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Larvicidal Activity of Tawa-tawa (*Euphorbia hirta*) and Oregano (*Origanum vulgare*) Ethanolic Leaf Extracts against Yellow Fever Mosquito (*Aedes aegypti*)

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Abstract Tawa-tawa *(Euphorbia hirta)* and Oregano *(Origanum vulgare)*, a well-known herbal plants endemic to tropical countries like the Philippines that are known to contain various bioactive components that had been used in traditional medicine. Despite its wide use and established efficacy, the plants' full potential has not yet been fully explored. Thus, this study sought to investigate the larvicidal activity of both plants as a potential larvicide for Yellow Fever Mosquitos *(Aedes aegypti)* which are known as diseases-carriers of yellow fever and dengue fever, specifically: determine their phytochemical properties, investigate the mortality rate of yellow fever mosquitoes exposed to both plant extracts, and ascertain if there is a significant difference in the mortality rate of the mosquitoes when exposed to varying concentrations of both plants extracts. The study employed a mixed-methods analysis particularly a qualitative phytochemical screening and quasi-experimental design. Plants extracts are acquired using the maceration technique and Wagner's test and Salkowski test were used to determine phytochemical properties. The study employed larvicidal bioassay on mosquito larvae that were exposed to the leaf extracts utilizing a container with a 50 mL volume of the solution with varying concentrations for three trials. Phytochemical screening revealed that both leaf extract contains alkaloids and has no terpenoids. One-way ANOVA revealed that at a significant level of 0.05, the p-value of 0.00, there has been a significant difference in the mortality rate of Yellow fever mosquitoes when exposed to Tawa-tawa and Oregano extracts; thus the study's null hypothesis was rejected. It is evident that both plant leaf extract was deemed effective as larvicides against mosquito larvae; however, further studies on dose analysis and lethal concentration may be considered to fully explore the plant extracts' full potential.

Keywords: *concentrations, disease carrier, larvicidal activity, larvicidal bioassay, leaf extract, mortality rate, mosquito larvae*

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1. Introduction

Mosquitos are flying insects that are well-known for their blood-sucking features. Mosquitoes are known to carry and transmit a wide range of diseases to humans and animals. These diseases can be viral, bacterial, or parasitic in nature, and some of the most common diseases transmitted by mosquitoes include malaria, dengue fever, Zika virus, chikungunya, yellow fever, and West Nile virus, among others [1]. When a mosquito bites an infected person or animal, it can pick up the disease-causing organism and then transmit it to another person or animal it bites.

In 2016, the World Health Organization has declared the mosquitoes as "public number one enemy". Moreover, Dengue is one of the diseases caused by mosquitoes that is endemic in over 100 countries including Asia, the Americas, Africa, and the Caribbean. Dengue fever cases have risen dramatically in recent decades, reaching the pandemic level [2]. It is estimated that around 50 million cases occur each year and more than 2.5 billion people are at risk of infection. The disease not only is onerous to healthcare but also adversely impacts the economy caused by illness, premature death, and increased healthcare costs [3].

Chemical control is said to be an effective way used generally by most of the people in controlling mosquito larvae [4]. However, the adverse effects of chemical insecticides based intervention measures for the control of mosquito vectors have received wide public apprehension because of several problems like insecticide resistance, the resurgence of pest species, environmental pollution, toxic hazards to humans, and non-target organisms. In addition, the continuous application of synthetic insecticides causes the development of resistance in vector species. These

problems have necessitated the need to explore and develop alternative strategies using eco-friendly, environmentally safe, bio-degradable plant products which are non-toxic to non-target organisms too [5].

Green larvicides are now being considered because plants are made up of bioactive components that are of low toxicity and biodegradability. Unlike conventional insecticide which is based on a single active ingredient, green insecticides comprise botanical variations of bioactive chemical compounds which act harmoniously on both behavioral and physiological processes [6]. Previous researches on the interactions between plants and insects revealed the potential use of plants in fundamental pest control programs [7].

Tawa-tawa also known as the asthma plant *(Euphorbia hirta)* and Oregano *(Origanum vulgare)* are both herbaceous plants that were used as herbal medicine or treatment, especially in the early remedies in treating fever and ailments. Tawa-tawa is a ground-hugging spurge with a reddish stem and dark green leaves that grow from early summer to fall, having more than 40 cm measurement. This plant exhibited significant larvicidal activity, antifeedant, and mosquito-repellent effect since the extracts of Tawa-tawa contain alcoholic properties, quercitrin, and polyphenols which might be responsible for the antifeedant effect and repellent activity [8].

Oregano *(O. vulgare)* on the other hand, is an herb from the mint family, it is used widely for centuries and was recognized for its antibacterial, antiviral, and antifungal activity in addition to its other beneficial properties [9]. Oregano is usually found in Asian and Mediterranean countries which are grown in mild climates. It also contains active ingredients known for their excellent antiseptic and insect-repellent properties such as α-terpinene, carvacrol, and thymol [3].

Furthermore, this study aimed to create an alternative larvicide made with natural materials rather than chemicals mosquito repellent. The study involved plant extracts that have the potential to kill mosquito larvae while elevating safety for the environment as well as the people while avoiding chemical hazards. Moreover, this study aims to provide information on the efficacy of tawa-tawa and oregano plant extract on larvicidal activity.

2. Methodology

2.1. Research Design

Quasi experimental design was utilized since this study involved statistical analysis and experimentation of Tawa-tawa *(E.hirta)* and Oregano *(O. vulgare)* leaf extract. The study also utilized a Qualitative research design for the phytochemical analysis. The study conducted two experimental groups with corresponding control group with three trials to attain the data by determining the mosquito larvae's mortality rate with varying concentrations.

2.2. Collection and Preparation of Plant Extract

A one (1) kilogram of Tawa-tawa (*E. hirta*) and Oregano (*O. vulgare*) leaves was collected at Barangay Linabo, Malaybalay City Bukidnon where it usually grows in wide grassland areas. The Tawa-tawa and Oregano leaves were dried for 7-10 days in the shade at the environmental temperatures (27-37°C daytime [7]. This study utilized the maceration method in extracting plant leaves. 1 kilogram of dried Tawa-tawa and oregano leaves was grind using an electric blender to attain a powdered substance and was extracted using the maceration method with a ratio of 1:3. Thus, 250 g of the powdered leaves was mixed with 750 ml of the solvent specifically 95% ethanol, and was left for 2 days approximately 48 hours. It was then filtered through a Buchner funnel with Whatman's number 1 filter paper [10].

2.3. Collection and Preparation of Mosquito *Culture*

The mosquito larvae were collected using a dropper at an unused household aquarium where mosquitoes hatched eggs to reproduce. Furthermore, rainwater was used for mosquito culture since it is a perfect environment for them to breed and hatch eggs. The mosquito larvae were placed in a 38×26 cm clear container. They were covered with a mosquito net for mosquito breeding to attain the same species of mosquitoes during the trials and experimentation. The mosquito larvae's specification was determined at the City Health Office laboratory. The mosquito larvae were fed with dog biscuits and yeast while not yet used for the experiment [10].

2.4. Phytochemical Testing

The presence of alkaloids was detected using Wagner's test. The 2 mL of dilute hydrochloric (HCI) acid was added to 2 mL of the ethanolic sample extract. After that, a drop of Wagner's reagent was then applied. The presence of reddish-brown color confirms the test is positive [11]. On the other hand, The Salkowski test was used to determine the presence of Terpenes in the leaf extract. 2 ml of chloroform and 2 mL of sulfuric acid were added to 5 mL of the ethanolic extract. The presence of Terpenoids is shown by the formation of the red-brown color [12].

2.5. Larvicidal Bioassay

The mosquito larvae were exposed to varying concentrations in each experimental group with 3 trials and a control group. Using the formula below,

$$\textit{volume percent concentration} = (\textit{volume of solute}) / (\textit{volume of solution}) \times 100\%$$

in a 12oz approximately 355 ml container added with the leaf extract and water to attain a specific concentration to be exposed to the mosquito larvae. For 100 % concentration, 50 ml of the plant extract was divided by 50 ml solution multiplied by 100. For 75% concentration, 37.5 ml of the plant extract was divided by 50 ml solution multiplied by 100. For 50% concentration, 25 ml of the plant extract was then divided by 50 ml solution multiplied by 100. For 25% concentration, 12.5 ml of the

plant extract was divided by 50 ml solution multiplied by 100. In experimental group 1, which utilizes pure Tawa-tawa extract, 3 trials were conducted with varying concentrations to be exposed to the mosquito larvae. In experimental group 2, which utilize pure Oregano extract, 3 trials were conducted with varying concentrations. Thus, in the control group, the mosquito larvae were exposed to water. In each trial, 10 mosquito larvae were used. The toxicity of the plant extract based on their concentration was determined using the mortality rate with the formula,

$$\text{Percent larval mortality } (\%) = \frac{\text{Number of dead larvae}}{\text{Number of the test larvae}} \times 100$$

This was observed for 24 hours time estimation for mosquito larvae exposure.

2.6. Statistical Tool

One-way Analysis of Variance (ANOVA) was used to compare varying concentrations of the two experimental groups to determine if the means were significantly different in the mortality rate between the experimental and controlled groups based on the concentrations exposed to the mosquito larvae.

3. Results and Discussion

3.1. Phytochemical Screening

Tawa-tawa (*E. hirta*) and Oregano (*O. vulgare*) plant has known to have insecticidal properties. Thus, phytochemical screening was done to determine its potential substance ideal for larvicidal activity tested against the Yellow Fever Mosquito for 24 hours' observation to attain the data needed to determine its mortality rate.

Table 1. Results of Phytochemical Screening

Plant	Phytochemical Constituent	Qualitative Test	Indication
Tawa-tawa (*Euphorbia hirta*)	Alkaloids	Wagner's Test for Alkaloids	+
	Terpenoids	Salkowski test for Terpenoids	-
Oregano (*Origanum vulgare*)	Alkaloids	Wagner's Test for Alkaloids	+
	Terpenoids	Salkowski test for Terpenoids	–

+ Presence of compounds; - Not detected.

Table 1 shows that Tawa-tawa and Oregano leaf extract has positive results in alkaloids since the presence of a redish to-brown color was observed using Wagner's test for alkaloids. On the other hand, a negative result for terpenoids was observed during the Salkowski test for terpenoids since the absence of the formation of red to brown color was not indicated during the phytochemical screening for terpenoids.

Furthermore, phytochemicals are a sort of natural pesticide derived from plant and floral resources. The use of phytochemicals in mosquito control has been widespread since the 1920s [13]. These compounds have been found to be efficient in killing mosquito larvae and are regarded as a safer and more ecologically friendly alternative to synthetic insecticides. The presence of alkaloids was likely to contribute to the larvicidal effect of Tawa-tawa and Oregano leaves.

In particular, the larvicidal effectiveness of four alkaloids derived from the plant *Alstonia scholaris* against mosquito larvae of *Aedes aegypti*. All four alkaloids were shown to have considerable larvicidal action, with LC50 values ranging from 4.4 to 23.9 ppm [14]. Terpenoids are not found in the Tawa-tawa and Oregano plants used in this study. However, a plant that does not have detectable levels of terpenoids may still have other bioactive compounds that contribute to its larvicidal activity. Additionally, variations in growing conditions can affect the production and presence of secondary metabolites, including terpenoids, in plants.

3.2. Larvicidal Activity of Tawa-tawa Leaves Extract

Table 2. Larvicidal Activity of Tawa-tawa *(Euphorbia hirta)* Leaf Extract against Yellow Fever Mosquito Larvae

Concentration %	Number of Mosquito larvae	Mortality Rate %			Mean	Standard Deviation
		T1	T2	T3		
Control	10	0	0	0	0	0.00
25	10	70	100	80	83.3	15.28
50	10	100	100	100	100	0.00
75	10	100	100	100	100	0.00
100	10	100	100	100	100	0.00

Table 2 presents the results of the larvicidal activity of tawa-tawa leaf extract. In the control group, the number of live mosquito larvae was constant which means that there were no deaths observed. In a similar study entitled "Larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against *Aedes aegypti"*, indicates similar results in their control group since no mortality was observed containing water alone since it is non-toxic [15]. As for 25% to 100% concentration, the mortality rate of the mosquito larvae increased by 70% to 100%. For trial 1 in 25% concentration, 7 out of ten mosquito larvae did not respond. While in trial 2, all of the mosquito larvae did not respond. Lastly, for trial 3, 8 out of 10 mosquito larvae did not respond which are considered dead. Moreover, from 50% to 100% concentrations, all of the mosquito larvae were observed dead, resulting a 100% mortality rate. 25% concentration has a mean of 83.33 and a standard deviation of 15.28. For 50%, 75%, and 100% concentration, a mean of 100 and standard deviation of 0.00 was indicated on the mortality rate results.

A high standard deviation indicates that the values are far from the mean (less precise) while lower standard deviation means that the values are close to the mean (more precise) [16]. Thus, in the control group, it shows no change in the mean and standard deviation since the mortality rate is 0. On the other hand, in 25% concentration, the standard deviation is 15.28 which is lower than its mean which is 83.33. This indicates the data in 25% is precise. In addition, the concentration of 50% to 100% also indicates precise data since it has a lower standard deviation of 0 than the mean of 100.

Furthermore, it also shows that as the concentration goes higher, the number of dead larvae also increases to

about 100%, which confirms the efficacy of the extract to eliminate the mosquito larvae. In a similar study, the ethanolic extract of rambutan leaves was used to kill *Aedes aegpyti* larvae. This result is supported by this study. 99% of the larvae were killed by the ethanolic extract of rambutan leaf [17]. The larvicidal effect of the Tawa-tawa leaf extract was attributed to the presence of alkaloid compounds.

3.3. Larvicidal Activity of Oregano Leaves Extract

Table 3. Larvicidal Activity of Oregano *(Origanum vulgare)* Leaf Extract against Yellow Fever Mosquito Larvae

Concentration %	Number Of Mosquito larvae	Mortality Rate %			Mean	Standard Deviation
		T1	T2	T3		
Control	10	0	10	0	3.33	5.77
25	10	80	90	80	83.33	5.77
50	10	100	100	100	100	0.00
75	10	100	100	100	100	0.00
100	10	100	100	100	100	0.00

The results of experiments investigating the effectiveness of Oregano as a larvicide are presented in Table 3. In the control group, 1 mosquito larvae did not respond which results a mean of 3.33 and a standard deviation of 5.77. This results to 10% mortality rate for the control group. Moreover, for 25% concentration, Trial 1 indicates an 80% mortality rate, while 90% in Trial 2, and 80% in Trial 3. The results in the mortality rate for 25% concentration indicate that 8 out of 10 mosquito larvae did not respond for Trials 1 and 3 while 9 out of 10 mosquito larvae for Trial 3 did not respond. As a result, 83. 33 is the mean and the standard deviation is 5.77. For 50%, 75%, and 100% concentration, a 100% mortality rate was observed which indicates that all mosquito larvae did not respond or died during the Larvicidal bioassay. A mean of 100 and a standard deviation of 0.00 was indicated in the results.

This means that as the higher the concentration of the leaf extract, the higher the mortality rate or death of mosquito was indicated. Hence, it also indicates that the leaf extract with 50% to 100% concentration, eradicated each and every mosquito larva from the containers. The duration of the larvae's death varied based on the extract concentration. This result is consistent with the study conducted in which the researchers assessed the efficacy of guava leaf ethanolic extracts against *Aedes aegypti* larvae. At a concentration of 1000 ppm, the ethanolic extract had a remarkable effect on the mosquito larvae, as all of them perished [18].

3.4. One-way Analysis of Variance (ANOVA)

Table 4. One-way Analysis of Variance (ANOVA) of the mortality rate of the mosquito larvae as exposed to Tawa-tawa (*Euphorbia hirta*) leaf extracts with varying concentrations

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	226.667	4	56.667	121.429	.000
Within Groups	4.667	10	.467		
Total	231.333	14			

*****Significant at 0.05 level.

The table above displays the findings of the One-way ANOVA analysis that was conducted to assess the mortality rate variations resulting from different concentrations of Tawa-tawa Leaves Extracts. The analysis generated the total sum of squares, which equals 231.333 and comprises the between groups sum of squares and the within groups sum of squares. The total degrees of freedom were 14, and it is the sum of the degrees of freedom between groups and the degrees of freedom within groups. The F statistic was calculated as the ratio of the mean square between groups to the mean square within groups. For this study, the critical F value is 121.429, and the F statistic value generated is much higher than the critical value, suggesting that the observed differences in means were significant. Moreover, the significance level of the study is 0.000, which was much lower than the significance level of 0.05, lending further support to the conclusion that the observed differences between the means of the groups were statistically significant.

In summary, the ANOVA results indicate that the concentration of Tawa-tawa Leaves Extracts has a significant effect on mortality rate, and this effect varies across different concentrations. The ANOVA results provide insights into the concentration-dependent effects of Tawa-tawa Leaves Extracts on mortality rates, which could guide the development and optimization of interventions that utilize the extract. The effects of Tawa-tawa Leaves Extracts on bacterial infections in mice. They found that the extract had a concentration-dependent effect on reducing mortality rates, with higher concentrations resulting in more significant reductions in mortality rates [19].

Table 5. One-way Analysis of Variance (ANOVA) of the mortality rate of the mosquito larvae as exposed to Oregano *(Origanum vulgare)* leaf extracts with varying concentrations

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	226.667	4	56.667	850.00	.000
Within Groups	.667	10	.067		
Total	227.333	14			

*****Significant at 0.05 level.

The table provided above presents the results of the One-way ANOVA to test the significance difference on the mortality rate of Oregano Leaves Extracts with varying concentrations. The results show that The total sum of squares is 227.333, which is the sum of the between groups and within groups sums of squares. The total df is 14, which is the sum of the df between groups and df within groups. The F statistic is the ratio of the mean square between groups to the mean square within groups. The critical F value for this study is 850.000, which is the value that the F statistic must exceed for the results to be considered statistically significant at a significance level of 0.05. In this study, the F statistic is much larger than the critical F value, indicating that there were significant differences between the means of the groups. The significance level of the study is 0.000, which is much lower than the significance level of 0.05, further supporting the conclusion that the differences between the means of the groups were statistically significant.

This means that at least one of the concentrations had a statistically significant effect on the response variable. This indicates that the concentration of the substance being tested has an impact on the larvae's response. The effects of different concentrations of *Bacillus thuringiensis* var. *israelensis* on the survival and development of mosquito larvae. As a result of their study, the number of mosquitoes surviving to the adult stage decreased with increasing *Bti* concentrations. This suggests that the concentration of the *Bti* had a significant impact on the survival and development of the mosquito larvae [20].

4. Summary, Conclusion, & Recommendations

4.1. Summary

The species of Yellow Fever Mosquito *(Ae. aegypti)* larvae was identified to attain the same species of mosquito during the experimentation; mosquito culture was done using a 38×26 cm clear container with rain water and was covered with a mosquito net. Moreover, Tawa-tawa *(E. hirta)* and Oregano *(O. vulgare)* have shown potential properties effective for larvicidal activity with lower environmental effects than chemical larvicides. The leaves of the plants were dried and extracted using the maceration extraction method. They were tested for phytochemical screening to determine the presence of alkaloids using Wagner's test and terpenoids using the Salkowski test. Positive results for alkaloids were observed in both Tawa-tawa and Oregano extracts. Meanwhile, a negative result for terpenoids was observed in both leaf extracts.

A larvicidal bioassay was done to determine the larvicidal activity and mortality rate of the Yellow Fever mosquito larvae as exposed to varying concentrations and a control group with three trials. The larvicidal activity consisted of experimental groups 1 and 2. In experimental group 1, it utilizes Tawa-tawa leaf extract; in experimental group 2, it utilizes Oregano leaf extract. Thus, the experiment has allotted 24 hours for observation. As a result, the mortality rate of Tawa-tawa leaf extract with varying concentrations shows high rates as the concentration increases.

Moreover, the mortality rate of Oregano leaf extract also increases as the concentration increases. Thus, One-way ANOVA was used to determine the significant difference in the mortality rate of Tawa-tawa and Oregano leaf extract, which has shown to be significant at 0.05 level. Following the qualitative analysis of phytochemical test results, the positive effects of the alternative larvicide occurred due to the active presence of alkaloids. This interpretation is supported by a study where the rising effect is likely to occur due to the presence of active substances such as saponins, flavonoids, and tannins on jackfruit leaves.

The study demonstrated the potential of Tawa-tawa and Oregano leaf extract as an effective alternative larvicide for eliminating mosquito larvae. The Tawa-tawa and Oregano ethanolic leaf extracts showed increasing efficacy as the concentration increased, with a percentage of 100 in the mortality rate observed at the highest concentration tested. This is congruent with the study, where they tested the larvicidal activity of cashew leaf extracts against the larvae of the mosquito *Culex quinquefasciatus*. The study found that as the concentration of the cashew leaf extracts increased, the mortality rate of the mosquito larvae also increased [21]. Hence, both Tawa-tawa and Oregano show positive effects in reducing mosquito larvae with its ethanolic extract, demonstrating a high degree of efficacy in killing mosquito larvae supported by the presence of alkaloids that has potential insecticidal activity.

4.2. Conclusion

The following conclusions were drawn from the study as answers to the stated problems:

The Tawa-tawa (*E. hirta)* and Oregano (*O. Vulgare*) leaf extracts contain alkaloids as a result of Wagner's test for alkaloids, in which concluded that the two leaf extract has potential insecticidal and larvicidal activity. However, a negative result was concluded for terpenoids using the Salkowski test for terpenoids. The results show that the larvicidal activity for Tawa-tawa leaf extract shows a 70% to 100% mortality rate with varying concentrations as exposed to the mosquito larvae for each trial. Furthermore, the larvicidal activity for Oregano leaf extract shows an 80% to 100% mortality rate with varying concentrations as exposed to the mosquito larvae for each trial. The result shows a significant difference in the One-way Analysis of Variance (ANOVA) of the mortality rate of the mosquito larvae as exposed to Oregano and Tawa-tawa leaf extracts with varying concentrations since the probability value is below the significant level. As concluded, the increase in concentration percentage increases the mortality rate or deaths of mosquito larvae. Therefore, Tawa-tawa and Oregano leaf extracts have effective larvicidal activity against Yellow Fever mosquito larvae. Thus, it can be used as an alternative larvicide to chemical insecticides that produce adverse effects on the environment and health.

4.3. Recommendations

In light of the study's findings and conclusion, the following recommendations are hereby presented:

1. It is recommended that future research on the larvicidal activity of plants should take into account the different growing conditions of the plants being studied. This is important because the presence and potency of secondary metabolites, such as terpenoids, can vary depending on factors such as soil composition, light exposure, and temperature. Therefore, it is essential to standardize the growing conditions and ensure that the plants being tested are cultivated under similar conditions, in order to obtain accurate and reliable results regarding their larvicidal activity.
2. Other parts of Tawa-tawa and Oregano should be also tested for its larvicidal activity to enhance the practicability of the findings.
3. The use of commercial larvicide as a variable in the study is also recommended to compare the

difference of a bio-larvicide that is derived from the plant extract.

4. It is recommended to do experimentations such as mixing the Tawa-tawa and Oregano extract to see the efficacy of the two plants as a larvicide.
5. Lethal Concentration is also recommended for the larvicidal activity to further determine the extract's toxicity as exposed to the mosquito larvae with varying concentrations.

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