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RESEARCH ARTICLE

MADRE DE CACAO (*Gliricidia sepium*) AND BOTTLEBRUSH (*Callistemon viminalis*) LEAVES EXTRACT AS MOSQUITO LARVICIDE

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ABSTRACT

Mosquitoes are major disease vectors, therefore keeping them under control is essential for human health. Insecticides have been shown to be effective at controlling mosquito populations, but insecticide resistance and environmental concerns are increasing. Looking for the health and environment hazards and cost of synthetic insecticides, there is a need to find a safe, practical and effective alternatives. This study aimed to determine the level of effectiveness and the presence of phytochemical analysis of Gliricidia sepium and Callistemon viminalis leaves extracts. It also included the larvicidal effect from the different extract formulations of Madre de Cacao and Bottlebrush at different instar stage of mosquito larvae. It further investigated whether there is no significant difference in the effectivity among the five different formulations of leaves extract. Results of the experiment revealed that 100% Madre de Cacao formulation were found to be very effective in killing mosquito larvae followed by 75%:25%, 50%:50%, 25%:75% while 100% Bottlebrush formulation came out as less effective. Likewise, Madre de Cacao and Bottlebrush contain sterols, flavonoids, alkaloids, saponins, glycosides, tannins and triterpene that have larvicidal impact on mosquito. Finally, a significant difference exists among the different treatments. Madre de Cacao and Bottlebrush maybe recommended as home-based mosquito larvicide which were found to be not hazardous to the health of people in extracting the leaves. A community may build and designate a certain area for plantation and development of Madre de Cacao tree and other plants that potentially source of insecticides specially mosquito larvae that can lessen the harmful effects of using commercial pesticides in controlling mosquitoes.

KEYWORDS

Aedes aegypti, Callistemon viminalis, Gliricidia sepium, Larvicidal effect.

1. INTRODUCTION

Mosquitoes act as a route for most of the life-threatening diseases in the country like malaria, fever, dengue fever, chikungunya, filariasis, and encephalitis. Most of the time, mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the Philippines and in the world. During an outbreak, the national and local government departments take the lead for large-scale mosquito control activities. In the Philippines, the Department of Health's (DOH) multi-pronged approach, which works in direct collaboration with local government unit (LGU's and medical centers aims to reduce cases of water-borne infectious diseases, leptospirosis, and influenza, including dengue (W.I.L.D.), which typically peak at the start of the rainy season. The DOH has noted that W.I.L.D. ailments crest essentially within the months of July and October due to fluctuating weather conditions, flooding, and accumulation of contaminated water, based on epidemiological data and analysis submitted (Department of Health, 2020).

Mosquitoes bear serious and well-known diseases such as malaria, arboviral encephalitis, dengue, chikungunya, and yellow fever, causing substantial morbidity and mortality in humans and domestic animals worldwide. Chemical insecticides have been commonly used for several decades to combat mosquitoes. Resistance against these chemical insecticides has been developed by mosquitoes and has adversely affected the environment. There is an urgent need to look for new eco-friendly alternatives that are more effective, secure and economical. An alternative to these insecticides is plant extracts with proven insecticidal properties. In terms of the importance of public health and causing diseases such as malaria, Japanese encephalitis, dengue fever and other fevers, mosquitoes are the single most vital gather of insects (Golding et al., 2015).

Plants contain a wide assortment of bioactive chemical compounds that can easily be biodegraded, target specific, lower bioaccumulation and low or even non-toxic for higher animals (Bernhoft, 2010). A complex of chemicals with unusual biological activity possesses phytochemicals extracted from plant sources Journal of Entomology and Zoology Studies. Larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth control, fecundity suppression, male sterility and smoke toxicity can function as phytochemicals derived from plant resources. Some of the extracts of plant leaves are tested on medically

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essential mosquitoes for their various insecticidal properties (Amir et al., 2017).

Madre de Cacao (*Gliricidia sepium*) could be a nitrogen-fixing tree. The toxic properties of its seeds and bark have given rise to the generic name "*Gliricidia*," which translates as "mouse killer." *Gliricidia sepium* has been reported to be narcotic, rodential, expectorant, suppurative and insecticidal (Duke and Wain, 2017; Abulude and Adebole, 2019). The Madre de cacao leaves are also proven to be a good source of coumarins, a toxic chemical that may kill practically all pests and insects (Rabena, 2007). It has a distinct scent that can attract rodents and, with the right amount, can eventually kill them. It is also said to have a wide range of applications as a pesticide and herbal medicine for humans and animals, according to indigenous knowledge (Tacio, 2019).

Several studies have documented the larvicidal activity of extracts from the leaves, flowers, and roots of several plants. *Gliricidia sepium* is a medium-sized leguminous tree belonging to the Fabaceae family and essentially referred to as *Gliricidia*. Afrormosin, medicarpin, tannin, and certain isoflavins are the active medicinal compounds found in *Gliricidia*. Tannin has antidiarrheic, antinephritic, bacterial, antidysentric, antiviral, cancer prevention, antimutagenic, pesticide, viricide properties, hepato-protective and psychotropic, according to studies (Akharay et al., 2012).

The efficacy of the extract of Madre de Cacao leaf as a larvicide for domestic mosquitoes it indicated that the leaf extract has the potential to be a natural mosquito larvicide and an alternative to industrial mosquito pesticide substitution. For the late third stage larvae of *Anopheles stephansi, Aedes aegypti*, and *Culex quinquefasciatus*, a study evaluated crude extracts (CE) of dried leaves, dried petioles, fresh leaves and stem bark harmful properties. All the crude extracts demonstrated toxicity to the three larval animals, with a dose of 16,000 ppm at or below 100 % mortality. The rate of mortality was dose dependent. Fresh leaves showed higher toxicity overall (Sharma et al., 2014).

The insecticidal, larvicidal and repellent activity of essential oils from *Callistemon viminalis, M. leucadendron*, and *H. suaveolenss* against *Chrysodeixis chalcitesss* and compared it with neem oil, with 100% larval mortality at 2 µg/ml for 6 h, the leaf essential oil showed higher biological activity than neem. The chemical composition and antibacterial movement investigation given 12 components from the CV leaves. 1,8-cineole and a-pinene were the main components (Ngom et al., 2016).

Dozens of phytochemical studies have been conducted on *Callistemon viminalis*. The extracts have been shown to be abundant in phenolics, triterpenoids, flavonoids, saponins, hormones, alkaloids, tannins, carbohydrates, amino acids and compounds of proteins (Ashmaway et al., 2014). The ornamental shrub *Callistemon viminalis* (Myrtaceae) is widely distributed in Asian countries. Anti-microbial properties have been identified and are commonly used for intestinal diseases and the insecticidal activity of this plant against mosquito larvae has been investigated (Jiji et al., 2012). The genus *Callistemon* has been phytochemically found to be high in triterpenoids, flavonoids, steroids and saponins.

2. Objectives of The Study

This study aimed to determine the level of effectiveness of Madre de Cacao (*Gliricidia sepium)* and Bottlebrush (*Callistemon viminalis)* leaves extract as mosquito larvicide using the different formulations: 25% Made de Cacao and 75 % Bottlebrush; 50% Madre de Cacao and 50% Bottlebrush; 75 % Madre de Cacao and 25% Bottlebrush; 100% Madre de Cacao and 100% Bottlebrush. It also indicated the phytochemical analysis components present in the Madre de Cacao and Bottle brush leaves extract as mosquito larvicide in terms of flavonoids, glycosides, saponins, tannins, alkaloids, triterpenes and sterols. It also included the Larvicidal effect of extract formulations of Madre de Cacao and Bottlebrush at different instar stage of mosquito larvae.

3. Materials and Methods

Leaves from Madre de Cacao and Bottlebrush were collected by cutting them from the branches and washed thoroughly with distilled water to clean dust or any particle stuck to them. The leaves of Madre de Cacao and Bottlebrush were extracted through chop and grind using mortar and pestle or electric blender and filtered it into a cheese cloth and squeeze to separate the juice extract into the container with different samples or formulations. Every sample was tested 20 mosquito larvae for the effectivity and the larvicidal effect of mosquito larvicide at the different instar stage with three replicates recorded the number of larvae mosquito die (mortality) and live when the extract added to their breeding sites within 24 hours (12/12 day/dark cycle) from the five formulations. The results of the leaves of Madre de Cacao and Bottlebrush were submitted to Analytical Service Laboratory at the DOST in Taguig for the chemical analysis (phytochemical) present for mosquito larvicide.

3.1 Rearing of Mosquito

Larvae of a mosquito were reared in a pail of tap water or rainwater in a dark place where mosquito larvae developed. The larvae obtained from this stock were used as subject in the study. Every day, the researcher observed when the egg of a mosquito will be developed into different stage of larvae. The larvae were collected or harvested using measuring spoon placed in 15 containers with 20 mosquito larvae each.

3.2 Testing of Effectiveness

Five treatments were used in the research. For every sample 25:75%, 50:50%, 75:25%, 100% extract from the leaves of Madre de cacao and Bottlebrush were test the effectiveness as mosquito larvicide at the different instar of mosquito larvae with three replicate each and recorded the number of dead larvae mosquito when the extracted sample were added to their breeding sites and were observed for every 12 hours.

3.3 Phytochemical screening of the plant extracts

The chemical analysis used for Madre de Cacao and Bottlebrush leaves extract was the Phytochemical that includes flavonoids, glycosides, tannins and saponins that present from the two leaves extract. Based on DOST, Manila the leaves of Madre de Cacao and Bottlebrush leaves were tested thru ethanol extract for 200 g to 300 g. The test was qualitative; thus, the report only showed positive or negative for presence of phytochemical components. No value of the amount of phytochemical component will be provided.

3.4 Statistical Tools in the treatment of Data

The data gathered from this study was analyzed using descriptive statistics such as frequency counts. Mean was also used to find out the level of acceptability of the developed product. Mean results were analyzed using One-Way Analysis of Variance (ANOVA) to evaluate the treatment effects and Tukey HSD as post hoc analysis was utilized for multiple comparison. To find out for the significant difference in the Effectiveness of Madre de Cacao and Bottlebrush Leaves Extract as Mosquito Larvicide, the (ANOVA) was utilized to know the significant difference in the level of effectiveness of Madre de Cacao leaf extract at different concentration and will be tested at .01 level of significance.

4. Results and Discussion

4.1 Phytochemical analysis

The result of the phytochemical analysis of fresh leaves of Madre de Cacao and Bottlebrush in Table 1.

Table 1: Phytochemical analysis of fresh leave s of Madre de Cacao and Bottlebrush

Constituents detected	Madre de Cacao (Replicate 1)	Madre de Cacao (Replicate 2)	Bottle-brush (Replicate 1)	Bottle-brush (Replicate 2)
Sterols	+	+	+	+
Triterpenes	-	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+

*Tests were done at the ITDI (Standard and Testing) DOST, Taguig, Manila

Table 1 shows the phytochemicals present in the ethanolic extract of *Gliricidia sepium* and *Callistemin viminalis*. Common to both extracts were present sterols, triterpenes, sterols flavonoids, alkaloids, saponins, glycosides and tannins. Madre de Cacao lacked triterpenes in one treatment. These compounds may contribute to the larvicidal activity against mosquito. Tannin toxicity in insects is hypothesized to be caused by a high amount of reactive oxygen species generation. Tannins have a significant negative impact on phytophagous insects. They have an impact

on insect growth and development by binding to proteins, limiting nutrition absorption efficiency, and producing midgut lesions. Tannins are astringent (mouth-puckering) bitter polyphenols that are effective insect pest deterrents (Barbehenn and Peter, 2011).

Most alkaloids have a bitter taste or are poisonous when ingested specially at high dose. Flavonoids were found to cause significant mortality in *Aedes aegypti* against III and IV larval instar and *Anopheles stephensi* isolated from crude extracts of *Vitex negundo* and *Andrographis paniculate* (Gautam et al., 2013). Saponins cause higher mortality, reduced food intake, weight loss, developmental delays, developmental abnormalities, and decreased reproduction in pest insects. However, the mechanism underlying these actions is still understood, but saponins are likely to have several functions. Saponins could either make food less appealing to eat (repellent/deterrent action), create digestive issues, cause molting disorders, or have harmful effects on cells, according to the main hypotheses (Tava and Avato, 2006).

The result of the phytochemical analysis of different formulations of aqueous extraction in Table 2.

Table 2: Phytochemical analysis of different formulations of aqueous extraction of the two plant extracts

Constituents detected	100% Madre de Cacao	100% Bottlebrush	75% Madre de Cacao: 25% Bottle-brush	50% Madre de Cacao: 50% Bottle-brush	25% Madre de Cacao: 75 % Bottle-brush
Sterols	-	-	-	-	+
Triterpenes	+	+	+	+	+
Flavonoids	-	+	-	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+

*Tests were done at the ITDI (Standard and Testing) DOST, Taguig, Manila

Table 2 shows the phytochemicals present in the ethanolic extract of the different formulations of leaves extract of Madre de Cacao and Bottlebrush. The – sign means absence of phytochemical components. The 100% Madre de Cacao extract lacked sterols and flavonoids same with the 75%:25% formulation. For the 100% Bottlebrush and 50%:50% formulation lacked sterols. For the 25%:75% formulation all phytochemical components sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides and tannins are present. A study revealed that saponins isolated from *Achyranthes aspera* have a high larvicidal effect against *Ades aegypti* and *Culex quinquefasciatus* and flavonoids isolated from the aqueous extract of the plant are effective in killing 80% of the tested *Culex chinensis* (Bagavan, 2008). Alkaloids, saponins and tannins are known to possess pesticidal properties

4.2 Larvicidal Efficacy of the Plant Extracts

Table 3: Mean and percentage mortality of *Aedes aegypti* mosquito larvae in the control and experimental groups after 24 hours of exposure.

Formulation	No. of mosquito larvae died			Average mosquito larvae used	Average mosquito larvae died	% Mor-tality	Level of effective-ness
	Trial 1	**Trial 2**	**Trial 3**				
25%:75% Madre de Cacao and Bottlebrush	9	8	10	20	9.00	45.00	Less effective
50%:50% Madre de cacao and Bottlebrush	14	15	13	20	14.00	70.00	Moderately effective
75%: 25% Madre de Cacao and Bottlebrush	18	17	18	20	17.67	88.35	Effective
100% Madre de Cacao	20	20	20	20	20.00	100.00	Very effective
100% Bottlebrush	10	9	10	20	9.67	48.35	Less effective

90% - 100% = Very Effective
80% - 89% = Effective
70% - 79% = Moderately Effective
40% - 69% = Less Effective
Below 40% = Not Effective

Table 3 presents the mean and percentage mortality as well as the level of effectiveness of the extract in killing the Aedes aegypti larvae. The researchers observed the variations in the mean and percentage mortality among the different formulations/concentrations of the plant extracts. The 25%:75% formulation exhibited the least percentage mortality while the 100% Madre de Cacao formulation manifested the highest percentage of mortality. This result shows the five extract formulations exhibited a formulation-dependent activity against mosquito larvae. This finding further implies that as the formulations of the extract from Madre de Cacao increases, the percentage mortality of the larvae also increases. Phytochemicals such as alkaloids, flavonoids, steroids, and tannins are found in the ethanolic stem extract of *Gliricidia sepium*. These phytochemicals have insecticidal and other pesticidal properties. Various investigations have revealed that this plant has antibacterial and parasite properties (Gutirrez, 2014). The percentage mortality effect of each concentration of the different extracts after 24 hours is illustrated better in the bar graph below.

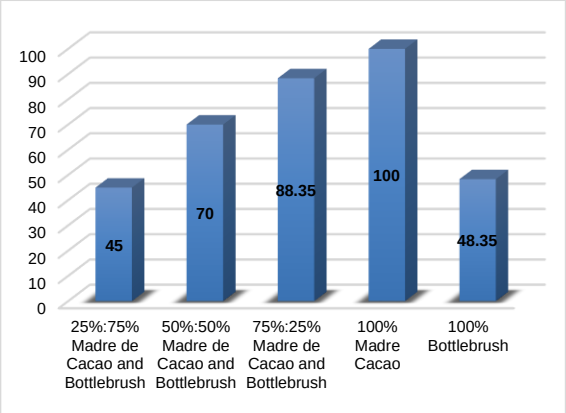

Figure 1: Percentage mortality effect of each concentration of the different extract after 24 hours.

Figure 1 indicates the phytochemical components presence in the 100% Madre de Cacao was very effective after 24 hours of exposure to the leaf extracts. Larvicidal activity were tested at different instar stage of mosquito larvae. Larvicides may contact poisons, stomach poisons, growth regulators, or (increasingly) biological control agents. According to research, sterol is a critical compound for most insects and mosquitos to complete their life cycle. Unfortunately, mosquitoes cannot synthesize the sterol, it depends on mammals for the same. During their larval stage, mosquitos consume sterol from plant decay in the form of phytosterol, which is then converted to cholesterol for further growth and reproduction. This conversion took place with the assistance of the sterol carrier protein 2 (SCP2). Plant-based inhibitors that inhibit sterol carrier protein activity (SCPI-Sterol carrier protein inhibitor) control mosquito populations (Kumar et al., 2010).

Table 2: Mean and percentage mortality of Aedes aegypti mosquito larvae in the control and experimental groups after 48 hours of exposure.

Formulation	No. of mosquito larvae died			Average mosquito larvae used	Average mosquito larvae died	% Mortality	Level of effectiveness
	Trial 1	Trial 2	Trial 3				
25%:75% Madre de Cacao and Bottlebrush	20	20	20	20	20.00	100	Very effective
50%:50% Madre de cacao and Bottlebrush	20	20	20	20	20.00	100	Very effective
75%: 25% Madre de Cacao and Bottlebrush	20	20	20	20	20.00	100	Very Effective
100% Madre de Cacao	20	20	20	20	20.00	100.	Very effective
100% Bottlebrush	10	10	11	20	10.33	51.65	Very effective

90% - 100% = Very Effective
80% - 89% = Effective
70% - 79% = Moderately Effective
40% - 69% = Less Effective
Below 40% = Not Effective

Table 2 shows the average and percentage mortality of *Aedes aegypti* mosquito larvae treated with the various formulations of the plant extracts and the control groups after 48 hours. It revealed that the least percentage mortality was observed in the 100% Bottlebrush extract (51.65%) while the highest percentage mortality was noted in the 25%:75% formulation, 50%:50% formulation and 75%:25% formulation and 100% Madre de Cacao extracts as 100% mortality rate. This finding means that the different formulations in the control and experimental groups were effective after 48 hours of treatment. The presence of the phytochemicals, Alkaloids, Tannins, Saponins, Sterols, Glycosides and Flavonoids, which are known to have a pesticidal properties have caused the high larvicidal activity of the five extracts.

All these phytochemicals from the different formulations with *Gliricidia sepium* may have caused its very potent larvicidal activity and its very strong unpleasant odor to manifest the mortality of the mosquito larvae, unlike the *Callistemon viminalis* that have less unpleasant odor compared to Madre de Cacao to have a lesser larvicidal activity effect. The synergistic effects of the two extracts may have caused their very efficient larvicidal activity against the mosquito larvae. Plants having larvicidal action, according to the study, might work together or independently (Ndung et al., 2014). In addition, alkaloids and tannins were said to be effective pesticides (Azmathullah et al., 2011). On the other hand, flavonoids possess bioactive properties such as antibacterial and insecticidal effects. When insect herbivores consume steroids, they disrupt the insect's molting cycle (Hopkins and Hunner, 2009). The percentage mortality effect of each formulation of the different extracts after 48 hours is illustrated in the bar graph below.

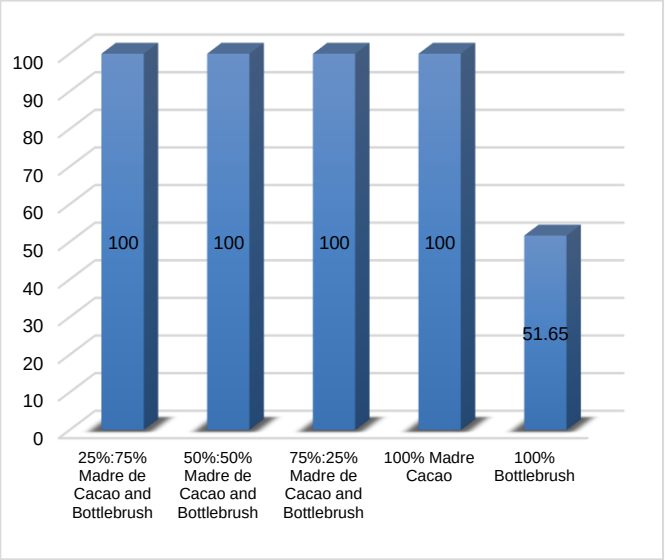

Figure 2: Percentage mortality effect of each concentration of the different extract after 48 hours

Figure 2 shows the mortality effect of the different instars stage of mosquito larvae from the extracts due to the components presents in it. It revealed that all the formulations were very effective except the 100% Bottlebrush. Based on the result of the % mortality, the 100% Bottlebrush had a great effect based on the extract and formulations and the components of the phytochemical analysis of the different formulations wherein lack sterols and flavonoids that affect the mortality rate, but not all the formulations. Flavonoid extract from the entire aerial part of *Andrographis paniculata* was found to be inactive against the selected larvae of *Aedes aegypti* even at 600 ppm, whereas it caused 70% mortality in *Anopheles stephensi* at 200 ppm (Gautam et al., 2013). Therefore, after 48 hours of exposure only 100% Bottlebrush was less effective. The table presented the summary of the percentage mortality rate of mosquito larvicide at different time interval.

Table 3: Larvicidal effect of extract formulations of Madre de Cacao and Bottlebrush at different instar stage of mosquito after 24 hours

Formulations	Stages of Mosquito Larvicide			
	1st	2nd	3rd	4th
25:75 Madre de cacao and Bottlebrush	slowly moving	slowly moving	lived	lived
50:50 Madre de Cacao and Bottlebrush	died	failing	failing	lived
75:25 Madre de cacao and Bottlebrush	died	died	slowly moving	slowly moving
100 Madre de Cacao	died	died	died	died
100 Bottlebrush	died	lived	lived	Lived

slowly moving- dragging
died – drown
failing – defeated
live - survive

Table 3 shows the larvicidal effect of formulations of Madre de Cacao and Bottlebrush at different instar of *Aedes aegypti* mosquito larvae. The most effective formulation is the 100% Madre de Cacao due to almost of the larvae are dead up to III instar stage. This was in line with the results of a research, which found that the change in body of larvae from normal conditions was caused by flavonoid compounds because it could cause weakness in larval nerves (Cania and Setyanimgrum, 2013). Phytochemicals from Madre de Cacao and Bottlebrush primarily affect the midgut epithelium and secondarily affect the gastric caeca and malpighian tubules in mosquito larvae and interfered with proper functioning of mitochondria more specifically at the portion transforming

sites and have suggested that saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most likely cause of larval death. Flavonoids have larvicidal effects because they impede the respiratory system and alter the electron transport process in the larval body, lowering ATP generation and decreasing oxygen utilization by mitochondria. Furthermore, tannins work in the larvae by inactivating enzymes and proteins (Sudjari et al., 2012).

Table 4: Larvicidal effect of extract formulations of Madre de Cacao and Bottlebrush at different instar stage of mosquito after 48 hours

Formulations	Stages of Mosquito Larvicide			
	1st	2nd	3rd	4th
25:75 Madre de cacao and Bottlebrush	died	died	died	died
50:50 Madre de Cacao and Bottlebrush	died	died	died	died
75:25 Madre de cacao and Bottlebrush	died	died	died	died
100 Madre de Cacao	died	died	died	died
100 Bottlebrush	died	died	died	slowly moving

slowly moving- dragging
died – drown
failing – defeated
live - survive

Table 4 shows that from the different formulations of Madre de Cacao and Bottlebrush, the different stages of *Aedes aegypti* mosquito larvae are mostly dead after 48 hours of exposure except the 4th stage of 100% Bottlebrush extracts, the larvicidal effect are slowly moving. The behavioral observations of the early IV instar larvae of *Aedes aegypti* treated with the leaf extract of Madre de Cacao and Bottlebrush revealed different movements in the larvae. Those symptoms were caused by nerve poisons revealed that the leaves extract could possibly act as cytolysin and had an impact on the neuromuscular system of larvae. The findings were supported by earlier studies, that the extract act as a nerve poison; though, the aggressive and uncoordinated movements along with other toxic symptoms were observed at relatively different time intervals (Kumar et al., 2010). The studies also revealed coiling movements in the treated larvae, as well as aggressive anal biting behavior, confirming the extract's neurotoxic impact on *Aedes aegypti* larvae. Mortality was recorded as which found that the change in body of larvae from normal conditions was caused by flavonoid compounds because it could cause weakness in larval nerves. The larvicidal activity of the aqueous extracts of the various plant parts considered in this study appears to be due to the presence of sterols, triterpenes, sterols flavonoids, alkaloids, saponins, glycosides and tannins.

Table 5: Test of significant difference in the effectivity from different formulations of Madre Cacao and Bottlebrush

		Sum of Squares	df	Mean Square	F	Sig.
After 24 Hrs.	Between Groups	279.600	4	69.900	131.063**	.000
	Within Groups	5.333	10	.533		
	Total	284.933	14			
After 48 Hrs.	Between Groups	224.267	4	56.067	841.000**	.000
	Within Groups	.667	10	.067		
	Total	224.933	14			

***Significant at p<.01*

Table 10 shows that there is a significant difference in the effectivity from different formulations of Madre Cacao and Bottlebrush both after 24-hour exposure with F (4, 10) = 131.063, p<.01 and 48-hour exposure with F (4, 10) = 841.000, p <.01.

5. Conclusion

Results of this present study on the larvicidal property of two plant extracts and their combined extracts revealed variable efficacy. Their difference has something to do with the plant extract used, the formulation of the extract, and the time of exposure. Likewise, the presence of secondary metabolites like sterols, flavonoids, alkaloids, saponins, glycosides and tannins, all of which have known insecticidal properties add more to the killing effect of the extracts. The mechanism of action of these bioactive agents is by disrupting the cuticle membrane of the *Aedes aegypti* mosquito larvae causing an irreparable damage and eventually causing death. The level of effectiveness from the different formulations after 48 hours exposure: 100% Bottlebrush was less effective, and the other formulations was very effective. Madre de Cacao and Bottlebrush leaf extracts used as mosquito larvicide are more preferable than the use of synthetic insecticides produced from plants which could pose serious health problem to human. A preservative may be added to the extracts to lengthen their shell life. Future researchers may conduct extraction of leaves, roots, stem and bark of Madre de Cacao and Bottlebrush and other potential use aside from its larvicidal effect.

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