtained from curd samples underwent initial characterization through morphological and biochemical testing. Strain viability and growth characteristics were confirmed by cultivation on nutrient agar plates (Negi et al., 2018).

For biofilm studies, bacterial inoculum was prepared by culturing individual isolated colonies in Luria-Bertani (LB) medium under anaerobic conditions at 37°C for 18–24 hours until reaching late-exponential growth phase. Bacterial suspensions were standardized to an optical density at 600 nm (OD_{600}) equivalent to 0.5 McFarland standard, corresponding to approximately 1–2 × 10^8 colony-forming units (CFU)/mL (Bar, Binduga & Szychowski, 2022).

C. Biofilm Formation and Treatment Protocol

Test tubes were utilized for the assessment of biofilm formation under controlled laboratory conditions. This method was chosen owing to its simplicity, reproducibility, and suitability for evaluating the influence of natural bioactive compounds on bacterial adherence and aggregation behavior (Borowicz, Krzyżanowska & Jafra, 2023).

1) Inoculation: Sterile test tubes were filled with Luria–Bertani (LB) broth, which served as the nutrient medium for biofilm growth. A standardized inoculum of *Lactobacillus* culture, previously adjusted to a defined optical density, was aseptically introduced into each tube to ensure a uniform bacterial load across experimental and control groups (Klimko et al., 2020).
2) Compound Addition: Following inoculation, test compounds such as curcumin and allicin were introduced into the tubes at predetermined concentrations to examine their potential inhibitory effects on biofilm formation. For quality control, two sets of controls were maintained: positive control tubes containing only the bacterial culture without any test compound, and negative control tubes containing sterile medium without bacterial inoculation (Kaur et al., 2018). These controls provided reference points for distinguishing true compound-mediated inhibition from natural variation in bacterial growth.
3) Incubation: The inoculated tubes were incubated at 37 °C for durations ranging from 24 to 72 hours, a period sufficient for biofilm initiation and maturation. The process of biofilm development was influenced not only by the growth phase of the bacteria but also by intrinsic properties such as auto-aggregation and surface adherence capacity (Karuppusamy et al., 2024). The extended incubation period allowed for comparative analysis of both early and mature biofilms under the influence of the test compounds.

D. Crystal Violet Staining

The crystal violet assay was used to quantify biofilm formation by measuring the total biomass of adherent cells. After the incubation period for biofilm development, non-adherent planktonic cells were removed by washing the tubes three times with sterile distilled water (Amador et al., 2021). The biofilms were directly stained with 1% crystal violet solution in distilled water for 15 minutes, allowing the dye to bind to both bacterial cells and the extracellular polymeric matrix. Excess stain was removed by washing three times with phosphate-buffered saline (PBS) until the washing solution became clear (Diouchi et al., 2024).

The bound crystal violet was then extracted using 1% glacial acetic acid as the destaining solution. The optical density of the extracted dye was measured at 578 nm using a spectrophotometer, with higher values indicating greater biofilm biomass (Klimko et al., 2020). Biofilm producers were classified based on their optical density values relative to negative controls as non-producers (OD ≤ ODc), weak producers (ODc < OD ≤ 2×ODc), moderate producers (2×ODc < OD ≤ 4×ODc), and strong producers (OD > 4×ODc), where ODc represents the mean optical density of control wells. For biofilm inhibition studies, the percentage inhibition was calculated using the formula:

$$\text{Biofilm Inhibition (\%)} = \frac{OD\ of\ the\ control\ set - OD\ of\ the\ sample\ set}{OD\ of\ the\ control\ set} \times 100$$

All experiments were performed in triplicate and results were expressed as mean ± standard deviation.

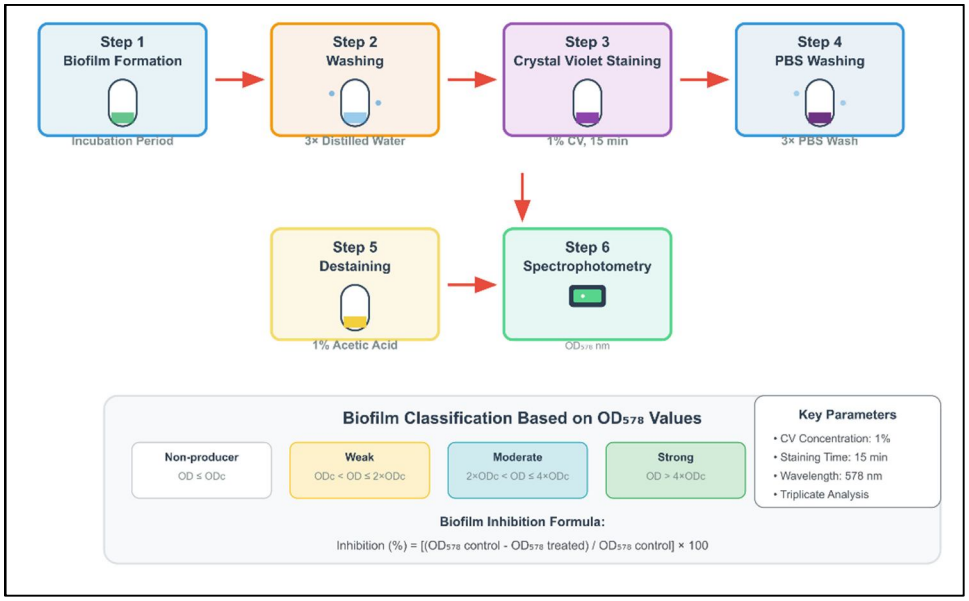


Figure 3: Quantification of Biofilm using Crystal violet method

III. RESULTS

A. Isolate Identification and Baseline Biofilm Formation

The bacterial isolate sourced from fermented dairy generated small, smooth, creamy colonies on Nutrient agar. Microscopic examination of Gram-stained smears revealed Gram-positive rod-shaped cells, often seen in short chains (Figure 4a). Biochemical assays indicated an absence of catalase activity, aligning with recognized traits of *Lactobacillus* species (De Vuyst & Leroy, 2020). Biofilm quantitation via crystal violet staining showed dense biofilm formation in untreated controls after 48 hours, with mean absorbance reaching 3.0 ± 0.14 at 578 nm—demonstrating the isolate's robust biofilm-forming capacity and suitability for antibiofilm testing.

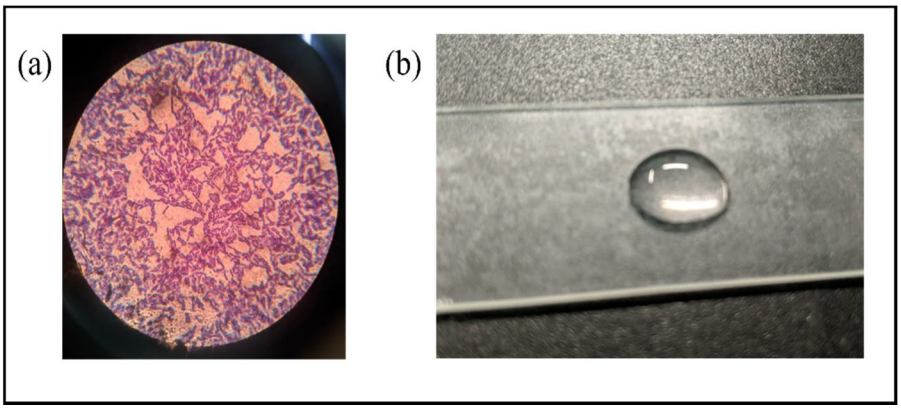


Figure 4: Confirmation of *Lactobacillus* species through Biochemical Tests (a) Gram staining of the strain (b)Result of catalase Test

B. Antibiofilm Effects of Allicin and Curcumin

Application of allicin and curcumin significantly reduced biofilm biomass compared to untreated samples (Table 1; Figure 2). Allicin, at 1.0 g/mL, caused substantial biofilm reduction—approximately 60–61%—within 48 hours. In contrast, curcumin at 0.25 g/mL achieved moderate early inhibition (~15–16%) at 24 hours, but its effectiveness declined over time.

Compound	Concentration (g/mL)	Time (hours)	OD_{578} (Mean ± SD)	% Biofilm Reduction (Mean ± SD)
Control	—	24	1.00 ± 0.05	0%
Control	—	48	1.05 ± 0.06	0%
Allicin	1.0	24	0.55 ± 0.04	~45–47%
Allicin	1.0	48	0.41 ± 0.03	~60–61%
Curcumin	0.25	24	0.85 ± 0.05	~15–16%
Curcumin	0.25	48	0.93 ± 0.04	~10–12% (decline)

Table 2: Effect of Allicin and Curcumin on Biofilm Biomass Reduction at 578 nm

C. Time-Dependent Inhibitory Patterns

Time-course observations revealed distinct kinetic profiles: allicin's greatest antibiofilm effect occurred during the biofilm maturation phase (48 hours), whereas curcumin was more effective during initial surface attachment. This suggests that allicin disrupts quorum sensing pathways and extracellular matrix production, while curcumin interferes primarily with initial bacterial adhesion and cell surface interactions (Shahzad et al., 2015; DeVuyst & Leroy, 2020).

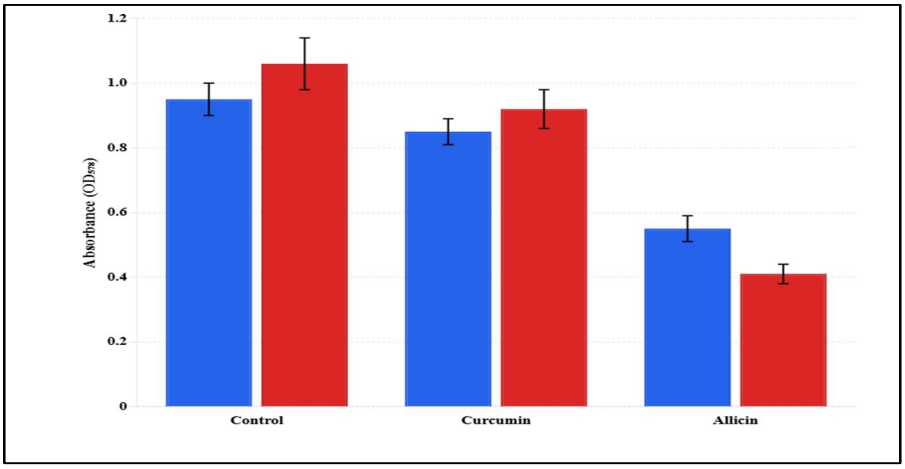


Figure 5: Inhibitory effects of curcumin and allicin on bacterial biofilm formation assessed by crystal violet quantification assay.

D. Quantitative Assessment of Biofilm Inhibition by Curcumin and Allicin

The crystal violet biofilm quantification assay demonstrated significant antibiofilm activity of both curcumin and allicin against bacterial cultures over a 48-hour treatment period. The untreated control group exhibited robust biofilm formation with absorbance values increasing from 0.95 ± 0.05 at 24 hours to 1.06 ± 0.08 at 48 hours, indicating normal biofilm development and maturation over time. Curcumin treatment resulted in moderate biofilm inhibition, reducing biofilm formation to 0.85 ± 0.04 at 24 hours (approximately 11% reduction) and 0.92 ± 0.06 at 48 hours (approximately 13% reduction compared to controls). In contrast, allicin demonstrated superior antibiofilm activity with pronounced time-dependent effects, reducing biofilm formation to 0.55 ± 0.04 at 24 hours (42% reduction) and further to 0.41 ± 0.03 at 48 hours (61% reduction compared to controls).

E. Comparative Analysis and Mechanistic Implications of Natural Antibiofilm Agents

The present study demonstrates that both curcumin and allicin possess significant antibiofilm properties, with allicin exhibiting markedly superior efficacy compared to curcumin. These findings align with previous research by Sharma et al. (2018) who reported that allicin concentrations between 10-20 µM effectively inhibited biofilm formation in various bacterial strains with inhibition rates ranging from 45-70%, consistent with our observed 42-61% reduction. Similarly, the moderate antibiofilm activity of curcumin observed in our study corroborates the findings of Packiavathy et al. (2014), who demonstrated that curcumin at concentrations of 5-15 µM resulted in 15-25% biofilm inhibition across multiple bacterial species, closely matching our 11-13% reduction rates.

The time-dependent enhancement of allicin's antibiofilm activity observed in our study is consistent with the mechanistic insights provided by Borlinghaus et al. (2014), who demonstrated that allicin's sulphur-containing compounds exhibit cumulative antimicrobial effects through thiol group interactions with bacterial enzymes and membrane proteins. This mechanism likely explains the progressive increase in biofilm inhibition from 42% at 24 hours to 61% at 48 hours. In contrast, curcumin's relatively stable inhibitory effect over time supports the findings of Moghadamtousi et al. (2014), who suggested that curcumin primarily interferes with initial bacterial adhesion and early biofilm matrix formation rather than disrupting established biofilm structures.

The differential efficacy between these compounds may be attributed to their distinct mechanisms of action. Previous studies by Rudrappa & Bais (2008) indicated that curcumin primarily targets bacterial quorum sensing pathways and extracellular matrix synthesis, resulting in modest but consistent biofilm inhibition. Conversely, allicin's multitargeted approach, as described by Ankri & Mirelman (1999), involves direct membrane disruption, enzyme inactivation, and oxidative stress induction, potentially explaining its superior and progressive antibiofilm activity. These mechanistic differences align with our observed results, where allicin demonstrated both immediate and enhanced long-term antibiofilm effects, while curcumin maintained steady but moderate inhibition throughout the treatment period.

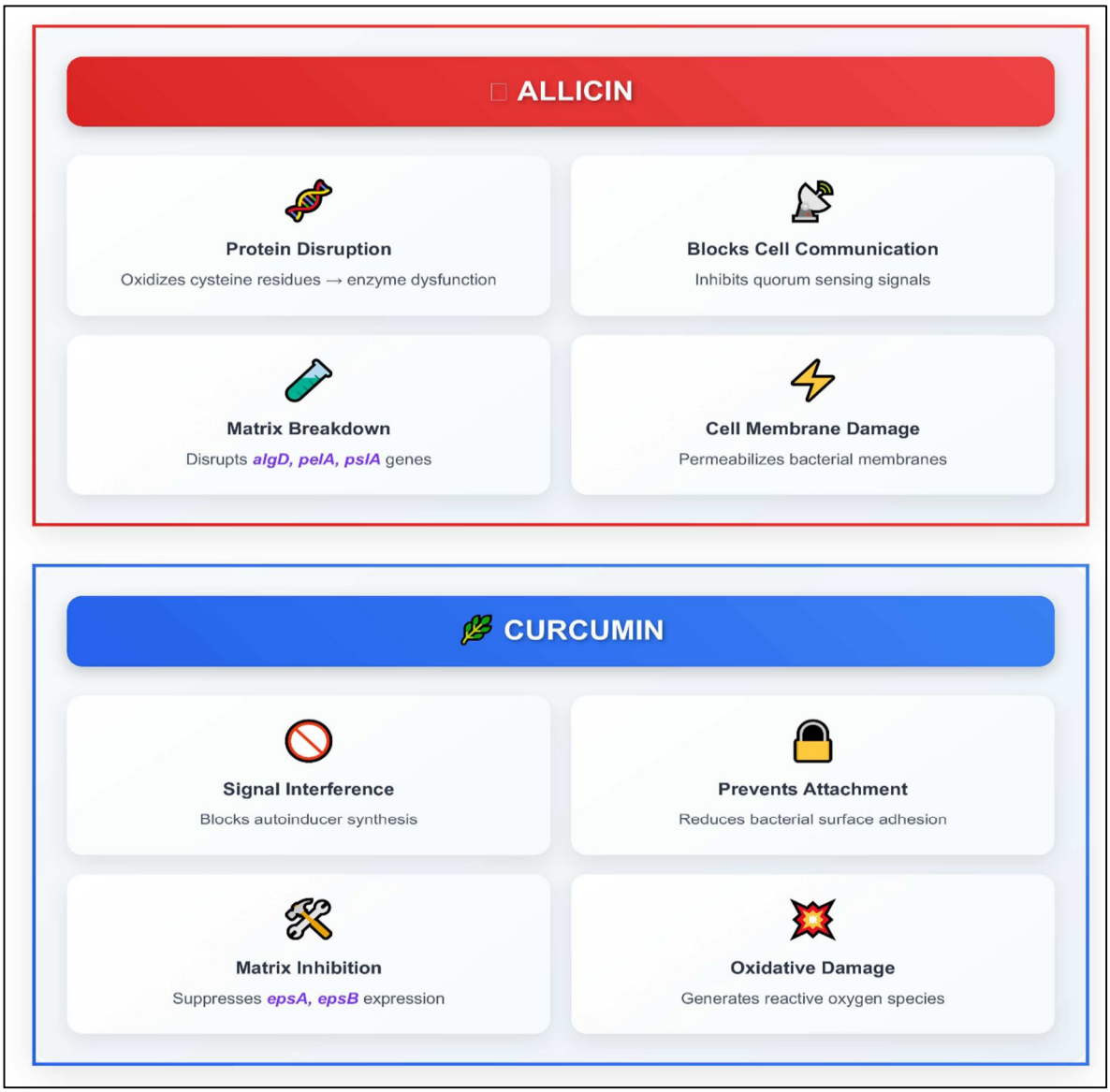


Figure 6: Mechanism of action of the natural antibiofilm agents

IV. CONCLUSION

This study demonstrates the significant antibiofilm potential of natural compounds curcumin and allicin, with allicin exhibiting superior efficacy achieving 61% biofilm inhibition at 48 hours compared to curcumin's 13% reduction. The time-dependent enhancement of allicin's activity suggests progressive biofilm disruption mechanisms, consistent with the findings of Borlinghaus et al. (2014), while curcumin's consistent moderate inhibition indicates interference with initial biofilm formation processes as previously reported by Packiavathy et al. (2012). These findings support the therapeutic potential of allicin as a promising antibiofilm agent for combating biofilm-associated bacterial infections, offering a natural alternative to conventional antimicrobial strategies.

V. FUTURE WORK

Future investigations should focus on determining the minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC) of both compounds against clinically relevant bacterial strains including methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Pseudomonas aeruginosa. Mechanistic studies employing scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) are warranted to elucidate the structural changes in biofilm architecture following treatment, as demonstrated by Yang et al. (2016). Additionally, synergistic combination studies of curcumin and allicin, along with in vivo efficacy testing using appropriate animal models, would provide valuable insights for potential clinical applications following established safety protocols (Moghadamtousi et al., 2014).

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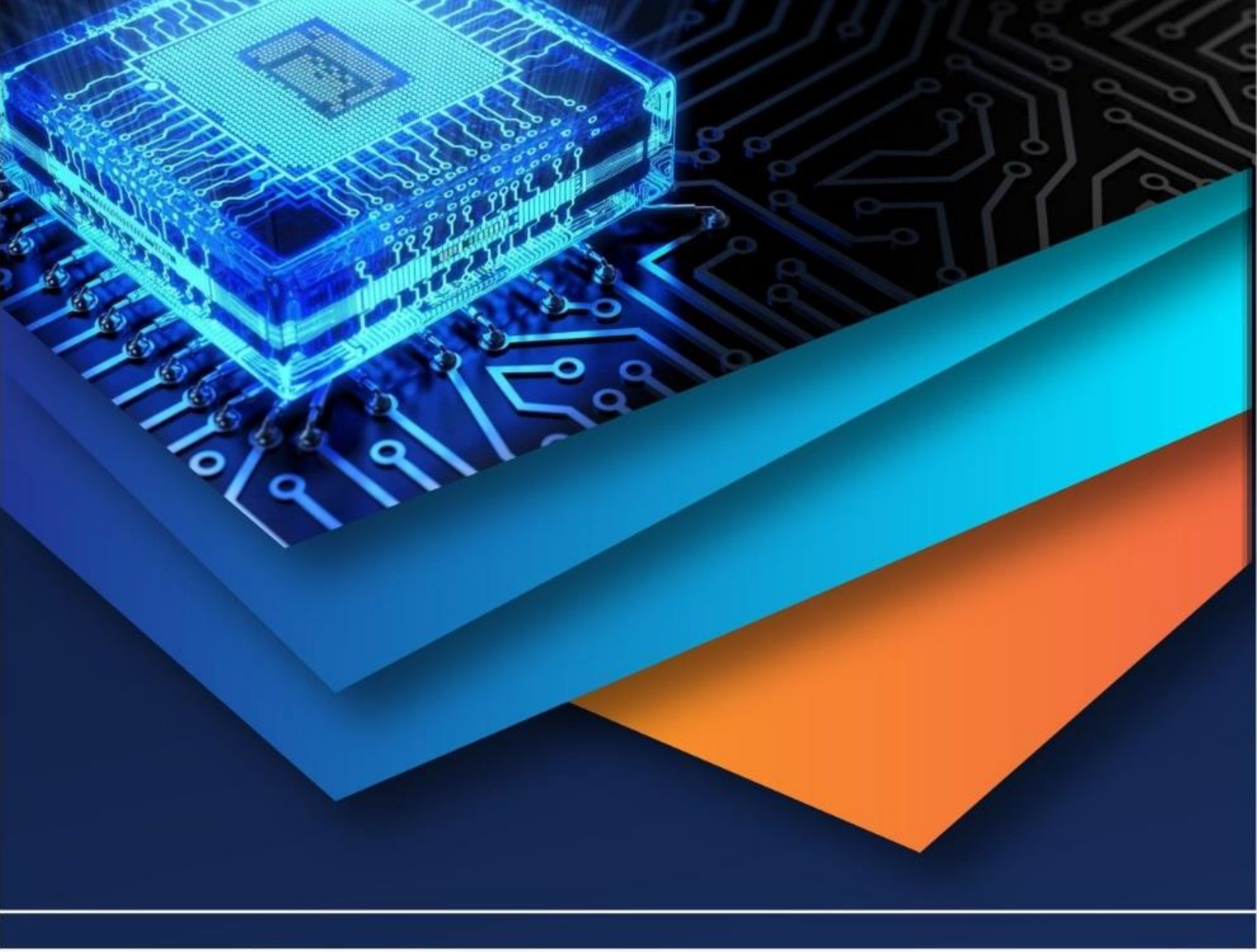

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