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COMPARATIVE ASSESSMENT OF THE ANTI-MICROBIAL PROPERTIES OF *Artocarpus altilis*, AND *Syzygium cumini* LEAVES AGAINST *Staphylococcus aureus* AND *Escherichia coli*

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ABSTRACT

A comparative assessment of the antimicrobial activity of two well-known and tropically distributed plants was conducted. Scientists and other researchers have been continuously investigating plants distributed all across the globe for medicinal properties so as to replace the chemically developed cures and treatment that are being used today. However, these plants and their extracts have not been developed for use in the medical field since adequate knowledge of the chemical makeup of these plants as well as clinical trials is still lacking. This study aimed to seek out the most antimicrobially active of the plants for testing and eventual use in medicine. The plant *Artocarpus altilis* and *Syzygium cumini* were investigated against a gram positive and a gram negative bacterium: *Staphylococcus aureus* and *Escherichia coli* respectively. Selective solvent extraction was used to extract the active compounds present in leaves. The solvents used were hexane, methanol and ethanol. The disc diffusion method was used to test antimicrobial activity of the plant extracts. The plant extracts were compared against a control and a reference, which were the solvents and Ampicillin respectively. Methanol and ethanol extracts of both plants showed zone of inhibition against the investigated microorganisms. However, the hexane extracts were unable to produce results. The results of this study noted that the ethanol extracts were more effective than the methanol extracts. According to this study, the most antimicrobially active plant is *Syzygium cumini* and the most inhibited bacteria is the gram positive bacteria: *Staphylococcus aureus.*

Contribution/Originality: This study aimed to seek out the most antimicrobially active of the plants for testing and eventual use in medicine.

1. INTRODUCTION

Plant remedies have been used for thousands of years to treat diseases and infections (Huffman, 2009). There is wide diversity of plants that have medicinal properties and this knowledge has been passed on through the generation (Estrada et al., 2011). The early man, when faced with various diseases, utilized the natural resources present around them to combat the symptoms and effects of various diseases (Mohanty & Pradhan, 2014). The primitive people characterized plants very simply based on what seemed to be good and useful versus what seemed not to be good and useful after trying them (Kunle, Egharevba, & Ahmadu, 2012). They tried various plants via application either topically or via ingestion as a concoction or by chewing to figure out what would cure/heal and

purify their bodies of the disease (Petrovska, 2012). The knowledge of the medicinal values and/or healing and purifying power of these plants was passed on orally from generation to generation (Estrada et al., 2011). Based on the knowledge that has been handed down from ancestors, a platform has been laid out for the scientific studies that are currently being conducted to find natural cures to various infectious diseases (Mohanty & Pradhan, 2014).

Guyana is undoubtedly filled with a wide variety of flora that can serve a wide array of purposes including medicinal. Even though many persons have made significant input into the pool of scientific investigations on the herbs of Guyana, there is still scope for more studies not only locate more plants that can be used in the fight against these diseases but also for clinical trials using these herbs. Scientists and other researchers have been continuously investigating plants distributed all across the globe for medicinal properties so as to replace the chemically developed cures and treatment that are being used today. However, these plants and their extracts have not been developed for use in the medical field since adequate knowledge of the chemical makeup of these plants as well as clinical trials is still lacking. *Artocarpus altilis* and *Syzygium cumini* are two plants that are widely distributed along the Coast of Guyana. The breadfruit plant parts are used to treat infections of the eyes and ears, diarrhea, stomachaches, skin diseases, sprains and for soothing the pain of sciatica (Mohanty & Pradhan, 2014). Jamun has been used for hundreds of years to treat diabetes. It has also been used ailments such as simple fevers, constipation, gastropathy, leucorrhoea and dermopathy (Gowri & Vasantha, 2010). This study investigated the anti-microbial potency of *Artocarpus altilis* and *Syzygium cumini* against *Staphylococcus aureus* and *Escherichia coli*.

2. METHOD

2.1. Collection of Plant Materials

This study was done from 6th June to 1st July, 2016 in the Department of Biology, University of Guyana, Turkeyen Campus. The leaves of the two plants to be investigated were collected. The *Artocarpus altilis* (breadfruit) leaves were collected from Walcott Street, West Bank Demerara. The *Syzygium cumini* leaves were collected from Abram Zuil on the Essequibo Coast.

2.2. Extraction

The leaves were first cleaned using distilled water and then blotted with newspaper. Selective solvent extraction was used. The leaves of *Artocarpus altilis*, and *Syzygium cumini* were soaked for 72 hours in hexane, methanol and ethanol. The extracts were thereafter filtered. The excess solvent was removed using a rotary evaporator. In order to prevent evaporation, the extracts were put into sample vials and sealed. The end product after drying via rotovaping was a paste substance (Jagessar, Ramchartar, & Spencer, 2015). 15mL of each extract was obtained.

2.3. Preparation of Extract Solution

The various concentrations for each were prepared by measuring a fixed amount of the extract and diluting with distilled water. First 2.5mL of *Artocarpus altilis* extract was measured using a graduated pipette and added to a 10mL vial after which the concentration was made by adding 7.5mL of distilled water to make up the 25% concentrated solution. In order to make up the 50% and 75% concentrated solutions the same procedure was followed using 5.0mL and 7.5mL of the plant extract respectively and adding the equivalent amount of water. The same procedure was followed using the other plant extract (*Syzygium cumini*).

2.4. Collection of Microorganisms

The two bacteria, gram positive; *Staphylococcus aureus* and gram negative; *Escherichia coli* were collected from the Georgetown Public Hospital (GPH) microbiology laboratory. The bacteria collected were stored in the refrigerator of the Department of Chemistry Annex, University of Guyana, Turkeyen Campus.

2.5. Inoculation of Microorganisms

The McFarland Turbidity Test was carried out to ensure the bacteria being inoculated was of the correct concentration. The 0.5 McFarland's standard was prepared in a test tube for use throughout this test procedure as reference.Two test tubes of saline solution were prepared, one for the testing of the *Staphylococcus aureus* bacteria and the other for the testing of the *Escherichia coli* bacteria.An inoculating loop was flamed until it became red, and then left to cool. Using the inoculating loop, bacteria was taken from the plate containing *Staphylococcus aureus* and added to one of the tubes containing saline solution. This solution was visually compared with the McFarland standard. Once the suspension was noted to have the same turbidity as the McFarland's standard it was used for inoculation of the agar plates to be used throughout the experiment. Steps 3 to 5 were carried out using the other bacteria; *Escherichia coli.*The inoculation steps were carried out twice for both bacteria in order to have enough bacteria throughout the course of the experiment. The tubes containing the inoculated solution were incubated at 37˚C (Sikarwar et al., 2014).

2.6. Reference and Control

An antibiotic was used as the reference. The antibiotic Ampicillin was used against the pathogenic bacteria: *S. aureus* and *E. coli.* The control experiment was solidified agar plates streaked with the respective bacteria cultures onto which discs treated with the respective solvents were placed using sterilized forceps.

2.7. Preparation of Agar

Nutrient agar was used to conduct this research project. 4200mL of Nutrient agar was made by placing 23g of the powdered substance into two different 1L volumetric flasks and 4.6g of the powdered substance into a 500mL volumetric flask, 500mL and 200mL of distilled water was then added to the two 1L flasks and the 500mL flasks respectively and the mixture was stirred while being boiled. Once all the powdered particles were thoroughly dissolved and the solution became translucent, the mixture was autoclaved at a temperature of 121˚C for 15minutes. The liquefied agar was cooled for approximately 3minutes and the plates will then be poured in a sterile environment and thereafter, allowed to solidify for 2hours. This was done 3 times, and then a final 500mL of agar was prepared in order to complete the 140 plates required. The plates were labeled. Under aseptic conditions, the microorganisms were then streaked onto separate plates. The discs were applied on the agar using sterile forceps. The plates were then incubated for 24hours at a temperature of 37˚C.

3. RESULTS

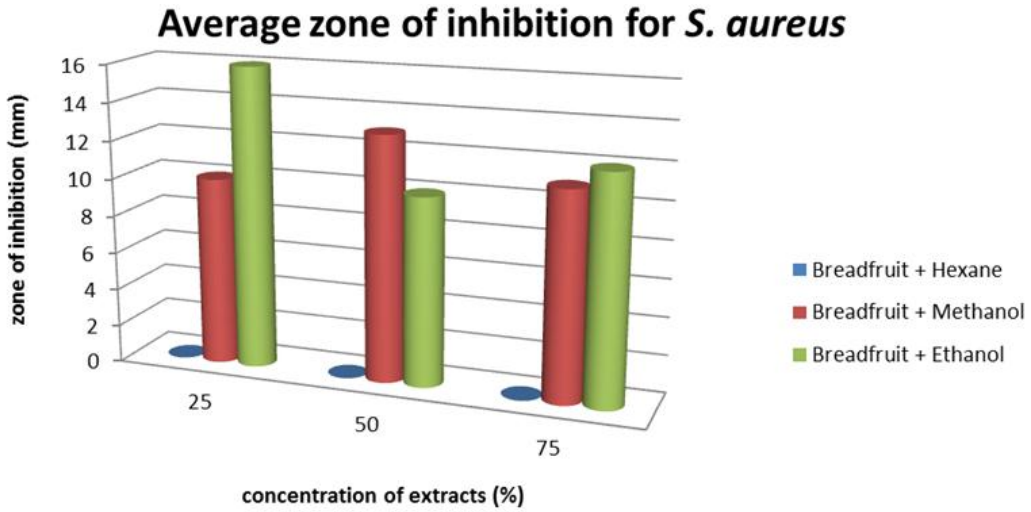

Figure-1. Graph showing average zone of inhibition for Breadfruit extracts against *S. aureus.*

Breadfruit extracts were first investigated against *Staphylococcus aureus*. The results in showed that 25% ethanol extracts gave the highest average zone of inhibition followed by 50% methanol and 75% ethanol extract. This indicated that the lowest plant extract concentration was most effective against the bacterium.

Breadfruit plant extracts when investigated against the gram negative, *Escherichia coli*, showed that the ethanol extracts were most effective against the bacterium with the 25% concentration giving the largest zones of inhibition followed by the 75% and 50% extracts in that order. This was followed by the methanol extracts in the same order: 25%, 75% and 50%.

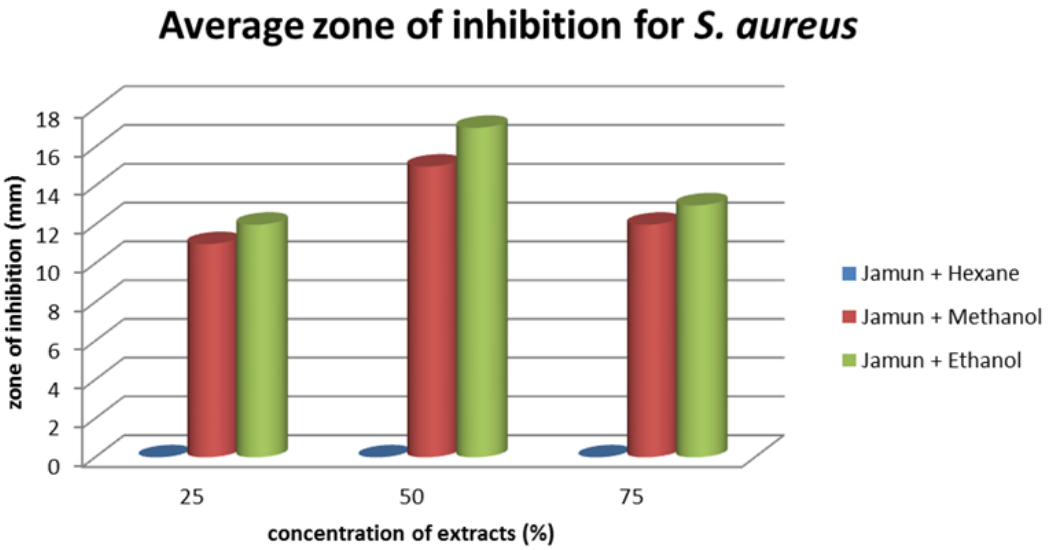

Figure-3. Graph showing average zone of inhibition for Jamun extracts against *S. aureus*.

Jamun plant extracts' antimicrobial activity was investigated against *S. aureus*. The ethanol extract was proven to be most effective against the bacterium in the 50% concentration and this was followed by the 50% methanol and the 75% ethanol extracts.

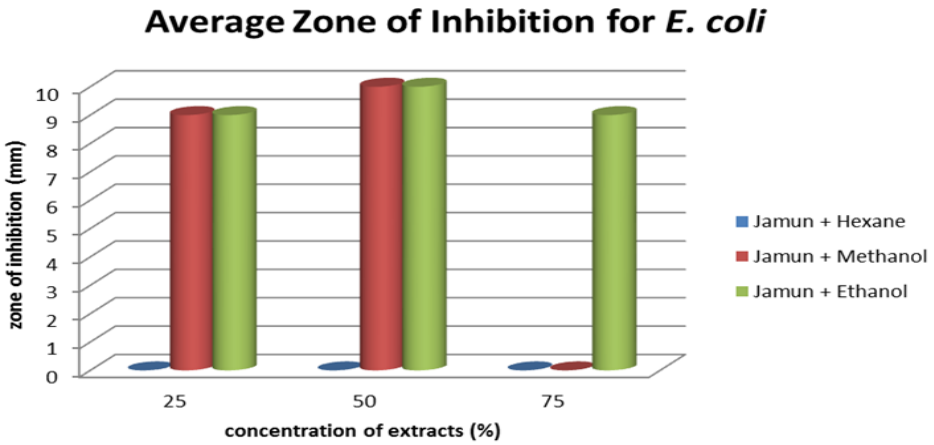

Figure-4. Graph showing average zone of inhibition for Jamun against *E. coli*.

When tested against *E. coli*, the methanol and ethanol extracts of Jamun both in the concentration 50% were most effective. The 25% methanol and ethanol extracts and 75% ethanol extracts followed.

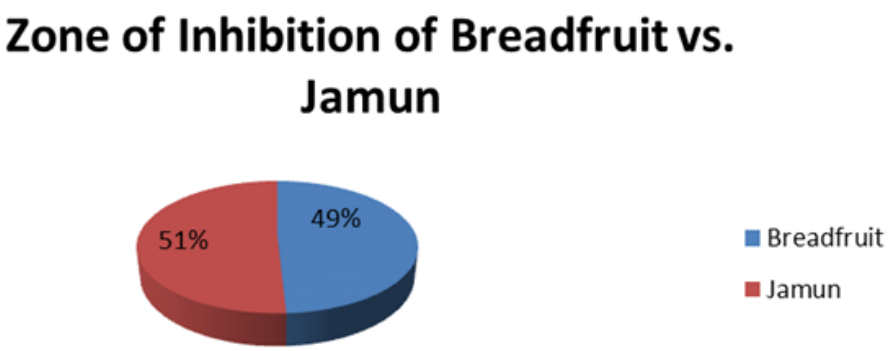

Figure-5. Pie chart showing zone of inhibition of Breadfruit vs. Jamun.

Overall the Jamun leaf extracts proved to be more potent against the microorganisms used in this investigation. When the zones of inhibition were measured, greater zones were noted in the plates containing the relative microorganism and Jamun extract discs.

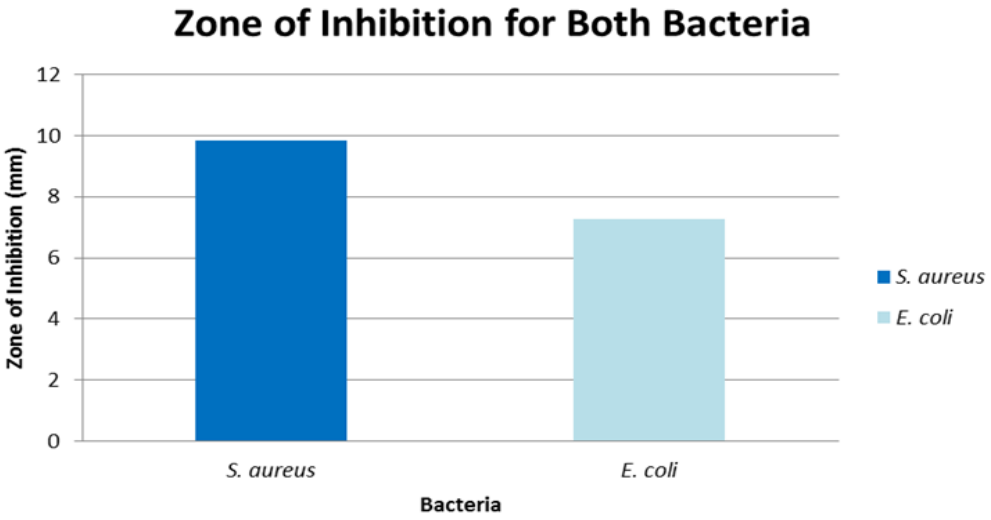

Figure-6. Graph showing average zones of inhibition for *S. aureus* vs. *E. coli.*

Greater antimicrobial activity was shown against the gram positive bacteria: *S. aureus* than against the gram negative: *E. coli* with a P-value = 0.000 proving it to be statistically significant.

Table-1. Showing ANOVA Analysis of Concentration versus concentration.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	22484.219	24	936.842	151.648	.000
Within Groups	1624.750	263	6.178		
Total	24108.969	287			

The ANOVA analysis produced a P-value = 0.000 which is less than 0.05. Therefore, there are significant differences between the different concentrations of plant extracts. The plant extracts; Breadfruit + Ethanol and Breadfruit + Methanol as well as Jamun + Ethanol and Jamun + Methanol were able to successfully inhibit the growth of both bacteria at various concentrations.

The Tukey's Post Hoc Test which carried out multiple comparisons showed that differences were present between several of the concentration groups. It also indicated what groups were significantly different from the other. A few of the most statistically significant groupings are represented in Table 2 above (for codes see Appendix Tables 2 and 3).

Table-2. Showing groups between which the most significance were seen.

Groups	P-value	Significance
7 vs 22	0.002	Stat. significant
7 vs 23	0.001	Stat. significant
7 vs 24	0.002	Stat. significant
7 vs 31	0.000	Stat. significant
7 vs 36	0.000	Stat. significant
14 vs 31	0.002	Stat. significant
14 vs 36	0.002	Stat. significant
15 vs 23	0.002	Stat. significant
15 vs 31	0.001	Stat. significant
15 vs 36	0.001	Stat. significant
16 vs 23	0.001	Stat. significant
16 vs 24	0.002	Stat. significant
16 vs 31	0.001	Stat. significant
16 vs 36	0.001	Stat. significant
18 vs 22	0.000	Stat. significant
18 vs 23	0.000	Stat. significant
18 vs 24	0.000	Stat. significant
18 vs 31	0.000	Stat. significant
18 vs 32	0.000	Stat. significant

Table-3. Showing ANOVA Analysis of Bacteria versus bacteria.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	454.010	1	454.010	64.539	.000
Within Groups	1927.508	274	7.035		
Total	2381.518	275			

When the ANOVA analysis was done, a P-value = 0.000 was obtained. This tells us that there were significant differences between the two bacterial strains: *Staphylococcus aureus* and *Escherichia coli.* The fact that the growth of one of the bacterium (*S. aureus*) was more affected by the plant extracts than the other bacterium (*E. coli*).

3.1. Discussion

The disc diffusion method was used throughout this experiment. When the Breadfruit extracts were investigated against *S. aureus* and *E. coli* the ethanol extracts were most effective followed by methanolic extracts. In studies carried out by Mohanty and Pradhan (2014) it was noted that the methanolic extracts were more effective against *S. aureus* than the other solvent extracts which were ethyl acetate and petroleum ether. Increased zones of inhibition were seen for *S. aureus* concentration of the plant extracts used was increased. Raman, Sudhahar, and Anandarajagopal (2012) used a wider variety of solvents, petroleum ether, chloroform, methanol, ethanol and water. They noted that the methanolic extracts were most effectively against *S. aureus* bacteria followed by the aqueous extract and the ethanol extract. *E. coli* showed the highest zone of inhibition when the petroleum ether extract was investigated and this was followed by the methanolic extracts. Mohanty and Pradhan (2014) attributed the antimicrobial activity of the Breadfruit leaves to the presence of a number of phytochemicals but more so to the tannins. Raman et al. (2012) stated that the phytochemicals that may be responsible for inhibiting the bacteria were tannins and flavanoids.

Jamun plant extracts' antimicrobial activity was investigated against *S. aureus* and the ethanol extract was most effective followed by the methanolic extract. Gowri and Vasantha (2010) showed that the highest zones of inhibition were with the methanolic extracts of Jamun leaves. In their study the solvents used were methanol and water. From our study it was noted that *E. coli* when investigated against the various Jamun extracts, both the methanolic and ethanol extracts were most effective in the 50% concentration. Researchers, Gowri and Vasantha

(2010) stated that inhibition noted may be due to the presence of tannins and several other phytochemicals found in the leaves of the Jamun plant. All hexane extracts were null against both bacteria.

The extracts of Jamun leaves was the more potent of the two plants investigated. The higher antimicrobial activity in Jamun leaf extracts may have been due to the fact that it contains some phytochemicals that are not present in the leaves of Breadfruit (Mohanty & Pradhan, 2014). Some of these very important phytochemicals are tannins, flavanoids, phenols, and plant steroids alkaloids among others.

Gowri and Vasantha (2010). These phytochemicals have a number of properties that are medically important such as anti-inflammatory, anti-cancer, antimicrobial etc. (Gowri & Vasantha, 2010).

The gram positive bacteria, *S. aureus* showed zones of inhibition greater than the gram negative, *E. coli*. This activity may be attributed to the fact that gram positive bacteria lack the outer membrane that is present in gram negative bacteria which inhibits certain chemicals, substances or particles from penetrating the cell (Asghar, 2011).

The results obtained from this research project can be attributed to the phytochemicals that were extracted by the solvents used. The solvent or solvents may have only been able to extract a few of the phytoconstituents of the plant.

All the parameters were tested and observed against a reference, Ampicillin. However, none of the zones of inhibition observed with plants extracts were greater than that of the reference.

4. CONCLUSION

The two plants investigated in this study possess medicinal properties. This research revealed that extracts of both *Artocarpus altilis* and *Syzygium cumini* are more effective against the gram positive bacteria, *S. aureus* than they are against gram negative, *E. coli*.

Overall *S. cumini* showed higher antimicrobial activity of the two plants against both pathogenic bacteria, hence it was the more effective plant in inhibiting the growth of the tested bacteria.

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