Original Research Article

Biofilm Reduction Capabilities of *Artocarpus Heterophyllus* Seeds, *Coriandrum Sativum, and Cynara Cardunculus* Flesh Aqueous Extracts on *Staphylococcus Epidermidis*

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ABSTRACT

Antimicrobial resistance is a growing issue globally, and this necessitates alternative methods of treatment such as Quorum Quenching (QQ). Quorum-sensing is a form of intracellular communication within microorganisms that is dependent on population density and influences certain traits of the species, such as biofilm production. Biofilm production is the primary focus of this study. Biofilm is a collection of microorganisms that aggregate into an extracellular slimy matrix that functions as a protective layer. This study investigated the antibiofilm capabilities of three novel aqueous plant extracts on *Staphylococcus epidermidis*: *Artocarpus heterophyllus* (jackfruit) seeds, *Coriandrum sativum* (cilantro), *and Cynara cardunculus* (artichoke) flesh. Minimum Inhibitory Concentration assays were conducted to establish the antibacterial capabilities of the plants, with *A. heterophyllus* and *C. cardunculus* displaying the highest percentage inhibition of 81.072% and 71.588% respectively. Sub-inhibitory concentrations of half the MIC were applied in a crystal violet biofilm assay to isolate the biofilm disruption properties. Quantitatively, optical density (OD) readings identified that *A. heterophyllus* seeds were the most effective aqueous extract out of the treatments tested, reducing the biofilm formation by 22.279 ± 0.834%. The findings demonstrate that *A. heterophyllus* seed extract reduces biofilm formation and exhibits measurable antibacterial activity against *S. epidermidis*. While the samples were crude extracts and limited in replication, this study supports the notion that *A. heterophyllus* seeds have a strong potential to act as an antibacterial agent against *S. epidermidis.* The findings demonstrate that *A. heterophyllus* seed extract reduces biofilm formation activity and exhibits measurable antibacterial effects against *S. epidermidis*.

Keywords: Biofilm; Quorum Sensing; Antimicrobial Properties; Aqueous Plant Extracts; Quorum Quenching; Antimicrobial Resistance; *Staphylococcus epidermidis*

INTRODUCTION

Antimicrobial Resistance (AR) is a growing issue within medicine (1). Antimicrobial resistance occurs when pathogenic microorganisms, such as bacteria, parasites, and viruses, develop the ability to resist antimicrobial drugs. As more microorganisms develop resistance, this field forms the basis of modern medicine, and ongoing research is being conducted. Antibiotics are a subclass of antimicrobials that focus specifically on treating bacterial infections (2). Antibiotic resistance can

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occur naturally as part of a bacterial species' evolution, but it can also be heightened by the overuse and misuse of antibiotics. For example, if one does not follow the dosage plan of an antibiotic, there is a chance that the bacteria are not completely eradicated and can evolve and multiply again. Medicinal herbs have been one of the longest-used antibiotics and have proven effective against certain bacteria (3).

Quorum sensing is a form of cellular communication that enables bacteria to share information about cell density, allowing them to adjust their gene expression accordingly (4). Bacteria produce autoinducers, usually made of peptides (in gram-positive) and traditionally made of acyl-homoserine lactone (in gram-negative). As bacteria multiply and more cells produce autoinducers, the extracellular concentration increases, eventually reaching a critical mass point. After critical mass, it becomes less favorable for autoinducers to keep diffusing out of the cell. Thus, the internal concentration increases, and the autoinducers bind to the intercellular receptors in increasing amounts, triggering a signaling cascade that directs gene expression. This process usually involves negative feedback to reduce further production of autoinducers and prevent overstimulation (5). Quorum-sensing signals are essential in the biofilm formation process: attachment, growth, microcolony formation, maturation, and dispersion (6). Quorum quenching (QQ), the inhibition of quorum sensing, has been a key focus in the study of antibacterial resistance, with the aim of understanding how to disrupt bacterial communication and thereby effectively preventing bacteria from evolving resistance to antibiotics. This paper explored how different novel plant extracts impact quorum sensing in *Staphylococcus epidermidis,* quantified by the biofilm formation.

In this study, QQ refers specifically to the disruption of the quorum sensing regulated behaviors, in particular, biofilm formation, without necessarily exerting a bactericidal effect. Because quorum sensing molecular pathways, such as the agr system, are identified as important regulators of biofilm formation (7), any reduction in biofilm at sub-inhibitory concentrations in this study was interpreted as evidence of quorum-quenching activity rather than general antibacterial action.

METHODS AND MATERIALS

Preparation of Plant Extract

Plants were selected after a thorough literature review of herbs that have not been well researched for their antimicrobial properties and/or biofilm-disrupting activity. The plants selected were jackfruit seeds, artichoke hearts, and cilantro. For jackfruit seeds, the process involved selecting and cleaning fresh seeds (62.5 g). The seeds were then blended with 100 mL water to form a "slurry" mixture. The mixture was stirred and heated using a magnetic stirrer on a hot plate at 75°C for 1.5 hours. Cilantro extraction involved cleaning and drying the plant, grinding it, then soaking cilantro (25g) in 100 mL of water for 1 hour at 75°C with occasional stirring, using a hot plate. Artichoke aqueous extraction involved cleaning and isolating the heart (the fruit flesh) (64 g), blending it with 100 mL distilled water into a slurry, then heating at 75°C with constant mixing for 2 hours.

All mixtures were strained through sterile cheesecloths, centrifuged at 10,000 rpm for 15 minutes, and concentrated using rotary evaporation. Dried crude extracts were collected and weighed. To standardize extract concentrations for antibacterial testing, masses equivalent to literaturereported MIC values (8, 9, 10)—derived from ethanol or hexane extracts tested against *Staphylococcus aureus*—were dissolved in 1 mL sterile water to prepare stock solutions (25 mg/mL for *C. sativum*, 64 mg/mL for *C. cardunculus*, and 62.5 mg/mL for *A. heterophyllus*). These literature MICs were used as preliminary reference points because no aqueousextract MIC values exist for *Staphylococcus epidermidis*, and aqueous extracts are generally less potent than organicsolvent extracts due to lower efficiency in extracting hydrophobic antimicrobial compounds. This approach provided a standardized staring concentration while acknowledging that solvent polarity and species-specific susceptibility difference may influence the antibacterial potency.

Minimum Inhibition Concentration (MIC) Assay

A liquid culture of *Staphylococcus epidermidis* was inoculated into Tryptic Soy Broth (TSB) medium and incubated at 37°C for 24 hours in an orbital shaking incubator. After diluting TSB, the liquid culture was brought to an Optical Density (OD) of 0.01.

Two MIC assays were performed to account for uncertainty in expected inhibitory range. The first assay utilized literature-based concentrations that were derived from *S. aureus* to provide an initial screening range. Because these values were based on organic-solvents extracts and different bacterial species, a second assay using twice the starting concentrations was conducted to determine whether higher aqueous-extract concentration

were required to observe inhibition in *S. epidermidis*. The expected inhibitory range was uncertain because aqueous extracts typically exhibit substantially lower antibacterial potency than ethanol or hexane-based extracts, and *S. epidermidis* may differ from *S. aureus* in susceptibility, making it unclear whether literature derived concentration would fall within an effective range for this organism and extraction method. This two-tiered approach ensured that MIC was not underestimated due to solvent dependent efficiency and species-specific susceptibility differences. Different starting concentrations were selected for each plant extract because the literature-reported MIC values differ vastly across species and solvents. Therefore, each extract required a specific mass to match its respective MIC equivalent starting point.

Literature-Based Values

Standardized stock solutions (25, 64, 62.6 mg/mL) were used as the starting concentrations. In a 96-well plate, serial two-fold dilutions were prepared in such a manner that the stock solution was positioned in Column 6 for each extract. Column 1 served as the extract-only control, and Column 12 served as the untreated, pure bacterial growth control. The plate was incubated in an orbital shaking incubator for 20 hours at 37°C and read for OD at 600nm. This assay served as initial screening step to approximate the inhibitory ranges based on literature values derived from different bacterial species and extraction solvents.

Higher Concentration Assay

A second MIC assay was performed at twice the standardized stock's concentrations to account for reduced potency expected from aqueous extraction and to validate whether higher concentrations were required for *S. epidermidis*. In this assay, Column 1 contained 200 µL of undiluted extract, column 12 contained 200 µL of bacterial culture cand columns 2-11 were prepared by serial two-fold dilution in PSB. The plate was incubated in an orbital shaking for 20 hours at 37°C and read for OD at 600nm.

MIC Percent Inhibition:

The MIC inhibition was calculated using Equation 1:

$$MIC\ \%\ Inhibtion = \left(1 - \frac{OD_{sample} - OD_{extract\ control}}{OD_{growth\ control} - OD_{extract\ control}}\right) \times 100 \quad \text{(Eq. 1)}$$

OD_{sample} = OD of each dilution (columns 2-11) from Tables 1 and 2

$OD_{extract\ control}$ = OD of the extract alone (column 1) from Tables 1 and 2

$OD_{growth\ control}$ = OD of untreated bacteria (column 12) from Tables 1 and 2

Equation 1 considers the turbidity of the plant extracts themselves while calculating the inhibition percentage to reflect the true antibacterial properties of the extracts.

Spot Assay

To further qualitatively support the notion that these plants exhibit antibacterial properties against *S. epidermidis*, 10 µL of solution from each column (2-11) of a given plant in the higher concentration MIC assay was added to a new 96-well plate, which was pre-filled with 90 µL of PSB. Then each column was serially diluted from a factor of 10^1 down to a factor of 10^4. Each column was then plated on LB Agar and incubated for 20 hours at 37°C.

Biofilm Assay

First, a liquid culture of *S. epidermidis* was prepared and incubated for 20 hours at 37°C**.** Then, it was diluted

Table 1. Optical density (OD measured at 600 nm) measurements for literature-based minimum inhibitory concentration (MIC) dilutions of three aqueous plant extracts tested against Staphylococcus epidermidis. *Extracts were serially diluted two-fold, with the literature MIC value positioned in column 6 of the 96-well plate. Column 1 contains extract control (no bacteria), used to correct extract turbidity. Column 12 contains growth control, untreated bacterial growth. OD600 readings reflect bacterial growth after 20 hours at 37°c. These raw values were later used in calculating percent inhibition and determining MIC for Staphylococcus epidermidis by implementing Equation 1.*

	1	2	3	4	5	6	7	8	9	10	11	12
C. sativum	0.226	1.421	1.396	1.326	1.369	1.357	1.338	1.377	1.418	1.345	1.374	1.587
C. cardunculus	1.829	1.883	1.508	1.281	1.528	1.257	1.226	1.338	1.236	1.233	1.175	1.621
A. heterophyllus	0.045	1.228	1.043	1.156	1.230	1.295	1.412	1.221	1.082	1.284	1.039	1.717

Table 2. Optical density (OD measured at 600 nm) measurements for higher concentration minimum inhibitory concentration (MIC) dilutions of three aqueous plant extracts tested against Staphylococcus epidermidis. *Extracts were serially diluted two-fold, with the literature MIC value positioned in column 6 of the 96-well plate. Column 1 contains extract control (no bacteria), used to correct extract turbidity. Column 12 contains growth control, untreated bacterial growth. OD600 readings reflect bacterial growth after 20 hours at 37°c. These raw values were later used in calculating percent inhibition and determining MIC for Staphylococcus epidermidis by implementing Equation 1. OVERFLOW indicates that the OD600 reading exceeded the measurable range of the plate reader.*

	1	2	3	4	5	6	7	8	9	10	11	12
C. Sativum	Overflow	3.313	2.460	1.978	1.765	1.798	1.625	1.624	1.545	1.526	1.497	1.036
C. Cardunculus	3.785	3.200	2.246	2.045	1.784	1.69	1.604	1.554	1.505	1.531	1.420	1.726
A. Heterophyllus	3.513	3.128	2.351	2.214	2.039	1.882	1.752	1.647	1.598	1.447	1.550	1.479

with TSB to an OD of 0.1. 150 µL of this solution was plated onto a special biofilm assay plate and incubated in an orbital incubator for 24 hours at 37°C. After this initial biofilm-formation period, any excess medium was gently removed. Half the determined MIC for each extract was prepared by diluting the MIC concentration 1:2 in sterile TSB, based on the highest percent inhibition value identified from the MIC assay. 150 µL of these sub-inhibitory treatments were added to the wells to isolate biofilm-disruption effects from general bactericidal activity and again incubated in an orbital incubator for 24 hours at 37°C. Afterwards, the plate was first fixed with 30% acetic acid and washed with PBS. Crystal violet was added to stain, and again the sample was washed with PBS. Lastly, the plate was fixed with methanol. Biofilm formation was quantified using crystal violent staining, followed by an OD measurement at 570nm. Percent biofilm inhibition was calculated using Equation 2.

$$Bioflim\ \%\ Inhibtion = \left(1 - \frac{OD_{sample}}{OD_{PBS\ control}}\right) \times 100 \quad \text{(Eq. 2)}$$

Statistical Analysis

All MIC assays and spot assays were each performed once per extract. These experiments were designed as preliminary screening assays to identify concentration values with observable and quantifiable antibacterial activity to qualitatively confirm inhibition patterns. Because these assays were not replicated, statistical significance testing and measure of variation could not be applied. However, the biofilm inhibition assay was performed 3 times for each treatment condition. These replicates allowed for calculations of descriptive statistics (Table 6) and a one-way ANOVA test from the calculated biofilm inhibition percentage values in Table 5.

RESULTS

Effect of Plant Extracts on Bacterial Growth: MIC Assay Results

Two MIC assays were conducted: one based on MIC values reported in literature against *Staphylococcus aureus* (based on alcohol-extracted plant materials), and a second at relatively higher concentrations (approximately twice the literature MIC) to account for differences in extraction method and solvent used. Ampicillin was included as an antibiotic control in the higher concentration of the MIC assay. Bacterial growth was measured by optical density (OD) at 600 nm. The raw OD readings for all serial dilutions for both assays, including extract-only controls (column 1) and untreated growth controls (column 12) are displayed in Tables 1 and 2 and are utilized for the calculation MIC % inhibition.

Percent inhibition was calculated for each dilution using Equation 1, and the resulting values for all extracts are summarized in Tables 3 and 4. These values were used to identify the MIC for each extract, which is defined as the dilution that exhibited the highest percent inhibition. The MIC values determined from this analysis were then subsequently used as treatment concentrations in the biofilm assay

Visual Confirmation of Antibacterial Activity: Spot Assay Results

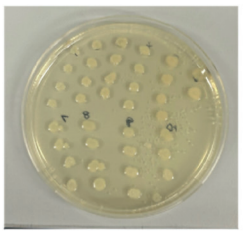
Figure 1 provides a qualitative visualization of antibacterial activity by showing the growth patterns of *S. epidermidis* after exposure to MIC-level extract concentrations. Ten-fold serial dilutions of treated cultures were spotted onto agar, and reductions in colony size or intensity across dilutions reflect the inhibitory trends observed in the MIC assay. This assay served to

Table 3. Percent inhibition values calculated from literature-based MIC assay for each extract across the serial dilutions. *Percent inhibition was calculated using Equation 1 and OD readings from Table 1, which accounts for extract turbidity by incorporating the extract-only control (column 1 and the untreated growth control (column 12). Values represent the degree of growth reduction/ suppression at each dilution of extract. The highest inhibition percentage value for each extract was used to determine the MIC that was utilized for the biofilm assay.*

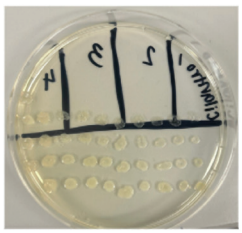
	2	3	4	5	6	7	8	9	10	11
C. sativum	12.200	14.034	19.177	16.018	16.899	18.295	15.43	12.417	17.381	15.65
C. cardunculus										
A. heterophyllus	29.246	40.211	33.55	29.127	25.239	18.24	30.203	37.977	25.897	40.555

Table 4. Percent inhibition values calculated from higher concentration MIC assay for each extract across serial dilutions. *Percent inhibition was calculated using Equation 1 and od readings from Table 2, which accounts for extract turbidity by incorporating the extract-only control (column 1 and the untreated growth control (column 12). Values represent the degree of growth reduction/suppression at each dilution of extract. The highest inhibition percentage value for each extract was used to determine the MIC that was utilized for the biofilm assay.*

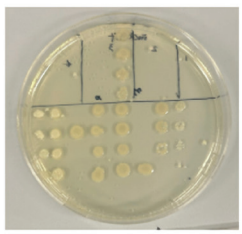
	2	3	4	5	6	7	8	9	10	11
C. sativum										
C. cardunculus	71.588	25.255	15.493							
A. heterophyllus	81.072	42.871	36.136	27.532	19.813	13.422	8.26	5.851		3.491



Artocarpus heterophyllus



Coriandrum sativum



Cynara cardunculus

Figure 1. ***Spot assay showing Staphylococcus epidermidis growth across ten-fold serial dilutions following treatment with MIC-level concentrations of each plant extract.*** *To qualitatively assess the antibacterial activity, a spot assay utilizing 10µL from columns 2-11 from higher concentration MIC assay was transferred into fresh PSB, serially diluted (10^1 to 10^4) an plated on LB agar. After 20 hrs. of incubation at 37°C colony size and spot intensity were visually evaluated. Reduced growth indicated stronger antibacterial properties. Each extract displayed the greatest reduction at the MIC level dilution.*

qualitatively confirm the quantitative inhibition patterns reported in Tables 3 and 4.

Effect of Sub-Inhibitory Concentrations on Biofilm Formation

The blue dye in Figure 2 represents the crystal violet stain. Given that *S. epidermidis* is a Gram-stain-positive bacterium, a greater number of colonies growing present translates to more crystal violet retained within the thick peptidoglycan cell wall, which theoretically should indicate a reduced biofilm disruption. All plant extracts exhibited a reduction in biofilm formation compared to PBS (control). Furthermore, *A. heterophyllus* represented the greatest reduction in biofilm formation. Biofilm formation was quantified using crystal violent staining, followed by an OD measurement at 570nm. Percent biofilm inhibition was calculated using Equation 2.

Raw absorbance values and the calculated inhibition percentages for all three replicates (n=3) are displayed in Table 5. Summary statistics for each extract (mean ± standard deviation, standard error of mean, and replicate number) are displayed in Table 6.

A one-way ANOVA showed a highly significant effect on percent biofilm inhibition ($F = 512.197$, $p = 1.767 \times 10^{-9}$). Since the primary objective of the experiment was to determine which extracts reduced biofilm formation

Table 5. Raw absorbance (OD_{570}) values and corresponding percent biofilm inhibition for three replicates of each treatment. *Biofilm formation of S. Epidermidis was quantified by using crystal violet staining. OD_{570} values (absorbance) represent the complete biomass of the biofilm, whereas the percent inhibition was a calculated value relative to the PBS negative control that was found by implementing Equation 2. Treatments were the three aqueous plant extracts: A. heterophyllus, C. sativum, C, cardunculus. PBS was the negative control (0% inhibition).*

	Absorbance	% Biofilm Inhibition
Replicate 1		
PBS	2.504	0.000
Artocarpus heterophyllus	1.969	21.366
Coriandrum sativum	2.351	6.110
Cynara cardunculus	2.503	0.0399
Replicate 2		
PBS	2.513	0.000
Artocarpus heterophyllus	1.935	23.000
Coriandrum sativum	2.298	8.556
Cynara cardunculus	2.505	0.318
Replicate 3		
PBS	2.510	0.000
Artocarpus heterophyllus	1.946	22.470
Coriandrum sativum	2.301	8.327
Cynara cardunculus	2.500	0.398

Table 6. Summary statistics for percent biofilm inhibition by each plant extract, including mean ± standard deviation (SD), standard error of mean, and number of replicates (n). *These statistics were used to perform the one-way ANOVA and Dunnet's post-hoc test comparing each extract to the PBS control. A. heterophyllus represents the highest and most consistent inhibition; C. sativum showed moderate inhibition, comparatively, and C. cardunculus showed negligible to minimal inhibition.*

	Mean % inhibition ± SD	SEM	n
PBS	0.000 ± 0.000	0.000	3
A. heterophyllus	22.279 ± 0.834	0.482	3
C. sativum	7.664 ± 1.351	0.780	3
C. cardunculus	0.252 ± 0.188	0.109	3

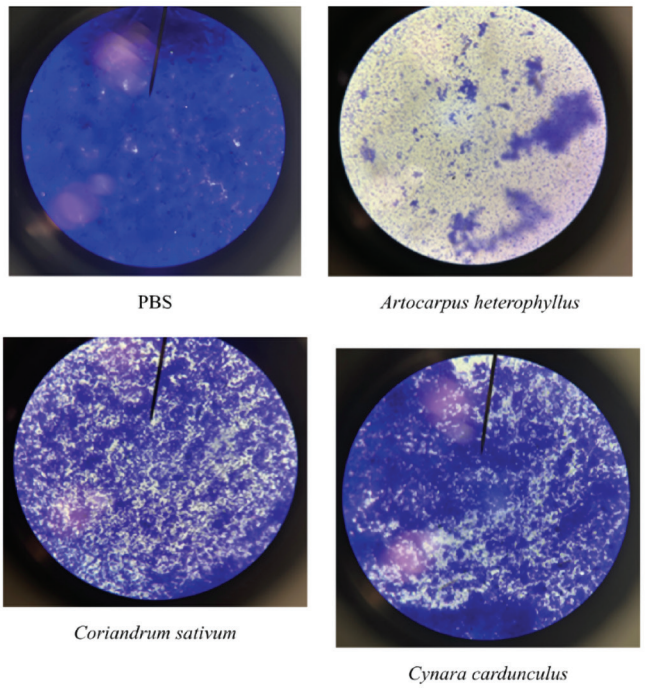


Figure 2. Crystal violet-stained Staphylococcus epidermidis biofilms following treat with each plant extract or the PBS negative control. *Biofilms were formed by incubating diluted S. epidermidis cultures (OD 0.1) for 24 hours then treated with half the MIC concentrations of each extract and incubated for an additional 24 hours. After fixation and staining, microscopic x40 images were captured to visualize the biofilm formation. Darker staining indicates thicker biofilm.*

the most comparative to the PBS control (the negative control), a Dunnett's post-hoc test was implemented. *A. heterophyllus* produced the greatest inhibition, with a mean reduction of 22.279%, which was highly significant when compared to PBS ($p < 0.0001$). *C. sativum* also showed statistically significant reduction (7.66% $p < 0.0001$, but to a lesser extent comparative to the *A. heterophyllus*. In contrast, *C. cardunculus* exhibited minimal inhibition (0.25%) and did not differ significantly from the PBS control ($p = 0.961$).

DISCUSSION

Antimicrobial Resistance is a growing field in biomedical sciences (1). Antibiotic resistance is major subset of antimicrobial resistance and has exhibited alarming trends in recent years. According to the World Health Organization, "between 2018 and 2023, antibiotic

resistance rose in over 40% of the pathogen-antibiotic combinations monitored, with an average annual increase of 5–15%" (11). Thus, there is an increasing demand for alternative treatments to combat the evolving pathogenic microorganisms. This study is believed to be the first to investigate the potential of three aqueous plant extracts: *Artocarpus heterophyllus* (Jackfruit) seeds, *Cynara cardunculus* (Artichoke) flesh, and *Coriandrum sativum* (cilantro) against the biofilm formation of *Staphylococcus epidermidis*, a process crucial to the vitality of various aggregations of microorganisms. Biofilm formation is known to be dependent on the quorum-sensing abilities of the microorganism (12). Quorum Sensing is a crucial form of intercellular communication that plays a crucial role in coordinating the population's density and controlling factors such as virulence, bioluminescence, and biofilm formation. Inhibiting this form of communication would lead to discord in the regulation of the population's density, and eventually cut off the synchronized bacterial growth, effectively ending the population and possibly preventing the species from evolving and growing resistance to the drug or source of treatment. This is the main principle behind quorum quenching research. The objective of this study was to determine whether natural aqueous extracts can disrupt biofilm formation and quantify the resulting percentage inhibition. Because biofilm formation in *S. epidermidis* is influenced by quorum-sensing pathways, reductions in biofilm at sub-inhibitory concentrations are consistent with quorum-quenching activity rather than direct bactericidal effects.

The Minimum Inhibition Concentration (MIC) assays were performed to determine the antibacterial potential of the plant extracts and, subsequently, were used to establish a sub-inhibitory level for the biofilm assay. The assay plates were read in an optical density (OD) plate reader at a wavelength of 600 nm, and the readings are displayed in Tables 1 & 2. Two assays were conducted to establish an understanding of the plant extracts and the impact of variations in concentration: one using concentrations reported in literature of these plant extracts obtained with non-aqueous solvents *against Staphylococcus aureus*, and a secondary assay at higher concentrations to account for differences in extraction method and solvent. The OD readings for both assays were used to calculate the MIC% value for each extract. It is important to note that *Equation 1*, used to calculate the MIC percent, takes into account the inherent turbidity of the plant extracts themselves to reflect accurate data on the extract's true antibacterial capabilities The results are as follows: *A. heterophyllus* showcased the highest inhibition percentage at 81.072%, followed by *C. cardunculus* at 71.588%, and lastly, *C. sativum* at 19.177%. While these percentages were lower than the Ampicillin (a known antibiotic) control, which achieved 100% at one dilution, the data established that the plant extracts do, in fact, contain compounds that contribute to antibacterial properties against *S. epidermidis*. The spot assay was also conducted to qualitatively support this finding, showing a general trend of a smaller colony size at the MIC value for each extract as can be seen in Figure 1.

The biofilm assay was a crucial component of the experiment, designed to measure biofilm disruption, which in turn gave insight into the quorum-quenching capabilities of the extracts. A crystal violet stain was used to quantify the reduction in biofilm production caused by each plant extract. The data confirms that the *A. heterophyllus* seeds (jackfruit seeds) disrupted biofilm production the most and therefore suggests a greater quorum quenching effect among the tested treatments, with a percent inhibition of 22.279 ± 0.834%. This was followed by *C. sativum* aqueous extract, 7.664 ± 1.3515, and lastly, the *C. cardunculus* flesh extract, 0.252 ± 0.188%, which showed almost negligible biofilm disruption at half its MIC value. The overall finding supports the notion that these plants have the potential to function as quorum quenching agents in medicine, especially *A. heterophyllus* seed extract, which demonstrates a potent capability in anti-biofilm formation as well as antibacterial properties against *S. epidermidis*. A previous study on the antibiofilm activity of an ethanolic extract of *A. heterophyllus* seeds against *Escherichia coli* and *Klebsiella pnuemoniae* reported higher inhibition values approaching nearly complete suppression of biofilm formation. However, the study also reported no inhibitory effect was detected against *S. aureus*, which was consistent with the antibacterial activity in the MIC assay (13). Taken collectively, these findings position *A. heterophyllus* at the upper end of the inhibition ranges previously reported for plant-derived anti-biofilm agents. Additionally, other plants extracts have also been tested for biofilm reduction against *S. aureus*, although most of these extracts used methanol extracts. Examples include: *Syzygium aromaticum* (clove) had a range of 54-65% biofilm reduction, *Camellia sinensis* (tea plant) had a range of 57-73%, and *Allium sativum* (garlic) had a range of 19-31% (14). These reported ranges provide a useful benchmark, indicating the levels of inhibition observed in this study fall within

the lower-moderate end of the spectrum for plant derived antibiofilm agents, particularly when compared to high performing extracts like *Camellia sinesis.*

While the study successfully demonstrates *A. heterophyllus* seeds' potential in quorum quenching and its antibacterial and antibiofilm properties, it was subject to certain limitations. First, the samples obtained were crude extracts; thus, specific bioactive compounds were not identified. Second, due to time and material constraints, multiple replications could not be performed, which limited the generalizability of the experiment and its statistical power. Lastly, because percent biofilm inhibition was calculated relative to the PBS negative control, the PBS group was constrained to 0% inhibition by definition. Thus, the PBS group exhibited no within-group variance, which may have contributed to a higher F-value in the one-way ANOVA. Future analysis could alternatively perform statistical analysis dependent on absorbance or re-run a one-way ANOVA on greater sample size.

CONCLUSION

This study successfully investigated the biofilm-disrupting potential of three novel aqueous plant extracts on *S. epidermidis*, reflecting their quorum-quenching and antibacterial properties. The MICs conducted proved that each plant extract presented some form of antibacterial activity. Additionally, by utilizing half the MIC value, a sub-inhibitory concentration was established to isolate their biofilm disruption (correlated to quorum quenching) effect from their general bactericidal activity. The quantitative data from the biofilm assay supported the notion that *A. heterophyllus* inhibited biofilm formation the most, 22.279 ± 0.834%. These results highlight the strong potential of aqueous plant extracts in quorum quenching-guided antibacterial medications that aid in limiting bacterial resistance. While the study was limited due to the use of crude extracts and limited replications, further research should aim to purify and perform spectrophotometry on specific bioactive compounds, such as phenolic or flavonoid compounds, within *A. heterophyllus* seeds to identify specific compounds that function as anti-biofilm and antibacterial agents in various bacterial strains, including *S. epidermidis.*

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CONFLICT OF INTEREST

The author declares that there are no conflict of interests related to this work

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