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PRELIMINARY EVALUATION AND ANTIMICROBIAL ACTIVITY OF *Barringtonia asiatica (L.) Kurz* EXTRACTS

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ABSTRACT. The study aimed to evaluate the antimicrobial activity of the leaves and fruit crude extracts from the *Barringtonia asiatica* plant against *S. aureus* and *E. coli.* The ethanolic extracts contained phenols, flavonoids, alkaloids, and saponins. The leaf extracts exhibited higher antibacterial activity than the fruit extracts and clear inhibitions were mainly observed in the first and second dilutions. Based on the results, there was a significant difference in the antimicrobial activities of the extracts against *S. aureus* but no significant difference was observed against *E. coli.* As for the traditional usage of *B. asiatica, a* personal interview was done by the researcher to the residents of Aplaya, Digos City. It turned out that out of 18 respondents, 55.55% claimed they've used the leaves while 38.88% used the seed/fruits and 11.11% utilized the roots as a treatment for ailments including stomach ache, goiter, skin, disease, bone fractures, cyst, and hernia. These manifested that *B. asiatica* has a potential antibacterial agent and demands further investigation to identify underlying mechanisms responsible for bioactivities.

Keywords: Antimicrobial activity, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Barringtonia asiatica Kurz (Lecythidaceae) with the common name "Bitoon" in the Philippines is known to contain active compounds that are known to cause the death of insect pests (Komansilan & Suriani, 2016). Selected plants contain some organic compounds that provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids. These compounds are synthesized by the primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions (Vaghasiya, *et al.,* 2011).

Phytochemicals which are bioactive compounds obtained from plants are widely applied in traditional herbal medicine and herbal medicines and are used by the local people to cure various diseases (Bansode and Salalkar, 2016). These phytochemicals are the chemicals present naturally in plants. Nowadays, phytochemicals become more popular due to their countless medicinal uses and they play a vital role against a number of diseases such as asthma, arthritis, cancer, etc., unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man-friendly medicines" (Banu and Cathrine, 2015). The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants (Vaghasiya, *et al.,* 2011).

Some studies were conducted to test plant materials as potential sources of medicine; thus, this study was proposed to check the presence of phytochemical compounds (saponin, tannin, phenols, alkaloids, flavonoids) and to know the antibacterial activity of *Barringtonia asiatica* fruit and leaf extracts against *E. coli* and *S. aureus* bacteria as there were local testimonies attesting the effectivity of this plant to cure various illness even goiter but no scientific basis has been found yet.

This study is very important because through knowing the compounds present in this plant, scientific-based knowledge gained can be used as baseline information for the possible development of this plant for herbal drugs that are considered to be "friendly medicine" which can be beneficial for everybody. The knowledge gained would certainly help people understand the right application and to what particular ailments/ diseases can it be applicable.

METHODOLOGY

Escherichia coli and *Staphylococcus aureus* were purchased from PNCM-UP Los Baños, Laguna. It was delivered directly to SPAMAST after 3-4 weeks of preparation of cultured specimen.

Extraction. Mature fruits and leaves samples were dried in an oven at 50°C for 48 hours followed by cutting into small pieces and pulverized by a hammer mill grinder Pulverized samples were packed and placed separately in small plastic bags which were stored in a tightly covered container.

The method of extraction used was that of Kumar *et al.,* 2010. Pulverized leaves and fruits samples (about 250g) were soaked in ethanol for 48 hours. After which, collected extracts were filtered and concentrated using a rotary evaporator under reduced pressure at 40-50°C.

Phytochemical Evaluation. Determination of the phytochemical compounds present in the leaves and fruit sample of *B. asiatica* was carried out using the methods used by Rahman et al. (2017).

Test for Flavonoids (Alkaline Reagent Test). A 2 ml of each extract was added with a few drops of 20% sodium hydroxide, formation of an intense yellow color was observed. To this, a few drops of 70%

dilute hydrochloric acid were added and the yellow color disappeared. The formation and disappearance of yellow color indicates the presence of flavonoids in the sample extract.

Test for Alkaloids (Hager's Test). 3 ml of extract was treated with 3 ml of Hager's reagent. Yellow-colored precipitate the presence of alkaloids.

Test for Saponins (Froth Test). Diluted the sample with water to 20 ml extract. Shake vigorously; the formation of 1 cm froth indicated the presence of saponins.

Test for Phenols (Lead acetate Test). Samples were treated with 3 ml of 10% lead acetate solution. A bulky white precipitate indicates the presence of phenols.

Protein (Biuret Test). Treat the samples with a few drops of 2% copper sulfate solution. Add 1 ml of ethanol followed by excess potassium hydroxide pellets. The formation of a pink color in the extract layer indicated the presence of protein.

Antibacterial Assay. The paper disc diffusion method was employed to evaluate the antibacterial activity of each diluted extract of B. asiatica fruits and leaves. Sterile paper discs were impregnated with the extracts (5.0×10^4 µg/mL, 5.0×10^3 µg/mL, 500µg/mL, 50µg/mL, and 5µg/mL) and partially dried and placed into the Plate count agar plates seeded with *S. aureus* and *E. coli*. Diffusion was done in triplicates and plates without treatment were used as control specimens under similar conditions.

Pure culture of *S. aureus* and *E. coli* was streaked in agar plates and incubated for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) which is the clear area around the paper discs.

Date Gathering for Indigenous Knowledge. Data was gathered through a research survey. A letter of permission was held by the researcher to the Barangay officials. A brief and thorough orientation about the main purpose of the study was discussed with the respondents and the survey was administered by questionnaire.

Statistical Tool Analysis. T-test was used to analyze the data expressed as means ± Standard deviation (SD) of measurements of growth inhibition zones of diameters obtained with those natural products. GraphPad Prism 8.1.1 (330) system was the tool used to analyze the results. Percentage computation was used to analyze the results of the IK survey using the formula below.

RESULTS AND DISCUSSION

Phytochemical

Phytochemicals are very important, for they contain health benefits for humans (Heneman and Zidenberg-Cherr, 2008).

Results showed in Table 1 that all tests for phytochemicals in both leaves and fruit samples exhibited the presence of each compound and it was high visibility of indicative results in phenol's test having +++ results for leaves and ++ for fruits.

Phytochemical compounds play a vital role in maintaining good human health, saponin for instance, according to Pacincu (2018), saponins are chemical compounds that occur in a wide range of herbs, seeds, and vegetables. They can also be found in starfish and sea cucumbers. In medicine, they're used in vaccine formulations to regulate immune function. Several studies conducted over the years confirm the health benefits of saponins. These chemicals may help reduce cholesterol levels, kill disease-causing bacteria, scavenge oxidative stress, and inhibit tumor growth.

According to the latest research, they improve lipid metabolism and may help prevent and treat obesity. The saponins and their biosynthetic intermediates display a variety of biological activities of interest to the pharmaceutical, cosmetic, and food sectors. Moses et al., (2014) added that saponins and their biosynthetic intermediates display a variety of biological activities of interest to the pharmaceutical, cosmetic, and food sectors. Ozkan et al., (2014) mentioned that phenolic compounds are reactive metabolites in a wide range of plant-derived foods and are mainly divided into four groups: phenolic acids, flavonoids, stilbenes, and tannins. They work as terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid oxidation. They are well-known nitrogen-containing natural bioactive compounds. Literature reveals that alkaloids contribute multiple biological activities and some alkaloids transform into active metabolites too (Debnath, 2018).

Table 1. Results of phytochemical screening for both leaves and fruit samples.

Phytochemical	Test	Leaves	Fruit	Indication
Saponin	Froth Test	+	+	1 cm froth
Flavonoid		+	+	*if NaOH is added yellow intense color formed then if acid it will turn into colorless
Alkaloid	Hager's Test	+	+	formation of yellow precipitate
Phenol	Lead Acetate Test	+++	++	white precipitate
Protein	Biuret Test	+	+	pink color layer

(+ *Presence, - Absence of the compound)*

Antibacterial Activity

According to Nayan and Vj (2011) the sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of the disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms. Below are the acquired results showing the antibacterial activity of each sample extract.

Results showed that leaves and fruit extracts exhibited antibacterial activity against *S. aureus* shown in Table 2 has a mean of 11.48 mm and 10.06 mm for leaf extracts in dilutions one and two; for fruit extracts, it showed a mean of 10.54 mm and 8.97 mm for dilutions one and two; for both extracts the rest of dilutions it showed lesser activity having a mean less than <8mm.

Table 2. Results of leaves and fruit extract antibacterial activity against *S. aureus*

Replicates	Zone of inhibition (mm) in Different Dilution Concentration µg/ml				
LEAVES	D1	D2	D3	D4	D5
R1	12.3	10.98	6.8	6.58	4.9
R2	11.28	9.84	8.76	4.98	4.94
R3	10.86	9.36	8.3	6.56	4.92
Total Mean	34.44	30.18	23.86	18.12	14.76
Grand Mean	11.48	10.06	7.95	6.04	4.92
Replicates					
FRUITS	D1	D2	D3	D4	D5
R1	11.26	9.2	8.56	6.56	4.96
R2	9.94	8.58	6.68	4.92	4.9
R3	10.42	9.12	8.38	6.62	4.96
Total Mean	31.62	26.9	23.62	18.1	14.82
Grand Mean	10.54	8.96	7.87	6.03	4.94

The graph below portrayed a clear trend of the results of inhibition of *S. aureus* bacteria when treated with the leaves and fruit samples. It shows a downtrend.

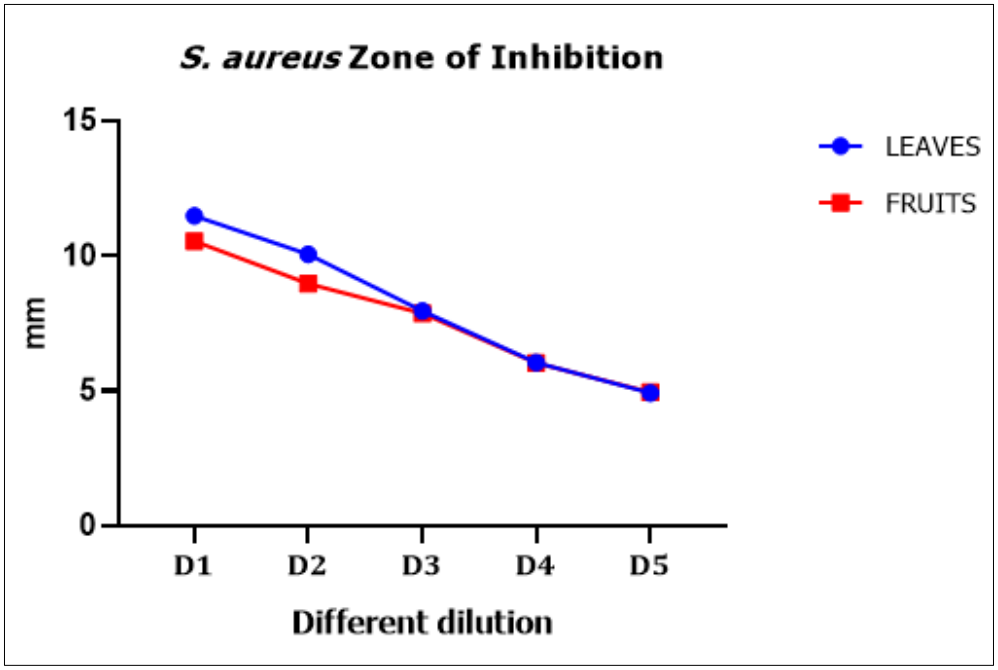

Fig. 2 Graphical representation showing the results for the zone of inhibition mean of each sample extract against *S aureus.*

Based on the results shown in Table 3, leaves and fruit extracts exhibited antibacterial activity against *E. coli* shown in Table 2 having a mean of 12.55 mm and 10.22 mm for leaf extracts in dilutions one and two; for fruit extracts it showed a mean of 11.69 mm and 9.48 mm for dilutions one and two; for both extracts the rest of dilutions it showed lesser activity having a mean less than <8mm.

It was mentioned by Kumar *et al.,* (2010) that a lower mean of inhibition or zero inhibition doesn't imply that tested samples are of no antibacterial activity, it could mean that samples don't exhibit reactions towards the tested bacteria.

Table 3. Results of leaves and fruits extracts antibacterial activity against *E. coli*

Replicates	Zone of inhibition (mm) in Different Dilution Concentration µg/ml				
LEAVES	D1	D2	D3	D4	D5
R1	13.32	10.5	7.08	6.74	4.96
R2	11.84	10.02	6.84	5.04	5
R3	12.5	10.14	6.68	5.06	4.96
Total Mean	37.66	30.66	20.6	16.84	14.92
Grand Mean	12.55	10.22	6.86	5.61	4.97
FRUITS	D1	D2	D3	D4	D5
R1	13.06	10.32	6.66	5	4.94
R2	11.16	9.08	6.64	4.96	4.92
R3	10.84	9.04	6.66	4.94	4.9
Total Mean	35.06	28.44	19.96	14.9	14.76
Grand Mean	11.68	9.48	6.65	4.96	4.92

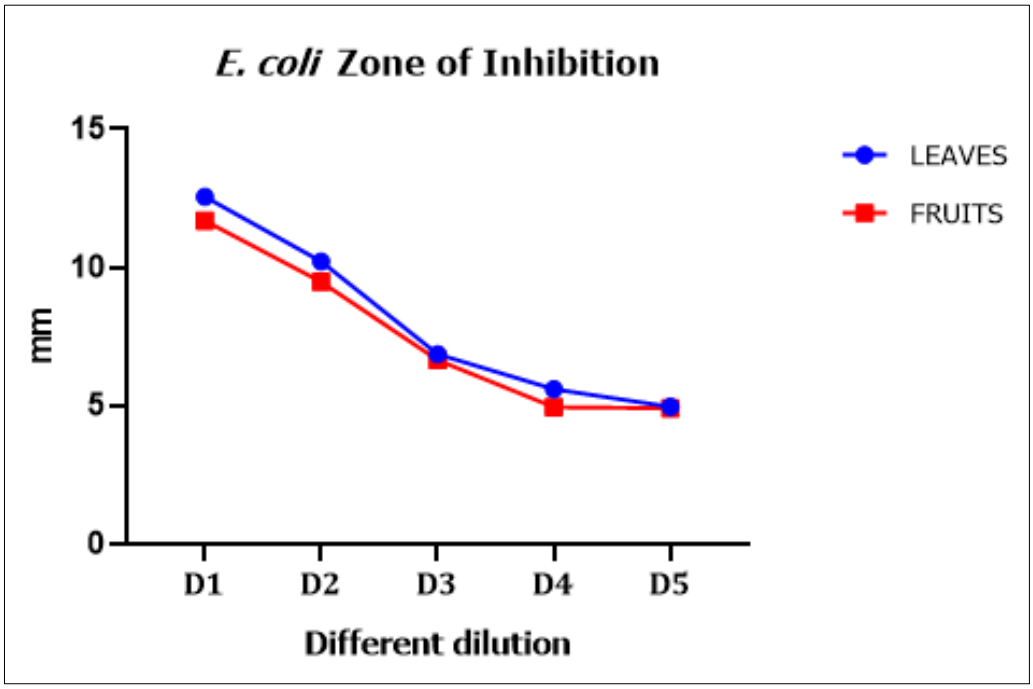

Fig. 3 Graphical representation showing the results for the zone of inhibition mean of each sample extract against *E. coli* bacteria.

Indigenous Knowledge

Based on the data gathered during the survey in Aplaya, Digos City. There were about 10% of the individuals said they were not familiar with this plant and its uses, however still a few of the residents of Brgy. Aplaya affirmed familiarity with this plant and answered the survey questionnaire.

Based on the results, 18 respondents answered the questionnaire, and out of it there were 5 males which comprise 28% of the total number of respondents, and 13 females which is 72.22% of the total number. There were 15 married respondents and 3 single-status respondents. It showed that out of 18, there were 66.66% who tried to use the plant for treating various ailments/diseases and there were 33.33% who weren't able to try it yet but are familiar that this plant contains medicinal capabilities. About how they used for treatment, there were 4 (22.22%) said they used "hunad" or wound bath, 9 (72.22%) mentioned that they used "hampol" or plaster and 1 (11.11%) said that they utilized it by drinking after they boil it. The most common part of the plant used for treating illness was the leaves (55.55%) followed by seed/fruit (38.88%) and lastly the roots (11.11%). According to the results gathered, the most common illnesses treated by this plant are stomach ache (38.88%), goiter (27.77%) skin disease (16.66%) bone fractures (5.55%), cyst (5.55%), and hernias (5.55%). Out of 18 respondents, there were 50% said they got their knowledge about its medicinal uses from friends, and another 50% said they got their knowledge from their ancestors.

Using the GraphPad Prism version 8.1.1 (330), it showed the results of the Paired T-test analysis between fruits and leaves extracts against *S. aureus* bacteria, which gives a calculated t-value of 1.717, with df=4 resulting in a P- value of 0.1611 in alpha level 0.05 (2.132) which is not significant. For the statistical analysis shown in Table 4, between fruits and leaves extracts against *E. coli* bacteria results showed a calculated t-value of 3.218, with df=4, resulting in a P-value of 0.3923 in alpha level 0.05 (2.132) which is significant.

Table. 4 .1. T-TEST analysis between fruits and leaves extracts against *S. aureus*

	Paired t test
P value	0.1611
P value summary	NS
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.717, df=4
Number of pairs	5

Table. 4 .2. T-TEST analysis between leaves and fruits extract against *E. coli*

	Paired t test
P value	0.3923
P value summary	S
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.218, df=4
Number of pairs	5

CONCLUSION

Barringtonia asiatica L. Kurz plant was proven to be positive in the phytochemical tests conducted and thus this plant has the potential to be used as a drug as mentioned by Picincu (2018), and that saponin has great effects on the human body. She said that these chemicals may help reduce cholesterol levels, kill disease-causing bacteria, scavenge oxidative stress, and inhibit tumor growth. Antibacterial activity of the samples was observed visibly in the leaves and fruit extracts when two results were compared. Most of the people who answered the survey said that they've used the leaves for treating various ailments and its effectiveness has been proven to be effective by them and their ancestors. The most common way of using it was by plastering it in the affected area.

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