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Evaluation of the larvicidal efficacy of *Artocarpus heterophyllus* (jackfruit) rags and rind ethanolic crude extracts against third to early fourth instar *Aedes aegypti* larvae

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Abstract

Artocarpus heterophyllus (jackfruit) is a shrub that has high phytochemical content in its different plant parts. However, 60% of its fruit remains underutilized. This study evaluated the larvicidal activity of the crude extracts of *A. heterophyllus* rags, rind, and combined rags and rind against third to early fourth instar *Aedes aegypti* larvae. Larvicidal bioassay was performed using concentrations ranging from 500–2500 ppm. Larval mortality data were recorded after 48 hours of exposure and were analyzed using Probit analysis. The *A. heterophyllus* rags and rind crude extracts showed high larval percent mortality rates of 70%-90%. The rind crude extract has the highest larvicidal efficacy since it has the lowest LC50 and LC90 values of 1136 ppm and 2500 ppm, respectively. The promising larvicidal activities shown by the treatments may be attributed to the abundance of triterpenes, saponins, tannins, and glycosides that was found using qualitative phytochemical analysis. Thus, the crude extracts of *A. heterophyllus* rags and rind may be used as alternatives to synthetic larvicides.

Introduction. - Dengue epidemics have increased for the past 20 years in both number and magnitude due to the rapid spread of the *Aedes aegypti* mosquito, the primary vector of dengue viruses, which thrives in highly urbanized areas [1]. The disease is one of the most significantly widespread mosquito-borne viral infections in humans [2]. Due to the lack of medical response against dengue viruses, effective vector control measures have become the sole weapon against dengue today [3]. The application of chemical insecticides to control the principal dengue vector, *Ae. aegypti*, is widespread. However, the development of resistance against these chemicals, the undesirable effects on non-target organisms, and the rise of environmental and health concerns led to the search for other alternative methods in controlling vector mosquitoes, such as the use of plants [4].

Plant-derived products have been used as insecticides in substitute for synthetic chemicals. They are less toxic, less prone to the development of resistance, and easily biodegradable [5]. The general classes of phytochemicals that they contain (sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins) are responsible for their insecticidal activity. These phytochemicals extend the postembryonic development of larvae and pupae and delay the formation of adult insects [6].

Artocarpus heterophyllus, also known as jackfruit, is a tree belonging to the mulberry family (Moraceae). It has naturalized in the tropics, particularly in Southeast Asia, and is considered an important crop in many countries such as the Philippines [7]. Only 15–20% of the ripe fruit is utilized as food [8], while 60% of the fruit which includes the rind, inner perdigones, and central core, are being thrown away as waste [9]. Thus, utilization of these parts must be done to convert these wastes into useful products [10].

Previous studies have focused on the pulp and seeds of the jackfruit and their phytochemical properties which reported that they have a high phytochemical content [11,12,13]. Moreover, the results of several studies have shown that high quantities of saponins, alkaloids, and flavonoids are present in jackfruits [11]. However, despite these pieces of information, none of the previous studies have isolated and examined the underutilized parts of jackfruit for their phytochemical content and larvicidal activity against dengue mosquito vectors,

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specifically, the rags and rind of the fruit.

This study aimed to evaluate the larvicidal effect of *A. heterophyllus* crude extracts in terms of mortality rate against *Ae. aegypti* larvae. Specifically, the study aimed to:

(i) count the dead and moribund *Ae. aegypti* larvae after the treatment of *A. heterophyllus* rags, rind, and 50:50 combination crude extracts.

(ii) determine the lowest concentration of *A. heterophyllus* rags, rind, and 50:50 combination crude extracts that would yield 50% and 90% mortality rates in the larval population; and

(iii) assess and confirm the presence and abundance of phytochemicals in *A. heterophyllus* rags and rind using qualitative phytochemical analysis.

Methods. - The study conducted was experimental in nature. After the acquisition, *A. heterophyllus* rags, white or pale yellow fibrous strands surrounding the flesh, and rind, the green or yellow spiky outer skin of the fruit, were thoroughly rinsed before oven drying and powderization. The ground samples were macerated for 24 hours with constant agitation and filtered using vacuum filtration. Rotary evaporation was performed to obtain the crude extracts used in the succeeding processes. A qualitative phytochemical analysis was performed on both rags and rind samples to assess the active components. The larvicidal effect of each crude extract, against *Ae. aegypti* larvae were investigated using a larvicidal bioassay. Probit analysis was utilized to determine the LC50 and LC90 values of the crude extracts. The number of mosquito larval deaths in set-ups was recorded and counted manually after 24 hours and 48 hours of exposure.

Preparation of Samples. The samples were collected from a jackfruit vendor in Calle Real, Iloilo, and sent to the Department of Agriculture (DA) for verification. The rags and rind portion were separated, cut into smaller pieces, rinsed with distilled water, and oven-dried at 40 °C for 48 hours. The dried samples were ground into fine particles and weighed using a digital analytical balance.

Extraction. One hundred grams (100 g) of the ground samples were macerated in 1000 mL of 95% ethanol in amber glass bottles for 24 hours at room temperature. The solutions were mixed in batches using a magnetic stirrer and agitated at varying intervals. They were then filtered via vacuum filtration using Whatman Filter Paper no. 41. Subsequently, the samples were placed in a rotary evaporator at 40 °C until the ethanol completely evaporated. Lastly, the extracts were subjected to a flame test to verify the absence of ethanol in the samples.

Collection and Acclimatization of Test Organisms. Twenty (20) third to early fourth instar mosquito larvae were used for both the preliminary and final testing. The first and second instars of the larvae were not utilized as they are considered too fragile to handle.

The *Ae. aegypti* larvae used in the study were bred and cultured by the Department of Science and Technology-Industrial Technology Development Institute (DOST-ITDI) Entomology Section Insectary according to the standard procedures and general guidelines set by World Health Organization (WHO).

Preparation of Test Set-ups. Larval populations containing 20 organisms in each 100 mL glass beakers with 50 mL dechlorinated water were established. Relatively smaller and moribund larvae were replaced.

Preparation of Treatments. As specified in the Guidelines for Laboratory and Field Testing of Mosquito Larvicides of WHO, the quantity of the stock solution was obtained by diluting 1.5 grams of each of the *A. heterophyllus* extracts, namely: pure rags crude extract, pure rind crude extract, and the 50:50 combination, with 1.5 mL 95% ethanol and 13.5 mL water to achieve 100000 ppm or 10% w/v crude extract-ethanol solution [14]. The stock solutions were placed in 140 mL beakers. Shaking and stirring of the solutions were done to dissolve the extract with the solvent.

Abate® 1SG mosquito larvicide and 1% ethanol in dechlorinated water were used as positive and negative controls, respectively. These were then applied to the mosquito larvae set-ups.

Larvicidal Bioassay. The WHO standard protocol for testing the mortality of mosquito larvicides was followed. Two (2) preliminary experiments were conducted to establish the range of lethal concentrations that would be effective in killing 10% to 90% of the larval population. Each preliminary test contained five (5) test concentrations with three (3) replicates each. The final test concentrations were obtained on the second preliminary test, ranging from 500–2500 ppm. Six (6) replicates were carried out for each concentration. A water depth between 5–10 cm was maintained to prevent undue mortality when soaked in deeper levels [14,15]. Mortality data was recorded after 24 and 48 hours of exposure for each test set-up. The mortality rates were calculated using the formula below:

$$Mortality\ rate = \frac{Total\ number\ of\ dead\ larvae}{Initial\ number\ of\ larvae\ present} \times 100\%$$

Larvae that fail to display any immediate activity when probed or prompted were identified as dead or moribund [14].

Qualitative Phytochemical Analysis. Two hundred grams (200 g) of oven-dried *A. heterophyllus* rags and rind samples were sent to DOST-ITDI. The presence and abundance of the phytochemical components, specifically alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and triterpenes were determined and assessed.

Data Analysis. Data from all replicates of each treatment were pooled for linear regression probit analysis using Microsoft® Excel® for Microsoft 365 MSO (16.0.13929.20360) 64-bit. The lethal concentrations to kill 50% and 90% of the larval

population, also known as LC50 and LC90 values, were determined by plotting the data points in a spreadsheet. The concentrations were then converted into logarithmic functions whereas the mean percentage of larval mortality adopted the corresponding probit values. A one-way Analysis of Variance (ANOVA) test was conducted at $\alpha = 0.05$ to statistically compare the treatments.

Waste Disposal. No chemical substances were disposed of down the drains. The containers with chemical wastes were placed in the chemical waste disposal box in the Biology Laboratory 2 of PSHS-WVC.

Stock solutions used in the larvicidal bioassay were disposed of in a separate container specifically for chemical waste while dead larvae were placed in biohazard containers. The standard procedures of the institution were followed. Beakers after testing were treated and sterilized with hot water.

Safety Procedure. The chemical and waste management were done according to the WHO Laboratory Safety Manual 4th Edition [16]. All laboratory chemicals and chemical wastes were treated with caution and exposure was minimized by observing laboratory safety measures such as wearing personal protective equipment (PPE) and conducting activities under the supervision of laboratory personnel. Chemical wastes were stored in properly-labeled closed containers. Extracts were properly stored in a 15 mL amber glass bottle.

Results and Discussion. - This study aimed to evaluate the larvicidal efficacy of the individual and combined crude extracts of *A. heterophyllus* rags and *A. heterophyllus* rind against third to early fourth instar *Ae. aegypti* larvae. This was done through a larvicidal bioassay. The data were collected after 48 hours for comparison and analysis.

Figure 1 shows the larvicidal activity of the three treatments against third to early fourth instar *Ae. aegypti* larvae by larvicidal bioassay. The three crude extracts showed significant toxicity based on the mean (%) mortality of the *Ae. aegypti* larvae. The mean (%) mortality of the three extracts increases as the concentration of treatments increases.

Two thousand five hundred (2500) ppm of *A. heterophyllus* rind extract has the highest mean (%) mortality. Five hundred (500) ppm of crude extracts of *A. heterophyllus* rags and of the combined *A. heterophyllus* rags and rind exhibited no larval death.

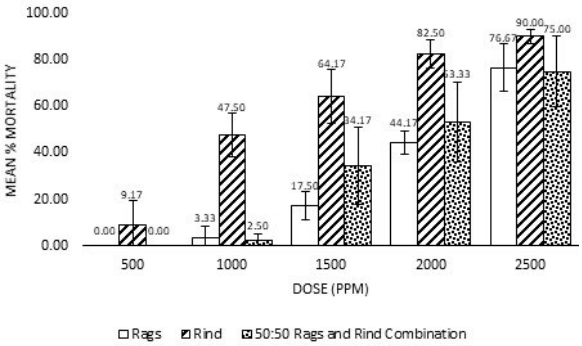

Figure 1. Larvicidal activity of the rind crude extract, rags crude extract, and 50:50 combination of rags and rind crude extract of *A. heterophyllus* against third and early fourth instar *Ae. aegypti* larvae after 48 hours.

Figure 2 shows the larvicidal activity of the positive control (Abate® 1SG larvicide) against third to early fourth instar *Ae. aegypti* larvae. The mortality of *Ae. aegypti* larvae was observed in all its concentrations with 0.3 ppm having the lowest mean (%) mortality at 10.00%, and 1.2 ppm having the highest at 96.67%. There was no larval mortality observed in the negative control treatment, 1% ethanol-dechlorinated water. The data show that all three crude extracts can be used as a larvicide. However, these are not as effective as the commercially available larvicide which is the positive control. Moreover, the results of the one-way ANOVA test showed that no significant difference exists between the groups determined by ($F(2,12) = 1.282$, $p = 0.3129$) at $\alpha = 0.05$. Thus, all extracts are equally effective.

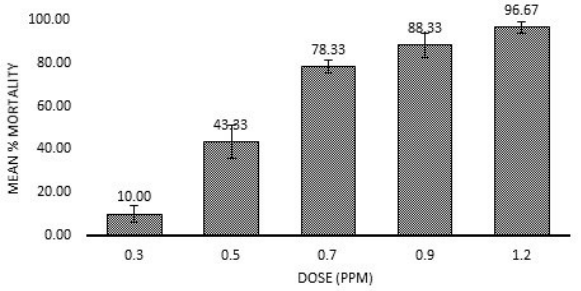

Figure 2. Larvicidal activity of the positive control, Abate® 1SG larvicide, against third to early fourth instar *Ae. aegypti* larvae after 48 hours.

Figure 3 shows the lethal concentrations of *A. heterophyllus* rags crude extract, *A. heterophyllu*s rind crude extract, and combined *A. heterophyllus* rags and rind crude extract against third to early fourth instar *Ae. aegypti* larvae after 48 hours of exposure.

The lowest LC50 and LC90 values were obtained from *A. heterophyllus* rind crude extract. The LC50 of *A. heterophyllus* rind crude extract was obtained at 1136 ppm while the LC90 was obtained at 2500 ppm. It was followed by combined *A. heterophyllus* rags and rind crude extract with LC50 and LC90 values of 1903 ppm and 3041 ppm, respectively. *A. heterophyllus* rags crude extract had its LC50 at 2012 ppm and LC90 at 3041 ppm.

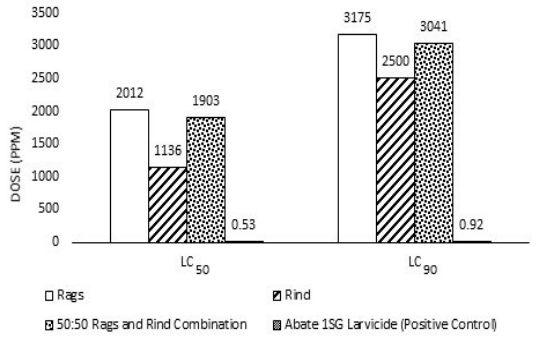

Figure 3. Lethal concentrations of different treatments against third to early fourth instar *Ae. aegypti* larvae after 48 hours of exposure period.

A. heterophyllus rind crude extract was the most effective larvicide among the three crude extracts for it has the lowest LC50 and LC90 values. The effectiveness of *A. heterophyllus* rind crude extract as a larvicide may be attributed to the abundance of known phytochemicals that are found in other plant-based larvicides in it.

A study conducted by Nair and Kavrekar [17] using the leaves of similar plant species was conducted and the results are more effective in terms of the LC50 and LC90. The LC50 and LC90 values of the treatment in the study are between 400 ppm and 600 ppm, and between 800 ppm and 900 ppm, respectively. The values mentioned are lower than the LC50 and LC90 values of the three treatments in this study [17]. Another study conducted by Pineda-Cortel et al. [18] made use of *Artocarpus blancoi* in different fractions and reported incomparable results with the result of the current study. Furthermore, the results of previous studies are more effective than the treatments used in this study.

The combination of crude extracts may exhibit synergistic or antagonistic effects. The combined extract used in this study, however, is only limited to one concentration which is 50:50. Other ratios were not studied in this study. Therefore, the best ratio to obtain a result that is more effective, in terms of the lethal concentration, than the individual extracts was not determined. The relationship of two crude extracts is synergistic when the quotient of the LC50 of the individual crude extract and of the combined plant crude extracts, also known as the synergistic factor, is greater than 1 [19]. The synergistic activity increases with the synergistic factor. In this study, the synergistic factor between the crude extracts of *A. heterophyllus* rags and the combined *A. heterophyllus* rags and rind crude extract is 1.057. On the other hand, the synergistic factor between the crude extracts of *A. heterophyllus* rind and the combined *A. heterophyllus* rags and rind is 0.597. This means that the crude extracts of *A. heterophyllus* rags and *A. heterophyllus* rind have an antagonistic relationship. This result is similar to the study of Grande et al. [20] where the combined extracts did not have an overall synergistic larvicidal effect against *Ae. aegypti* larvae. The LC50 and LC90 values of the combined extracts fell under the LC50 and LC90 values of the individual extracts. Based on the study, the failure of the combined extracts to have a synergistic effect on each other was due to the possible unforeseen chemical reactions between the phytochemicals that the individual extracts contain. However, this result differs in study of Yuan et al. [19] where the individual extracts exhibited a synergistic activity because the combined extracts were found to be a more effective larvicide than the individual extracts.

Table 1 shows the results of the qualitative phytochemical analysis of *A. heterophyllus* rags and rind. The results showed that *A. heterophyllus* rags crude extract does not contain sterols, flavonoids, and alkaloids. However, the rags extract has traces of saponins and glycosides, a moderate amount of tannins, and an abundance of triterpenes. The results also showed that *A. heterophyllus* rind crude extract does not contain sterols and alkaloids. Despite this, the rind extract contains traces of flavonoids and glycosides, and an abundance of triterpenes, saponins, and tannins.

Table 1. Qualitative phytochemical analysis of *A. heterophyllus* rags and *A. heterophyllus* rind samples.

Phytochemical	*A. heterophyllus* rind	*A. heterophyllus* rags
Sterols	(-)	(-)
Triterpenes	(+++)	(+++)
Flavonoids	(+)	(-)
Alkaloids	(-)	(-)
Saponins	(+++)	(+)
Glycosides	(+)	(+)
Tannins	(+++)	(++)

Traces (+), Moderate (++), Abundant (+++), Absent (-)

Saponins are known to decrease the digestive enzyme activity of mosquito larvae [21] and attack the cuticle membrane of the larvae [22]. Moreover, flavonoids attack the nerves and respiratory system of mosquito larvae [21]. Lastly, tannins form complexes with the digestive enzymes in the gut of insects which reduce the digestion efficiency and inhibit the growth of insects [22]. Previous studies have concluded that the abundance of saponins and tannins in plant extracts is responsible for the larvicidal activity of the plant extract [18,23,24].

In this study, the positive larvicidal activity exhibited by the three crude extracts was attributed to the presence of phytochemicals. Similar to previous studies, saponins, flavonoids, and tannins disrupted the normal body functions of larvae. The abundance of saponins and tannins in *A. heterophyllus* rind crude extract made it more effective than the other two crude extracts having a lesser amount of the mentioned phytochemicals present. Also, flavonoids are present in *A. heterophyllus* rind crude extract but not in *A. heterophyllus* rags crude extract. This is also a factor as to why *A. heterophyllus* rind crude extract is more effective as larvicide at a lower concentration than *A. heterophyllus* rags crude extract. Furthermore, the disruption of the normal body functions of the larvae decreased the health of the larvae and eventually caused the mortality of the larvae.

Limitations. The qualitative phytochemical test of DOST-ITDI only included a predetermined list of phytochemical components that were to be assessed in the plant samples. Thus, limiting the assessment to seven (7) components. The presence nor abundance

of other phytochemicals was not confirmed through the said test.

Conclusion. - The current study found *A. heterophyllus* rags, *A. heterophyllus* rind, and combinations of *A. heterophyllus* rags and rind crude extract as equally effective larvicides against third to early fourth instar *Ae. aegypti* larvae. The individual *A. heterophyllus* rind crude extract is the most efficient among the three treatments because it requires the least amount of concentrations to result in 50% and 90% mortality rates in the larval population. Phytochemicals were also found to be present in *A. heterophyllus* rags and rind, with the rind containing more phytochemicals. This is the reason why *A. heterophyllus* rind is more effective than *A. heterophyllus* rags. Therefore, the crude extracts of *A. heterophyllus* rags, *A. heterophyllus* rind, and a combination of *A. heterophyllus* rags and rind can be used as an alternative to synthetic larvicides.

Recommendations. - The researchers recommend that more replicates of each concentration for each treatment are to be used so as to eliminate outliers in the results. Other vector species may also be utilized as test organisms and various parts of the plant such as the leaves, bark, and seeds may be maximized for future studies involving a similar research design. The incorporation of essential oils in the treatments may also be considered. Furthermore, this study serves as a basis for future analyses on the active compounds present in *A. heterophyllus* rind and rags. Thus, it is recommended that an in-depth approach be conducted to further explore and identify the phytochemical components present in *A. heterophyllus*.

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