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Cytotoxicity Analysis and Antibacterial Activity of Jackfruit (*Artocarpus heterophyllus*) Rind Extract on *Staphylococcus aureus* and *Salmonella spp.* Bacteria

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Abstract Antibacterial resistance has become a worldwide public health concern, prompting the search for alternative antibacterial agents. Novel antibacterial agents have already been studied; however, their cytotoxicity must be evaluated first to guarantee their safety on normal cell lines. This study examines the cytotoxicity and antibacterial activity of jackfruit (*Artocarpus heterophyllus*) rind extracts on *Staphylococcus aureus* and *Salmonella spp.* This study utilized Brine Shrimp Lethality Assay for the cytotoxicity test while the Kirby-Bauer method for the antibacterial test. The results of the study indicated that the *A. heterophyllus* rind extract had an LC50 value of 559.4, which is considered toxic according to Meyer's criteria of toxicity and is categorized as low toxic according to Clarkson's criteria of toxicity. The study also found that the lethality of the rind extract was concentration dependent. Furthermore, the results showed that the rind extracts of *A. heterophyllus* did not successfully prevent the growth of *S. aureus* and *Salmonella spp.*, as all samples of the rind extract yielded a diameter of 0 mm. Further research on the cytotoxicity and antibacterial activity of jackfruit rinds using more concentrations and various solvents for the extraction method, as well as for the diluent, is recommended.

Keywords: *antibacterial activity, brine shrimp lethality assay, cytotoxicity, gram-positive, gram-negative, jackfruit rind extract*

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1. Introduction

Jackfruit, also known as *Artocarpus heterophyllus*, is a tropical climacteric fruit of the Moraceae family native to India's Western Ghats and widely distributed in Asia, including the Philippines. Various studies have been conducted using *A. heterophyllus*' tree bark, fruit, leaves, peels, or even seeds [1]. The medicinal properties of *A. heterophyllus* are diverse [2], and this fruit contains a high concentration of phytochemicals that have antioxidant properties. Jackfruit seeds could fight HIV strains and boost immune response [3]. The seeds have an antimicrobial activity that helps prevent foodborne diseases [4]. In the industries that process jackfruit, a significant amount of trash is produced from inedible portions like peel. Unfortunately, only a few studies have examined the potential for turning these wastes into value-added products, leading to significant waste management and environmental issues.

Meanwhile, bacterial pathogens have developed antibacterial resistance and evolved strategies for colonizing and invading human organs [5]. *Staphylococcus aureus* is one of the most well-known and widespread bacterial pathogens, causing many uncomplicated skin infections and hundreds of thousands to millions of more severe, invasive infections [6]. Additionally, *Salmonella spp.* is a gram-negative, facultative anaerobe that causes foodborne illness [7]. Antibacterial resistance has become a public health concern worldwide, leading to the search for alternative antibacterial agents [8]. Natural plant products, such as fruits, have shown broad-spectrum antibacterial activity, but their cytotoxicity should be assessed to ensure human safety [9].

The main objective of this study is to investigate the cytotoxicity and antibacterial activity of *A. heterophyllus* rind extracts. This study aims to determine whether the

extract of jackfruit rind had any toxicity using the brine shrimp lethality assay and to establish its effectiveness as an antibacterial agent against *S. aureus* and *Salmonella spp*. In order to achieve this objective, the study used a quantitative design that utilized an experimental approach. The data for the study were gathered through a series of experiments that were conducted in a controlled environment.

2. Materials and Methods

2.1. Plant Material

The identified unripe jackfruit (*A. heterophyllus*) was obtained from the Malaybalay City Agriculture Office. After acquiring the said fruit, it was then peeled, and its rinds were washed thoroughly with distilled water. The rinds had been cut into small pieces.

2.2. Methods

The identified unripe jackfruit (*A. heterophyllus*) rind extracts were extracted using different methods for certain tests. For the cytotoxicity test, the decoction method was used, while maceration was used for the antibacterial test.

2.2.1. Maceration Process

The small pieces of rinds were sun-dried for three days [10]. After drying, the small pieces of sun-dried rinds were pounded from well to fine powder using a portable grinder. The powdered rinds were soaked in ethanol (99%) using a 1:10 ratio. The ethanolic extract was then filtered using filter paper and had been separated into a flask, and the ethanolic extract was weighed. Finally, it was sent to Central Mindanao University (CMU) for extraction using a rotary evaporator. Afterward, 0. 5 g of rind extract was dissolved using distilled water to produce a final concentration of 50 mg/ml. The solution was then diluted into four different concentrations: 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL.

2.2.2. Decoction Process

For the preparation of the plant extract, 150 ml of distilled water was added to 15 g of powdered jackfruit rinds to achieve a 1:10 [11]. Afterward, it was heated until boiled, and then it was filtered using Whatman no. 1 filter paper. From the stock solutions, six concentrations were drawn, and they were transferred into pre-marked test tubes to give a series of chosen concentrations of; 0 ppm, 100 ppm, 500 ppm, 1000 ppm, 10000 ppm, and 100000 ppm, respectively. 10 ml of alternative seawater was added to the test tubes, and 15 brine shrimps were introduced to each test tube using a Pasteur pipette. Three tests were conducted per concentration. The brine shrimps would then be left for 24 hours before counting the survivors and calculating their percentage of mortality.

2.2.3. Bacteria Used

The *S. aureus* and *Salmonella spp.* were obtained and cultured for 24 hours at the Laboratory of Veterinary Medicine at CMU.

2.2.4. Cytotoxicity Test

For the cytotoxicity testing, a brine shrimp hatchery tool set was prepared, the container was filled with 3/4 cup of distilled water, and the air pump was turned on. To create an artificial seawater, a teaspoon of rock salt was added to the water, and a pinch of baking soda was added as well to maintain its pH level. A half teaspoon of Ocean Star International (OSI) brine shrimp eggs with a hatching rate of 90% had been placed in the hatchery. In 24 hours, the nauplii had been harvested, and it had been placed in a vial for preparation for the cytotoxicity testing.

2.2.5. Antibacterial Test

For the antibacterial testing procedure, the rind extract of jackfruit (*A. heterophyllus*) was tested against *S. aureus* and *Salmonella spp.* using agar disk diffusion assay or the Kirby-Bauer test. Ten petri dishes and bent glass rods had been prepared and sterilized. Mueller-Hinton agar was mixed and stirred with distilled water in a beaker. The solution would then be sterilized in an autoclave at 121 degrees Celsius for 15 minutes and let to cool down for 15 minutes. The agar solution had been poured into the petri dish, and it had been left for five minutes to harden. The cultured *S. aureus* and *Salmonella spp.* samples were poured and streaked in the petri dish using the spread plate method. The paper discs were soaked into the four different concentrations of rind extract as well as in the positive and negative control treatments, and each treatment had five replicates. Afterward, five paper discs were used on each quintet of the petri dish for the positive and negative controls as well as the various concentrations of the rind extract. The petri dishes were then incubated at 37 degrees Celsius for 24 hours. After its incubation, the diameter zone of inhibition was measured in millimeters using a caliper.

2.3. Data Analysis Procedure

For the antibacterial test, the inhibition zone was measured using the Zone of Inhibition Testing or Kirby Bauer's Test. The diameter had been measured using a caliper on the underside of the petri dish. The mean diameter of the zone of inhibition was recorded and interpreted in accordance with the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol by [20].

In assessing the cytotoxicity of the rind using Brine Shrimp Lethality Assay, the study would utilize equation 1 [12].

$$Mortality\ \% = \frac{\#\,dead\ \ nauplii}{\#\ of\ \ dead\ \ nauplii + \#\ of\ \ alive\ nauplii} \times 100 \quad (1)$$

The lethal concentration (50%) or LC50 would also be calculated using the probit analysis as displayed in Equation 2

$$y = ax + b \quad (2)$$

wherein;
y = Probit value of LC50
a = Coefficient value of X variable 1
x = logarithm value of LC50
b = Coefficient intercept value

3. Results and Discussion

3.1. Cytotoxicity Test Results

The number of survivors was counted in each concentration of the rind extract. Table 1 summarizes the mortality rate of brine shrimp nauplii treated with different concentrations of *A. heterophyllus* rind extracts.

It is shown in Table 1 that as the concentration of the rind extract increases, the mortality rate of the brine shrimp nauplii has also increased. Figure 1 shows that the mortality rate of the nauplii is directly proportional to the concentration, and it is similar to [13]. The maximum mortality (100%) was detected at 100 000 ppm. Furthermore, a linear regression equation was used in this study and yielded an LC50 value of 559.4 ppm. The estimated LC50 values obtained by the probit regression analysis can be compared to either Meyer's or Clarkson's toxicity criteria [14]. In accordance with Meyer's criteria, the LC50 value of 559.4 ppm is considered toxic since it is <1000 ppm. However, using Clarkson's criteria, 559.4 ppm is specifically categorized as low toxic since the said value is within the range of 500-1000 ppm.

Table 1. Mortality rate of brine shrimp nauplii after treating with various concentrations of *A. heterophyllus* rind extracts

Concentration (ppm)	Number of Surviving Nauplii (after 24 h)			Total Number of Nauplii Survivors	Mortality Rate (in %)	LC_{50} (ppm)
	R1	R2	R3			
0	15	15	15	45	0.00	559.4
100	10	10	9	29	35.56	
500	6	10	9	25	44.00	
1000	7	6	7	20	55.56	
10 000	2	4	5	11	75.56	
100 000	0	0	0	0	100.0	

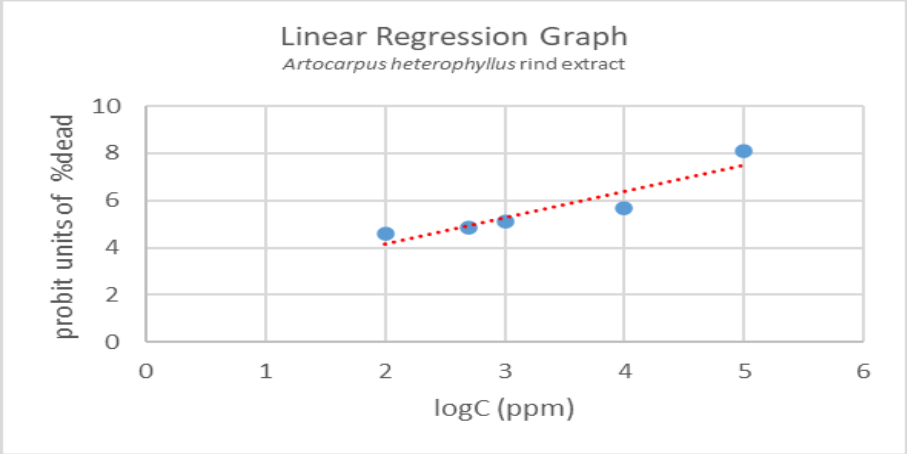

Figure 1. Linear Regression Graph

The results of this study coincide with the study of other authors. Reference [15] shows the LC50 of the combined crude acetone extracts (CCAE) of *Plumbago zeylanica L.*, *Limonia acidissima L.*, and *Artocarpus heterophyllus Lam*, also using the BSLA, to be <1000 ppm (157 ppm). However, it is not congruent with the study of others since it is discovered that the seed extracts of *A. heterophyllus* show no toxicity in the BSLA [16]. This may be due to the different bioactive compounds present in both the seed and rind extracts or the solvents utilized for extracting, as well as the extraction method used.

The mean of dead brine shrimp nauplii treated with various concentrations of *A. heterophyllus* rind extracts are summarized in Table 2 and is further compared and analyzed using One-Way ANOVA in Table 3.

Table 2. Mean of dead brine shrimp nauplii after treating with various concentrations of *A. heterophyllus* rind extracts

Concentration (ppm)	Number of Dead Nauplii (after 24 h)			Total Number of Dead Nauplii	Mean of Dead Nauplii
	R1	R2	R3		
0	0	0	0	0	0.00
100	5	5	6	16	5.33
500	9	5	6	20	6.67
1000	8	9	8	25	8.33
10 000	13	11	10	34	11.33
100 000	15	15	15	45	15.00

It is evident in the table above that the control group, which has a concentration of 0 ppm, provided no deaths of brine shrimp nauplii, which means that all nauplii survived in this environment. On the other hand, *A. heterophyllus* rind extracts with a concentration of 100 ppm and 500 ppm produced a total number of 16 and 20 dead nauplii with a mean of 5.33 and 6.67, respectively. Meanwhile, the extracts with 1000 ppm, 10 000 ppm, and 100 000 ppm yielded total numbers of 25, 34, and 45 dead nauplii with a mean of 8.33, 11.33, and 15.00 in the given order.

Table 3. Summary of One-Way ANOVA on the mean of the dead brine shrimp nauplii

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	398.4	5	79.69	65.2	<0.000	3.11
Within Groups	14.67	12	1.2222			
Total	413.1111	17				

Significant at 0.05 level.

One-Way ANOVA was the statistical tool used to determine the significant difference between the mean of dead brine shrimps using different concentrations of the rind extract. The results provided an F value of 65.2 and an F crit value of 3.11, which indicates that F > F crit. The p-value is also less than the 0.05 level of significance ($p<0.05$); therefore, there is a significant difference between the means of dead brine shrimp nauplii after applying various rind extract concentrations, which verifies the concentration-dependent behavior of the *A. heterophyllus* rind extract.

Jackfruit contains phytonutrients like isoflavones, lignans, and saponins that have anti-cancer properties and reduce the development of cancer cells, and these compounds could potentially cure or prevent lymphoma cancer [17]. Jackfruit rinds may also contain these phytonutrients, as its extract showed an LC50 value that is <1000 ppm. Even the twigs of *A. heterophyllus* have natural products with cytotoxic abilities against certain human cancer cell lines [18]. While the jackfruit seed extract showed no toxicity in BSLA, its seed extract was still able to have cytotoxic activity on human cancer cell lines without harming normal human cell lines [16].

3.2. Antibacterial Test Results

In each petri dish, the inhibition diameters were measured. Table 4 summarizes the findings of the zone of inhibition using various treatments and concentrations of *A. heterophyllus* rind extract and Figure 2 and Figure 3 show the various cultures.

Table 4. Zone of Inhibition using various treatments and concentrations of *A. heterophyllus* rind extracts

Antibiotic/Extract	Bacteria/Isolate	Zone of Inhibition (mm)					Interpretation
		R1	R2	R3	R4	R5	
Rind Extracts (25 mg/mL)	*S. aureus*	0	0	0	0	0	Resistant
	Salmonella spp.	0	0	0	0	0	Resistant
Rind Extracts (50 mg/mL)	*S. aureus*	0	0	0	0	0	Resistant
	Salmonella spp.	0	0	0	0	0	Resistant
Rind Extracts (75 mg/mL)	*S. aureus*	0	0	0	0	0	Resistant
	Salmonella spp.	0	0	0	0	0	Resistant
Rind Extracts (100 mg/mL)	*S. aureus*	0	0	0	0	0	Resistant
	Salmonella spp.	0	0	0	0	0	Resistant
Positive Control (Doxycycline)	*S. aureus*	28	28	28	28	28	Susceptible
	Salmonella spp.	0	0	0	0	0	Resistant
Negative Control (Distilled Water)	*S. aureus*	6	6	6	6	6	Resistant
	Salmonella spp.	0	0	0	0	0	Resistant

It is evident in the table above that all four samples of jackfruit rind extract with five replicates did not produce a zone of inhibition in both gram-positive *S. aureus* and gram-negative *Salmonella spp*., therefore having a diameter of zero. However, the positive (Doxycycline) and negative control (Distilled Water) produced a diameter of 28 mm and 6 mm on *S. aureus*, respectively. The raw data of this test is usually in the form of a zone size or Minimum Inhibitory Concentration (MIC) [19]. For this study, the zone size was used in reporting the data, and it was then interpreted whether it was Susceptible, Intermediate, or Resistant in accordance with the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol [20].

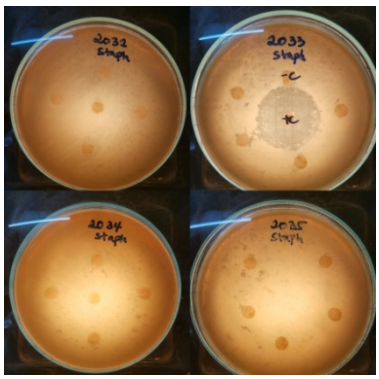

Figure 2. *Staphylococcus aureus* after treating with various treatments

Figure 3. *Salmonella spp*. after treating with various treatments

Both *S. aureus* and *Salmonella spp*. have shown resistance in the four concentrations (25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL) of jackfruit rind extract as well as the negative control, which means that they did not respond to the given treatments. Although the distilled water yielded a diameter of 6 mm, it is still considered as resistant since it did not reach the range of ≥13 to ≥29 mm nor 11-19 mm to be interpreted as susceptible or intermediate, respectively. It can also be seen in the table that both positive and negative controls influence the gram-positive *S. aureus*, while it does not have any effect on *Salmonella spp*. This supports the claim that gram-positive bacteria are more fluent to eliminate than gram-negative bacteria due to their unique structure, which causes major morbidity and mortality worldwide [21].

This outcome may be a result of some factors, such as the preparation of the samples, which affected the antibacterial activity of the rind extract against the bacteria. The jackfruit leaves have already shown an antibacterial effect on *S. aureus* and *Salmonella spp.* [22,23]. The dilution method used in this study might have affected the results since in the study of De Sousa et al., they used the serial microdilution technique [22] while this study used a simple dilution method with larger amounts of concentrations, making it difficult for the rind extract to move into the nutrient agar since the zone of inhibition varies depending on variables such as disk potency, diffusibility of the agent, amount of inoculum, and media type [24]. This further supports the claim that the size of the zone is also influenced by the size and weight of the antibiotic molecules [25]. Another factor that could have affected the results is that the diluent used is distilled water only. The utilization of distilled water as a diluent may be more efficient than using ethanol. However, the choice of diluent can still have an impact on the antibacterial activity of the extract [26].

4. Conclusion

The cytotoxic potential of jackfruit rind was investigated in this study using the Brine Shrimp Lethality Assay (BSLA) at various concentrations. The mortality rate of the brine shrimp nauplii is directly proportional to the concentration of the jackfruit rind extract, indicating that the extract's lethality is concentration-dependent. At 100,000 ppm, the highest mortality rate (100%) was observed, and the LC50 value obtained from the regression analysis was 559.4 ppm, which is toxic according to Meyer's toxicity criteria and low toxicity range based on Clarkson's criteria.

There is also a significant difference in the means of dead brine shrimp nauplii after applying different rind extract concentrations. These findings support the possibility that jackfruit rinds contain phytonutrients with cytotoxic properties against cancer cell lines.

Furthermore, the results of the study for *A. heterophyllus* antibacterial activity revealed that none of the four samples of jackfruit rind extract exhibited any antibacterial activity against either of the tested bacterial strains, as evidenced by the lack of any zone of inhibition. The positive control (Doxycycline), on the other hand, showed a zone of inhibition on *S. aureus* with a mean diameter of 28 mm, indicating its effectiveness in inhibiting the growth of the bacterial strains. The negative control (Distilled Water) was able to create a zone of inhibition of 6 mm on *S. aureus*; however, the bacteria was still able to show resistance to it. As a result, the jackfruit rind extract used in this study possesses no significant antibacterial activity against *S. aureus* and *Salmonella spp.*

Lastly, at various concentrations, the antibacterial potential of *A. heterophyllus* rind extract was tested against two bacterial strains, *S. aureus* and *Salmonella spp*. The results show that the extract had no significant antibacterial activity against either of the two strains at any of the concentrations tested. Both bacterial strains were resistant to the negative control, while the positive control was effective against the bacteria *S. aureus* but not against *Salmonella spp*.

5. Recommendations

Based on the findings of the study, the cytotoxicity and antibacterial activity of jackfruit rind extract suggest that fruit rinds could be a source of bioactive compounds with antimicrobial properties, and further research can be conducted to explore the cytotoxicity and antibacterial activity of other fruit rinds; such as pineapple, watermelon, durian, miracle fruit, or orange, on Gram-positive and Gram-negative bacteria. The use of various extraction methods, such as microwave-assisted extraction or ultrasound-assisted extraction, to determine their efficiency in extracting bioactive compounds from fruit rinds is also recommended.

In addition, it is necessary to test different concentrations and combinations of bioactive compounds extracted from fruit rinds to identify the most effective formulation for inhibiting the growth of particular bacterial strains. This is because the bioactive compounds found in fruit rinds may have varying levels of antimicrobial activity against various bacterial strains. By testing different concentrations and combinations of these compounds, researchers may determine the most effective formulation that can target the specific bacteria of interest. Furthermore, the use of fruit rinds as a natural alternative to conventional antibiotics is a promising avenue for promoting environmentally friendly and sustainable antimicrobial treatments. This is because fruit rinds are a plentiful agricultural waste material that is easily available and can be processed using sustainable extraction methods.

Lastly, conventional antibiotics are usually produced using non-renewable resources, such as petroleum-based chemicals, in energy-intensive processes. This endangers the environment and sustainability. Additionally, antibiotic overuse and misuse have resulted in the emergence of antibiotic-resistant bacteria, posing a threat to public health. As a result, natural alternatives, such as fruit rinds, must be investigated in order to reduce reliance on conventional antibiotics and reduce the risk of antibiotic resistance. However, more research is needed to determine the safety of fruit rind extracts on human cells. This will help ensure that the extracts can be used in antimicrobial treatments. Moreover, researchers can

investigate ways to incorporate fruit rind extracts into various products, such as disinfectants or soaps, to improve their efficacy. By doing so, the full potential of fruit rinds as a natural alternative to conventional antibiotics could be unlocked. Other methods for cytotoxicity analysis, such as MTT assay, Alamar blue, and Inflammatory markers, are also suggested in order to obtain cellular-level results.

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