Journal of Prevention, Diagnosis and Management of Human Diseases
Vol: 06, No. 01, Jan-June 2026
ISSN: 2799-1202, DOI: 10.55529/jpdmhd.61.13.25

Research Paper

Inhibition of quorum sensing in microbial escherichia coli and pseudomonas sp. biofilms using yellow bell (allamanda cathartica) leaf extract

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Article Info

Article History:
Received: 22 November 2025
Revised: 04 February 2026
Accepted: 10 February 2026
Published: 25 March 2026

Keywords:
Quorum Sensing Inhibition
Pseudomonas Aeruginosa
Escherichia Coli
Biofilms
Yellow Bell

ABSTRACT

Background: Antimicrobial resistance is an escalating global health threat, necessitating alternative strategies such as quorum sensing (QS) inhibition. Quorum sensing governs bacterial virulence and biofilm formation, making its inhibition a promising therapeutic target.
Objective: To evaluate the anti-quorum sensing and antibiofilm activity of ethanol extract from Allamanda cathartica leaves against Escherichia coli and Pseudomonas sp.
Methods: Allamanda cathartica leaves were collected, processed, and extracted using ethanol. Multiple concentrations of the extract were tested alongside positive and negative controls. Anti-QS susceptibility assays and biofilm formation experiments were conducted under laboratory conditions. Inhibition zones were measured and analyzed using one-way Analysis of Variance (ANOVA).
Results: A statistically significant difference in inhibition zones was observed across treatment concentrations for both E. coli and Pseudomonas sp. ($p < 0.05$, one-way ANOVA). Higher extract concentrations produced greater inhibition, demonstrating dose-dependent anti-QS activity and suppression of biofilm formation.
Conclusion: Allamanda cathartica leaf extract demonstrates potential as a natural, accessible, and cost-effective quorum sensing inhibitor. Further research testing the extract against additional bacterial strains is recommended to fully characterize its antibacterial scope.

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1. INTRODUCTION

1.1 Background of the Study

The global challenge of Antimicrobial resistance is one of the most serious dangers present in modern medicine and public health systems globally. Over the past two decades (2000-2024), the creation and spread of multidrug-resistant organisms (MDROs) have increased at an unprecedented rate, posing a significant public health concern [1]. Antimicrobial resistance (AMR) has increased in recent years as antibiotics have become more widely used in clinical practice. Drug-resistant bacterial infection dramatically raises the death rate, hospitalization time, and treatment expense, which grows with age [2].

Escherichia coli, a member of the Enterobacteriaceae family, is known to cause a variety of diseases in people and animals. Pathogenic E. coli strains are frequently recognized as a cause of sickness in children and the efficacy of current antimicrobials, which were formerly considered the last choice, is beginning to decline [3]. Because of the extensive presence of E. coli and its continued evolution of antibiotic resistance, humans and animals are gravely at danger of infection. Antibiotic use is expected to increase by 67% by 2030, putting a financial strain on healthcare systems. Extended-Spectrum Beta-Lactamase (ESBL) E. Coli, a prominent multidrug- resistant bacteria, is responsible for generating severe infections in both hospital and community settings [4].

Pseudomonas aeruginosa, a Gram-negative bacteria, is known for its flexibility and opportunistic behavior. The complex antibiotic resistance systems which create biofilms together with their ability to create ongoing infections in animal and human bodies make this condition a major obstacle for medical facilities according to research from [5]. Multidrug-resistant Pseudomonas aeruginosa (MDRPA) which spreads through hospitals creates a critical public health threat because it increases patient suffering and death rates while driving up healthcare expenses. P. aeruginosa acts as an opportunistic pathogen which creates various health care-associated diseases that include pneumonia respiratory infections urinary tract infections and surgical site or wound infections together with bacteremia [6].

Quorum Sensing (QS), also termed as Density Sensing, regulates numerous physiological functions in bacteria. Quorum-sensing mechanisms exist in both Gram- negative and Gram-positive bacteria, however, the signal molecules used to communicate information differ. Bacteria regulate the behavior of the entire bacterial population by manufacturing and secreting signal molecules (also known as self-inducing molecules). When the concentration of signal molecules exceeds a given threshold in relation to bacterial population density, the expression of specific genes can be activated to regulate bacterial population adaptability [7]. The active QS system of Gram-negative bacteria operates through a complex system that involves N-acyl homoserine lactones and quinolone signals which function as interconnected control mechanisms. Similarly, Gram-positive pathogens use peptide-based QS systems, specifically the accessory gene regulator system, to control pathogenicity and biofilm formation. QS's crucial role in bacterial pathogenesis has made it a more appealing target for therapeutic interventions [8].

Allamanda cathartica L. (A. cathartica) is also known as The Golden Trumpet and The Buttercup Flower and The Yellow Bell. The Apocynaceae family contains these tropical shrubs and vines as its members. In traditional medicine, A. cathartica is used to treat a range of illnesses. It has anticancer, antioxidant, antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory, anti-diabetic, and cathartic properties. Apart from these pharmacological actions, species of A. Cathartica has also been recorded to be used as an anti-ascites, antihypertensive, diuretic and emetic, antipyretic, laxative, and to cure jaundice. The most commonly used plant parts are leaves, stem bark, flowers, roots, stem, sap, seeds, and branches, in that order [9]. People claim that the plant's distilled extract, which they have studied, provides treatment for cancer and fungal and bacterial infections and colic and extreme stomach discomfort. It is used to treat

jaundice and enlarged spleen caused by malaria. The cathartic has antibacterial and perhaps anticancer properties [10].

In this study, we will investigate into the anti-quorum sensing attributes of Allamanda cathartica and its extract, which will be used to suppress specific virulence factors in Escherichia coli and Pseudomonas aeruginosa. Thus, it is being considered whether the herb that is widely cultivated in and around the Bayugan Area could be used as an alternative to allopathic therapy.

2. RELATED WORK

2.1 Allamanda Cathartica as Antimicrobial

For many years, nature served as the principal source of medical medicines. Historically, plant products derived from barks, leaves, flowers, roots, fruits, and seeds have been used to treat a variety of ailments for centuries [11]. As bacteria become more resistant to synthetic antibiotics, many studies have looked into medicinal plants as possible sources of antibacterial compounds [12]. The recent studies show that the medical establishment needs to adopt better procedures to handle their existing antibiotic resistance crisis phytochemical makeup of A. Along with its direct antibacterial actions, cathartica may disrupt quorum sensing [13] undertook a systematic review of Allamanda cathartica Linn.

People have used Apocynaceae for a long time to treat a number of health problems because it protects the liver, lowers blood sugar, fights cancer, and fights bacteria [14]. Allamanda cathartica stands as a perennial shrub belonging to the Apocynaceae family which scientists have studied for its unique biological active substances and its possible medicinal applications. The extract contained carbohydrates and alkaloids and saponin glycosides and proteins and tannins and flavonoids and amino acids according to the complete phytochemical analysis.

The plant provides multiple health benefits through its antioxidant properties and its ability to reduce inflammation and promote wound healing and its antibacterial effects. Because more people are interested in using natural ingredients to treat cancer [15]. The plant demonstrates the ability to eliminate both bacterial organisms and cancer cells. The plant serves as a treatment for jaundice and diabetes and high blood pressure and polio. The species provides all its economically valuable and medically useful plant parts which serve as components for production of a powerful laxative solution [16].

2.2 Pseudomonas Aeruginosa

Antimicrobial resistance (AMR) is one of the most important issues affecting healthcare systems worldwide. The process of microbial resistance develops when bacteria viruses fungi and parasites undergo evolutionary changes that enable them to survive pharmaceutical treatments designed to eliminate them. In a study [17], Antibiotic resistance is becoming a persistent pandemic of bacterial illnesses over the world. In 2016, the Drugs for Neglected Diseases initiative and the World Health Organization (WHO) formed the Global Antibiotic Research and Development Partnership to speed up the development of new antibiotics to treat multidrug-resistant bacterial illnesses [18].

Pseudomonas aeruginosa is one of the leading causes of hospital-acquired and chronic infections, and it is associated with high antibiotic resistance, morbidity, and death. Indeed, antibiotic-resistant P. aeruginosa infections are predicted to cause over 300,000 deaths each year, and they are at the top of the WHO priority list for new antibiotic research and development. Pseudomonas aeruginosa is a rod-shaped Gram-negative bacterium that can cause a variety of illnesses in people with both healthy and weakened immune systems. This microbe is usually referred to as an opportunistic pathogen [19].

2.3 Escherichia Coli

Antibiotics have played a critical role in the treatment of infectious diseases, increasing patients' quality of life and life expectancy. However, their abuse and misuse have aided in the evolution of antimicrobial resistance (AMR), which happens when germs withstand the fatal effects of antibiotics [20]. E. Coli are Gram-negative bacteria that are widely found in the gastrointestinal systems of humans and animals. In beef cattle, E. coli are normally innocuous, although some strains might cause diarrhea in

newborn calves. Likewise, most E.

Although most coli strains in people are harmless, a few can cause sickness [21]. Although Escherichia coli is a typical intestinal microbiota, certain pathogenic strains are a major cause of clinical infections globally, notably urinary tract infections (UTIs). This accounts for up to 85-90% of community-acquired cases [22]. Antibiotics can be used to treat bacterial infections, however, microorganisms like E. Coli can develop resistance to certain antibiotics, making infections more difficult to treat. E. coli was expected to die in 4.95 million people around the world in 2019 because of antimicrobial resistance (AMR). Coli is one of the main species that is involved [23].

2.4 Quorum Sensing

Quorum sensing is the means by which bacteria talk to each other, which depends upon the number of cells. It enables coordination with many virulence factor determinants, allowing bacteria to invade the host and acquire antibiotic resistance. The signal-response principle governs the operation of QS systems [24]. Quorum sensing enables cells to communicate with one another while it controls gene expression based on the number of cells present in the environment. The system operates through its continuous production and release of auto-inducers which serve as small signaling molecules to establish a communication system [25].

QS controls vital cellular functions which include biofilm production and development of virulence factors which enhance the pathogenicity of disease-causing organisms while increasing their ability to resist treatment and produce harmful substances.

2.5 Statement of the Problem

In this study, the effectiveness of Allamanda cathartica leaf extract in inhibiting quorum sensing among Escherichia coli and Pseudomonas sp. biofilms is investigated. Specifically, this research seeks to answer the following questions

Is it that Allamanda cathartica leaf extract inhibits quorum sensing in E. coli and Pseudomonas species biofilms when applied at different concentrations?

- Control set-up
- Positive control (ethanol)
- Negative control (distilled water)
- 1 µL leaf extract
- 2 µL leaf extract
- 3 µL leaf extract
- 4 µL leaf extract
- 5 µL leaf extract

Is there a significant difference in the quorum sensing inhibitory effects of Allamanda cathartica leaf extract between Escherichia coli and Pseudomonas sp.?

Is there a significant difference in the effects of Allamanda cathartica leaf extract on the motility of Escherichia coli and Pseudomonas sp. in terms of:

- Swimming motility
- Swarming motility
- Twitching motility

2.6 Significance of the Study

The outcome of this research and data gathered may be beneficial to:

Students: This study could serve as a guide and reference in conducting future research works.

Specialists: Specialists who study ethno-botanical sources as anti–quorum sensing agents may use the findings of this research as baseline information, providing evidence on a potential new therapeutic direction for the treatment of bacterial infections.

Public: The outcome of this study may provide an alternative aid in addressing microbial infections that are difficult to treat due to antibiotic resistance. The plant, previously valued mainly for ornamental purposes,

may help reduce the spread of diseases caused by E. coli and Pseudomonas sp. in the community.

2.7 Conceptual Framework

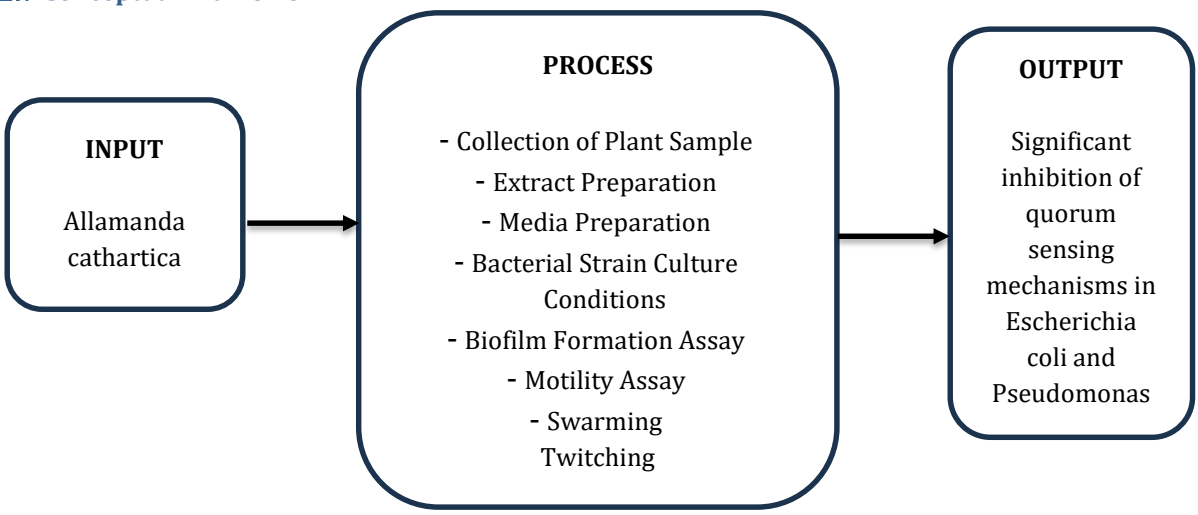

Figure 1. The Conceptual Framework of the Study

Figure 1 Shows The Flow Of The Focus Of The Study Where Allamanda Cathartica Is The Input That Leads To The Significant Inhibition Of Quorum Sensing Mechanisms In Escherichia Coli And Pseudonomas sp.

2.8 Definition of Terms

Allamanda Cathartica: A. cathartica is a flowering plant belonging to the family Apocynaceae, commonly cultivated as an ornamental plant in tropical and subtropical regions. In this study, its leaf extract is used to evaluate its potential to inhibit quorum sensing in Escherichia coli and Pseudomonas sp.

Biofilm: A biofilm is a community of microorganisms that adhere to a surface and to each other. It is associated with many microbial infections and is regulated by quorum sensing, which this study aims to inhibit

Escherichia Coli: E. coli is a gram-negative, rod-shaped bacterium, some strains of which are pathogenic. In this study, it is used to assess the quorum sensing inhibitory effect of A. cathartica leaf extract and to compare results with Pseudomonas sp.

Pseudomonas sp: Pseudomonas sp. refers to a group of gram-negative, aerobic, motile bacteria, some of which are pathogenic. This bacterium is examined to evaluate and compare the inhibitory activity of A. cathartica leaf extract with E. coli.

Quorum Sensing: Quorum sensing is a bacterial communication process that regulates group behaviors such as biofilm formation, virulence, and motility based on cell density. In this study, quorum sensing is targeted for inhibition using Allamanda cathartica leaf extract.

3. METHODOLOGY

In this chapter, the methods as well as the materials being used were properly discussed.

3.1 Experimental Flow

The researchers collected the essential materials used before and during the experiment: bacterial strains (Escherichia coli and Pseudomonas sp.), Luria-Bertani (LB) agar, LB broth, agar plates, Petri dishes, test tubes, cotton plugs, leaf extract from Allamanda cathartica, ethanol, and distilled water.

Materials were obtained from Bayugan National Comprehensive High School (BNCHS), and the bacterial strains were sourced from the Veterinary Medicine Laboratory at Central Mindanao University

(CMU). All experiments were conducted at CMU, specifically in the Microbiology Laboratory of the College of Arts and Sciences (CAS) Building.

3.2 Preparation

3.2.1 Collection of Plant Sample

The Yellow Bell (Allamanda cathartica) leaves was obtained freshly from Maygatasan in the locality of Bayugan City. The leaves was identified, confirmed and verified in the Department of Horticulture by Prof. Patria P. Solidum, In-charge, Tissue Culture Laboratory, CMU.

3.2.2 Preparation of Materials

Test tubes, erlenmeyer flasks, and such lab equipment were cleaned and sterilized before experimentation.

3.2.3 Extract Preparation

The plant leaves were washed twice with tap water. They were then sun-dried for 3- 4 days at ambient temperature (t > 35^\circ \text{C}). The dried leaves were pounded into a fine powder using a mortar and pestle. The powdered substance was submerged in 95% ethanol for a 24-hour period at a 1:2 ratio. The mixture was filtered through Whatman No. 1 filter paper and the resulting liquid was gathered in an Erlenmeyer flask.

3.2.4 Media Preparation

The bacterial medium consisted of 10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl. Swarm and twitch media were prepared with 200 mL of water and 5% (wt/vol) agar. The media were diluted, covered with aluminum foil, and sterilized in an autoclave. They were then dried overnight prior to bacterial inoculation.

3.3 Bacterial Strain Culture Conditions

Bacterial strains of (Escherichia coli and Pseudomonas sp.) were cultured in LB (Luria-Bertani) medium and incubated at 37°C for 24 h.

3.4 Assays

3.4.1 Biofilm Formation Assay

The cultured Escherichia coli and Pseudomonas sp. were diluted in fresh AB medium (1:20) to be placed in test tubes. The different concentrations of Yellow Bell leaf extract (1 µL, 2 µL, 3 µL, 4 µL and 5 µL) were incorporated into the test tubes using pipette tips. The dilutions were placed into the test tube rack and were incubated at 37°C for 24 h. After the 24 hour incubation period, 4 mL of crystal violet solution are added inside the test tubes to stain the biofilm cells for 30 minutes. Stained cells were washed with deionized water to remove unbound crystal violet whereas bounded crystal violet was eluted in 70% ethanol. The OD of the eluted ethanol samples were measured using photo spectrometry.

3.4.2 Motility Assay

Motility assays were conducted using polystyrene Petri dishes (82 mm diameter). Bacterial inoculation was performed in a laminar flow hood to prevent contamination by extraneous agents.

3.4.2.1 Swarming

The bacterial inoculum was applied to the center of the agar surface using a sterile toothpick to observe motility across the plate. The diameters of the swarming zones were measured after incubating the inoculated swarm media at 37^\circ \text{C} for 24 hours.

3.4.2.2 Twitching

A bacterial colony was inoculated deeply into the agar using a sterile toothpick, ensuring contact

with the petri dish interface. The plates were incubated at 37^\circ \text{C} for 24 hours. To measure twitching motility diameter, the agar was gently removed with forceps, and the plates were stained with 0.1% (wt/vol) crystal violet for 1 minute before rinsing.

3.5 Statistical Analysis

The collected data were analyzed using one-way Analysis of Variance (ANOVA) with Statistical Package for the Social Sciences (SPSS) software.

3.6 Disposal

After the experiments, reusable materials were autoclaved and stored securely in containers. Disposable items were decontaminated properly. The utilized experimental subjects will be returned to the Biology Laboratory at CMU.

4. RESULTS AND DISCUSSION

This section presents the analysis and interpretation of the data obtained in the study. The discussion of the results is organized in accordance with the order of the Statement of the Problem.

4.1 For Pseudomonas Aeruginosa

Table 1. Result of the Zone of Inhibition of Pseudomonas sp. under Five Treatment Conditions

Treatment	Pseudomonas sp.		Mean
	R1	R2	
T1: Control	0.007	0.000	0.0035
T2: Ethanol	0.003	0.003	0.003
T3: 5µL Yellow Bell Extract	0.005	0.008	0.0065
T4: 25µL Yellow Bell Extract	0.025	0.032	0.0285
T5: 100µL Yellow Bell Extract	0.040	0.022	0.031

Table 1 shows each of the five treatments: Control, Ethanol, 5µL Yellow Bell Extract, 25µL Yellow Bell Extract, and 100µL Yellow Bell Extract, shows clear evidence that each treatment can prevent an anti-quorum sensing activity. The mean zone of inhibition for each of the five treatments in its two replications is shown above. With the exception of the third treatment, which has a higher result value than the others, all of the treatments exhibit comparatively similar outcomes.

Table 2. Analysis of Variance of the Mean Zone of Inhibition of Pseudomonas sp. under Five Treatment Conditions

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F	P-Value
Between Groups	.002	4	.000	12.827	0.015**
Within Groups	.000	4	.000		
Total	.002	8			
Level of Significance of the Test: α = 0.05					

Table 2 demonstrates that the alternative hypothesis that there is a substantial difference in the zone of inhibitions of Pseudomonas aeruginosa under the five treatment conditions has been accepted and the null hypothesis has been rejected.

Table 3. Result of the Zone of Inhibition of Es Cherichia Coli under Five Treatment Conditions

Treatment	Escherichia Coli		Mean
	R1	R2	
T1: Control	0.003	0.013	0.023
T2: Ethanol	0.004	0.003	0.0035
T3: 5µL Yellow Bell Extract	0.008	0.005	0.0065
T4: 25µL Yellow Bell Extract	0.041	0.028	0.0095
T5: 100µL Yellow Bell Extract	0.038	0.040	0.039

The outcomes of the five treatments Control, Ethanol, 5µL, 25µL, and 100µL of Yellow Bell Extract in the Zone of Inhibition of Escherichia coli are displayed in Table 3. It demonstrates how anti-quorum sensing activity can be inhibited by the treatments.

Table 4. Analysis of Variance of the Mean Zone of Inhibition of Escherichia Coli under Five Treatment Conditions

	Sum of Squares	Degree of Freedom	Mean Square	F	P-Value
Between Groups	.002	4	.001	20.412	.003
Within Groups	.000	5	.000		
Total	.002	9			

Table 4 demonstrates that the alternative hypothesis that there is a substantial difference in the zone of inhibitions of Escherichia coli under the five treatment conditions is accepted and the null hypothesis is rejected based on the results.

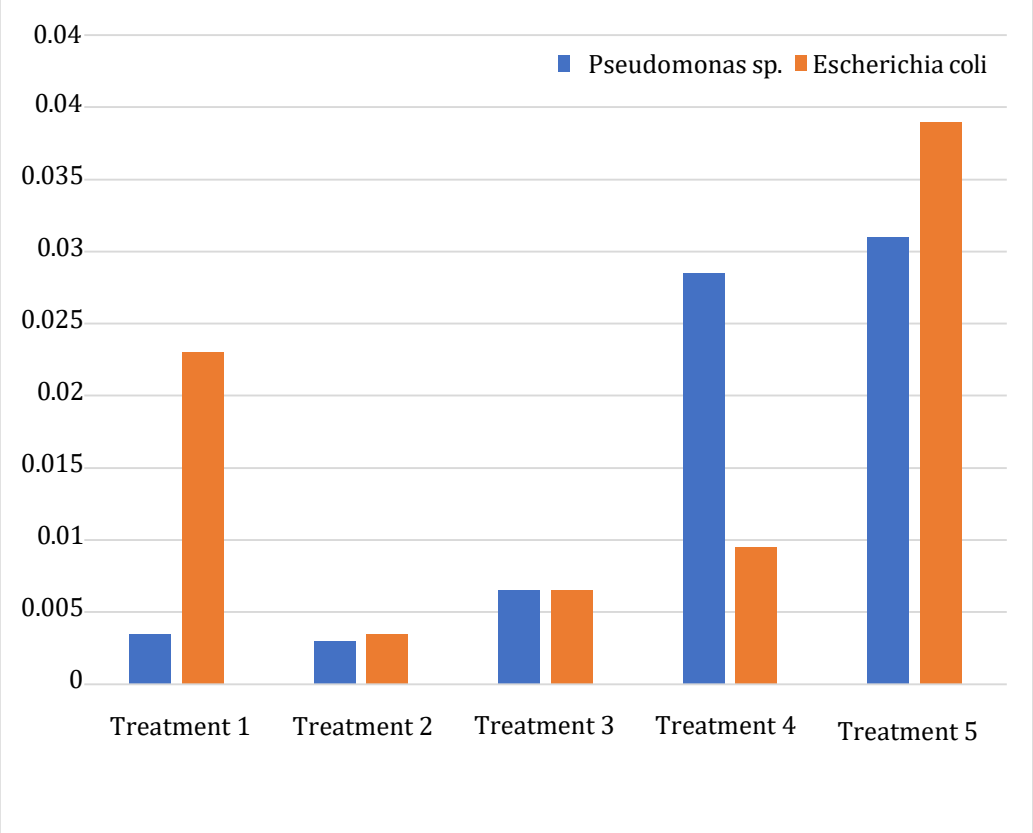

Figure 2. Mean Zone of Inhibition if Pseudonomass P. and Escherichia Coli

Figure 2 shows the Graphical Presentation of the Mean Zone of Inhibition of Pseudomonas sp. and Escherichia coli with the following legends.

Treatment 1 = Control Treatment 2 = Ethanol

Treatment 3 = 5 µL of Yellow Bell Leaf Extract Treatment 4 = 25 µL of Yellow Bell Leaf Extract Treatment 5 = 100 µL of Yellow Bell Leaf Extract.

The means of each treatment for the three test organisms show distinct variations. For Pseudomonas sp., the recorded means were 0.0035 for Treatment 1, 0.003 for Treatment 2, 0.0065 for Treatment 3, 0.0285 for Treatment 4, and 0.031 for Treatment 5.

In comparison, Escherichia coli exhibited means of 0.023 for Treatment 1, 0.0035 for Treatment 2, 0.0065 for Treatment 3, 0.0095 for Treatment 4, and 0.039 for Treatment 5. These values highlight the significant differences in the zone of inhibition between Pseudomonas sp. and Escherichia coli under the five treatment conditions.

4.2 Summary of Findings, Conclusions and Recommendations

This chapter presents the summary of the findings derived from the study, as well as the conclusions drawn from the results and the corresponding recommendations based on the outcomes of the research.

4.3 Summary of Findings

Using the anti–quorum sensing susceptibility assay, zones of inhibition were observed and measured on the assay plates. Statistical analysis using Analysis of Variance (ANOVA) revealed a significant difference in the zones of inhibition exhibited by Pseudomonas sp. and Escherichia coli across the five treatment conditions.

5. CONCLUSION

The study discovered an affordable natural substitute which can be obtained easily after researchers conducted extensive testing and analysis work of their research materials. Based on the findings of the study, the following conclusions were drawn.

1. A statistically significant difference exists in the zones of inhibition of Pseudomonas sp. and Escherichia coli under the five treatment conditions.
2. Yellow Bell (Allamanda cathartica) leaf extract exhibits the ability to inhibit biofilm formation in Pseudomonas sp. and Escherichia coli.

Acknowledgments

We would like to express our sincere gratitude to the parents of the authors, and to Bayugan National Comprehensive High School. Special thanks to sir Orvin A. Lobitos, our research adviser for his guidance and unwavering support. Also, to our school principal, Ma'am Minda I. Teposo emotional, and deep understanding on our research endeavor.

Funding Information

This research was funded by personal monetary contribution by the authors.

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Cris Anne L. Avila	✓	✓	✓		✓	✓		✓	✓	✓	✓		✓	✓
Novie A. Plondaya	✓	✓		✓			✓		✓	✓		✓	✓	
Shelah V. Berongoy		✓	✓	✓	✓			✓	✓	✓		✓	✓	✓
Precious Pearl L. Mibolos	✓	✓		✓		✓	✓	✓		✓	✓		✓	✓
Jesson C. Cagampang	✓		✓	✓	✓		✓	✓	✓	✓	✓			✓

C: **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that their research work has no financial interests that could create conflicts. The authors were grouped together at the start of the semester, and they are the only ones who wrote this paper.

Informed Consent

The authors obtained written consent from all participants before they joined the study. The authors needed to obtain written consent from a parent or guardian because they were underage during the study, which required them to receive an explanation about the potential dangers involved.

Ethical Approval

This study was approved by the School Research Committee through oral defense and presentation. According to The Code of Professional Ethics Electronic and Computer Engineers, all operations must be conducted in a fair and honest manner.

Data Availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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How to Cite: Cris Anne L. Avila, Novie A. Plondaya, Shelah V. Berongoy, Precious Pearl L. Mibolos, Jesson C. Cagampang. (2026). Inhibition of quorum sensing in microbial escherichia coli and pseudomonas sp. biofilms using yellow bell (allamanda cathartica) leaf extract. Journal of Prevention, Diagnosis and Management of Human Diseases (JPDMHD), 6(1), 13-25. https://doi.org/10.55529/jpdmhd.61.13.25

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