

Exploring the antimicrobial and antibiofilm activities of *Artocarpus heterophyllus* Lam. against *Pseudomonas aeruginosa* PAO1

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Abstract. *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen accountable for severe illness and mortality among immunocompromised individuals due to its extraordinary resistance to antibiotics. The regulation of pathogenic traits in *P. aeruginosa* is governed by a quorum sensing (QS) signaling molecule named acyl homoserine lactone (AHL). The present study investigated the inhibitory effects of the compound, *Artocarpus heterophyllus* Lam (*A. heterophyllus*), on the number of AHL-dependent components produced by *P. aeruginosa* (PAO1). *A. heterophyllus* is considered as a beneficial natural compound with notable inhibitory effects on several pathogens. The present study investigated the antimicrobial and antibiofilm activities of *A. heterophyllus* against PAO1 through *in vitro* experiments, including minimum inhibitory concentration tests, biofilm inhibition assays and growth curve analysis. The results demonstrated that the methanolic extract of *A. heterophyllus* at a concentration of 10 mg/ml effectively inhibited PAO1. Additionally, the extract exhibited a 55.12% biofilm inhibitory effect at the lowest sub-inhibitory concentration of 2.5 mg/ml, without affecting planktonic cell growth. On the whole, the findings of the present study indicate the prospective significance of *A. heterophyllus* as an anti-pathogenic agent, which is effective in reducing QS-dependent infection in PAO1.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*), a Gram-negative, aerobic pathogen, infects both immunocompetent and immunocompromised hosts. It can lead to a variety of community-acquired illnesses, including pneumonia, osteomyelitis-causing puncture wounds, folliculitis, urinary tract infections, septicemia, endocarditis and otitis externa (swimmer's ear) (1). Exopolysaccharide, lytic enzymes, such as protease and elastase, pyocyanin pigment formation and motility are all necessary for the pathogenic activity of *P. aeruginosa* (2-4).

Quorum sensing (QS) depends on protein systems that synthesize auto inducer (AI). *P. aeruginosa* uses four primary QS systems, each of which is linked with its unique autoinducer: 3-oxododecanoyl-L-homoserine lactone (3-oxo-C12-HSL), N-butanoyl homoserine lactone (C4-HSL), 2-heptyl-3-hydroxy-4-quinolone (*Pseudomonas* quinolone signal) and 2-(2-hydroxy-phenyl)-thiazole-4-carbaldehyde (integrated quorum sensing signal) (5). When these acyl-homoserine lactone (AHL) molecules bind to their respective receptors, LasR and RhIR induce the expression of pathogenic traits (2,6). The RhII/RhIR system regulates rhamnolipid expression, while the LasI/LasR system also significantly contributes to virulence factors (7). The synchronized expression of these factors in response to QS enables bacteria to adapt to shifts in population density and environmental conditions. Targeting and disrupting these QS systems with antimicrobial compounds may be a promising approach with which to combat *P. aeruginosa* infections. The production of virulence factors frequently reduces the efficacy of a number of antibiotics, resulting in increased bacterial pathogenicity and a mortality rate of 18 to 61% in patients (8).

Natural products have long been a source of novel pharmacological compounds, and there is a renewed interest in examining them as potential candidates for drugs, particularly in the fight against antimicrobial resistance (9). Plant-based bioactive compounds can suppress disease pathogenesis-related genes by disrupting QS-associated virulence factors and preventing biofilm formation. Research findings indicate that natural products exert synergistic effects when

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combined with antibiotics against microbial pathogens (10,11). A wide range of natural products with medicinal properties, including flavonoids (e.g., curcumin and quercetin), quinones (e.g., plumbagin), alkaloids (e.g., piperine), triterpenoids and essential oil phenols (e.g., eugenol and thymol), have been shown to be effective against certain bacteria (9). Some of these products have been demonstrated to be potent antibacterial and antibiofilm agents; they can also inhibit cell attachment and adhesion, and suppress the production of virulence factors, inhibit polymer matrix formation, and thus disrupt the QS network (12,13). Therefore, determining potent QS inhibitors is essential, ideally sourced from natural sources.

Artocarpus heterophyllus Lam. (*A. heterophyllus*), belonging to the family Moraceae, produces the largest edible fruit among evergreen trees, commonly known as jackfruit. This species yields more fruit than any other fruit tree species. However, despite its abundance, the leaves of the jackfruit tree (*A. heterophyllus*) are often regarded as agro-industrial waste. Only a small proportion of the total biomass is utilized, primarily as cattle fodder, and occasionally, for managing asthma, diarrhea and dermatitis. Traditional medicine has extensively utilized the fruits, leaves and bark of the jackfruit tree due to their various medicinal properties. These include anticarcinogenic, antibacterial, antifungal, anti-inflammatory, wound-healing and hypoglycemic qualities (14). According to research, *A. heterophyllus* contains bioactive compounds, such as alkaloids, flavonoids, phenolic acids and terpenoids, which are known for their antimicrobial properties. These compounds have the potential to limit the growth and survival of pathogenic microbes, either individually or in combination (15). It has been previously demonstrated that flavonoids, particularly artonin and artocarpesin, exhibit anti-plasmodial activity (16). Additionally, the methanolic extract of dried *A. heterophyllus* leaf powder has been shown to exhibit potent antibacterial properties against a variety of microbes, including *Escherichia coli* (*E. coli*) and *Salmonella enterica* (*S. enterica*) (17). Furthermore, ethyl acetate extracts from unutilized parts of the jackfruit have been found to exhibit optimal antibacterial activity against *Xanthomonas axonopodis*, with the peels exhibiting the most potent inhibitory effects, followed by the fiber and the core exhibiting the least potent effects (18). Additionally, it has been reported that *A. heterophyllus* exhibits antimicrobial activity against certain foodborne pathogens (19). In a previous study, it was found that the seed powder extract of *A. heterophyllus*, which is used in the green synthesis of silver nanoparticles (AgNPs) from an aqueous solution of silver nitrate (AgNO₃), contains jacalin (20). This lectin constitutes more than half of the proteins in the jackfruit crude seed extract and has a variety of biological activities. The resulting AgNPs demonstrate potent antibacterial activity against both Gram-positive and -negative bacteria, suggesting potential applications in nanomedicine (20). In the study conducted by Sato *et al* (21), it was found that artocarpin, extracted from *A. heterophyllus*, demonstrated potent antibacterial activity against cariogenic bacteria, with minimum inhibitory concentration (MIC) values ranging from 3.13 to 12.5 µg/ml. At this MIC, the compound effectively inhibited the growth of cariogenic bacteria (21). Furthermore, Sun *et al* (22) found that artocarpin exerted selective cytotoxic effects on human colon cancer

cells. It attenuated anchorage-independent growth, inhibited colon cancer cell growth and caused G1 phase cell cycle arrest, followed by apoptotic and autophagic death (22).

It has also been shown that isolated bioactive compounds from *A. heterophyllus* fruits exert anti-inflammatory effects. Jackfruit contains flavonoids, which can inhibit the production of inflammatory chemicals from mast cells, neutrophils and macrophages (23). Prakash *et al* (24) documented in their study that *A. heterophyllus* contains compounds, such as morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cycloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, betulinic acid, artocarpanone and heterophyllol. These compounds have been found to be beneficial in treating fever, boils, wounds, skin diseases, convulsions, diuretic conditions, constipation, ophthalmic disorders and snake bites (24). Fernando *et al* (25) found that a hot water extract of *A. heterophyllus* leaves significantly improved glucose tolerance in both normal and diabetic subjects when taken orally in doses of 20 g/kg.

Numerous studies have focused on *A. heterophyllus* as an achievable source of starch. Tulyathan *et al* (26) reported that jackfruit seeds contain ~20% starch on a dry basis, the recovery yield of starch extracted from jackfruit seeds was ~77%, indicating that it could be a valuable source of starch for the food and pharmaceutical industries (26). These properties highlight the potential of *A. heterophyllus*. The primary aim of the present study was to examine the effects of *A. heterophyllus* on *P. aeruginosa* (PAO1). To the best of our knowledge, the anti-quorum sensing (anti-QS) properties of *A. heterophyllus* are unexplored in relation to *P. aeruginosa*.

The present study aimed to investigate the antimicrobial and antibiofilm properties of *A. heterophyllus* against PAO1. The objective was to evaluate the effectiveness of *A. heterophyllus* in preventing and disrupting biofilm formation by this common pathogen.

Materials and methods

Sample collection. In the present study, which was conducted from January to October, 2023, *A. heterophyllus* was obtained from an indigenous botanical garden in Chennai, Tamil Nadu, India. A qualified botanist examined the plant and confirmed its authenticity. The leaves were cleaned with water and allowed to dry naturally for 1 week. To prepare the extract, 10 g *A. heterophyllus* leaf powder were mixed with 50 ml methanol and distributed across two maceration containers for 48 h, with occasional shaking using a shaker. Following the extraction process, the resulting suspension was filtered through No. 1 filter paper (Whatman, HiMedia Laboratories, LLC) and placed over a funnel lined with a white muslin cloth. The methanol solvent was then evaporated from the filtrate using a hot water bath set precisely at 50°C. The dehydrated filtrate was measured, and the dried substance was weighed before being stored at 4°C for later use.

Bacterial strain and growth condition. The culture of *Pseudomonas aeruginosa* (PAO1) samples utilized in the present study was generously provided by Dr Busi Siddhardha from Pondicherry University, Puducherry, Tamil Nadu, India.

The samples were sub-cultured in Luria Bertani (LB) broth (HiMedia Laboratories, LLC). PAO1 cultures were then incubated at 37°C in a shaking incubator set at 100 rpm for 24 h. Characteristic growth patterns were observed on LB agar and Nutrient agar. To verify the identity of PAO1, a preliminary identification was conducted by laboratory personnel at Saveetha Dental College and Hospital in Chennai, Tamil Nadu, India, utilizing the VITEK 2 automated system. As previously described by David H. Pincus (BioMérieux, Inc.), the biochemical reactions of the bacterial isolate were compared to a comprehensive database to provide accurate identification (27). In addition, various phenotypic tests, including Gram staining, catalase, oxidase, motility and citrate utilization, were conducted and documented based on standard microbiological investigations, as previously described by others (28). The bacterial cultures underwent routine sub-culturing for experimental use.

Antimicrobial activity and antibiotic susceptibility testing (AST). The agar well-diffusion method, an established technique, was employed to evaluate the antibacterial activity of *A. heterophyllus* (29). The bacterial culture of PAO1 was spread onto Mueller Hinton agar (MHA) (HiMedia Laboratories, LLC) using a swab moistened with the bacterial suspension. Subsequently, a well with a diameter of 8 mm was punched into the MHA medium using a sterile cork borer, and a well was filled with 50 μ l *A. heterophyllus* extract, while water served as a control in another well. The plate was then incubated upright at 37°C for a 24 h. Following incubation, the zone of inhibition around the wells was measured using a Vernier caliper on a mm scale to detect the antibacterial activity of the *A. heterophyllus* extract.

Subsequently, the antibiotic susceptibility of PAO1 was assessed using the Kirby-Bauer disk diffusion method (30). Using this standard technique, the authors were able to determine the susceptibility of PAO1 to various antibiotics. Initially, the culture of PAO1 was evenly distributed onto MHA plates using a sterile swab saturated with the bacterial suspension. Subsequently, these plates were subjected to testing with a comprehensive panel of conventional antibiotics, including colistin, tobramycin, ciprofloxacin, azlocillin, aminoglycosides, netilmicin, piperacillin and carbenicillin (HiMedia Laboratories, LLC).

Evaluation of MIC. A 2-fold broth dilution method was used to determine the MIC of *A. heterophyllus* extract against PAO1. The assessment was carried out at concentrations ranging from 10 to 0.01 mg/ml. The MIC for the methanol extract was determined using established protocols (31,32). In brief, 20 μ l PAO1 broth culture with the cell mass equivalent of 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml) was filled in tubes containing LB broth. Following the successive dilution with *A. heterophyllus* extract, each of the tubes underwent incubation at 37°C for 24 h. Following incubation, 40 μ l 2,3,5-triphenyl tetrazolium chloride (HiMedia Laboratories, LLC) was added to each tube to check for color changes and confirm the results. The minimum concentration that caused no growth (no color change) was recorded as the MIC. Additional antibiofilm studies were carried out in accordance with these findings.

Crystal violet biofilm inhibition assay. To determine the effects of *A. heterophyllus* extract on PAO1 biofilm formation, the crystal violet staining assay was used (32). An overnight culture of PAO1 (20 μ l) was added to a microtiter plate containing 180 μ l fresh LB medium, and the *A. heterophyllus* extract was added in a concentration-dependent manner (ranging from 5 to 0.009 mg/ml). The mixture was incubated for 48 h at 37°C. Following incubation, the surface-adherent biofilm was stained with a 0.1% crystal violet solution (HiMedia Laboratories, LLC) at room temperature for 2 min, and the planktonic cells were washed away with sterile distilled water. After 10 min, the CV-bound biofilm was eluted in 200 μ l 70% ethanol. A UV-Vis spectrophotometer (JASCO UV/Vis, India) was used to determine the concentration of the eluted CV by measuring the crystal violet intensity at 520 nm. The percentage of inhibition was then calculated using the following equation: Control optical density (OD) 520 nm-treated OD 520 nm/control OD 520 nm x100.

Bacterial growth curve. The growth curve analysis was conducted following previously established protocols (33). The concentration of 2.5 mg/ml *A. heterophyllus* was selected based on the crystal violet biofilm inhibition assay, which indicated that this concentration inhibited PAO1 biofilm formation by 55.12%. Subsequently, the present study wished to confirm its influence on bacterial growth at the same concentration through growth curve experiments to determine whether it inhibited growth as well. To encapsulate, the present study delved into the growth dynamics of PAO1 bacteria under dual conditions, with or without the presence of *A. heterophyllus*, at a concentration of 2.5 mg/ml. The cultures were meticulously incubated at 37°C, with hourly recordings of OD at 600 nm spanning a duration of up to 24 h.

Statistical evaluation. Each experiment was carried out three times, with statistical significance rigorously demonstrated for both the growth curve analysis and biofilm quantification. The Student's t-test with GraphPad prism 10.1.0 software (Dotmatics) served as the tool for statistical analysis. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Bacterial identification of PAO1. The VITEK 2 system accurately confirmed the identity of the bacterial strain as PAO1 with high confidence. The biochemical profile aligned with the expected characteristics for PAO1, thus validating the initial identification methods (34). The morphological profiling of the bacterial isolates further supported the findings, revealing distinct morphotypes consistent with PAO1. Notably, Gram staining revealed the characteristic Gram-negative, rod-shaped morphology of PAO1 (Fig. 1A). Additionally, the identity of PAO1 was further confirmed by positive results in catalase (Fig. 1B), oxidase (Fig. 1C), motility (Fig. 1D) and citrate utilization (Fig. 1E) tests.

Antimicrobial susceptibility and AST. The present study initially examined the antimicrobial activity of *A. heterophyllus* extract against PAO1, indicated by a measured zone of

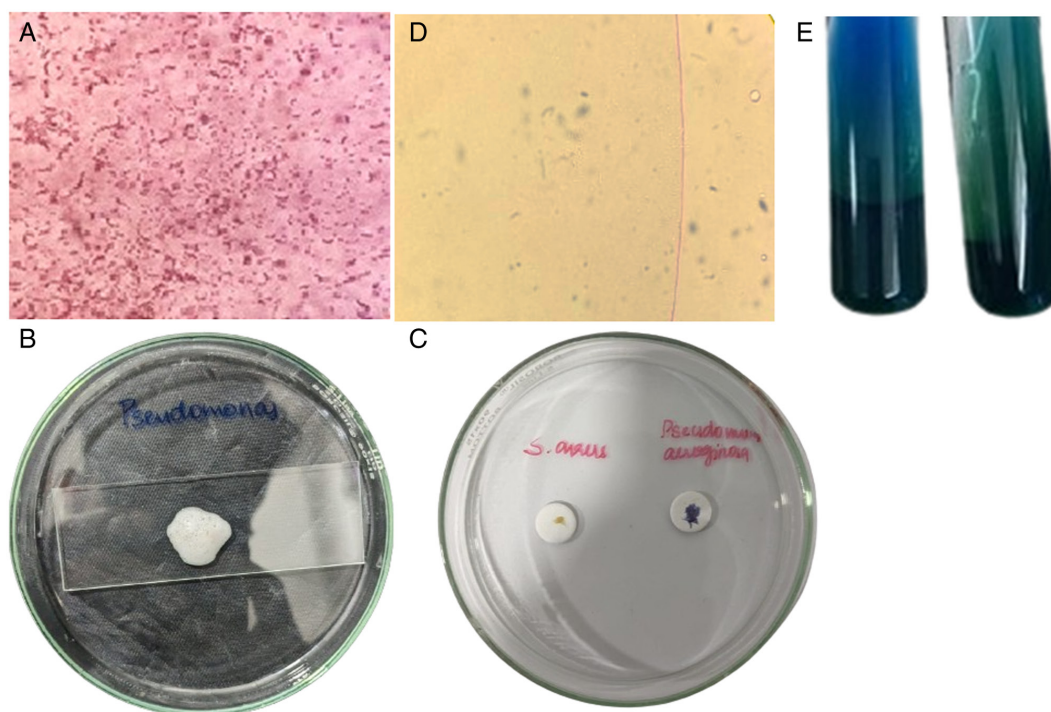


Figure 1. Identification of PAO1 using various biochemical tests. (A) Gram staining reveals the Gram-negative, rod-shaped morphology of PAO1 at a magnification of x40. (B) The catalase test revealed a positive reaction for PAO1, indicated by bubble formation. (C) The oxidase test confirmed the presence of cytochrome oxidase enzyme in PAO1, indicated by a positive reaction (+ve PAO1) compared to a negative control (-ve *S. aureus*). (D) The motility test demonstrated the motile nature of PAO1 using the hanging drop method at a magnification of x100. (E) The citrate utilization test revealed a positive result for PAO1, as evidenced by the color change in the medium (+ve) compared to the control. PAO1, strain of *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.

inhibition with a recorded diameter of 8 mm, highlighting its potent efficacy (Fig. 2). Subsequent AST, conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) Guidelines 2022 (35), unveiled PAO1 resistance to a majority of tested drugs, including colistin, ciprofloxacin, piperacillin and azlocillin (Table I).

Antibacterial activity of *A. heterophyllus* at the MIC level. By employing a 2-fold serial dilution method, the present study explored the antibacterial potential of the *A. heterophyllus* extract across a spectrum ranging from 10 to 0.019 mg/ml. Notably, at the end point concentration of 10 mg/ml, the inhibition of PAO1 growth was observed (Table II), suggestive of the promising antimicrobial efficacy of *A. heterophyllus* against PAO1. This prompted further investigation into the anti-biofilm properties of the *A. heterophyllus* extract at sub-MIC concentrations.

Effect on biofilm formation. The present study investigated the ability of *A. heterophyllus*, at concentrations ranging from 5 to 0.009 mg/ml, to inhibit PAO1 biofilm formation using 0.1% crystal violet dye on a static microtiter plate. The findings indicated that the *A. heterophyllus* extract significantly reduced biofilm formation when PAO1 was exposed to concentrations of 2.5 and 1.25 mg/ml, resulting in reductions of 55.12 and 26%, respectively. In the control, no biofilm inhibition was observed (without *A. heterophyllus* extract) (Fig. 3). Therefore, conducting a bacterial growth curve analysis will further assess the efficacy of the *A. heterophyllus* extract at these same concentrations.



Figure 2. Antimicrobial activity of *A. heterophyllus* extract against PAO1. (A) The antimicrobial activity of *A. heterophyllus* extract is demonstrated by a zone of inhibition measuring 8 mm in diameter against PAO1. (B) Water serves as the control, showing no inhibition zone. PAO1, strain of *Pseudomonas aeruginosa*; *A. heterophyllus*, *Artocarpus heterophyllus*.

Bacterial growth curve analysis. The growth curve was analyzed both with and without *A. heterophyllus*. The results revealed that *A. heterophyllus* at a concentration of 2.5 mg/ml did not inhibit bacterial growth (Fig. 4). The spectrophotometric analysis revealed no noticeable disparity between the control and treated bacterial cells at 600 nm. These findings suggest that *A. heterophyllus* does not exert inhibitory effects

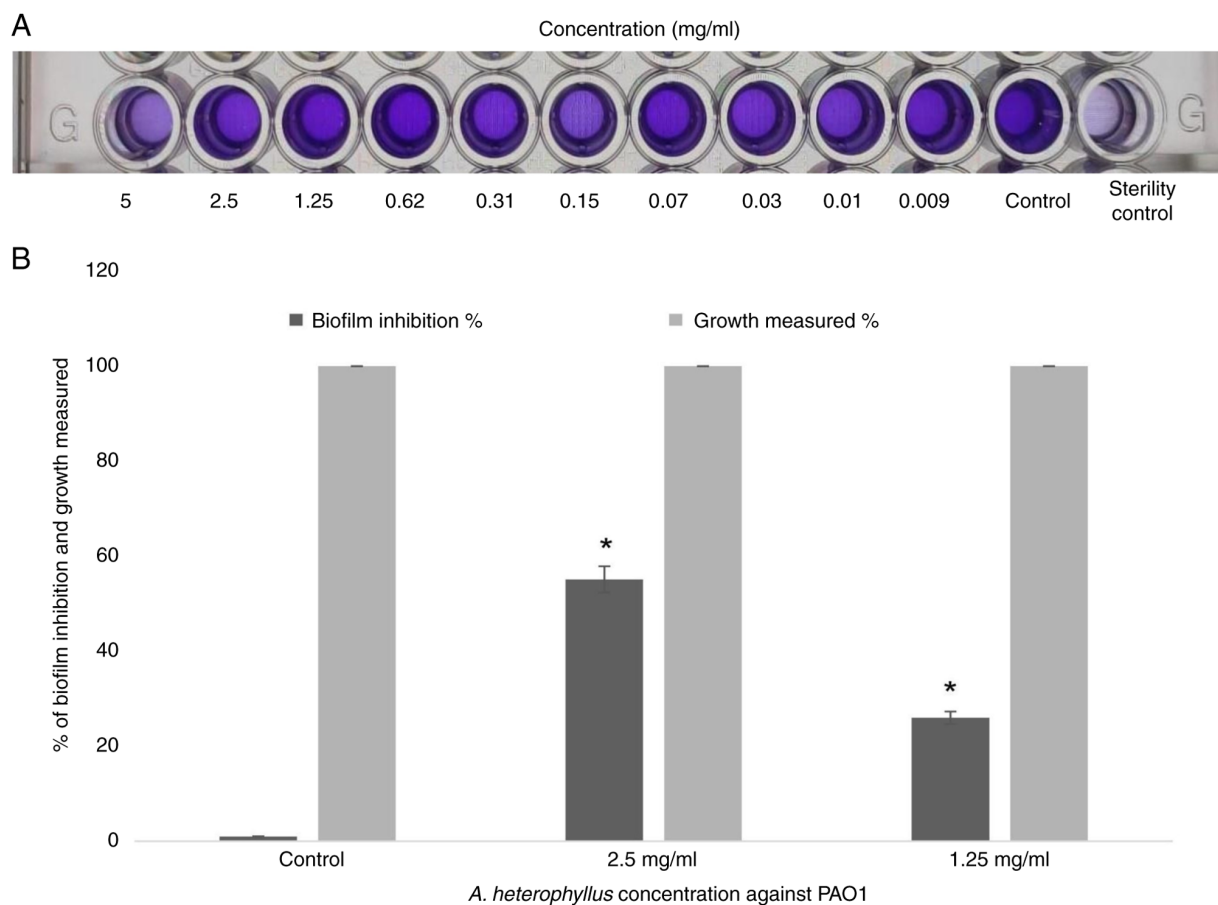


Figure 3. (A) Crystal violet biofilm inhibition assay. *A. heterophyllus* extract inhibited PAO1 biofilm at sub-inhibitory concentration of 2.5 and 1.25 mg/ml. (B) Graphical representation; 55.12% of biofilm inhibition in PAO1 when treated with 2.5 mg/ml and 26% biofilm inhibition when treated with 1.25 mg/ml *A. heterophyllus* extract. PAO1, strain of *Pseudomonas aeruginosa*; *A. heterophyllus*, *Artocarpus heterophyllus*. *P<0.05, significant biofilm inhibition.

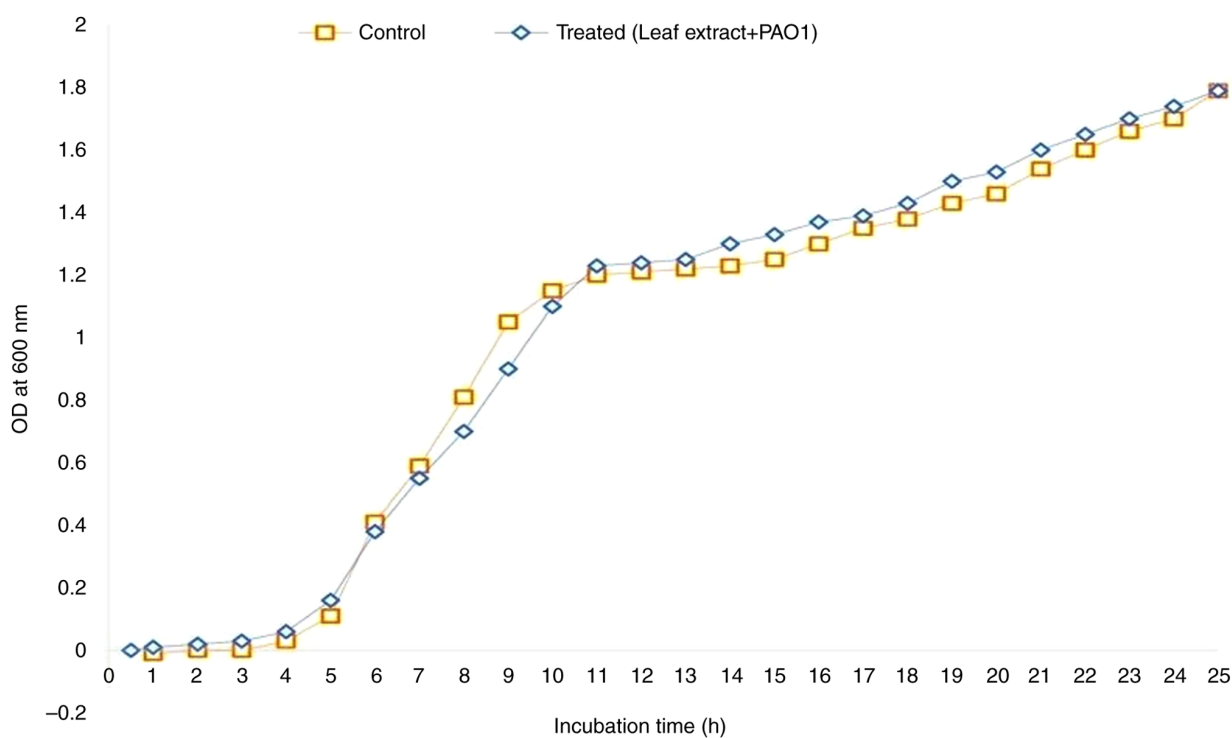


Figure 4. Growth curve analysis. PAO1 grown without (control) and in the presence of *A. heterophyllus* extract at the concentration of 2.5 mg/ml. PAO1, strain of *Pseudomonas aeruginosa*; *A. heterophyllus*, *Artocarpus heterophyllus*.

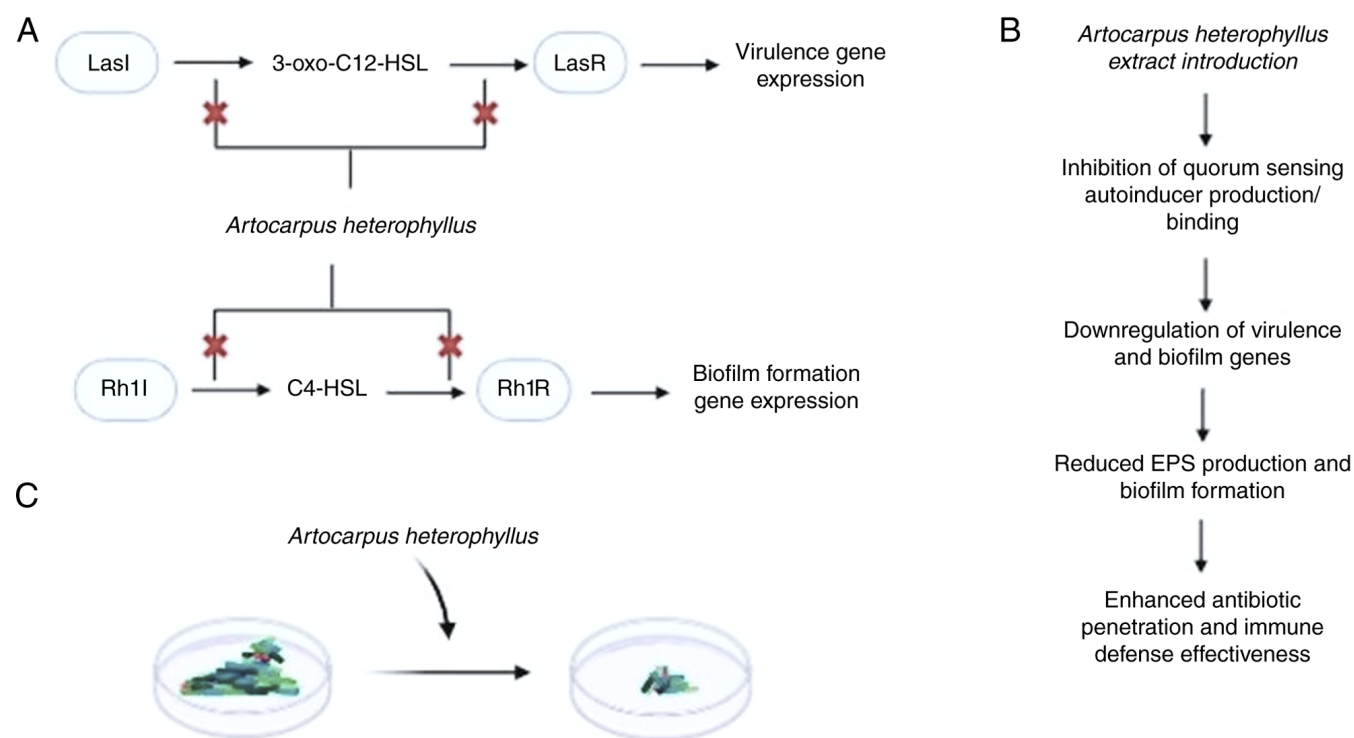


Figure 5. Inhibition of PAO1 biofilm formation by *A. heterophyllus*. (A) Disruption of PAO1 gene expression by *A. heterophyllus* extract. Treatment with the extract results in significant downregulation of key virulence genes in PAO1. (B) Flowchart outlining the impact of *A. heterophyllus* on gene expression and biofilm formation. (C) Comparison of biofilm formation in treated (*A. heterophyllus*) vs. untreated PAO1 cultures, demonstrating a reduction in biofilm mass and density. PAO1, strain of *Pseudomonas aeruginosa*; *A. heterophyllus*, *Artocarpus heterophyllus*.

Table I. Antibigram of PAO1 against several antibiotics.

Serial no.	Antibiotics	<i>P. aeruginosa</i> (PAO1)
1	Tobramycin	22.3±2.9
2	Azlocillin	R
3	Aminoglycosides	15±0.9
4	Netilmicin	18±1.1
5	Piperacillin	R
6	Colistin	R
7	Ciprofloxacin	R
8	Carbenicillin	16±1.1

Values represent the mean inhibition zone diameter (mm) ± standard deviation for *P. aeruginosa* (PAO1) isolates tested. R, resistance to the antibiotic; *P. aeruginosa*, *Pseudomonas aeruginosa*.

on bacterial growth at a concentration of 2.5 mg/ml under the tested conditions. However, it does exhibit inhibitory effects specifically on biofilm formation.

Discussion

Gram-negative bacteria primarily cause infections and form biofilms due to the activity of QS signaling molecules. However, treating infections caused by biofilm-forming *P. aeruginosa* poses a considerable challenge due to bacterial resistance to

Table II. Minimum inhibitory concentration.

Serial no.	Two-fold dilution concentration (mg/ml)	Growth measured ^a
1	10	-
2	5	+
3	2.5	+
4	1.25	+
5	0.62	+
6	0.312	+
7	0.156	+
8	0.078	+
9	0.039	+
10	0.019	+

Methanol extract of *A. heterophyllus* inhibited PAO1 growth at a final concentration of 10 mg/ml. ^aThe growth measured refers to the presence (+) or absence (-) of visible growth in the microbial culture following exposure to the respective two-fold dilution concentrations (mg/ml) of *A. heterophyllus*. *A. heterophyllus*, *Artocarpus heterophyllus*.

traditional antibiotics (36). The AHL molecule plays a crucial role in the pathogenesis of *P. aeruginosa*, and inhibiting its activity holds promise for mitigating the pathogenicity of this pathogen.

The present study evaluated the potency of *A. heterophyllum*, which contains bioactive compounds, such as alkaloids, flavonoids, phenolic acids and terpenoids, and examined whether it has the ability to reduce AHL-dependent factors production in PAO1. In the study by Khan *et al* (17), *A. heterophyllum*, which contains flavonoids, was shown to exert an inhibitory effect on several bacteria, including *E. coli* and *S. enterica* (17). Similarly, a recent study by Alam *et al* (37) demonstrated that the plant-derived extract, *Berginia ciliata*, which also contains flavonoids, inhibited the pathogenic bacteria PAO1 (37).

The present study evaluated the antibiofilm activities of *A. heterophyllum* extract against PAO1. The preliminary findings indicated that *A. heterophyllum* effectively inhibited biofilm formation at the lowest concentration of 10 mg/ml. The current data support the findings of the study by Sivag nanasundaram and Karunanayake (38), which reported that *A. heterophyllum* had potent bactericidal activity against *E. coli* at 3 mg/ml, and the presence of phytosterols and terpenoids was reported (38). In another study, it was found that artocarpin, extracted from *A. heterophyllum*, inhibited the growth of *Streptococcus mutans* at an MIC of 1.95 µg/ml by altering cell membrane permeability, leading to the release of intracellular proteins (39). The study by Sato *et al* (21) demonstrated that artocarpin exhibited potent antibacterial activity against cariogenic bacteria, with MIC values ranging from 3.13 to 12.5 µg/ml. At these MIC levels, artocarpin was able to inhibit the growth of cariogenic bacteria (21).

In the present study, *A. heterophyllum* extract inhibited QS-dependent biofilm formation in PAO1 at concentrations below the MIC. The crystal violet biofilm inhibition assay revealed that at a concentration of 2.5 mg/ml, the *A. heterophyllum* extract significantly reduced biofilm formation (Fig. 3). Vijayaraghavan *et al* (40) reported that peel waste from jackfruit was identified as a source for anaerobic biohydrogen production and exhibited potential for removing toxic dyes and chemicals from wastewater released by the textile and pharmaceutical industries. Moreover, Majik *et al* (41) reported that natural pyrrolidine alkaloid (R)-Bgugaine extracted from *Arisarum vulgare*, which decreased the biofilm density by 83%, also inhibited the pyocyanin pigmentation, LasA protease and rhamnolipid production of PAO1 (41). Similarly, Vijayakumar and Ramanathan (42) reported that the tropical plant *Musa acuminata* contains flavonoids and 5-hydroxymethylfurfural, which inhibited *P. aeruginosa* biofilm formation at a concentration of 400 µg/ml. Furthermore, methanolic extract from the leaves and stems of *A. heterophyllum* contains chromones and flavonoids that have anti-proliferative activity. These compounds have been shown to exert significant inhibitory effects against various human cancer cells, with IC₅₀ values ranging from 0.36±0.02 to 22.09±0.16 µM (43). A previous study found that flavonoids act to inhibit bacterial movement and reduce biofilm formation in *P. aeruginosa*, while also inhibiting the production of bacterial toxins in *Staphylococcus aureus* (44).

In the present study, growth curve analysis conducted in the presence of *A. heterophyllum* extract at a concentration of 2.5 mg/ml, as depicted in Fig. 4, indicated that *A. heterophyllum* did not impede bacterial growth. These results suggest

that, under the conditions tested, *A. heterophyllum* does not exert inhibitory effects on bacterial growth at the specified concentration. However, it is noteworthy that *A. heterophyllum* does demonstrate inhibitory effects specifically on biofilm formation. Rashmi *et al* (45) conducted a growth curve experiment using antibiofilm concentrations. The results of their study revealed that the growth pattern of *P. aeruginosa* with and without treatment of *Alternaria alternata* extract at three concentrations did not impede bacterial growth. However, the extract exhibited inhibitory effects specifically on biofilm formation (45).

Taken together, the results of the present study suggest that the methanol extract of *A. heterophyllum* inhibits the QS system in PAO1 by targeting the LasI/LasR and RhII/RhlR systems, possibly due to the presence of bioactive compounds. However, additional research is warranted to identify active components within *A. heterophyllum* extract that may harbor anti-QS and anti-biofilm producing properties.

In conclusion, *A. heterophyllum* was found to exhibit notable potential in disrupting the QS pathways in PAO1. Specifically, it targets the LasI/LasR and RhII/RhlR systems. By inhibiting the production of autoinducer molecules, such as 3-oxo-C12-HSL and C4-HSL, or by preventing their binding to the LasR and RhlR receptors, *A. heterophyllum* downregulates the expression of genes critical for virulence and biofilm formation (Fig. 5A). This interference leads to a reduction in extracellular polymeric substance production and impaired biofilm development (Fig. 5B). Additionally, the extract introduction enhances antibiotic penetration and immune defense effectiveness (Fig. 5C).

The results of the present study suggest that the significant inhibition of the QS system in PAO1 by *A. heterophyllum* is likely due to its bioactive compounds, such as alkaloids, flavonoids, phenolic acids and terpenoids. The methanol extract of *A. heterophyllum* demonstrates considerable potential in restoring the effectiveness of antibiotics by facilitating their penetration through the compromised biofilm structure of PAO1. By degrading the biofilm matrix, this extract can render the infectious bacteria more accessible to immune defense.

Considering the broad availability of jackfruit in India and the versatile use of all of its parts, including wood and latex, which are known to have therapeutic properties, there is significant potential for scientific research into its medicinal benefits. The present study highlights the importance of exploring plant-based compounds for enhancing current antimicrobial therapies and addressing the challenges posed by *P. aeruginosa* biofilm-associated infections. Further research with various formulations would be beneficial in improving the pharmacological therapeutic applications of the identified compounds, particularly their anti-QS and anti-biofilm properties. Despite being historically neglected, jackfruit is gaining recognition for its medicinal properties, and continued research is essential to unlock its full potential as an antibiotic adjuvant. In conclusion, *A. heterophyllum* could serve as a valuable antibiotic adjuvant, offering promising strategies to prevent or treat chronic infections caused by *P. aeruginosa*. This underscores the importance of plant-based compounds in addressing the growing challenge of antibiotic resistance and biofilm-associated infections.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MS collected, managed the data and participated in the writing of the manuscript. AV and NNP participated in writing the proposal, performing data collection and in the writing of the manuscript. RVG and PSG were involved in data curation, data analysis and in revising the manuscript. RVG and PSG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Kerr KG and Snelling AM: *Pseudomonas aeruginosa*: A formidable and ever-present adversary. *J Hosp Infect* 73: 338-344, 2009.
- Bjarnsholt T and Givskov M: Quorum-sensing blockade as a strategy for enhancing host defences against bacterial pathogens. *Philos Trans R Soc Lond B Biol Sci* 362: 1213-1222, 2007.
- Adonizio A, Kong KF and Mathee K: Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrob Agents Chemother* 52: 198-203, 2008.
- De Kievit TR: Quorum sensing in *Pseudomonas aeruginosa* biofilms. *Environ Microbiol* 11: 279-288, 2009.
- Lee J and Zhang L: The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein Cell* 6: 26-41, 2015.
- Pesci EC, Pearson JP, Seed PC and Iglewski BH: Regulation of las and rhl quorum sensing in *Pseudomonas aeruginosa*. *J Bacteriol* 179: 3127-3132, 1997.
- Mukherjee S, Moustafa D, Smith CD, Goldberg JB and Bassler BL: The RhlR quorum-sensing receptor controls *Pseudomonas aeruginosa* pathogenesis and biofilm development independently of its canonical homoserine lactone autoinducer. *PLoS Pathog* 13: e1006504, 2017.
- Chimi LY, Bisso BN, Njateng GSS and Dzoyem JP: Antibiotic-potentiating effect of some bioactive natural products against planktonic cells and biofilms, and virulence factors of *Pseudomonas aeruginosa*. *Biomed Res Int* 2023: 9410609, 2023.
- Atanasov AG, Zotchev SB, Dirsch VM; International Natural Product Sciences Taskforce and Supuran CT: Natural products in drug discovery: Advances and opportunities. *Nat Rev Drug Discov* 20: 200-216, 2021.
- Hemaiswarya S, Kruthiventi AK and Doble M: Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 15: 639-652, 2008.
- Bisso Ndezo B, Tokam Kuaté CR and Dzoyem JP: Synergistic antibiofilm efficacy of thymol and piperine in combination with three aminoglycoside antibiotics against *Klebsiella pneumoniae* biofilms. *Can J Infect Dis Med Microbiol* 2021: 7029944, 2021.
- Guzzo F, Scognamiglio M, Fiorentino A, Buommino E and D'Ambrosia B: Plant derived natural products against *Pseudomonas aeruginosa* and *Staphylococcus aureus*: Antibiofilm activity and molecular mechanisms. *Molecules* 25: 5024, 2020.
- Cowan MM: Plant products as antimicrobial agents. *Clin Microbiol Rev* 12: 564-582, 1999.
- Ranasinghe RASN, Maduwanthi SDT and Marapana RAUJ: Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): A review. *Int J Food Sci* 2019: 4327183, 2019.
- Saha RK, Jamiruddin M and Acharya S: Comparative analysis of lectins isolated from seed and testa of *Artocarpus heterophyllus* LAM. *Int J Curr Res Chem Pharma Sci* 2: 65-75, 2015.
- Vazhacharickal JP, Sajeshkumar NK, Mathew JJ, Kuriakose AC, Abraham B, Mathew RJ, Albin AN, Thomson D, Thomas RS, Varghese N and Jose S: Chemistry and medicinal properties of jackfruit (*Artocarpus heterophyllus*): A review on current status of knowledge. *Int J Innov Res Rev* 3: 83-95, 2015.
- Khan MR, Omoloso AD and Kihara M: Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia* 74: 501-505, 2003.
- Adan AA, Ojwang RA, Muge EK, Mwanza BK and Nyaboga EN: Phytochemical composition and essential mineral profile, antioxidant and antimicrobial potential of unutilized parts of jackfruit. *Food Res* 4: 1125-1134, 2020.
- Loizzo MR, Tundis R, Chandrika UG, Abeysekera AM, Menichini F and Frega NG: Antioxidant and antibacterial activities on foodborne pathogens of *Artocarpus heterophyllus* Lam. (moraceae) leaves extracts. *J Food Sci* 75: M291-M295, 2010.
- Jagtap UB and Bapat VA: Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. seed extract and its antibacterial activity. *Ind Crops Prod* 46: 132-137, 2013.
- Sato M, Fujiwara S, Tsuchiya H, Fujii T, Iinuma M, Tosa H and Ohkawa Y: Flavones with antibacterial activity against cariogenic bacteria. *J Ethnopharmacol* 54: 171-176, 1996.
- Sun G, Zheng Z, Lee MH, Xu Y, Kang S, Dong Z, Wang M, Gu Z, Li H and Chen W: Chemoprevention of colorectal cancer by artocarpin, a dietary phytochemical from *Artocarpus heterophyllus*. *J Agric Food Chem* 65: 3474-3480, 2017.
- Wei BL, Weng JR, Chiu PH, Hung CF, Wang JP and Lin CN: Antiinflammatory Flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J Agric Food Chem* 53: 3867-3871, 2005.
- Prakash O, Gupta R and Banarasi B: *Artocarpus heterophyllus* (Jackfruit): An overview. *Phcog Rev* 6, 353-358, 2017.
- Fernando MR, Wickramasinghe S, Thabrew MI, Ariyananda PL and Karunanayake EH: Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. *J Ethnopharmacol* 31: 277-282, 1991.
- Tulyathan V, Tananuwong K, Songjinda P and Jaiboon N: Some physicochemical properties of jackfruit (*Artocarpus heterophyllus* Lam) seed flour and starch. *Sci Asia* 28: 37-41, 2002.
- Pincus DH: Microbial identification using the bioMérieux Vitek® 2 system. *Encyclopedia of Rapid Microbiological Methods*. Bethesda, MD: Parenteral Drug Association, ppl-32, 2006.
- Holt JG and Krieg NR: *Bergey's Manual of Systematic Bacteriology*. Vol 2. Williams and Wilkins Publishers, Baltimore, 2001.
- Ganesh PS, Veena K, Senthil R, Iswamy K, Ponmalar EM, Mariappan V, Girija ASS, Vadivelu J, Nagarajan S, Challabathula D and Shankar EM: Biofilm-associated agr and sar quorum sensing systems of *Staphylococcus aureus* are inhibited by 3-hydroxybenzoic acid derived from *Illicium verum*. *ACS Omega* 7: 14653-14665, 2022.
- Hudzik J: Kirby-bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*, 2009.
- Packiavathy IASV, Agilandewari P, Musthafa KS, Karutha Pandian S and Veera Ravi A: Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Res Int* 45: 85-92, 2012.

32. Venkatramanan M, Sankar Ganesh P, Senthil R, Akshay J, Veera Ravi A, Langeswaran K, Vadivelu J, Nagarajan S, Rajendran K and Shankar EM: Inhibition of quorum sensing and biofilm formation in *Chromobacterium violaceum* by fruit extracts of *Passiflora edulis*. ACS Omega 5: 25605-25616, 2020.
33. Ganesh PS and Rai RV: Inhibition of quorum-sensing-controlled virulence factors of *Pseudomonas aeruginosa* by *Murrayakoenigii* essential oil: a study in a *Caenorhabditis elegans* infectious model. J Med Microbiol 65: 1528-1535, 2016.
34. Moehario LH, Tjoa E, Putranata H, Joon S, Edbert D and Robertus T: Performance of TDR-300B and VITEK®2 for the identification of *Pseudomonas aeruginosa* in comparison with VITEK®-MS. J Int Med Res 49: 300060521989893, 2021.
35. CLSI. Performance standards for antimicrobial susceptibility testing, M100. 32nd edition. Clinical and Laboratory Standards Institute, Wayne, PA, 2022.
36. Pang Z, Raudonis R, Glick BR, Lin TJ and Cheng Z: Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. Biotechnol Adv 37: 177-192, 2019.
37. Alam K, Farraj DAA, Mah-E-Fatima S, Yameen MA, Elshikh MS, Alkufeidy RM, Mustafa AEMA, Bhasme P, Alshammari MK, Alkubaisi NA, et al: Anti-biofilm activity of plant derived extracts against infectious pathogen-*Pseudomonas aeruginosa* PAO1. J Infect Public Health 13: 1734-1741, 2020.
38. Sivagnanasundaram P and Karunanayake KOLC: Phytochemical screening and antimicrobial activity of *Artocarpus heterophyllus* and *Artocarpus altilis* leaf and stem bark extracts. OUSL J 9: 1-17, 2015.
39. Daud NNNNM, Septama AW, Simbak N and Rahmi EP: The phytochemical and pharmacological properties of artocarpin from *Artocarpus heterophyllus*. Asian Pac J Trop Med 13: 1-7, 2020.
40. Vijayaraghavan K, Ahmad D and Ibrahim MKB: Biohydrogen generation from jackfruit peel using anaerobic contact filter. Int J Hydrogen Energy 31: 569-579, 2006.
41. Majik MS, Naik D, Bhat C, Tilve S, Tilvi S and D'Souza L: Synthesis of (R)-norbugaine and its potential as quorum sensing inhibitor against *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 23: 2353-2356, 2013.
42. Vijayakumar K and Ramanathan T: *Musa acuminata* and its bioactive metabolite 5-hydroxymethylfurfural mitigates quorum sensing (las and rhl) mediated biofilm and virulence production of nosocomial pathogen *Pseudomonas aeruginosa* in vitro. J Ethnopharmacol 246: 112242, 2020.
43. Liu YP, Yu XM, Zhang W, Wang T, Jiang B, Tang HX, Su QT and Fu YH: Prenylated chromones and flavonoids from *Artocarpus heterophyllus* with their potential antiproliferative and anti-inflammatory activities. Bioorg Chem 101: 104030, 2020.
44. Biharee A, Sharma A, Kumar A and Jaitak V: Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia 146: 104720, 2020.
45. Rashmi M, Meena H, Meena C, Kushveer JS, Busi S, Murali A and Sarma VV: Anti-quorum sensing and antibiofilm potential of *Alternaria alternata*, a foliar endophyte of *Carica papaya*, evidenced by QS assays and in-silico analysis. Fungal Biol 122: 998-1012, 2018.



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