Research Article

Determination of Potential Anti Quorum Sensing Activity of *Eleusine indica* Ethanolic Crude Extract against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Aim/Background: The study assesses the potential anti-quorum sensing ability of the ethanolic crude extract of the plant Eleusine indica and its ability to inhibit biofilm formations in two clinically significant bacteria, *Escherichia coli* and *Staphylococcus aureus*. **Materials and Methods:** To prove its anti-quorum sensing capability, antimicrobial susceptibility testing was done by placing filter paper disks loaded with *Eleusine indica* with varying concentrations (25%, 50%, 75% and 100%) on a MH agar inoculated with the two bacteria. Subsequently, biofilm inhibition testing was conducted to evaluate the extract's effect on the bacteria's biofilm formation by combining the *Eleusine indica* crude extract with overnight cultures of the bacteria in a 96-well plate then measuring its optical density. Descriptive statistics were employed to analyze the optical density data, with values higher than the positive control indicating increased biofilm formation. **Results:** Antimicrobial susceptibility testing showed that no antimicrobial activity was present in the extract for the two bacteria. Moreover, results revealed that *Eleusine indica* crude extract did not inhibit biofilm formation in gram-positive *Staphylococcus aureus*, while all concentrations (25%, 50%, 75% and 100%) successfully inhibited biofilm formation in gram-negative *Escherichia coli*. **Conclusion:** These findings suggest that Eleusine indica may possess anti-quorum sensing properties against certain bacteria. Further research on the overall mechanism of *Eleusine indica* and its differential response on different clinically significant bacteria is warranted.

Keywords: Anti-quorum sensing, Biofilm, Eleusine indica.

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INTRODUCTION

Worldwide cases of antibiotic resistance have grown at an alarming rate, to the point wherein it has been widely considered as a cause for concern in the medical field. Stated in their 2019 report, the Centers for Disease Control and Prevention (CDC) emphasized the severity of this issue, linking antibiotic resistance to at least 1.27 million deaths worldwide.[1] However, while considering the gravity of this problem, several studies have noted that the urgency to alleviate this matter remains low until now. Therefore, this alarming scenario necessitates immediate action to diminish the burden of antimicrobial resistance. In response to this pressing challenge, quorum quenching emerged as a novel and promising strategy. Quorum quenching, as proposed by Bhardwaj *et al.* (2013), can attenuate bacterial virulence by inhibiting bacterial receptors, resulting in the inactivation of mechanisms related to quorum-sensing.[2] As mentioned in the study of Barcena, *et al.* (2022) Quorum Sensing (QS) pertains to the innate communication system of bacteria that

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promotes coordination among isolates to carry out colony-wide functions, biofilm formation being one if its most notable functions.[3] This process involves the activation and expression of virulence factor through signaling molecules. Once it reaches or exceeds its threshold value in the presence of high concentrations of AI or oligopeptide, bacterial responses are promoted, specifically, its antibiotic production and virulence factors such as pigment, fluorescence, sporulation, motility and biofilm.[3-5]

With all that being said, anti-quorum sensing or quorum quenching is basically a novel therapeutic process wherein it disrupts or interferes with the communication system among bacteria to prevent the emergence of disease in its host. Additionally, it also has the ability to target several components of the quorum sensing through different ways, including inhibiting the quorum sensing signal synthesis, degradation of quorum sensing signals itself, blockage of QS receptors by anti-quorum sensing antibodies and a combination of anti-quorum sensing agents with antibiotics.[6] With the help of quorum-sensing inhibitors, these can completely interfere with their communication system, thus limiting the resistance of bacteria since QS inhibitors impose a minimum selective pressure as compared to our conventional antimicrobial or antibiotic medications.[3] All that being said, this process may provide a new strategy to eradicate antibiotic resistance since studies show that microbial communication through quorum sensing is significant in developing resistance against a multitude of antibiotics.[7]

Eleusine indica, known locally as "Paragis" or goosegrass, is a tufted and spreading annual grass plant that belongs to the Poaceae family.[8] This botanical plant can grow easily in various places and is abundant in many tropical places worldwide, including the Philippines.[9] The study of Sukor *et al.* (2023) showed that *E. indica* contains various plant-derived compounds that might exhibit anti-quorum sensing properties such as embelin, vitexin and other phyto-compounds.[10] In a study by Dwivedi and Singh (2016), results showed that embelin has significant biofilm-inhibiting capabilities on strong biofilm-forming isolates.[11] In connection, some, if not all gram-positive organisms undergo the same pathway for QS regulation with embelin, hence testing the anti-quorum sensing capabilities of *E. indica* can be a potential anti-quorum sensing phytochemical of these microorganisms, such as on *S. aureus*. Similarly, gram-negative organisms also undergo the same pathway for QS regulation. With this study using *E. coli*, which is a gram-negative organism, vitexin can be a potential phytochemical of *E. indica* to determine whether it potentially has the same effect.[12] With this interconnection, the possibility of using *E. indica*'s phytochemicals, particularly embelin and vitexin, in the inhibition of the quorum sensing functions of the two aforementioned bacteria can be further studied as a possible alternative treatment to complement other medication, aiding the prevalence of antibiotic resistance. As stated in the study of Barcena *et al.* (2022), the potential use of *E. indica*, specifically its ethanolic crude extract, as a quorum-sensing inhibitor can also be used in medical settings, particularly in coating the dressings and catheters that cause various nosocomial infections.[3] As such, understanding how quorum sensing works and harnessing the possibility of using anti-quorum sensing mechanisms of various alternative sources emerges as a pivotal approach in addressing antibiotic resistance, laying the groundwork for exploring the therapeutic properties of *E. indica*.

Escherichia coli, belonging to the Enterobacteriaceae family, is known to be a facultative anaerobic, gram-negative bacillus.[13,14] While it is usually considered a resident normal flora of the intestines in humans and is commonly found in hospitals and long-term care facilities, due to certain virulence factors, numerous strains of *E. coli* are also known to be major agents of common diseases that affect various systems of the body such as meningitis, gastroenteritis, pneumonia, urinary tract infections, bacteremia and peritonitis. Hence, the fact that it is common in hospital settings and a causative agent of certain diseases makes *E. coli* a relatively significant bacteria to study. Relatively, the gram-positive *Staphylococcus aureus*, which is also considered a resident normal flora in the anterior nares of numerous adults, is a well-known agent of numerous bacterial infections such as bacteremia, septic arthritis, pulmonary infections, endocarditis, urinary tract infections and various hospital-acquired infections.[15] Additionally, *Staphylococcus aureus* is also known for its antibiotic resistance. For instance, there is a 60% resistance of ciprofloxacin against *S. aureus*.[16] With this, healthcare workers and immunocompromised individuals are at risk. Moreover, being a causative agent of both bacterial and hospital-acquired infections, *Staphylococcus aureus* imposes a great threat on the health of healthcare workers and those who are immunocompromised, making it a relevant species of bacteria to study. Statistically speaking, the annual reports of the Antimicrobial Resistance Surveillance Program of the Philippines mentioned that among other bacteria, *E. coli* and *S. aureus* remain consistently included in the three most common bacterial isolates in different specimens from sentinel satellite hospitals.[17] Moreover, aside from their prevalence, both

aforementioned bacteria are also capable of forming biofilms, in addition to various virulence factors that contribute to their pathogenicity.[18,19] By definition, biofilms are defined as the accumulation of bacteria that function as a community, surrounded by an extracellular matrix.[20] Also, it protects microorganisms by producing Extracellular Polymeric Substances (EPS) to create an optimal internal environment, enabling bacteria to survive in extreme environmental conditions.[18,19]

Furthermore, given the context of the prevalence of antibiotic resistance and its alarming rise in the medical field, the study made by the researchers will determine if the *E. indica* ethanolic crude extract possesses anti-quorum sensing properties by examining its biofilm inhibition capacity. Conversely, this study will examine the effects and significance between various concentrations of the aforementioned crude extract against two of the most prevalent bacterial isolates contributing to the issue of antibiotic resistance in the Philippines while also aiming to analyze how the effect of the *E. indica* crude extract differs against the two aforementioned bacteria.

MATERIALS AND METHODS

Sample Collection

The *E. indica* plant was obtained from Barangay Sapalibutad, Angeles City, Pampanga. After procurement, the plant was sent to the Far Eastern University-Herbarium for identification and authentication purposes. Likewise, the collection of bacterial isolates was obtained from the Medi Linx Laboratory in Quezon City, Metro Manila. The *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacterial isolates were cultivated in sheep blood agar for the antimicrobial susceptibility testing. Additionally, the inoculum suspension was prepared in trypticase soy broth which was used for biofilm inhibition testing.

Sample Preparation: Plant Extraction

The collected *E. indica* leaves were processed for extraction by the researchers at the laboratory of Far Eastern University with 95% ethanol as the solvent. The extraction process also includes using a rotary evaporator in the aforementioned facility to remove the excess solvents from the sample. The ethanolic crude extract was subjected to a flame test to ensure that the extract did not contain any residue of ethanol. The procedure for plant extraction used the study of Barcena *et al.* (2022) and Velasco, Fernando and Judan Cruz (2020) but with modification.[3,21] As for storage consideration, the methods of Velasco, Fernando and Judan Cruz (2020) were used.[21] The procedure starts with the use of distilled water for proper washing of the leaves and then followed by air-drying for seven days. Once it was completely dried, the dried leaves underwent blender homogenization followed by three days of soaking of homogenized leaves in 95% ethanol (1:10 w/v ratio). Using a Whatman filter paper no.4, the solution was filtered into a round bottom flask. For 40ºC at 100 rpm, the filtrate in the flask was heated until the solvent evaporated completely using a rotary evaporator. The extract was stored in an amber bottle with a stopper, stored at 0-5ºC.

Phytochemical Testing

The obtained ethanolic crude extract of *E. indica* was given to the Department of Science and Technology-Industrial Technology Development for phytochemical testing.

Antibacterial Susceptibility Testing

The Antimicrobial Susceptibility Test was performed to rule out the possibility of *E. indica* possessing antibacterial properties and to ensure its validity of indirectly measuring the potential anti-quorum sensing property since this study requires a resistant outcome result to increase the accuracy of the subsequent assays. Mueller-Hinton (MH) agar plates were used for the antimicrobial susceptibility testing and inoculated evenly with the *E. coli* and *S. aureus*. For our positive control, erythromycin (disk content of 15 micrograms) was used for *S. aureus*. On the other hand, for the positive control of *E. coli*, cefoxitin (disk content of 30 micrograms) was used. As for the negative control, the researchers utilized sterile distilled water as stated in the research of Barcena *et al.* (2022).[3] This was conducted three times.

Inoculum Preparation and Standardization

Bacteria were transferred from Sheep Blood Agar (SBA) to Normal Saline Solution (NSS) with the use of the inoculating loop (0.05 mm) to compare it to a standard 0.5 McFarland. According to Mister and Lehman (2022), the 0.5 Mcfarland is prepared using a 99.5 mL of 1% H2SO4 (sulfuric acid) mixed with 0.5 mL of 1.175% $BaCl_2$ (barium chloride).[22] The 1.5×10^8 CFU/mL turbidity of a solution is equivalent to the standard inoculum suspension for the disk diffusion method. A vortex mixer was also used to mix both of the solutions to have an even and uniform distribution of precipitate and bacterial isolates in 0.5 McFarland and NSS, respectively. These are all protocols mentioned by Mister and Lehman (2022).[22]

Inoculation of Impregnated Disk in MHA

Once both of the NSS reached the standard turbidity of 1.5×10^8 CFU/mL, it is then subjected for inoculation in the Mueller Hinton Agar (MHA) for the antimicrobial susceptibility testing. The researchers used a sterile cotton swab for each bacterial isolate. It was streaked or swabbed evenly three times and a final rim was to end the inoculation. Based on Barcena *et al.* (2022) and Bubonja-Šonje, Knežević, and Abram (2020), the blank filter paper disks measuring 6 mm were loaded and soaked with 20 micrograms of *E. indica* ethanolic crude extract with different concentrations (25%, 50%, 75% and 100%) for 2 hrs and dried for 2 hrs to evaporate the solvent. Once it dried, the impregnated disks with different concentration were placed on the Mueller Hinton Agar and is subjected for 18-24 hrs incubation at 37°C.[3,23]

Measure the Zone of Inhibition

Following incubation, the measurement of inhibition zone was done using vernier calipers. Specifically, the breakpoint cut-off was observed using the criteria of the Clinical and Laboratory Standards Institute (CLSI) to see if each of the concentrations used is susceptible, intermediate, or resistant.

Anti-Quorum Sensing Assay: Biofilm Inhibition Testing

This test followed the protocol of Velasco, Fernando, Judan Cruz (2020) and Mister and Lehman (2022) with modifications.[21,22] This was used to assess if the ethanolic crude extract of *E. indica* can inhibit the biofilm formation to indirectly measure the anti-quorum sensing property. The researchers prepared the inoculum by transferring these bacteria from the SBA to Trypticase Soy Broth (TSB) using the inoculating loop (0.05 mm) and incubating it for 24 hrs under 37°C. A 96 well-plate was used to conduct the biofilm inhibition testing wherein a 180 microliter of overnight culture of *Escherichia coli* and *Staphylococcus aureus*, together with the 20 microliters of ethanolic crude extract of *E. indica*, with different concentrations (25%, 50%, 75% and 100%) was transferred in each well of the 96-well plate. The researchers' utilized cinnamaldehyde (20 microliters) as a positive control while sterile distilled water (20 microliters) for the negative control. Afterward, it was incubated at 30°C for 40 hrs without shaking to promote adherence of biofilm on the sides of the wells. After the incubation period, each well is subjected to washing for five times to remove the presence of planktonic cells with the use of 150 microliters of distilled water and then left for 45 min of air drying. Once dried, 150 microliters of 1% crystal violet were added to the wells to stain the adhered biofilm for 45 min. Once discarded, it was washed five times with 150 microliters of sterile distilled water.

Biofilm Inhibition Testing: Quantification of Anti Quorum Sensing Activity using Microplate Biofilm Formation

To destain the crystal violet that was added, 200 microliter of 95% ethanol was used to each stained well. Then, 100 microliters from each well were transferred using a micropipette to a new 96-well plate. The level of crystal violet in each well was quantified using a microplate reader and measured the optical density at 630 nm. Moreover, the biofilm inhibition was quantified and calculated using the percent inhibition formula.[24] In line with this, the percent biofilm inhibition was rated between 0% to 100% in which a percent inhibition within 0% to 50% is considered having weak biofilm inhibition activity. In comparison, a percent inhibition of more than or equal to 50% is considered having strong biofilm inhibition activity.

Formula of Percent Biofilm Inhibition

$$\%\ \text{Inhibition} = \left[\frac{(\text{OD}_{\text{Negative control}} - \text{OD}_{\text{Sample}})}{\text{OD}_{\text{Negative control}}}\right]\times 100.$$

Statistical Analysis

The results from different experimental protocols were tabulated and analyzed. For data analysis, mean and standard deviation under descriptive statistics were used. This is to compare the various concentrations (25%, 50%, 75% and 100%) of *E. indica* ethanolic crude extract that is used against *Staphylococcus aureus* and *Escherichia coli* to the positive and negative control. Likewise, descriptive statistics will be used to calculate the percent inhibition of the various concentrations used against the aforementioned microorganism.

RESULTS

Phytochemical Analysis

To know the different phytochemical constituents of *E. indica*, the extract is subjected to different tests to know whether they contain the specified phytochemicals. Table 1 presents the phytochemicals being found, the test result and the method used to determine their presence or absence.

The phytochemical analysis showed that only Sterols tested negative among all the phytochemicals being looked for. The tests for all other phytochemicals,

including flavonoids, triterpenes, saponins, alkaloids, tannins and glycosides were all positive.

Table 1: Phytochemical analysis of *Eleusine indica* crude extract.

Test Parameter	Result	Test Method
Sterols	-	Liebermann-Burchard Test
Triterpenes	+	Liebermann-Burchard Test
Flavonoids	+	Shinoda Test
Alkaloids	+	Mayer's Test
Saponins	+	Froth Test
Glycosides	+	Fehling's Test
Tannins	+	Ferric Chloride Test

Antimicrobial Susceptibility Testing for *Staphylococcus aureus* and *Escherichia coli*

This study uses Erythromycin as a positive control for *S. aureus* with a zone of inhibition measurement of 26.5 mm in diameter. According to CLSI guidelines, the susceptibility range of erythromycin for all Staphylococci should be equal or more than 23 mm for it to be susceptible, on the other hand, a measurement below or equal to 13 mm is considered as resistant.[25] Based on the gathered results of the antibacterial susceptibility testing on *S. aureus*, the said bacteria expressed a consistent resistance of less than or equal to 13 mm in all concentrations (25%, 50%, 75%, 100%) of *E. indica* extract. Table 3 shows the different measurements of the zone of inhibition for all four concentrations of *E. indica* when tested on *Staphylococcus aureus* wherein all concentrations did not exhibit any significant inhibition compared to the positive control used in the study. Therefore, the test indicates that the extract used appeared no antibacterial activity in the *S. aureus* isolate.

The positive control used of the *Escherichia coli* bacterial isolate was cefoxitin which showed a zone of inhibition of 24 mm. According to the CLSI, the susceptibility range of cefoxitin for Enterobacterales is equal to or more than 18 mm to be considered susceptible.[25] A measurement of less than or equal to 14 mm is considered to be resistant. The results of the antimicrobial susceptibility testing on *Escherichia coli* showed consistent resistant (≤ 14 mm) results in all concentrations (25%, 50%, 75%, 100%) of the *E. indica* extract as seen in Table 2, indicating that the extract itself showed no direct antimicrobial activity towards the bacterial isolate.

Table 2: Antimicrobial susceptibility testing result of *Escherichia coli*.

Plate 1	Measurement	Interpretation
Positive control (Cefoxitin)	26.5 mm	Susceptible
Negative control	0 mm	Resistant
Plate 2		
25%	0 mm	Resistant
50%	5 mm	Resistant
75%	7 mm	Resistant
100%	5 mm	Resistant
Plate 3		
25%	0 mm	Resistant
50%	5 mm	Resistant
75%	6 mm	Resistant
100%	6 mm	Resistant
Plate 4		
25%	0 mm	Resistant
50%	5 mm	Resistant
75%	0 mm	Resistant
100%	6 mm	Resistant

Table 3: Antimicrobial susceptibility testing result of *Staphylococcus aureus*.

Plate 1	Measurement	Interpretation
Positive control (Erythromycin)	24 mm	Susceptible
Negative control	0 mm	Resistant
Plate 2		
25%	0 mm	Resistant
50%	0 mm	Resistant
75%	0 mm	Resistant
100%	0 mm	Resistant
Plate 3		
25%	0mm	Resistant
50%	0 mm	Resistant
75%	0 mm	Resistant
100%	0 mm	Resistant
Plate 4		
25%	0 mm	Resistant
50%	0 mm	Resistant
75%	0 mm	Resistant
100%	0 mm	Resistant

Biofilm Assay for *Escherichia coli* and *Staphylococcus aureus*

The optical density was measured using a microplate reader to assess the biofilm formation inhibitory activity of the four concentrations of *E. indica* ethanolic

crude extract against gram-positive and gram-negative bacteria, *Staphylococcus aureus* and *Escherichia coli*. The biofilm assay was performed in triplicates hence, the mean of the optical densities was used to compare the wells treated with the different concentrations of *E. indica* ethanolic crude extract and the wells treated with Cinnamaldehyde for the positive control and distilled water for the negative control. The presence of biofilm formation inhibition activity may be evaluated by obtaining an optical density of a lower value than the positive control.

As shown in Table 4, the mean optical densities of the negative control, positive control, 25%, 50%, 75% and 100% concentrations tested against *E. coli* are 0.713, 0.083, 0.079, 0.067, 0.059 and 0.053 respectively. The data obtained in all four concentrations of the plant extract against *E. coli* appear to have a lower value compared to the positive control. This implies that all four concentrations of the plant extract possess a significant biofilm formation inhibition activity against the gram-negative bacteria, *E. coli*. Furthermore, the percent inhibition of the positive control, 25%, 50%, 75% and 100% concentrations of *E. indica* ethanolic crude extract against the biofilm formation of *Escherichia coli* are 88.35%, 88.92%, 90.60%, 91.72%, 93.27% respectively as seen in Figure 1.

On the other hand, the mean optical densities of the negative control, positive control, 25%, 50%, 75% and 100% concentrations are 0.568, 0.099, 0.448, 0.405, 0.346 and 0.311 respectively. As shown in Figure 1, The percent inhibition of the positive control, 25%, 50%, 75% and 100% concentrations against the biofilm formation of *Staphylococcus aureus* are 82.57%, 21.13%, 28.70%, 39.08%, 42.25% respectively. The data obtained in all four concentrations of the plant extract against *S. aureus* in Table 5 appear to have a higher value compared to the positive control. This implies that all four concentrations of the plant extract do not possess any biofilm formation inhibition activity against the gram-positive bacteria, *Staphylococcus aureus*.

Table 4:Descriptive statistics of *Escherichia coli.*

	Mean	Standard Deviation
Negative Control	0.713	0.0548
Positive Control	0.083	0.0046
Ei25	0.079	0.0016
Ei50	0.067	0.0037
Ei75	0.059	0.0037
Ei100	0.053	0.0042

Table 5: Descriptive statistics of *Staphylococcus aureus.*

	Mean	Standard Deviation
Negative Control	0.568	0.0532
Positive Control	0.099	0.0127
Ei25	0.448	0.0160
Ei50	0.405	0.0131
Ei75	0.346	0.0082
Ei100	0.311	0.0131

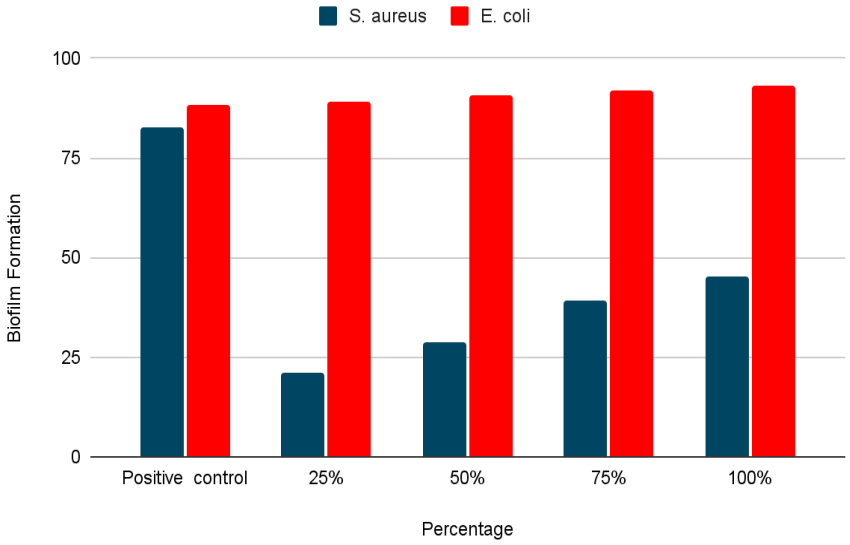

Figure 1: Comparison of Biofilm Percent Inhibition of *Eleusine indica* ethanolic crude extract against *Escherichia coli* and *Staphylococcus aureus.*

DISCUSSION

Antimicrobial Susceptibility Testing for *Staphylococcus aureus* and *Escherichia coli*

The researchers conducted AST to rule out the antimicrobial property that might inhibit the biofilm formation of the two selected bacteria using the various concentrations of *E. indica* crude extract. The experimental results showed that the crude extract of *E. indica* did not exhibit any significant antimicrobial activity toward these bacteria. The test was conducted in three trials which all showed consistent results, further confirming the absence of any antimicrobial activity to the *E. indica* ethanolic crude extract. Although other studies involving *Eleusine indica* extract may appear to contradict the outcome of this study by showing the antimicrobial properties present in the plant extract, the extraction methods should be taken into account. In a study by Morah and Odey (2020) where *E. indica* essential oils showed both antibacterial and antifungal properties on chosen bacteria, including both *E. coli* and *S. aureus*, the study used a different extraction method through the use of steam distillation methods to obtain the essential oils.[26] In another study made by Alaekwe *et al.* (2015), methanol and chloroform extraction methods are used to show the antimicrobial properties of the *E. indica* extract.[27] It can be seen that both studies used different extraction methods in comparison to the methods used by the researchers in this study, implying that the method of extraction had a significant effect on the composition and activity of the plant extract.

Moreover, in this research, a rotary evaporator separates the ethanol solvent to obtain the pure extract from *E. indica* leaves. In addition, a flame test is also applied as a confirmatory test to guarantee that the ethanolic crude extract is free of alcohol that can interfere with the possible results from biofilm inhibition testing. Following these methods, the researchers were able to procure an extract that indicated a negative result in both the antibacterial susceptibility test and flame test. These gathered results qualify the ethanolic method of extraction for the biofilm inhibition assay to detect and evaluate the potential of *E. indica*'s anti-quorum sensing property. Therefore, ethanolic extraction method for *E. indica* is eligible for biofilm inhibition testing to detect its potential anti-quorum sensing activity against two of the selected bacteria for this study.

Biofilm Inhibition Testing for *Escherichia coli*

In a study made by Escobar-Muciño *et al.* (2022), it was mentioned that one of the natural quorum sensing inhibitors are triterpenes and flavonoids wherein these anti-quorum sensing inhibitors can also disrupt the communication system among bacteria.[28] In a research conducted by Ettebong, Ubulom and Obot (2020), vitexin and schaftoside are the main agents of flavonoids present in the *E. indica* plant.[29] On the other hand, isoscaftoside, another flavonoid classification, was also found in the *E. indica* plant.[10] These aforementioned phyto-compounds were confirmed to exhibit anti-quorum sensing properties against bacteria.[4]

Moreover, alkaloids and saponins, which are considered naturally-occurring organic phyto-compounds found in various plants and fungi, have also been studied for their potential to inhibit quorum sensing in various bacteria. According to Asfour (2018), alkaloids and saponins can disrupt quorum sensing activity by various mechanisms, which involve inhibition of signal molecule synthesis and inhibition of signaling pathways.[4] For instance, Cankaya and Somuncuoglu (2021) stated that saponins can disrupt the communication system of various bacteria, particularly *E. coli* by interfering with its signaling molecule synthesis, which leads to the reduction of biofilm formation and virulence.[30] In line with this, a study conducted by Al Zubairi *et al* (2011) mentioned that *E. indica* leaves contain the aforementioned phytochemicals.[31] According to Barcena (2022), they have also found that the alkaloids, which are present in the crude extract, were able to significantly reduce the quorum sensing-mediated assembly and colony-wide virulence factor production, which includes secretion of toxins, motility and biofilm formation.[3]

Lastly, tannins and glycosides are some of the well-known natural quorum-sensing compounds that could significantly impact a bacteria's ability to form biofilms.[4] In line with this, Tannins have been shown to suppress and disrupt quorum sensing activity and decrease gene expression that involves the production of virulence factors in bacteria by disrupting cell membranes and inhibiting quorum sensing pathways.[32] Furthermore, a strong link was shown by Neumann *et al.* (2022) between the concentration of tannins and the capability of *E. coli* to form biofilms, highlighting the outstanding role of tannins in regulating the formation of bacterial biofilm formation.[33] Furthermore, Vikram *et al.* (2013) conducted a research that reveals B-sitosterol glucoside, a type of glycoside, being the most potent phytochemical component in inhibiting *E. coli*'s virulence factor, which are biofilm formation and motility.[34] This indicates that glycosides, like tannins, exhibit the ability to interfere and disrupt with bacterial quorum sensing mechanisms and its formation of biofilm, suggesting their potential as novel anti-quorum sensing agents.

The effectiveness of *E. indica* crude extract to *E. coli* can be related to the anti-quorum sensing phyto-compounds

present in the crude extract. This justifies the effectiveness of *E. indica* crude extract to *E. coli* thus inhibiting quorum sensing-mediated biofilm formation. Consequently, the collective findings from the aforementioned research support the data obtained from the biofilm assay in this study. It is found that the *E. indica* extract used in this study contains the aforementioned phytochemicals which contribute to its potential anti-quorum sensing activity by inhibiting biofilm formation, as confirmed by the phytochemical analysis report obtained from the DOST-ITDI.

Biofilm Inhibition Testing for *Staphylococcus aureus*

The phytochemicals like luteolin, quercetin, sesquiterpenes, kaempferol, naphthoquinones and aromatic acids are found to inhibit biofilm formation in *S. aureus*.[35] These aforementioned phytochemicals were not present in the *E. indica* plant, which further proves the possibility that its weak inhibition is caused by insufficient phytochemicals needed for significant reduction.[10] In addition, 1,4-Naphthoquinone (1,4-NQ), which is another phytochemical that is not present in *E. indica* plant, was found to reduce the formation of quorum-mediated biofilms and preformed biofilms of various Multidrug-Resistant (MDR) strains of *S. aureus*. In line with this, Wu *et al.* (2024) also observed that other natural compounds upregulate agrA and downregulate icaA expression, 3-HBA aromatic acids target the Agr and SarA systems and specifically, sesquiterpenes even inhibit the key genes expression that is involved in virulence factor production and formation of quorum-mediated biofilm in *S. aureus*.

In this study, testing for the aforementioned phytochemical constituents was not performed due to the scope and limitations of this study. This may contribute to the factors that caused the inability of the *E. indica* crude extract to reduce the formation of quorum-mediated biofilm of the *S. aureus* (a gram-positive bacteria). Additionally, the quorum-sensing pathway differs between the pathway used by the gram-positive bacteria and the pathway of the gram-negative bacteria.[4] This factor may also be one of the reasons why the various concentrations of *E. indica* crude extract inhibit the biofilm formation of *E. coli* while it does not inhibit the biofilm formation of *S. aureus*.

CONCLUSION AND RECOMMENDATION

Anti-quorum sensing activity has been continuously being studied as a potential alternative to aid medical health issues like antibiotic resistance and to create an innovative medication or combined therapies since the discovery of quorum sensing in many clinically significant bacteria. A biofilm assay was performed to assess the quorum sensing-mediated biofilm formation inhibition activity of different concentrations of *E. indica* ethanolic crude extract. With the data obtained from the aforementioned assay, it can be concluded that the results of the biofilm assay obtained imply that *E. indica* crude extract even at different concentrations is not effective at inhibiting the biofilm formation of *S. aureus* while all four concentrations, 25%, 50%, 75% and 100% are effective in inhibiting the biofilm formation of *E. coli*. The researchers recommend that further research be done to test the efficacy of the *E. indica* ethanolic crude extract on other clinically significant bacterial isolates. Additionally, further studies can be done in order to isolate the specific phytochemical compounds present in the *E. indica* ethanolic crude extract. It is also recommended to explore the possible factors that caused the plant extract to not inhibit the biofilm formation of *S. aureus*. Furthermore, the researchers recommend that the potential of *E. indica* in clinical settings be explored further. To further assess *E. indica*'s potential toxicological effects and its overall effects on a living organism, *in vivo* studies are recommended. Moreover, further research about *E. indica* and its synergistic effects when combined with conventional antibiotics used to treat clinically significant bacteria would also further assess the extract's efficacy. By conducting *in vivo* testing and further exploring its therapeutic potential, it could pave the way for *E. indica* to become a conventional natural medicine to treat certain bacterial infections. These recommendations given by the researchers can provide further evidence of the efficacy of the *E. indica* ethanolic crude extract as an alternative in controlling the growth of bacterial isolates in clinical applications, which can have a significant effect in expanding the possible therapeutic treatments for said bacteria.

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AUTHORS CONTRIBUTION

Authors Alona Marie C. Santos and Tristan Josef E. Tinte composed the introduction of the study, which offered a brief overview of the topic, highlighting important terms and the significance of conducting it. The methodology section was divided into two parts: authors Eziah C. San Pascual, Michelle Angelica T. San Jose, Jaimie L. Trinidad and Reign Chloe C. Zapanta conducted the Biofilm Inhibition Testing, while authors Adara Abrianne D. Vidal, Lester John C. Zabala, Alona Marie C. Santos and Tristan Josef E. Tinte performed the Antimicrobial Susceptibility Testing, both involving data extraction, interpretation and its discussion. Author Daniel H. Bercede provided critical evaluation of the paper's overall trajectory and study concept. All authors collectively gathered the articles used in the study and contributed to material acquisition, plant extraction and proofreading of the final manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The use of human or animal subjects was not implemented in the study made by the authors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AST: Antimicrobial Susceptibility Testing; **$BaCl_2$:** Barium Chloride; **CDC:** Centers for Disease Control and Prevention; **CFU/mL:** Colony Forming Units per Milliliter; **CLSI:** Clinical and Laboratory Standards Institute; **DMSO:** Dimethylsulfoxide; *E. coli*: *Escherichia coli*; **EPS:** Extracellular polymeric substances; *E. indica*: *Eleusine indica*; **DOST-ITDI:** Department of Science and Technology-Industrial Technology Development Institute; **MHA:** Mueller-Hinton agar; **mm:** Millimeters; **MDR:** Multidrug-resistant; **NSS:** Normal Saline Solution; **OD:** Optical Density; **RPM:** Revolutions per minute; **SBA:** Sheep Blood Agar; *S. aureus*: *Staphylococcus aureus*; **TSB:** Trypticase soy broth; *Staphylococcus aureus* **ATCC 25923:** *Staphylococcus aureus* American Type Culture Collection 25923; **H_2SO_4:** Sulfuric Acid; *Escherichia coli* **ATCC 25922:** *Escherichia coli* American Type Culture Collection 25922; **QS:** Quorum sensing; **1,4-NQ:** 1,4-Naphthoquinone; **3-HBA:** 3-hydroxybutyric acid.

SUMMARY

This paper is an experimental research article that explores the anti-quorum sensing properties of *E. indica* ethanolic crude extract against two of the selected biofilm-forming bacteria, *E. coli* and *S. aureus*. This was determined by conducting antimicrobial susceptibility testing to determine if the *E. indica* ethanolic crude extract exhibits antimicrobial properties. Subsequently the biofilm inhibition testing was conducted to identify if the said extract can significantly reduce the formation of biofilm (*E. coli* and *S. aureus*) and the concentration at which it was found to be the most effective. The findings showed that no antimicrobial properties were observed in both bacteria. Furthermore, results revealed that all concentrations (25%, 50%, 75% and 100%) of *E. indica* ethanolic crude extract significantly inhibit the biofilm formation of *E. coli*. Specifically, 100% concentration was the most effective among the concentrations used. On the contrary, the biofilm formation of *S. aureus* was not significantly reduced and requires further studies. Therefore, this research revealed that the *E. indica* plant can disrupt the quorum-sensing activity of certain gram-negative bacteria.

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