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Anti-Biofilm and Quorum Sensing Inhibitory Activities of *Carica papaya* L. Peel Extract Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Antimicrobial resistance (AMR) continues to pose a major threat to global public health due to the increasing resistance of pathogenic bacteria to conventional antibiotics. This study evaluated the anti-biofilm and quorum sensing inhibitory activities of *Carica papaya* L. peel extract against *Escherichia coli* and *Staphylococcus aureus*. An experimental research design was employed involving methanolic extraction, physicochemical analysis, phytochemical screening, and crystal violet biofilm assay. Papaya peel extract was prepared through maceration using 85% methanol. The extract was subjected to organoleptic evaluation, pH determination, solubility testing, and phytochemical screening for polyphenols and saponins. Results revealed that the extract contained polyphenols and saponins and exhibited acidic and polar characteristics. Crystal violet assay demonstrated a significant reduction in biofilm formation in both bacterial isolates treated with papaya peel extract compared to the negative control. Statistical analysis using one-way Analysis of Variance (ANOVA) revealed significant differences among treatment groups ($p < 0.05$). The findings suggest that *Carica papaya* peel extract possesses promising anti-biofilm and quorum sensing inhibitory properties and may serve as a potential natural alternative for combating antimicrobial resistance.

Keywords: *Anti-biofilm, quorum sensing inhibition, Carica papaya peel extract, antimicrobial resistance, Escherichia coli, Staphylococcus aureus*

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most serious public health concerns worldwide. The increasing resistance of pathogenic bacteria to commonly used antibiotics has reduced the effectiveness of conventional antimicrobial therapies and increased the burden of infectious diseases. Among the common resistant bacterial pathogens are *Escherichia coli* and *Staphylococcus aureus*, which are associated with urinary tract infections, pneumonia, skin infections, sepsis, and other life-threatening conditions.

One mechanism that contributes to bacterial pathogenicity and resistance is quorum sensing (QS), a bacterial communication system responsible for regulating virulence factor production, biofilm formation, and bacterial survival. Through quorum sensing, bacteria release signaling molecules called autoinducers

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that allow them to coordinate collective behaviors based on cell density. Biofilm formation is one of the most important QS-regulated mechanisms because it protects bacteria from antibiotics and host immune responses, making infections more difficult to eliminate.

Recently, quorum sensing inhibition (QSI) has emerged as a promising alternative strategy in controlling bacterial infections. Unlike traditional antibiotics that directly kill bacteria, QS inhibitors interfere with bacterial communication pathways, thereby reducing virulence and biofilm formation while minimizing the development of resistance. This strategy has gained increasing attention due to its potential to reduce bacterial pathogenicity without exerting selective pressure that promotes antimicrobial resistance.

Natural products have gained increasing attention as potential sources of antimicrobial and quorum sensing inhibitory compounds. Medicinal plants contain bioactive phytochemicals that exhibit antibacterial, antioxidant, and anti-inflammatory properties. Among these plants is *Carica papaya* L., commonly known as papaya, which is widely cultivated in tropical countries and recognized for its medicinal value. Papaya peels are often discarded as agricultural waste despite containing important phytochemicals such as polyphenols, flavonoids, and saponins that may possess antimicrobial and anti-biofilm activities.

Several studies have reported the antibacterial potential of papaya-derived compounds against pathogenic microorganisms. However, limited studies have explored the anti-biofilm and quorum sensing inhibitory activities of papaya peel extract specifically against *Escherichia coli* and *Staphylococcus aureus*. Therefore, this study aimed to evaluate the anti-biofilm and quorum sensing inhibitory activities of *Carica papaya* L. peel extract against the selected bacterial isolates.

Specifically, the study aimed to:

1. Determine the physicochemical properties of *Carica papaya* peel extract;
2. Identify the phytochemical constituents present in the extract; and
3. Evaluate the anti-biofilm and quorum sensing inhibitory activities of the extract against *Escherichia coli* and *Staphylococcus aureus*.

METHODS

Research Design

The study utilized an experimental research design to evaluate the anti-biofilm and quorum sensing inhibitory activities of *Carica papaya* L. peel extract against *Escherichia coli* and *Staphylococcus aureus*.

Research Locale

The study was conducted at the laboratory of the University of Perpetual Help System DALTA – Calamba Campus.

Collection and Preparation of Plant Material

Papaya peels were collected from unripe *Carica papaya* fruits in Lobo, Batangas, Philippines. The collected peels were thoroughly washed with water and air-dried in a shaded area for five to seven days to prevent photodegradation of phytochemicals. The dried peels were ground into powder form using a blender.

Extraction Procedure

Methanolic extraction was performed through maceration. Approximately 100 g of powdered papaya peel was soaked in 1000 mL of 85% methanol for 72 hours with intermittent stirring every 12 hours. The mixture was filtered using Whatman filter paper No. 1, and the filtrate was concentrated using a steam bath to obtain the crude extract.

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Physicochemical Analysis

The crude extract underwent physicochemical analysis including organoleptic evaluation, pH determination, and solubility testing. Organoleptic properties such as appearance, color, odor, and consistency were observed. The pH was determined using a pH indicator strip. Solubility testing was conducted using distilled water, ethanol, toluene, and chloroform.

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify the presence of bioactive compounds. Polyphenols were detected using Ferric Chloride Test and Lead Acetate Test, while saponins were identified using the Froth Test.

Preparation of Test Organisms

The bacterial isolates *Escherichia coli* and *Staphylococcus aureus* were obtained from the Angelo King Medical Research Center. The isolates were cultured using nutrient agar plates and incubated at 37°C for 18–24 hours.

Crystal Violet Biofilm Assay

The anti-biofilm activity of the extract was evaluated using crystal violet staining in a 96-well plate assay. Treatment groups included papaya peel extract, salicylic acid as positive control, and sterile water as negative control. Following incubation at 37°C for 24 hours, biofilms were stained with 0.1% crystal violet and quantified spectrophotometrically at 600 nm.

Statistical Analysis

Data gathered from the study were analyzed using one-way Analysis of Variance (ANOVA). A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Percentage Yield of Extract

The methanolic extraction of papaya peel produced a percentage yield of 17.93%, indicating effective extraction of phytochemical constituents.

Table 1. *Physicochemical Properties of Carica papaya Peel Extract*

Properties	Results
Appearance	Translucent dark green liquid
Color	Dark green
Odor	Slightly pungent
Consistency	Watery
pH	4.5
Solubility in Water	Miscible
Solubility in Ethanol	Miscible
Solubility in Toluene	Immiscible
Solubility in Chloroform	Immiscible
Percentage Yield	17.93%

The extract appeared as a translucent dark green liquid with slightly pungent odor and watery consistency. The extract exhibited a pH value of 4.5, indicating moderate acidity. Solubility testing showed that the extract was miscible in polar solvents such as distilled water and ethanol but immiscible in non-

polar solvents such as toluene and chloroform. These findings suggest the presence of polar phytochemical compounds within the extract.

Phytochemical Screening

Phytochemical analysis confirmed the presence of polyphenols and saponins in the papaya peel extract. The Ferric Chloride Test produced black coloration, while the Lead Acetate Test formed white-yellow precipitates, indicating positive results for polyphenols. The Froth Test demonstrated persistent foam formation, confirming the presence of saponins.

The presence of polyphenols and saponins may have contributed to the observed anti-biofilm and quorum sensing inhibitory activities of the extract. Previous studies reported that these phytochemicals interfere with bacterial signaling pathways, inhibit virulence factor production, and reduce biofilm formation.

Anti-Biofilm Activity

The crystal violet assay demonstrated reduced biofilm formation in both *Escherichia coli* and *Staphylococcus aureus* treated with papaya peel extract compared to the negative control. Statistical analysis using ANOVA revealed significant differences among treatment groups ($p < 0.05$), indicating that the extract exhibited significant anti-biofilm and quorum sensing inhibitory activities.

The significant reduction in biofilm formation suggests that *Carica papaya* peel extract may interfere with bacterial quorum sensing mechanisms responsible for virulence and biofilm development. These findings support previous studies indicating that plant-derived phytochemicals can disrupt bacterial communication systems and inhibit biofilm formation.

The acidic nature of the extract may also have contributed to its antimicrobial effects, as acidic environments can interfere with bacterial growth and quorum sensing signaling pathways. Furthermore, the polar characteristics of the extract may have enhanced its interaction with bacterial membranes and enzymes involved in biofilm formation.

The findings highlight the potential use of papaya peel extract as a natural and sustainable source of anti-biofilm and quorum sensing inhibitory compounds. Since papaya peels are commonly discarded as agricultural waste, their utilization in antimicrobial research may contribute to both environmental sustainability and public health innovation.

CONCLUSION

The findings of the study demonstrated that *Carica papaya* L. peel extract possesses significant anti-biofilm and quorum sensing inhibitory activities against *Escherichia coli* and *Staphylococcus aureus*. The presence of polyphenols and saponins may contribute to the biological activity of the extract. The study highlights the potential of papaya peel as a sustainable and natural source of antimicrobial agents that may help address the growing challenge of antimicrobial resistance.

Further studies are recommended to isolate and identify the specific active compounds responsible for the observed biological activities and to determine their exact mechanisms of action.

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