



Larvicidal Activity of Selected Plant Extracts against the Dengue vector *Aedes aegypti* Mosquito

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Abstract

The larvicidal effects of leaf and stem/bark extracts of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* were tested on the larvae of the dengue-vector, *Aedes aegypti*. Phytochemical screening of the extracts was conducted to determine the active toxic compounds. Various concentrations (20 mg/mL, 40 mg/mL and 60 mg/mL) of the plant extracts were tested against third instar larvae of *A. aegypti*. Phytochemical screening revealed the presence of alkaloids, flavonoids and steroids in the leaf and bark extracts of *Jatropha curcas* while the leaf and bark/stem extracts of *Citrus grandis* and *Tinospora rumphii* are rich in alkaloids, saponins, tannins, flavonoids and steroids. These compounds are known to possess insecticidal and larvicidal properties causing the mortality of insects and other pests. All plant extracts showed significant larvicidal activity against *A. aegypti* mosquito larvae at 0.05 level of significance. *Tinospora rumphii* leaf extract is the most effective mosquito larvicide which is manifested by the highest percentage mortality on the larvae of 90% and 93% after 24 and 48 hours respectively; with an LC_{50} and LC_{90} values of 10 mg/mL and 46 mg/mL respectively after 48 hours of exposure. *Citrus grandis* bark and *Tinospora rumphii* stem extracts showed a significant difference on the increased of the mortality of mosquito larvae with increasing concentrations of the plant extracts at 0.05 level of significance. The high larvicidal activity of *Tinospora rumphii* leaf is supported by the abundance of phytochemicals which show synergistic effects in terms of larvicidal action to mosquito larvae. The larvicidal activities of the three plants differ according to the plant species and part used which is supported by the presence of several bioactive chemicals.

Keywords: Larvicidal activity, *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii*, *Aedes aegypti*.

Introduction

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human¹. They do not only transmit parasites and pathogens but they also source of allergic reaction that includes local skin and systemic sensitivity². The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever where *Aedes aegypti* is one of the mosquito species responsible for the transmission of these vector borne diseases¹. World Health Organization (WHO) stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease.

Dengue is one of the most significant viral diseases transmitted by *Aedes aegypti* because it afflicts humans worldwide whose symptoms ranging from mild fever to a severe and potentially life threatening hemorrhagic disease. *Aedes aegypti* is of supreme concern because of its wide distribution and close association with humans³. *Aedes aegypti* is present in heavy polluted areas like Asia, America and some Pacific Islands and infested about 2/3 of the world's population⁴. The reported dengue cases in the Philippines from January to

June 2012 have already reached 32,193 nationwide, 3.8% higher for the same period last year⁵.

Due to the pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years⁶. The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. 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 larvicide⁷.

However, the use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end⁸. Synthetic larvicides also disrupt natural biological control systems that sometimes results into a widespread development of resistance⁷. This phenomenon has triggered and urged the development of alternative techniques using natural products.

Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other

organisms and the environment^{9,10,7}. Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly¹¹.

Jatropha curcas (Tuba-tuba) plant is reported to be toxic and found to inhibit and control insects such as aphids and maize weevil. The plant seeds are also used by local folks as fish poison. On the other hand, *Citrus grandis* (Pomelo) leaves are burn together with dried plants by local folks in order to drive away the mosquitoes. *Tinospora rumphii* (Panyawan) vine is used by farmers as organic plant pesticides and is believed to have antimicrobial and parasitical activity.

The aim of this study is to determine the larvicidal activity of the stem/bark and leaf extracts of *Jatropha curcas* (Tuba-tuba), *Tinospora rumphii* (Panyawan) and *Citrus grandis* (Pomelo) against the dengue-carrying mosquito, *Aedes aegypti*.

Material and Methods

Preparation of the Plant Samples: The collected plant samples were segregated as stem, bark and leaves and then washed with tap water and rinsed with distilled water. The plant samples were air dried for 48 hours at room temperature. Dried leaves of the plant samples were cut and pulverized using an electric blender while the dried stem and bark of the plant samples were chopped using a kitchen knife and pulverized using an electric blender.

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 then concentrated in a rotary evaporator.

Rotary Evaporation and Phytochemical Analysis: The methanolic plant extracts were sent to the Chemistry Laboratory of MSU-IIT, Iligan City for the rotary evaporation and phytochemical analysis. Plant samples have undergone rotary evaporation to make it highly concentrated until it is semi-solid in form. The phytochemical screening¹² was done following the standard procedure as described by Harborne (1998).

The methanolic extracts of the stem/bark and leaf of *Jatropha curcas* (Tuba-tuba), *Tinospora rumphii* (Panyawan) and *Citrus grandis* (Pomelo) have undergone rotary evaporation to make it highly concentrated until it is semi-solid in form. Evaluation of the major phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, anthraquinone, and cyanogenic glycos was conducted.

Collection of Mosquito Larvae: Mosquito larvae were gathered from the water present in the exposed coconut shells in Villaflor, Oroquieta City, Misamis Occidental. Mosquito larvae were brought to a small room enclosed with a mosquito

net and then transferred to a molder with clear tap water. The larvae were kept in a small room which is enclosed with a mosquito net for safety precautions in case of the fast emergence of the larvae into an adult mosquito.

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 a mosquito larvae was drop in a slide to be able to view the specimen in the compound microscope.

The target mosquito larva in this study was the third instar larva of dengue carrying mosquito *Aedes aegypti*. *Aedes aegypti* larvae 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 aegypti* mosquito larvae were separated from the other mosquito species and were placed in a water- filled plastic molder.

Mosquito Larvicidal Bioassay: The efficacy of the plant extracts as larvicide against the dengue-vector *Aedes aegypti* mosquito was evaluated in accordance with the guidelines of World Health Organization¹³. Batches of 20 third-instars larvae of *Aedes aegypti* were placed in a small plastic container with 50 ml dechlorinated water and lay in the netted area in the Laboratory room at 30-32°C. For the control group, the mosquito larvae were exposed to 60 mg/mL methanol since it is the solvent used in the extraction of different plant samples. The experimental group is the methanolic extracts of the stem/bark and leaf of *Jatropha curcas*, *Tinospora rumphii* and *Citrus grandis* with 20 mg/mL, 40 mg/mL, 60 mg/mL concentrations. These concentrations were chosen after the pre-test//pre-treatment conducted. Identification of the mosquito larvae were done by tapping it with a needle in the siphon or cervical area. 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 treatment, and the percentage mortality was computed.

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

Statistical Analysis: The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead of mosquito larvae, Analysis of Variance

(ANOVA), to determine the significant difference on the mortality of mosquito larvae between the control and the experimental groups, Scheffe Test to test the degree of variability between the control and different concentrations of the plant samples, and Probit Analysis to calculate LC₅₀ and LC₉₀ values to determine Lethal concentrations of the plant extracts on *Aedes aegypti* mosquito larvae after 24 and 48 hours of treatment.

Results and Discussion

Phytochemical Screening: Table 1 shows the phytochemicals present in the methanolic extracts of bark/stem and leaf of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii*. *Jatropha curcas* leaves and bark contain alkaloids, steroids and flavonoids. However, the leaves of *Jatropha curcas* contain less flavonoid than those of the bark. The leaf and bark extracts of *Citrus grandis* and *Tinospora rumphii* contain alkaloids, saponins, flavonoids, steroids and tannins. The phytochemicals of the plants serve as huge storage of compounds that have biological action¹⁴. Alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties¹⁵. Hegde *et al.*¹⁶ reported the antimicrobial properties of *Punica granatum* and revealed the presence of tannins, flavonoids, and saponins. Another study conducted by Srinivas *et al.*¹⁷ conducted a phytochemical screening and antimicrobial activity of leaf extract of *Wrightia tomentosa* revealed that the plant contains alkaloids, steroids, tannins, etc. and showed antimicrobial activity. In addition, compounds such as flavonoids, alkaloids,

tannins and saponins in the plants are responsible for the insecticidal and toxicity to other animals^{18,19}.

Saponins are known by their toxicity to harmful insects²⁰. Saponins isolated from *Achyranthes aspera* through bioassay-guided fractionation possessed a larvicidal efficacy against *A. aegypti* and *C. Quinquefasciatus*²¹. Moreover, Flavonoids isolated from water extracts of *Annona squamosa* is effective as insecticides against mosquito killing 80% of *C. Chinensis*²².

Larvicidal Efficacy of Plant Extracts: The larvicidal activity of the methanolic extracts of the plants against the larvae of the dengue-vector, *Aedes aegypti* mosquito was determined through mosquito larval bioassay. The mortality of the mosquito larvae were noted in the 20 mg/mL, 40 mg/mL and 60 mg/mL concentrations of the plant extracts after 24 and 48 hours of exposure. Table 2 shows the average and percentage mortality of *Aedes aegypti* mosquito larvae treated in three various concentrations of plant extracts and the control group after 24 hours of treatment. Variations of the death percentage of mosquito larvae among the plant extracts were observed. On the other hand, the least percentage mortality was observed in the control group (1.67%) which is extremely low than those in the experimental groups. The 60 mg/mL concentration of *Tinospora rumphii* leaf extract shows the highest percentage of mortality equal to 90 % of the mosquito larvae. On the other hand, *Jatropha curcas* leaf extract shows the least percentage of mortality compared to other plant samples.

Table-1
Phytochemicals present in the Methanolic Extracts of the Different Plants

Plant Extract	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Anthraquinone	Cyanogenic glycosides
<i>Jatropha curcas</i> leaves	+++	-	+	+++	-	-	-
<i>Jatropha curcas</i> bark	+++	-	+++	+++	-	-	-
<i>Citrus grandis</i> leaves	+++	+++	+++	+++	+++	-	-
<i>Citrus grandis</i> bark	+++	+++	+++	+++	+++	-	-
<i>Tinospora rumphii</i> leaves	+++	+++	+++	+++	+	-	-
<i>Tinospora rumphii</i> stem	+	++	+++	+++	+	-	-

Legend: (+) present, (-) absent

Table – 2
Mean and Percentage Mortality of *Aedes aegypti* mosquito larvae in the Control and Experimental Group after 24 hours

Plant Species	Parts Used	Concentration (mg/mL)	Mean Mortality	Mean Percentage
<i>Jatropha curcas</i>	Leaves	20	2.33	11.67%
		40	2.67	13.33%
		60	7.67	38.33%
	Bark	20	5.33	26.67%
		40	2.00	10.00%
		60	14.67	73.33%
<i>Citrus grandis</i>	Leaves	20	8.33	41.67%
		40	10.00	50.00%
		60	13.33	66.67%
	Bark	20	5.33	26.67%
		40	11.33	56.67%
		60	16.00	80.00%
<i>Tinospora rhumpii</i>	Leaves	20	11.33	56.67%
		40	17.67	88.33%
		60	18.00	90.00%
	Stem	20	6.33	31.67%
		40	8.67	43.33%
		60	17.33	86.67%
Control			0.33	1.67%

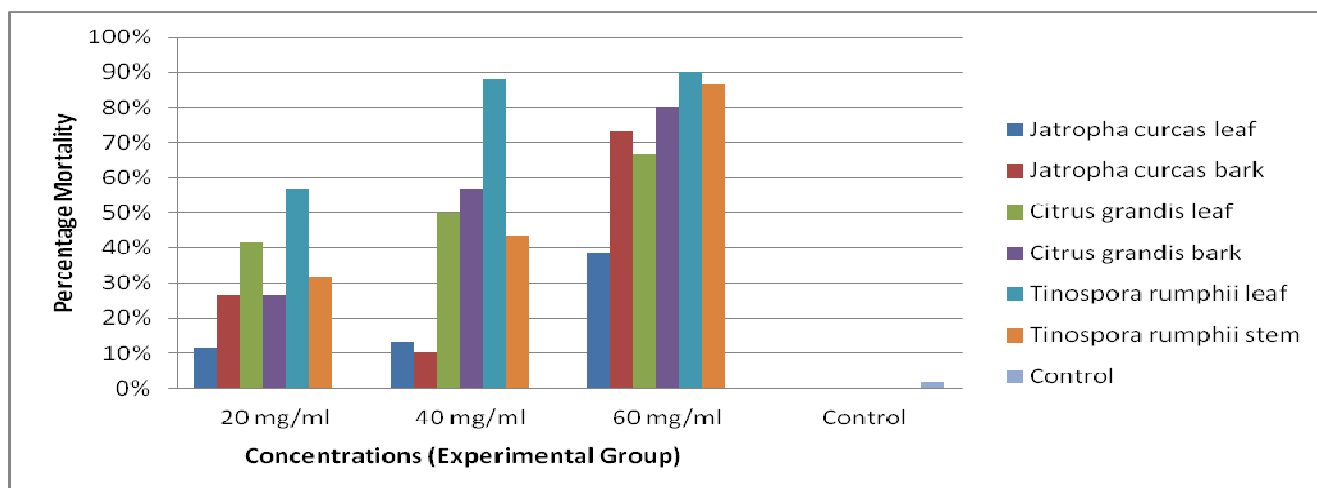


Figure – 1

Percentage Mortality of *Aedes aegypti* larvae treated with the various concentrations of the plant extracts and the control group after 24 hours

Furthermore, the plant extracts exhibited a concentration-dependent activity against mosquito larvae since the percentage mortality (figure 1) were observed to increase with increasing concentrations of the plant extracts except in the two concentrations (20 mg/mL and 40 mg/mL) of *Jatropha curcas* bark. The increase of percentage mortality of the treated mosquito larvae is supported by the presence of phytochemicals in the plant extracts which have pesticidal activities.

Table 3 shows the average and percentage mortality of *Aedes aegypti* mosquito larvae treated with various concentrations of the plant extracts and the control group after 48 hours.

Differences of the percentage mortality among the plant extracts were also observed. The least percentage mortality was noted in the control group (3.33%) which is extremely lower compared to those in the experimental groups. It reveals that all tested concentrations of the three plant extracts caused mortality of mosquito larvae in comparison to those in the control group. Result also indicates that 40 mg/mL and 60 mg/mL concentrations of *Tinospora rumphii* leaf extract have the highest percentage of mortality among the various concentrations of the plant extract. The plant's high larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins which are known to have insecticidal and pesticidal properties. These

phytochemicals present in the *T. rumphii* leaf extract could show synergistic effects in terms of larvicidal action of mosquito larvae. This is supported by the previous study on the larvicidal efficacy of the three plant extracts, *Murraya paniculata*, *Cleistanthus collinus*, and *Carica papaya* against *C. quinque-fasciatus*. Results showed the presence of phytochemicals such as steroids, alkaloids, terpenes, saponins, etc. that are accountable for the positive effects. Other studies have also reported that plants that contain larvicidal activity could act in combination or independently²³.

On the other hand, 40 mg/mL concentration of *Jatropha curcas* bark extract shows the least percentage mortality in the

experimental group. The plant's low mortality effect on mosquito larvae is due to the absence of saponins and tannins in the leaf and bark extracts of the said plant. It is further noted that the percentage mortality increased with increasing concentrations of the three plant extracts (figure 2). Moreover, the mortality of mosquito larvae was also increased in relation to the time of exposure. The larvicidal activity of the highest concentration (60 mg/mL) of the plant extracts on *A. aegypti* mosquito larvae within 48 hours of exposure showed the following order i.e. *T. rumphii* leaves > *C. grandis* bark > *T. rumphii* stem > *C. grandis* leaves > *J. curcas* bark > *J. curcas* leaves.

Table-3
Mean and Percentage Mortality of *Aedes aegypti* mosquito larvae treated with the various concentrations of the plant extracts and the Control Group after 48 hours

Plant Species	Parts Used	Concentration (mg/mL)	Mean Mortality	Mean Percentage
<i>Jatropha curcas</i>	Leaves	20	7.00	35.00%
		40	7.67	38.33%
		60	12.33	61.67%
	Bark	20	6.33	31.67%
		40	4.33	21.67%
		60	15.67	78.33%
<i>Citrus grandis</i>	Leaves	20	16.33	81.67%
		40	16.33	81.67%
		60	16.67	83.33%
	Bark	20	7.67	38.33%
		40	15.67	78.33%
		60	18.00	90.00%
<i>Tinospora rumphii</i>	Leaves	20	14.00	70.00%
		40	18.67	93.33%
		60	18.67	93.33%
	Stem	20	8.67	43.33%
		40	12.33	61.67%
		60	17.33	86.67%
Control			0.67	3.33%

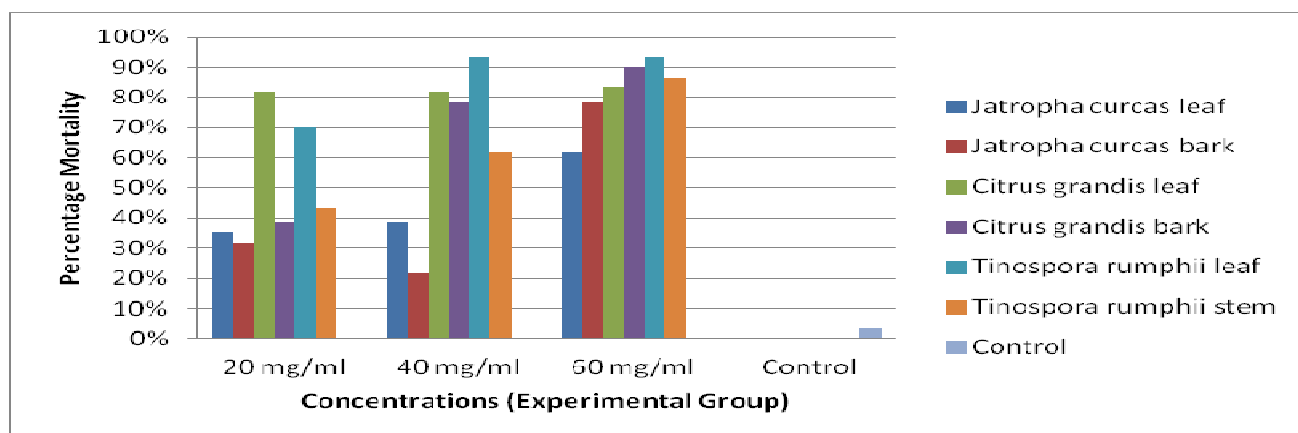


Figure - 2

Percentage Mortality of *Aedes aegypti* larvae treated with the various concentrations of the plant extracts and the control group after 48 hours

Table 4 presents the One-Way ANOVA results on the mortality of *Aedes aegypti* mosquito larvae among the control group and those treated with 20 mg/mL, 40 mg/mL and 60 mg/mL concentrations of the various plant extracts. All plant extracts showed a significant difference on the increase of mortality on the mosquito larvae in relation to the control group. This reveals that the mortality of mosquito larvae is not due to the solvent used in extracting the plant samples but instead, the mortality of mosquito larvae is due to the toxic compounds found in the plants. This result denotes that the plant extracts are susceptible to the larvae of the dengue-vector *Aedes aegypti* mosquito.

Table 5 shows the results of the Scheffe test on the Mortality of *Aedes aegypti* mosquito larvae treated on the various concentrations of the plant extracts and the control group after 48 hours of exposure. Results show that the mortality of mosquito larvae in all concentrations (20 mg/mL, 40 mg/mL and 60 mg/mL) of the leaf and stem extracts of *Tinospora rumphii* and the leaf extract of *C. grandis* are significantly different compared to the control. On the other hand, the 40 mg/mL and 60 mg/mL concentrations of bark extract of *C. grandis* showed significant difference compared to the control group. It means that these plant extracts are effective as mosquito larvicides and are potent against the third instar larvae of *Aedes aegypti*.

Moreover, the results also exhibited that there is a significant difference on the mortality of mosquito larvae between the

control group and the 60 mg/mL concentration of the various plant extracts. This result denotes that higher concentration of the plant extracts would lead to greater number of mortality in the mosquito larvae. Furthermore, it implies that the efficacy of the plant extracts as larvicide is not due to the use of methanol as the extracting solvent. Hence, it is attributed to the different phytochemicals present in these plant extracts.

In addition, the Scheffe test results show that there is a significant difference in the mortality of mosquito between the 20 mg/mL and 60 mg/mL concentrations of the bark extracts of *C. grandis* and *T. rumphii*. This implies that the extract of these plants are more susceptible to the larvae of *Aedes aegypti* mosquito in a higher concentration.

Determination of LC₅₀ and LC₉₀ values: The lethal concentrations (LC₅₀ and LC₉₀) values of the three plant extracts on *Aedes aegypti* mosquito larvae after 24 hours of exposure are summarized in Table 6. *Tinospora rumphii* leaf extract reveals the lowest LC₅₀ and LC₉₀ values of 16 mg/mL and 57 mg/mL, respectively. It shows that *T. rumphii* leaf extract is the most effective in terms of pesticidal activity compared to the other two plant samples. On the other hand, *Jatropha curcas* leaf extract shows the least effective among the plant samples since it has the highest LC₅₀ and LC₉₀ values of 88 mg/mL and 321 mg/mL, respectively. Results show that *T. rumphii* is highly lethal to *A. aegypti* larvae which were followed by *C. grandis* and *J. curcas* respectively.

Table- 4
ANOVA Results on the Mortality of *Aedes aegypti* mosquito larvae treated on the various concentrations of the Plant Extracts and the Control group after 48 hours of exposure

Variable Compared	F-computed	P-value	Implications
Control vs. <i>Jatropha curcas</i> leaf extracts	7.260	0.011*	<ul style="list-style-type: none"> At 0.05 level of significance F critical = 4.06618 * implies that there is a significant difference
Control vs. <i>Jatropha curcas</i> bark extracts	4.885	0.032*	
Control vs. <i>Citrus grandis</i> leaf extracts	140.083	0.000*	
Control vs. <i>Citrus grandis</i> bark extracts	22.078	0.000*	
Control vs. <i>Tinospora rumphii</i> leaf extracts	37.078	0.000*	
Control vs. <i>Tinospora rumphii</i> bark extracts	34.122	0.000*	

Table - 5
Scheffe Test Results on the Mortality of *Aedes aegypti* mosquito larvae treated on the various concentrations of the Plant Extracts and the Control group after 48 hours of exposure

Variables Compared	P- values					
	<i>J. curcas</i> leaf	<i>J. curcas</i> bark	<i>C. grandis</i> leaf	<i>C. grandis</i> bark	<i>T. rumphii</i> leaf	<i>T. rumphii</i> bark
Control vs. 20 mg/mL	0.177	0.610	0.000*	0.103	0.001*	0.011*
Control vs. 40 mg/mL	0.126	0.847	0.000*	0.002*	0.000*	0.001*
Control vs. 60 mg/mL	0.012*	0.040*	0.000*	0.001*	0.000*	0.000*
20 mg/mL vs. 40 mg/mL	0.995	0.969	1.000	0.059	0.205	0.275
20 mg/mL vs. 60 mg/mL	0.288	0.237	0.988	0.017*	0.309	0.007*
40 mg/mL vs. 60 mg/mL	0.388	0.128	0.988	0.811	0.989	0.103

At 0.05 level of significance, *implies that there is a significant difference

It is evident from the result that the various concentrations of the three plant extracts were the main cause of mortality in *A. aegypti* larvae. Similar study was conducted and reported that the seed extract of *Clitoria ternatea* showed larvicidal activity against the larvae of *A. Stephensi*, *A. aegypti* and *C. quinquefasciatus*³⁸. Moreover, the extract of *Cassia obtusifolia* leaf had larvicidal efficacy with LC₅₀ and LC₉₀ values were 52.2 and 108.7 mg/mL, respectively against *A. Stephensi*²⁴.

Table 7 shows the lethal concentrations (LC₅₀ and LC₉₀) values of the plant extracts on *Aedes aegypti* mosquito larvae after 48 hours of exposure. *T. rumphii* leaf extract reveals the most effective as mosquito larvicides since it has the lowest LC₅₀ and LC₉₀ values of 10 mg/mL and 46 mg/mL respectively. However, *J. curcas* bark extract shows the least larvicidal activity among the three plant samples with the highest LC₅₀ and LC₉₀ values of 44 mg/mL and 206 mg/mL respectively. Results show that the leaf extract of *T. rumphii* is highly lethal followed *C. grandis* and *J. curcas* respectively. However, bark extract of *C. grandis* is highly lethal followed by the stem extract of *T. rumphii* and bark extract *J. curcas* respectively. The results of the LC₅₀ and LC₉₀ significantly decreased in relation to the time of exposure with the least value after 48 hours of exposure. It means that increasing the period of exposure increases the mosquito larvae mortality.

Nowadays, controlling mosquitoes are mainly focussed against larvae and only against adults when necessary. Fighting against adult mosquito is impermanent, insufficient and could cause

environmental pollution, while controlling them in larval stage is more confined in space and time resulting in efficient outcomes. In addition, it is a valuable control means since they are slow mobile and their habitat can be simply identified¹⁴.

Results of the present study with the extracts from different parts of these plants exhibited variable larvicidal efficacy. These larvicidal activities differ based on the plant species and the part used. The presence of several bioactive chemicals like alkaloids, saponins, tannins, flavonoids and steroids can be attributed to the susceptibility of the plant extracts as killing agent against mosquito larvae. A study conducted by Rawani *et al.* showed the larvicidal activity of *Carica papaya*, *Murraya paniculata* and *Cleistanthus collinus* extracts against *Culex quinquefasciatus* and revealed the presence phytochemicals such as saponins,steroids, alkaloids, terpenes, etc. that are accountable for their larvicidal efficacy potential²⁵.

Phytochemicals of plants possess a broad scope of biocontrol potential. Roopa and Wadje²⁶ stated that some of these substances serve as plant defence mechanisms against microorganisms, insects and herbivores. In addition, Padhi and Mahapatra²⁷ who established the antibacterial activity of the leaf extracts of *Mimusops elengi* and revealed the presence of alkaloids, steroids, saponins, flavonoids, etc. that are potential for their affectivity. Another studies on the antibacterial activity²⁸ of the leaf of *Colocasia esculenta* showed the presence of tannin, saponins, steroid, alkaloids, etc.

Table - 6
Lethal concentrations (LC₅₀ and LC₉₀) values of the Plant Extracts on *Aedes aegypti* mosquito larvae after 24 hours of treatment

Plant Extracts	Parts Used	LC ₅₀ values (mg/mL)	Lower and Upper Bounds	LC ₉₀ values (mg/mL)	Lower and Upper Bounds
<i>Jatropha curcas</i>	leaf	88	50 - 247	321	141 - 2504
	bark	53	31 - 108	193	97 - 1009
<i>Citrus grandis</i>	leaf	34	19 - 58	123	69 - 453
	bark	32	17 - 56	117	66 - 427
<i>Tinospora rumphii</i>	leaf	16	6 - 27	57	32 - 139
	stem	33	18 - 56	119	66 - 438

Table - 7
Lethal concentrations (LC₅₀ and LC₉₀) values of the Plant Extracts on *Aedes aegypti* mosquito larvae after 48 hours of exposure

Plant Extracts	Parts Used	LC ₅₀ values (mg/ml)	Lower and Upper Bounds	LC ₉₀ values (mg/ml)	Lower and Upper Bounds
<i>Jatropha curcas</i>	leaf	43	23 - 89	199	94 - 1718
	bark	44	24 - 95	206	96 - 1858
<i>Citrus grandis</i>	leaf	11	2 - 23	54	28 - 152
	bark	19	7 - 35	89	48 - 371
<i>Tinospora rumphii</i>	leaf	10	2 - 20	46	23 - 127
	stem	23	9 - 41	107	57 - 516

Saponins are plant's bioactive compounds with significant insecticidal action and other biological properties. In addition, saponins are responsible for amplifying pest insects' death intensity, lowering food intake, retardation in development, instability in development and declining reproduction²⁹. Moreover, saponins are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking with the cuticle membrane of the larvae, eventually disturbing the membrane, which is the main cause for larval death³⁰. Another study reported that saponin extracted from fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegomyia aegypti*³⁰. On the other hand, flavonoids revealed an extensive scope of biocontrol potential such as antimicrobial and insecticidal activities. Important phenolics in terms of insecticidal, repellent and feeding deterrent functions are flavonoids which are characteristics of higher plants^{31, 23}.

Alkaloids and tannins are also known to possess medicinal and pesticidal properties¹⁵. Alkaloids derived from *Piper longum* fruit³² showed larvicidal activity against *C. Pipiens*. Another study reported the action of tannins isolated from *Hemides musindicus*, *Gymnemasylvestre* and *Eclipta prostrate* that causes mortality in *Culex quinquefasciatus* larvae³³. Steroids derived from *Ocimum bacillum* showed larvicidal activity against *Culex quinquefasciatus*¹³.

Kumar and Sharma (2008) stated *J. Curcas* are known to be toxic, explaining the larvicidal activity shown in the present study³⁴. Moreover, *Jatropha* contains a compound known as phorbol ester that exhibit toxic activity against various types of insects³⁵. Hence, the efficacy of *J. curcas* extracts as larvicides could be attributed of the presence of phorbol esters as toxic components, in addition to alkaloids, flavonoids, and steroids which revealed in the phytochemical screening. On the other hand, the relatively lower larvicidal activities of *J. curcas* shown in the present study indicate that the toxic phorbol esters and other compounds are highly accumulated in *J. curcas* seeds, rather than in other parts of the plant including the bark and leaves.

T. rumphii has been used as pesticide against rice black bugs, rice green leafhoppers, and rice stem borers³⁶. In addition, a study reported that *T. rumphii* is an effective dewormer for goats. Findings of his study showed that the crude extract of *T. rumphii* vine at 40 mL/kg body weight significantly reduced the eggs per gram by 85.6 percent in naturally infected goats³⁷.

A study on the potential of citrus seed extracts against *Aedes albopictus* showed that citrus seed extracts have good larvicidal potential against *Ae. Albopictus* larvae with rough lemon and lemon being the most effective in terms of LC₅₀, LT₅₀ and percent mortalities¹⁴. Citrus plants also contain limonoids which work both as toxicant and feeding deterrents³⁸. Limonoids affect the egg laying process of insects due to nutritional disruption which ultimately induce antifeedant effects³⁹. Thus, the

larvicidal activity of *Citrus grandis* is due to limonoids, in addition to alkaloids, saponins, steroids, flavonoids and tannins.

The present study indicates that the methanolic extracts of stem/bark and leaves of *Jatropha curcas*, *Citrus grandis*, and *Tinospora rumphii* possess larvicidal activity against *Aedes aegypti* mosquito larvae. The numbers of the dead of the larvae were affected by increasing the concentrations of the plant extracts. Moreover, *Tinospora rumphii* leafextract has the highest percentage of mortality among the various concentrations of the plant extracts. The larvicidal activity of the three plants is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins and other compounds which possess insecticidal and larvicidal effects of insects and other pests. Hence, the use of these plant extracts is one of the economic and environmental friendly alternatives to chemical larvicides for mosquito larvae control.

Conclusion

The study on the larvicidal activity of the methanolic leaf and stem/bark of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* against the dengue-vector, *Aedes aegypti* mosquito showed that the leaf and bark methanolic extracts of *Jatropha curcas* contains alkaloids, flavonoids and steroids while the leaf and bark/stem methanolic extracts of *Citrus grandis* and *Tinospora rumphii* are rich in alkaloids, saponins, tannins, flavonoids and steroids. These compounds are known to possess insecticidal and larvicidal activities of insects and other animals.

The three plant samples showed larvicidal activity to *Aedes aegypti* mosquito larvae which is manifested by a high percentage of mortality in comparison to those in the control group. The mortality of mosquito larvae between the various concentrations of the plant extracts and the control group is also significantly different at 0.05 level of significance. Furthermore, *Tinospora rumphii* leaf extract shows the most effective larvicide among the various plant extracts with the percentage mortality of 90% and 93% in 24 and 48 hours of exposure respectively.

The plant's high larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins which showed combination effects in terms of larvicidal action to mosquito larvae. Moreover, *Citrus grandis* bark and *Tinospora rumphii* stem extracts showed significant difference on the increased of the mortality of mosquito larvae with increasing concentrations of the plant extracts at 0.05 level of significance. *Tinospora rumphii* leaf extract is the most effective in larvicidal activity compared to the other extract which is manifested by the lowest LC₅₀ and LC₉₀ values of 10 mg/mL and 46 mg/mL respectively after 48 hours of exposure. On the other hand, the leaf and bark of *J. curcas* is the least lethal to mosquito larvae among the three plant samples.

The larvicidal activities of the three plants differ according to the plant species and part used. The presence of several bioactive chemicals like alkaloids, saponins, tannins, flavonoids and steroids can be attributed to the susceptibility of the plant extracts as killing agent against mosquito larvae.

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