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Larvicidal activity of *Citrofortunella microcarpa* (Lemonsito) and *Carica papaya* (papaya) extracts against the dengue-vector mosquito, *Aedes* sp.

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Abstract
This study investigated the larvicidal activity of the methanolic leaf extract of *Citrofortunella microcarpa* and *Carica papaya* against the dengue-vector, *Aedes* sp. mosquito. Qualitative phytochemical screening was performed to identify the bioactive chemicals present in the extract and a larvicidal assay was performed against the third instar larvae of *Aedes* sp. to determine the efficacy of the plant extracts at 6 mg/mL, 12 mg/mL and 18 mg/mL concentration. Data on the mortality of mosquito larvae after 24 and 48 hours were analyzed using the arithmetic mean, Tukey test and *t*-test. Results from the assay revealed that both plants possessed a larvicidal potential. However, lemonsito demonstrated a concentration dependent effect and a significantly higher larvicidal activity at 0.05 level of significance compared to papaya. This larvicidal property can be attributed to the presence of known insecticidal and larvicidal phytochemicals in both plants extracts namely: the alkaloids, flavonoids, saponins, steroids and tannins. In addition, fatty acid was identified to be present in lemonsito extract which may produce a synergistic effect with other abundant phytochemicals hence a higher larvicidal activity.

Keywords: Biolarvicide, bioassay, dengue, phytochemical

1. Introduction
Mosquitoes serve as vector for various diseases which cause destructive effects to human [1]. They do not only transmit parasites and pathogens but they can cause allergic responses that include local skin reactions and systemic reactions [2]. One of the most significant diseases transmitted by *Aedes* sp. mosquito is dengue because it afflicts humans worldwide. Dengue is a viral disease causing mild to severe fever and potentially life threatening hemorrhagic disease. Every year it is estimated that 390 million people are infected dengue and 96 million show clinical manifestation [3]. In the Philippines, reported cases of dengue from January to October, 2015 have already reached 124, 728 nationwide which is 42.3 percent higher compared to the same reporting period in 2014 [4].

Due to the pathogenic diseases and serious harms caused by mosquitoes particularly *Aedes* sp., controlling them has been the primary subject of several new researches over the past few years [5]. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults by using synthetic larvicides or botanical extracts as an alternative larvicide [6]. The use of larvicides is commonly practiced since mosquitoes at larval stage are easier to locate and eliminate before they emerge into adult [7].

Current research trends use plant extracts as alternative larvicides because they contain a wide range of bioactive chemicals with selective and have little or no harmful effect on non-target organisms and the environment [8]. Instead of using synthetic larvicides, the use of these botanical derivatives in controlling mosquito could reduce the cost and environmental pollution [9]. Chowdhury and Ghosh (2008) [10] also stated that the presence of the major phytochemical constituents such as steroids, alkaloid, terpenoids, saponins amino acids, phenolics, flavonoids is attributed to its larvicidal efficacy.

Previous studies on *Citrofortunella microcarpa* (lemonsito) extract mixed with onion extract revealed an effective cockroach killer [11]. *Carica papaya* leaf extract was also used as natural pesticides against aphid, termites, small insects and caterpillars [12]. Another study on the chloroform extract of *C. papaya s*howed insecticidal activities against *S. frugiperda* [13].

Several studies showed the effects of *Citrofortunella microcarpa* (lemonsito) and *Carica papaya* (papaya) extracts to other insects but little is known about their larvicidal efficacy against mosquito. No study was conducted that compares the effect of *Citrofortunella microcarpa* (lemonsito) and *Carica papaya* (papaya) on the mortality of *Aedes* sp. larvae. Hence, this study investigates the larvicidal activity of the methanolic extracts of *Citrofortunella microcarpa* (lemonsito) and *Carica papaya* (papaya) leaves against the dengue vector, *Aedes* sp. mosquito larvae. This study may lead to the revelation of new natural larvicide that will help control the population of *Aedes* sp. mosquito thus, aiding humans in the prevention of the transmission of dengue and other pathogens carried by this mosquito.

2. Materials and Methods

2.1 Preparation of the Plant Samples

Plant samples, leaves, were washed with tap water and then rinsed with distilled water. The plant samples were air dried for 48 hours at room temperature. Dried leaves of the plant samples were cut into small pieces and pulverized using an electric blender.

2.2 Extraction of Plants

Two hundred fifty grams of pulverized plant samples were placed in a glass container. The samples were soaked with methanol in the ratio of one gram of samples is to one ml of methanol (1:1) and were left to stand for 48 hours and then filtered. The resulting filtrates were concentrated in a rotary evaporator.

2.3 Rotary Evaporation and Phytochemical Analysis

The methanolic plant extracts of *Citrofortunella microcarpa* (lemonsito) and *Carica papaya* (papaya) were sent to the Research Laboratory of Cebu Doctors University, Cebu City for rotary evaporation and in the Chemistry Laboratory of MSU-IIT, Iligan City for the phytochemical analysis. Plant samples had undergone rotary evaporation to make it highly concentrated until it is semi-solid in form. The phytochemical screening was done following the standard procedure as described by Harborne (1998) [14]. Evaluation of the major phytochemical analysis such as alkaloids, flavonoids, tannins, saponins, steriods, anthraquinone and cyanogenic glycocides was conducted.

- **Alkaloid**

10 grams of each plant extract were evaporated into a syrupy consistency over a steam bath with 5 mL of 3 M HCl; and the filtrate was treated with Mayer's Wagner's reagent.

- **Flavonoids**

10 grams of the plant samples that were evaporated and the residue was defatted by treating it with 95% n-Hexane until the extract was almost colorless. The hexane extract was discarded. Its residue was taken up with 80% alcohol and added with hydrochloric acid. The appearance of color red within 10 minutes demonstrated positive test for flavonoids.

- **Tannins**

10 grams of evaporated plant extract was added with 10 ml boiled distilled water, and 5 drops of 10% $FeCl_3$ (ferric chloride) was added to the filtrate. The positive result of tannins was indicated by the presence of white precipitate.

- **Saponins**

The crude methanolic extracts was added with 10 mL distilled water and shaken vigorously to record froth formation.

- **Steroids**

20 grams of sample was extracted in 10 mL methanol. Five (5) mL of this methanolic extract were treated with 2 mL glacial acetic acid containing 1 drop of 5% $FeCl_3$ solution. This solution was carefully transferred to the surface of 1 mL concentrated of H_2SO. The formation of reddish brown ring at the junction of two liquids was the indication of the presence of steroids.

- **Anthraquinone**

10 grams of the plant samples was evaporated until it was almost dry over a steam bath. The residue took up with 10 mL distilled water and filtered. The filtrate was extracted twice with 5 mL portions of benzene. Benzene extract was divided into portion, the other one served as control while the other was treated with 5 mL ammonia solution. The appearance of color pink in the lower alkaline layer indicated the presence of Anthraquinone.

- **Cyanogenic glycocides**

2-5 grams of crushed plant material was moistened with distilled water and added with a few drops of chloroform to enhance enzyme activity. One (1) mL of 1 % emulsin solution was added to ensure hydrolysis of glycoside. The test tubes was tightly covered with cork from which was suspended with a piece of picrate paper that must not touch the inner side of the test tube. The tubes were warmed up at 35-40°C or kept at room temperature for three hours. Appearance of various shades of red within 15 minutes measured the relative concentration of cyanogenic glycoside.

2.4 Identification of Mosquito Larvae

Larvae of a mosquito can be identified from any other aquatic insects since it has a combination of two characters, they have no legs and the thorax is wider than the head or abdomen. The three divisions of the body part mosquito larvae are head, thorax and abdomen. The structure of three body regions serves as the basis for identifying the mosquito larvae. The mosquito larva was identified using a compound microscope. A small amount of water with mosquito larvae was dropped in a slide to be able to view the specimen in the compound microscope.

The mosquito larvae used in this study were the third instar larva of dengue carrying mosquito *Aedes* sp. They can be distinguished from any other mosquito larvae since it normally has a single hair, a three branch hair tufts on each side of the air tube. When the hair tuft has two or more branches all branches arise from the same socket. Other species have two or more hairs, branches and hair tufts on each side of the air tube or siphon.

Identified *Aedes* sp. mosquito larvae were separated from the other mosquito species and were placed in a water- filled plastic molder.

2.5 Mosquito Larvicidal Bioassay

Larvicidal activity of the plant extracts against the *Aedes* sp. mosquito was evaluated in accordance with the guidelines of World Health Organization [7]. Batches of 25 third-instars larvae of *Aedes* sp. were placed in a small plastic container with 50 mL dechlorinated water. The bucket containing the larvae was kept in a netted enclosure in the Laboratory at room temperature (30 ± 2^0C). For the negative control group, the mosquito larvae were exposed to methanol solution at 12 mg/ML concentration since it is the solvent used in the extraction of different plant samples to determine whether or not its residues that maybe found in the crude plant extracts had a significant influence to the larvicidal property of the plants in study. While for the positive control a commercially available mosquito larvicide temephos at 8.0 ppm was utilized. The experimental group is the methanolic extracts of the leaves of *Citrofortunella microcarpa* (lemonsito) and *Carica papaya* (papaya) with three (3) various concentrations: 6 mg/mL, 12mg/mL and 18 mg/mL. The three various concentrations were identified after the range finding testing activity in accordance with the WHO guidelines in larvicidal bioassay. Dead mosquito larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting the number of dead larvae after 24 and 48 hours of exposure, and the percentage mortality were reported from the average of three replicates.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{ x } 100$$

2.6 Statistical Analysis

The statistical tools that were used in this study are the following: Arithmetic Mean to get the average number of dead of mosquito larvae; Tukey test to determine if there are significant differences on the mortality of mosquito larvae between the experimental and control group; and *t* – Test to determine which plant extract was more effective in killing the mosquito larvae.

3. Results and Discussion

3.1 Phytochemical Content

Qualitative phytochemical screening carried out on both methanolic plant extracts of *Citrofurtunella microcarpa* and *Carica papaya* leaves revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins (Table 1). However, anthraquinone and cytogenic glycosides are absent in both plants. In addition, *C. microcarpa* contains fatty acid which was not detected from *C. papaya* leaves extracts.

These chemicals are known to be responsible for the larvicidal potentials of various plants against insect vector including the moquitoes by influencing their behavior, growth and survival (Mann and Kaufman 2012). Several studies on larvicidal efficacy of plant extracts in Ghosh *et al*. (2012), Gopiesh Khanna and Kannabiran (2007), Gutierrez *et al*. (2014) [15, 16, 17] attributed this property to the presence of one or more of these phytochemicals. Plants are traditionally sources of natural insecticide as they have evolved various mechanisms to increase their survival against herbivory. Plants defend themselves by producing secondary metabolites which are toxic to herbivores.

Table 1: Phytochemicals present in the Methanolic Leaf Extracts of *Citrofurtunella microcarpa and Carica papaya*

Plant Extract	Alkaloids	Anthra-quinone	Cyanogenic glycosides	Flavonoids	Saponins	Steroids	Tannins
**Citrofurtunella microcarpa*	+++	-	-	+++	+	+++	+++
Carica papaya	+++	-	-	+++	+++	+++	+++

*****Positive for the presence of fatty acid
Legend: (+) present, (-) absent

Alkaloids, phenols including flavonoids and tannins and terpenoids are known to have insecticidal properties [18]. Alkaloids are among the widely utilized pesticides as they show insecticidal properties even at low concentration. The mode of action varies with the structure of their molecules, but many are reported to affect acetylcholinesterase (AChE) or sodium channels [19]. Earlier study on *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostate* also attributed the larvicidal activity of these plant extracts to the presence saponin and tannin. Saponin is thought to interact with the cuticle membrane of the larvae, ultimately disarranging the membrane causing larval death. At the same time the antioxidant property of may cause deficiency of dissolved oxygen thus the death of the larvae [20].

While the study of Permalsamy *et al*. (2015) [21] on the mode of action of flavonoids and fatty acids in *Milletia pinnata* revealed that flavonoids karanjachromene, pongamol and pongatorene strongly inhibited mosquito larval cholinesterase (ChE). Findings on fatty acids indicated that unsaturated fatty acid oleic acid and saturated fatty acid palmitic acid acted mainly on AChE, while unsaturated fatty acids elaidic acid and the saturated fatty acids arachidic acid and behenic acid caused a considerable increase in cAMP, indicating that their insecticidal action might be due to interference with octopaminergic system.

3.2 Biolarvicidal Assay

3.2.1 Mean and Percentage Mortality

Larvicidal efficacy of both methanolic plant extracts against *Aedes* sp. was determined through mosquito larval assay. Table 2 shows the mean and percentage mortality of mosquito larvae after 24 hours of exposure from the various concentrations of plant extracts at 6mg/mL, 12 mg/mL and 18 mg/L concentration and the positive and negative control group for comparison.

Table 2: Mean and Percentage Mortality of *Aedis* sp. Mosquito Larvae in the Experimental and Control Group after 24 hours

Plant Extract	Concentration (mg/ml)	Mean Mortality	Mean Percentage
Citrofurtunella microcarpa	6	3.00	12.00%
	12	21.67	86.67%
	18	24.67	98.67%
Carica papaya	6	7.67	30.67%
	12	7.67	30.67%
	18	9.33	37.33%
Positive Control		4.00	16.00%
Negative Control		1.00	4.00%

Result shows that mortality increased with the increasing concentration of *C. microcarpa* (Figure 1) and highest (98.67%) in 18mg/ml. While in *C. papaya* the mortality of *Aedes* sp. larvae at different concentration does not exhibit a similar trend. The very low mortality (4%) of larvae in the negative control indicated that methanol did not influence the larvicidal property of *C. microcarpa* and *C. papaya* plant leaf extracts. On the other hand, mortality of larvae among the experimental groups is much higher than the positive control (8.05 ppm of temephos). Larvicidal activities of these plant extracts can be associated with the presence of known insecticidal phytochemicals. The higher larvicidal potential of *C. microcarpa* may be attributed to the presence of fatty acids. However, test for specific bioactive components and quantifying their amounts maybe performed in order to understand the varying effects of these plant extracts to *Aedes* sp. larvae.

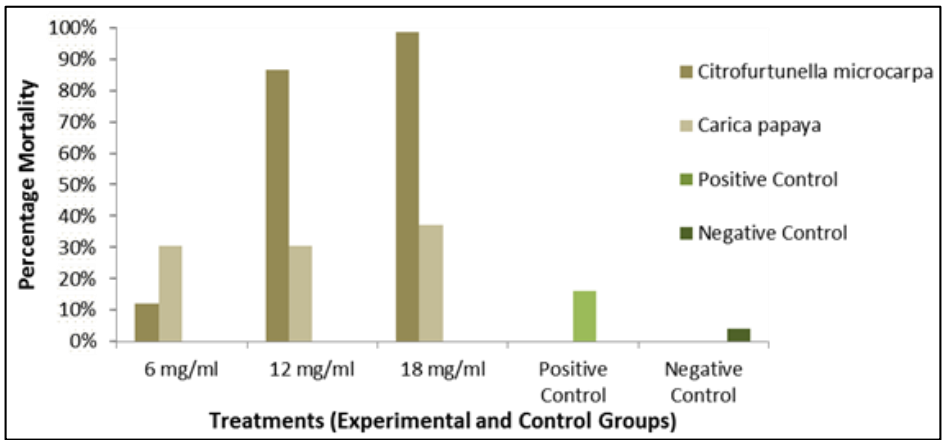

Fig 1: Percentage Mortality of *Aedis* sp. Larvae Treated with the Various Concentrations of Leaf Extracts and the Control Group after 24 hours

Table 3 shows the average mortality of *Aedes* sp. larvae treated with various concentrations of leaves extracts and with the positive and negative control group after 48 hours. Varying larval mortality is observed from the result. But this is consistent with the previous observation. Larval mortality is highest (98.67%) in 18mg/ml of *C. microcarpa* extract and lowest (4%) in the negative control.

Table 3: Mean and Percentage Mortality of *Aedis* sp. Mosquito Larvae in the Experimental and Control Group after 48 hours

Plant Extract	Concentration (mg/ml)	Mean Mortality	Mean Percentage
Citrofurtunella microcarpa	6	6.33	25.33%
	12	23.33	93.33%
	18	25.00	100.00%
Carica papaya	6	8.67	34.67%
	12	10.00	40.00%
	18	10.33	41.33%
Positive Control		9.67	38.67%
Negative Control		1.67	6.67%

Larvicidal activity of *C. microcarpa* exhibited a concentration dependent characteristic (Figure 2). This result is similar with the study of Gutierrez *et al*. (2014) [17] on the plant extracts of *Jatropha curcas, Citrus grandis, and Tinosphora rumphii*. Increasing the concentration of plant extract increases the efficacy against the larvae of *Aedes* spp. larvae. However, this is not demonstrated by *C. papaya* (Figure 2). Higher larvicidal activity of *C. microcarpa* compared to *C. papaya* may be partly explained by the synergistic effect of several secondary metabolites including fatty acids. Studies on synergistic, antagonistic and additive toxic effects of binary mixtures including phytochemicals showed that larvicidal efficiency of cedarwood, lavender, peppermint, clove, garlic and eucalyptus oil against *Aedes aegypti* was increased after adding citronella oil, in concentrations ranging from 0.5% to 15 % [22].

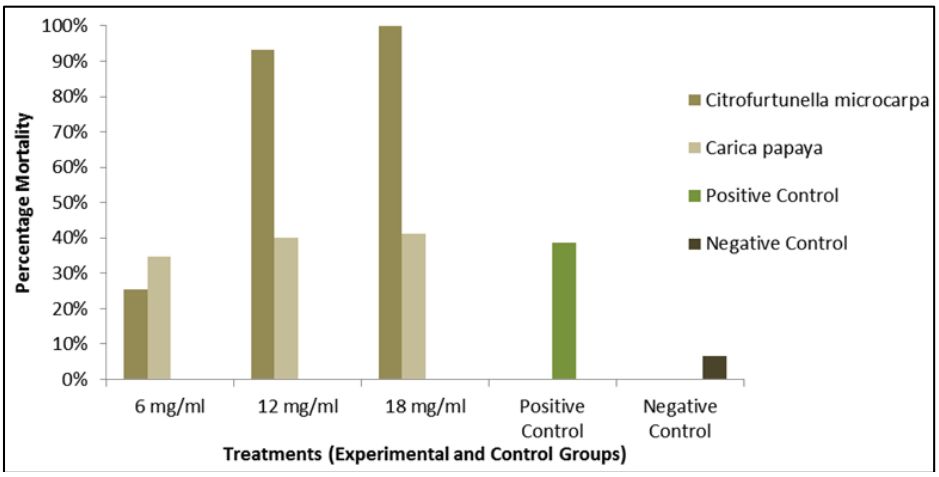

Fig 2: Percentage Mortality of *Aedis* sp. Larvae Treated with the Various Concentrations of Leaf Extracts and the Control Group after 48 hours

3.2.2 Tukey Test Results on the Mortality of *Aedes* sp. Mosquito Larvae Using Various Concentrations of *Carica papaya*

The findings from table 4 revealed that using 6 mg/ml, 12 mg/ml and 18 mg/ml concentrations of papaya leaf extract resulted on the same number of mosquitoes killed during the experiments. The three different treatments yielded comparable effects in terms of effectiveness of killing larval mosquitoes.

Table 4: Summary of All Pairwise Comparison Results Showing the Differences on the Mortality of *Aedes* sp. Mosquito Larvae Using Various Concentrations of *Carica papaya* Leaf Extracts with the Tukey Test

Compared Treatments	Mean Difference	*p* – Value	Interpretation
6 mg/ml versus 12 mg/ml	-0.67^{ns}	0.979	No Significant Difference
6 mg/ml versus 18 mg/ml	-1.67^{ns}	0.638	No Significant Difference
6 mg/ml versus Negative control	6.83*	0.000	Significant Difference
6 mg/ml versus Positive control	1.33 ^{ns}	0.795	No Significant Difference
12 mg/ml versus 18 mg/ml	-1.00 ^{ns}	0.914	No Significant Difference
12 mg/ml versus Negative control	7.50*	0.000	Significant Difference
12 mg/ml versus Positive control	2.00 ^{ns}	0.476	No Significant Difference
18 mg/ml versus Negative control	8.50*	0.000	Significant Difference
18 mg/ml versus Positive control	3.00 ^{ns}	0.139	No Significant Difference
Negative control versus Positive control	-5.50*	0.003	Significant Difference

p-Value < α = 0.05– Significant at α = 0.05 (*)
p-Value > α = 0.05- Not Significant at α = 0.05 (^{ns})

Moreover, the treatments with 6 mg/ml, 12 mg/ml and 18 mg/ml concentrations of papaya leaf extract also revealed a comparable effect with the positive treatment in terms of efficiency of killing mosquitoes. This result implies that *C. papaya* can be a potential larvicide substitute of commercially available chemical-based larvicides used in controlling the population of *Aedes* spp. mosquitoes. However, the three treatments using 6 mg/ml, 12 mg/ml and 18 mg/ml dosage of papaya solutions are found to be more effective than the negative treatment on its effect of killing mosquito larvae which confirms that methanol did not influence the larvicidal property of *C. papaya* extract.

3.2.3 Tukey Test Results on the Mortality of *Aedes* sp. Mosquito Larvae Using Various Concentrations of *Citrofurtunella microcarpa*

Table 5 shows that the results of using 12 mg/ml concentration of *C. microcarpa* leaf extract is comparable to 18 mg/ml concentration (*Mean Difference* = -2.33, *p-value* = 0.408) since it yields the same effect in terms of its efficacy of killing mosquito larvae. On the other hand, the use of 6 mg/ml of *C. microcarpa* leaf extract also exhibits certain degree of larvicidal activity but with a lesser efficiency as compared to 12 mg/ml or 18 mg/ml dosage of lemonsito solutions (*Mean Difference* = -17.83, *p-value* = 0.000, and *Mean difference* = -20.17, *p-value* = 0.000). This implies that proper concentration of this extract should be applied in their breeding sites to effectively control mosquito larvae and thus the vector of dengue. One important advantage of using these plant extracts is that, they are relatively safer and economical contrary to the commercially available chemical-based larvicides.

Moreover, findings also revealed that the use of 12 mg/ml or 18 mg/ml concentration of lemonsito extract is more effective than the positive control considering the number of mosquitoes killed during the experiments. It implies that a better mosquito-killer option can be developed out of lemonsito plant utilizing either of these concentrations for public utility and protection against feasting mosquitoes. In addition, this plant is locally grown and widely distributed which makes it less costly, readily available and environment-friendly since it is free from harmful chemicals that may have negative effects to other organisms and to humans.

Table 5 also shows that the use of 6 mg/ml of *C. microcarpa*

extract had the same effect on positive control in terms of the number of mosquito larvae killed. Meanwhile, mortality of mosquito larvae is significantly lower in the negative control than the use of either 12 mg/ml or 18 mg/ml concentration of *C. microcarpa* extract which suggests that larvicidal property of the extract is not due to methanol but may be attributed to bioactive contents of the plant.

From the findings, it can be deduced that the use 12 mg/ml dosage of lemonsito extract is comparable to an 18 mg/ml lemonsito ingredients in terms of eradicating mosquito larvae. Using either 12 mg/ml or 18 mg/ml dosage of lemonsito extract can kill a large number of mosquitoes even higher than the other types of solutions in the experiment. The result suggests that lemonsito has high larvicidal potential thus makes a better substitute to chemically-based larvicides intended to improve dengue vector mosquito management system without compromising the environment.

Table 5: Summary of All Pairwise Comparison Results Depicting the Differences on the Number of Mosquitoes Died Using *Citrofurtunella microcarpa* Across Five Different Treatments with the Tukey Test

Compared Treatments	Mean Difference	*p* – Value	Interpretation
6 mg/ml versus 12 mg/ml	-17.83*	0.000	Significant Difference
6 mg/ml versus 18 mg/ml	-20.17*	0.000	Significant Difference
6 mg/ml versus Negative Control	3.33 ^{ns}	0.126	No Significant Difference
6 mg/ml versus Positive Control	-2.17 ^{ns}	0.478	No Significant Difference
12 mg/ml versus 18 mg/ml	-2.33 ^{ns}	0.408	No Significant Difference
12 mg/ml versus Negative Control	21.17*	0.000	Significant Difference
12 mg/ml versus Positive Control	15.67*	0.000	Significant Difference
18 mg/ml versus Negative Control	23.5*	0.000	Significant Difference
18 mg/ml versus Positive Control	18.00*	0.000	Significant Difference
Negative Control versus Positive Control	-5.50*	0.005	Significant Difference

p-Value < $\alpha = 0.05$ - Significant at $\alpha = 0.05$ (*)

p-Value > $\alpha = 0.05$ - Not Significant at $\alpha = 0.05$ (^{ns})

3.2.4 *t*-Test Comparing the Effectiveness between Carica papaya and Citrofortunella microcarpa Against the Dengue-Mosquito Larvae

The findings (table 6) show that using *C. microcarpa* extract results into a significantly higher mortality of mosquito larvae than using *C. papaya extract*, (*t – value* = -3.497, *p – value* = 0.002, and $\alpha = 0.05$). This implies that lemonsito is more effective than papaya solution in terms of its larvicidal potential. However, both can be indigenous solution to our problem with controlling mosquito larvae. Utilization may now depend on its availability in the locality. The presence of various phytochemicals known for its larvicidal property including flavonoids, tannin, saponins may be responsible for this characteristic as also indicated in the study of Khanna *et al*. (2007), Permalsamy *et al*. (2015) and Ghosh (2013) [20, 21, 23]. Nevertheless, lemonsito exhibits a more potent larvicidal property than papaya which may be attributed by the synergistic effect of fatty acid present to other bioactive components. There is inadequate knowledge regarding the interaction and mode of action on these phytochemicals, thus future studies are needed to understand further their effects.

Table 6: Comparative Analysis on the Effectiveness between Papaya and Lemonsito Solutions in Terms of its Capacity of Killing Dengue-Mosquito Larvae across Treatments and Replicates

Compared Treatments	*t* – value	*P* – Value	Interpretation
Papaya Versus Kalamansi Solutions	-3.497*	0.002	Significant Difference

p-Value < $\alpha = 0.05$- Significant at $\alpha = 0.05$ (*)

p-Value > $\alpha = 0.05$- Not Significant at $\alpha = 0.05$ (^{ns})

Moreover, the use of natural products in insect management has a particular advantage. Völlinger (1987) [24] suggested that while it is not exempted from evolving resistance to its natural bioactive components but the presence of numerous and more complex compounds compared to synthetic pesticides, delays the build-up of resistance. Many phytochemicals are unexplored with target sites selective to insects but safe to the environment and humans.

3.2.5 *T*-Test Comparing the Mortality of Mosquito Larvae between 24 Hours and 48 Hours of Treatment

Table 7: Differences on the Number of Mosquitoes Died Between 24 Hours and 48Hours of Treatments with *Carica papaya* Extract

Paired Treatments	*t* – value	*P* – Value	Interpretation
24 Hours versus After 48 Hours of Treatments with Papaya Solutions	-3.26^{ns}	0.083	No Significant Difference

p-Value < $\alpha = 0.05$ - Significant at $\alpha = 0.05$ (*)

p-Value > $\alpha = 0.05$- - Not Significant at $\alpha = 0.05$ (^{ns})

Table 8: Differences on the Number of Mosquitoes Died between 24 Hours and 48Hours of Treatments with *Citrofurtunella microcarpa* Extract

Paired Treatments	*t* – value	*P* – Value	Interpretation
24 Hours versus After 48 Hours of Treatments with Kalamansi Solutions	-2.040^{ns}	0.178	No Significant Difference

p-Value < $\alpha = 0.05$ - Significant at $\alpha = 0.05$ (*)
p-Value > $\alpha = 0.05$ - Not Significant at $\alpha = 0.05$ (^{ns})

As depicted from the findings (table 7 and 8), the number of mosquitoes died 24 hours and after 48 hours of treatment during the experiment remains the same for papaya extract (*t – value* = -3.26, *p – value* of 0.083) and lemonsito extract (*t – value* of -2.040, *p – value* of 0.178) at 0.05 level of significance. This implies that the most number of mosquitoes died after 24 hours of treatments with these plant extracts. Hence, the first 24 hours is more critical in the application of these plant extracts to ensure optimum result in the eradication of mosquito larvae.

4. Conclusion

Papaya and lemonsito are found out to show certain larvicidal potential comparable to commercially available larvicide based on the mortality of the dengue-vector *Aedes* sp. mosquito larvae. However, lemonsito exhibited a relatively higher potential, in fact it exceeded the larvicidal activity of a commercial larvicide- temephos at the concentration used in the experiment.

The larvicidal property of these plants may be attributed to the presence of alkaloids, flavonoids, saponins and tannins which are known to possess insecticidal and larvicidal characteristics. While both plants are confirmed to possess these same phytochemicals, fatty acid was also detected to be present in lemonsito extract but not in papaya extract. The interaction of fatty acid and other phytochemicals may contribute to the significant difference in the effectiveness of lemonsito extract compared to papaya extract in the mortality of *Aedes* sp. larvae.

However, further studies may be conducted to clearly understand the possible synergistic action and mode of action of fatty acids and other phytochemicals to explain further the effectiveness of lemonsito. Test for specific bioactive components and quantifying their amounts maybe performed in order to understand the varying effects of these plant extracts to *Aedes* sp. larvae. Moreover, the findings of the study showed that optimal result in controlling mosquito larvae using papaya and lemonsito extract may be observed in 24 hours. Thus, application of these plant extracts to effectively control mosquito larvae may take into important consideration the time element.

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