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Larvicidal activity of *Gmelina arborea* fruit extract against dengue vector *Aedes aegypti* mosquito

Jomel C. Montero

Department of Science, Technology, and Engineering (STE), San Miguel National Comprehensive High School-Magroyong, San Miguel, Surigao del Sur, Philippines

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Abstract

Aedes aegypti, the principal vector of dengue virus has caused alarming health worldwide. There are available commercial mosquito insecticides, but the vectors develop resistance to these and cause harm and pose negative effects on living organisms and the environment. This study was conducted to evaluate the larvicidal activity of *Gmelina arborea* fruit extract as a biological control agent against 4th instar *A. aegypti* mosquito larvae. This study applies the control group and variation of treatment using the different concentration of *G. arborea* fruit extract (25, 50, 75, and 100 mg/L respectively) exposed to 15 batches of 10 4th instars *A. aegypti* larvae. The data obtained were analyzed using the mean percentage, One Way-ANOVA at 0.05 level of significance and probit analysis. Based on the results, the 100 mg/L concentration of fruit extract obtained the 100% mortality while the control group has no observed mortality of *A. aegypti* larvae after 24 hours of treatment. Moreover, there is a significant difference ($F=33.88$, $p<0.05$) between the control and the experimental group with varying concentration of *G. arborea* fruit extract. The lethal concentration (LC_{50} and LC_{90}) values of *G. arborea* fruit extractwere 56.23 mg/L and 74.13 mg/L which means it is toxic to the mosquito larvae sample. The results showed great potential for the production of organic insecticides from the *G. arborea* fruit extract to control the *A. aegypti*, a vector of the dengue virus.

* **Corresponding Author:** Jomel C. Montero ✉ jomelmontero25@gmail.com

Introduction

Aedes aegypti is commonly known as yellow fever mosquito. According to the World Health Organization (2017), *A. aegypti* mosquito is the main vector that transmits viruses that causes dengue. They do not only transmit parasites and pathogens but they are also the source of allergic reaction that includes local skin and systematic sensitivity (Cheng *et al.,* 2003). Thus, this international agency declared the mosquito as *"Public enemy number one"*. In fact, the increasing number of dengue infections has become one of the main health concerns in tropical and subtropical countries worldwide, wherein over 2.5 billion people with 40% of the world's population is severely affected, composed of 50-100 million infections every year and about 2.5% of affected people died (WHO, 2014). In the recent report of the World Health Organization (2019), the Philippines as of June 1, 2019 got the highest cumulative number of dengue cases of 83, 570 with 354 deaths compared to last year; thus, resulting to the declaration of dengue outbreak in the country. In Malaysia, the cumulative number of dengue cases as of June 15, 2019 reached 56, 819 including deaths which is next after the Philippines. For this reason, a continuous search of the biological control agent that would eventually reduce the risk brought by a mosquito is deemed necessary.

Mosquito control agent has been found beneficial in preventing the proliferation of mosquito-borne disease and in improving the quality of the environment and public health (Gosh, Chowdhury and Chandra, 2012). The use of an anti-larval agent is the ultimate objective of public health to reduce the number of adults of this vector mosquito.The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target (Benelli, Jeffries and Walker, 2016). Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide (Tiwary*et al.,* 2007 as cited by Gutierrez*et al.,* 2014). In the early years, the use of synthetic chemical larvicides and insecticides are said to be effective and has an immediate action towards mosquito control health (Gosh, Chowdhury and Chandra, 2012).But the excessive, continual and unsystematic use of synthetic chemicals has led to more alarming problems like resistance to insecticide, concern for environmental sustainability, high cost, harmful effect on human health and other non-target organism, higher rate of biological magnification through ecosystem, and increasing insecticides resistance on a global scale (Arnason, Philogene and Morand, 1989; Hedlin *et al.,* 1997;Manthivanan *et al.,* 2000; Das Ng, Goswami and Rabha, 2007;Tiwary *et al.,* 2007; Kulkarni and Veeranjaneyula, 2010). Thus, many researchers nowadays are seeking organic ways and the used of the plant as a natural mosquito control agent became an alternative (Warikoo, Wahab and Kumar, 2011).

One of the popular and abundant plants with medicinal properties and a large number of phytoconstituents is *Gmelina arborea* (Nayak, Dinda and Ellaiah, 2013; Lawal, 2016; Iswarya, Sridevi and Mayavel, 2017). The tree has a high alkaloid content particularly the fruits, stem bark, and roots (Oparaeke, 2006; Offor, 2014).This plant reviewed by (Arora and Tamrakar, 2017) exhibits pharmacological and biological activities such as antidiuretic (Nayak, Dinda and Ellaiah, 2013), antidiarrhoeal (Abdulkarim *et al.,* 2005), antipyretic (Pravat *et al.,* 2011; Panda, Das, and Tripthathy, 2015), antianalgesic (Gangwar *et al.,* 2013), antioxidant (Saleem *et al.,* 2005;Sinha *et al.,* 2006; Syamsul, Takeshi, Toshisada, 2008; Patil, Kadam and Ghosh, 2009;Chantal, 2009;Pandey and Kulkarni, 2010; Attanayake *et al.,* 2015), antidiabetic (Dixit and Sudurshan, 2011; Kulkarni and Addepalli, 2011; Pattanayak *et al.,* 2011; Kulkarni and Veeranjaneyulu, 2013;Kulkarni and Addepalli, 2013), anthelmintic (Ambujakshi andShyamnanda, 2009; Panda, Das and Tripthathy, 2015), antibacterial (Alagesaboopathi, 2011;El-Mahmood, Doughari and Kiman, 2010; Idu, Ovuakporie and Ndana, 2015), antifungal (Valsaraja *et al.,*1997; Kawamura, Ohara and Nishida, 2004; Kawamura and Ohara, 2005; Idu, Ovuakporie and Ndana, 2015), cardiopotective (Vijay

et al., 2011), insecticidal (Oparaeke, 2006), antiulcer (Giri *et al.,* 2009), gastro-protective (Chellappan and Pemiah, 2014), anticancer (N'gaman *et al.,* 2014; Sahu *et al.,* 2015), antihyperlipidemic (Punitha*et al.,* 2012) and immunomodulatory activity (Shukla *et al.,* 2010). The chemical component found in *G. arborea* issuitable and adaptive to ecological conditions. Hence, it is very interesting to investigate its promising potential as an eco-friendly biological control agent against the mosquito.

In this study, the used of fruit from *G. arborea* is the main subject of this investigation, since this tree is very abundant in the Philippines and its fruit is being considered as waste.

Also, many studies have proved that *G. arborea* has phytochemicals such as alkaloids and steroids that have the potential to be an insecticide. However, no studies had been reported regarding the larvicidal activity of its fruit extract against *A. aegypti*. Hence, this gap in the literature has triggered the researcher of this study to examine its efficacy and to identify what concentration would be effective in the mortality of mosquito larvae.

The present study aims to examine the larvicidal activity of *G. arborea* fruit extract against *A. aegypti*to determine its efficacy as an alternative natural product to control the breed of *A. aegypti*, the main vector in transmitting viruses that caused dengue.

Materials and methods

Collection and extraction of fruit samples

Fresh ripe fruits (1 kg) of *G. arborea* were collected at Barangay Siagao, San Miguel, Surigao del Sur, Philippines and brought to the Science Laboratory of San Miguel National Comprehensive High School for extraction. The collected fruit samples were washed thoroughly with tap water and rinsed with distilled water. The *G. arborea* fruit samples were dried at room temperature for 24 hours. Then the fruits were sliced into smaller pieces using a sterilized kitchen knife and ground eventually using a blender.

Collection of mosquito larvae sample

The Ovicidal-Larvicidal Trapping (OL) method was used in this study to employ the collection of mosquito larvae. There were 12 OV Traps filled with ¾ tap water installed in the different classrooms of San Miguel National Comprehensive High School. These OV Traps are made of tin can coated with black paint with 'lawanit' paddle on it.

The paddle was used in catching the eggs of Mosquito. Installation of OV Traps follows the procedure of the Department of Health (DOH) and Department of Science and Technology (DOST). If there were already small eggs on the surface of the water, the eggs were transferred from the bucket to a jar at room temperature using freshwater and the paddle is provided for easier catch until they reach their 4th instar larval stage of development. After seven days, the larvae were taken from the jar using the fishnet and used as a subject in the study.

Identification of Aedes aegypti larvae

To verify the collected mosquito samples used in the study were *A. aegypti,* the comb scales were examined under a compound light microscope. Comb scales of *A. aegypti* were a single row and pitched fork in shape (Rueda, 2004).

The identification of *A. aegypti* species was done at the Science Laboratory of San Miguel National Comprehensive High School and the certification of *A. aegypti* sample was done by Shaira P. Tibre, RMT, Medical Technologist of San Miguel Community Hospital, Tina, San Miguel, Surigao del Sur, Philippines. The identified 4th instars *A. aegypti* mosquito larvae were separated from the other mosquito species and were placed in a water-filled plastic molder (Gutierrez *et al.,* 2014).

Mosquito larvicidal bioassay

The efficacy of fruit extract as a larvicide against the dengue-vector *A. aegypti* mosquito was evaluated based on the guidelines of the World Health Organization (2005) and the method conducted by (Gutierrez *et al.,* 2014; Florence and Solomon, 2016).

A total of 15 batches of 10 4th instars of *A. aegypti* larvae were placed in a beaker with 200 mL dechlorinated water and placed in an isolated area in the Science Laboratory at room temperature. Small, unhealthy or damaged larvae were removed and replaced. For the control group, the mosquito larvae were exposed to dechlorinated water (negative control) or with no concentration of *G. arborea* fruit extract (0 mg/L). On the other hand, the experimental group contains the different concentration of *G. arborea* fruit extracts namely: 25 mg/L, 50 mg/L, 75 mg/L, and 100 mg/L concentrations. These concentrations were prepared from the 1000 mg/L stock solutions by dissolving 100 mg of crude extract in dechlorinated water until the volume raised to 100 mL. From this, different dilutions of 25-100 mg/L were prepared in 200 mL dechlorinated water in 250 mL beaker.

Three (3) replicate cups were used for each concentration and the control group, which is 200 mL dechlorinated water.

The effects of the different fruit extract concentration were monitored carefully through the counting of the number of dead mosquito larvae after 24 h of exposure to the treatments.

Moribund larvae are counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to movewhen they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed (WHO, 2005). The percentage mortality of *A. aegypti* was computed using Abbott's formula:

$$\text{Mortality (\%)} = \frac{\text{\% mortality treated group} - \text{\% mortality control group}}{\text{\% mortality control group}} \times 100$$

Disposal of mosquito samples

The *A. aegypti* larvae were subjected to hot water after the experimentation that is a traditional way of killing mosquito larvae (Buckner, 1934 as cited by Lumabao, 2016). Also, liquids with mosquito larvae were thrown in the cemented dry ground to ensure the total death of the organisms (Lumabao, 2016).

Statistical analysis

The statistical tools that were used analyzing the data are the following:

Mean percentage*:* This tool was used to get the percentage mortality of mosquito larvae.

One-Way ANOVA:This was used to test the significant difference in the mortality of mosquito larvae between the control group and the experimental group.This was done by using IBM-SPSS version 21.

Probit analysis: This was used to examine the lethal concentration (LC_{50} and LC_{90}) values of the fruit extracts on *A. aegypti* mosquito larvae after 24 hours of treatment. This was performed by using the Microsoft excel 2016.

Results and discussion

The larvicidal activity of the fruit extract of *G. arborea* against *A. aegypti,* a vector of dengue virus was performed through mosquito larvicidal bioassay.

The mortality of the mosquito larvae was observed and noted from the different concentration (25 mg/L, 50 mg/L, 75 mg/L, and 100 mg/L).

Table 1. Significant difference between the mortality of *A. aegypti* treated at different concentration and the control group after 24 hours of exposure.

Variable Compared	F-Computed	P-value	F-Critical	Decision	Interpretation
Control Group vs *Gmelina arborea* Fruit Extract	33.88	0.000	3.4781	Reject Ho	Significant

Figure 1 presents the mean percentage mortality of *A. aegypti* mosquito larvae with various concentrations of the fruit extract and the control group after 24 hours of treatment. In this study, the variation of the death percentage of mosquito larvae was observed. The control group (0 mg/L) obtained 0 % mortality which means no mosquito larvae died after 24 hours of exposure.

Table 2. Lethal Concentration (LC 50 and LC 90) values of the *G. arborea* fruit extract on *A. aegypti* mosquito larvae after 24 hours of treatment.

Fruit Extract	LC_{50} *(mg/L)*	LC_{90} *(mg/L)*	Regression Analysis	Multiple R	R^2	Standard Error
Gmelina arborea	56.23	74.13	Y=10.39 x – 13.19	0.91	0.82	1.03

On the other hand, the experimental group (100 mg/L) obtained the highest percentage of mortality equal to 100% of the mosquito larvae. Similar findings of Nayak (2014) reported the larvicidal activity of *A. reticulata* leaf crude extract at different concentration shows 100 % mortality rate of larvae was observed at 5, 10, 25, 50, 100 and 200 ppm concentrations of crude extract. The present result coincides to the 100% mortality of *A. aegypti* larvae at 100 mg/L after 24 h exposure. This implies that all sample of mosquito larvae died under 100 mg/L or 100 ppm concentration. Furthermore, the different fruit extract concentration exhibited a concentration-dependent activity against mosquito larvae, since, the percentage mortality observed in figure 1 shows an increasing concentration.

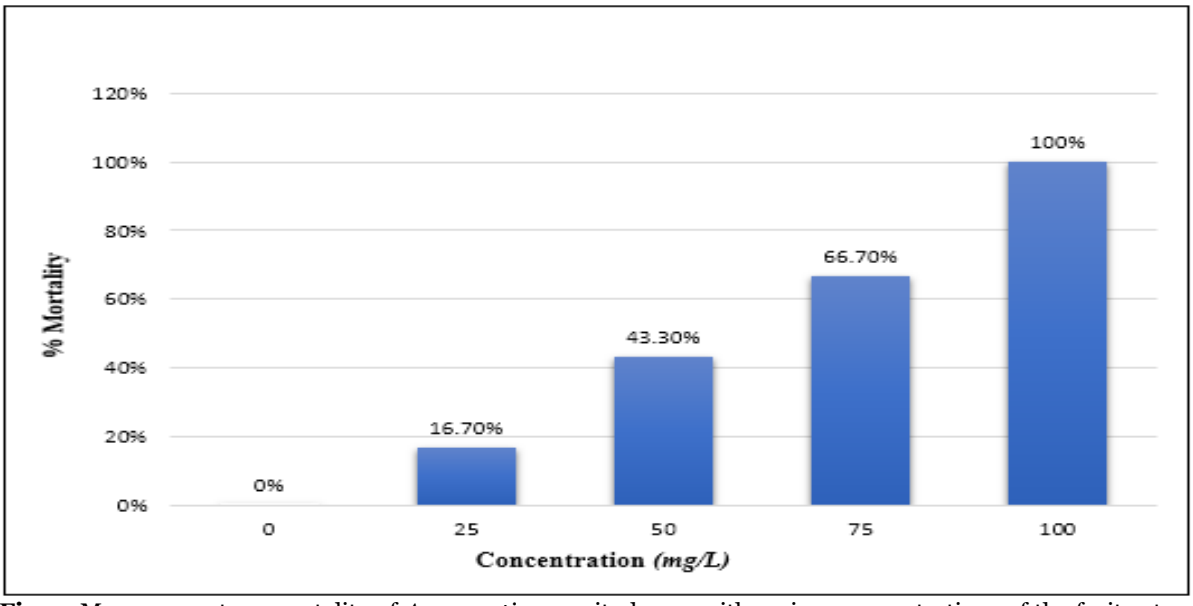

Fig. 1. Mean percentage mortality of *A. aegypti* mosquito larvae with various concentrations of the fruit extract and the control group after 24 hours.

The observed increase of percentage mortality of mosquito larvae is supported by the presence of phytochemicals such as alkaloids and steroids which have insecticidal and pesticidal activity (Oparaeke, 2006; Lawal, 2016). A similar study revealed that *G. arborea* is less attacked by insect pest all through a season due to its high alkaloid content and tannin content. Liquid from the fruits has been found toxic to larvae of moths and butterflies (Okweche *et al.*, 2015). Oparaeke (2006) reported the insecticidal potential of *G. arborea* extract that there is a considerable reduction of the target pests compared to the unsprayed one. Hence, the present result supported the previous findings that the extract produced from *G. arborea* has a great ability to eradicate mosquito larvae considering the high content of alkaloid and tannin embedded in its fruits. This manifests that this fruit extract could be

developed as natural larvicides against *A. aegypti* mosquito larvae.However, similar study may be conducted using a bigger number of mosquito larvae and other solvents with varying concentration tofurther validate its efficacy in the combat of *A. aegypti* mosquito larvae.

The table 1 shows the result of the One-Way ANOVA on the mortality of *A. aegypti* mosquito larvae among the control group and the experimental group treated with 25 mg/L, 50 mg/L, 75 mg/L, and 100 mg/L concentrations of the various fruit extract. The *G. arborea*fruit extract showed a significant difference ($F=33.88$, $p<0.05$) on the increase of mortality on the mosquito larvae in relation to the control group. This implies that the mortality of mosquito larvae is not attributed to the solvent used in changing the concentration of the fruit extract but the mortality of the fruit extract is possibly due to the toxic chemical constituents found in the fruit extract.This result manifests that the fruit extracts obtained from *G. arborea* are susceptible to the larvae of the dengue-vector *A. aegypti* mosquito.This indicates further that these fruit extracts are effective to be developed as mosquito larvicide which is potential to be utilized against the fourth instar larvae of *A. aegypti*.Hence, to reduce the risks of using commercial insecticides in eradicating mosquitoes, the use of *G. arborea* fruit extract as natural larvicide is recommended. The lethal concentration (LC_{50} and LC_{90}) values of the *G. arborea* fruit extracts on *A. aegypti* are presented in Table 2. The results revealed the LC_{50} and LC_{90} values of 56.23 mg/L and 74.13 mg/L, respectively. The results revealed that the different fruit extract concentration applied in the experiment is toxic to the mosquito larvae (Gutierrez *et al.,* 2014).Hence, it is evident from the result that the various concentrations of *G. arborea* fruit extract were the main cause of mortality on *A. aegypti* mosquito larvae. This result corroborates the finding of (Florence and Solomon, 2016) that the leaf extract of *G. asiatica* L. possesses the larvicidal potential to be used as an eco-friendly approach for the control of vectors *A. aegypti.* A similar study conducted and reported that the leaf extract of *Jatropha curcas, Citrus grandis, and Tinospora rumphii* possessed larvicidal activities. Among these plants, *Tinospora rumphii* leaf extract is the most effective mosquito larvicide which is manifested by having the highest percentage mortality of 90% and 93% with an LC_{50} and LC_{90} values of 10 mg/mL and 46 mg/mL respectively against *A. aegypti* larvae (Gutierrez *et al.,* 2014). Therefore, since the fruit extract of *G. arborea* is a potent larvicide, its uses in eradicating and killing mosquito are highly recommended especially on wet places prone to the breeding of mosquitoes. However, as revealed in Table 2, the correlation coefficient (R^2) of *G. arborea* fruit extract obtained the value of 0.82 and its relationship can be expressed as-13.19 against the mosquito larvae of *A. aegypti*.The result indicated that the percentage of mortality is directly proportional to the different concentration (ranging from 25 to 100 mg/L) of the fruit extract asshown in the regression equation (based on probit analysis). This showsthat the larvicidal activity of the fruit extract is concentration-dependent which explains that by increasing the concentration of *G. arborea,* the higher percentage of mortality will be observed among mosquito larvae after 24 h exposure. This finding is also similar to the previous findings of (Govindarajan *et al.,* 2012 ; Sukhthankar *et al.,* 2014 Florence and Solomon, 2016; Anjum *et al.,* 2016) which showed that the mortality of the mosquito larvae is directly proportional to the dose and duration of treatment.

Conclusion

Based on the results of the study, it was found that the 100 mg/L concentration of *G. arborea* fruit extract exhibited high mortality count to *A. aegypti* larvae.

The variation of the fruit extract concentration is significantly susceptible to the mortality of *A. aegypti* larvae. These occurrences in the observation also showed that the lethal concentration (LC_{50} and LC_{90}) values of the fruit extract are toxic against the tested mosquito larvae. With these findings, a great potential for the production of organic insecticides from the *G. arborea* fruit extract for the management of the dengue-vector *A. aegypti* mosquito.

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