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A Study on Larvicidal Activity of Medicinal Plant Extracts against *Aedes aegypti*

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Abstract:

Mosquitoes are vectors harbouring infection and are responsible for transmission of various dreadful diseases, causing millions of deaths every year. Random use of chemical insecticides has resulted in the development of resistance, resulting in reviving vectorial capacity. Such chemicals have also given rise to many serious environmental issues. To prevent the spreading of mosquito borne diseases and to improve the quality of environment and public health, mosquito control is essential, that are feasible and at the same time effective. This has led to the search for biologically active compounds in plants, which are having several advantages over the chemical insecticides for controlling vector species. The present attempt is to assess the larvicidal ability of the methanol and aqueous leaf extracts of nine medicinal plants extracts against mosquito larvae, Aedes aegypti. Mortality percentages, statistical analysis for determining significant differences and LC_{50} were calculated. Larvicidal activity was found to be increasing at varying concentration and retention time for every plants selected. Out of the 18 test samples (methanol and aqueous medicinal leaf extracts) Lantana camara offers promised as a potential biocontrol agent against A. aegypti particularly in its markedly larvicidal effect. It shows that Lantana camara methanolic leaf extract is the most effective in terms of larvicidal activity (lowest LC_{50} value of 148.466 ppm after 72 hours of incubation). Further studies on the isolation and identification of the active compounds involved and their mode of action and field trials are needed to recommend L. camara as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

Keywords: Lantana camara, larvicidal activity, Aedes aegypti, LC50

1.Introduction

Mosquitoes transmit several public health problems, such as malaria, filariasis, and dengue causing millions of deaths every year. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. The use of herbal products is one of the best alternatives for mosquito control (Nandita, 2008).

The biting Diptera are two-winged flying insects that suck blood from humans and animals. In many parts of the world their biting is a considerable trouble. They are carriers of a number of diseases, mostly in the tropics, causing ailment and death on a large scale. The most important group of biting Diptera is the mosquitoes, which have a long, slender body and long, needle-shaped, penetrating mouthparts. Others include the blackflies, phlebotomine sandflies, tsetse flies, biting midges, horseflies and stable flies, which generally have shorter biting mouthparts and more tough bodies. They are of limited importance as vectors of human disease.

The mosquito *Aedes aegypti*, an important vector of arboviruses such as dengue fever, urban yellow fever, and chikungunya is a holometabolous insect processing a life cycle with four instar stages: Egg, four larval instars, pupa, and adult. The eggs are laid singly on moist surfaces just above or near the water line in temporary pools and other habitats where the water level rises and falls. *A. aegypti* is more closely associated with human residence and uses indoor breeding sites, including water storage tanks and jars inside and outside houses, and roof channels, leaf axils, bamboo stumps and temporary containers such as jars, drums, used car tyres, tin cans, bottles etc. All these habitats typically contain relatively clean water and theyreach the terrestrial environment only as an adult. Its preference for humans as a host is an important factor for transmission. Thus, environmental evaluation at the household level is necessary for dengue control.

Boundless use of conventional pesticides in the water sources, however, introduces many risks to people and the environment. Repellents such as vaporizers, diethytoluimide, and herbs are widely used in the country to combat mosquito nuisance and malaria. These repellents are harmful to human health, and their use should be avoided and discouraged. Although symptoms disappear shortly after withdrawal, those who do not suffer acute toxicity symptoms and continue to use these repellents for long run may suffer neurotoxic and immunotoxic hazard.

Plants are rich sources of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against a number of species including specific target-insects and are eco-friendly. Several secondary metabolites present in plants serve as a

defense mechanism against insect attacks. These bioactive chemicals may act as insecticides, anti-feedants, moulting hormones, oviposition disincentives, repellents, juvenile hormone mimics, growth inhibitors, anti-moulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance and are easily biodegradable (Srinivasan *et al.*, 2013).

Natural products of plant origin with insecticidal properties are generally preferred as an alternative source of mosquito control agents since they are relatively less harmful and also biodegradable. Plant based products do not cause any hazardous effect on ecosystem. Many recent researches has proved that effectiveness of phytochemicals, such as saponins, steroids, flavonoids, essential oils, alkaloids and tannins, are potential mosquito larvicides. Plant's secondary metabolites and their constructed derivatives provide alternative source in the control of mosquitoes.

The interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control. Plant derived compounds have received greater attention from scientists and more than 2000 plant species are already known to have insecticide properties. The objective of the present study was to evaluate the larvicidal activity of plant extracts and the resistance of *Aedes aegypti* mosquito larvae towards selected medicinal plants.

2. Materials and Methods

The present study has been carried out to assess the larvicidal activity of methanol and aqueous extracts of nine medicinal plants belonging to varied taxonomic groups.

2.1. Collection and Preparation of Plant Extracts

The leaves of *Azadirachta indica, Eupatorium perfoliatum, Glyricidia sepium, Lantana camara, Phyllanthus amarus, Mimosa pudica, Justicia adhatoda, Ocimum sanctum* and *Strychnos nuxvomica* were collected, washed thoroughly and shade dried. The washed and air dried plant materials were chopped properly. For the preparation of extracts, approximately 5 grams of leaves were taken and ground using methanol and distilled water using a mortar and pestle. The extract was filtered out and the filtrate was made up to 50 ml and retained as stock solution for further experimentation.From this stock solution, different concentrations were prepared and used for larvicidal bioassay.

2.2. Selection, Collection and Culture of Mosquito Species

Vector species, *Aedes aegypti* was selected for the present study. Mosquito larvae, collected from controlled breeding sites maintained with plastic vessels kept at varying distances around households, were used in the present study. Fig 1 and 2 shows the morphology of *A. aegypti* larva. Collected larvae were pooled in the laboratory and subjected to species level identification using standard reference manual (Dr. Peter Jupp, 2014). The larvae of *A. aegypti* were maintained at room temperature. The breeding cups were covered with wire mesh to avoid contact with foreign mosquitoes. Mean room temperature of $27 \pm 20C$ and a relative humidity of 70-80 per cent were maintained. The larvae were observed for the different instar stages, and the third instar larvae were used throughout the experiment.



Figure1:A. aegypti third instar larva

Figure 2: Siphonal setae characteristic of A. aegypti

2.3. Larvicidal Bioassay

Bioassay for the larvicidal activity was carried out using WHO procedure with minor modifications. From the stock solution of 10%, 1% solution was prepared by proper dilution. Concentrations of 500, 1000 and 2000 ppm was prepared from this. Both methanolic and aqueous extracts were diluted in this manner. Ten healthy larvae were released into each 200 ml disposable glasses containing 100 ml of distilled water and test concentration. Mortality was observed for 24, 48 and 72 hours after treatment. A total of three replicates

were carried out. Controls were run simultaneously. Dead larvae were those incapable of rising to the surface or without the characteristic diving reaction when the water is disturbed. Dead larvae were removed as soon as possible in order to prevent decomposition, which may cause rapid death of the remaining larvae. No mortality was observed with the negative control. Mortality percentage was calculated using the formula,

Mortality (%) =
$$\frac{Number of dead larvae}{Number of larvae introduced} \times 100$$

2.4. Statistical Analysis

The Arithmetic Mean and percentage were evaluated for dead mosquito larvae, Normality of data evaluated with Jarque- Bera test and opted parametric test for further statistical analysis of data, Analysis of Variance (ANOVA) followed by Tukey analysis to determine the significant difference on the mortality of mosquito larvae between selected plants and between three different concentrations (500 ppm, 1000 ppm, 2000 ppm) analysed, Paired t test to determine the significant difference between methanol and aqueous extracts of the plant samples, and Probit Analysis to calculate LC_{50} value to determine lethal concentration of the medicinal plant extract showing highest larval mortality data on *Aedes aegypti* mosquito larvae after 24, 48 and 72 hours of treatment for both methanol and aqueous extracts. (Statistical analysis were done using PAST version 2- 17c software (Hammer *et al.*, 2001) and LC_{50} using MINITAB statistical software, 2010).

3. Results and Discussion

Mosquito control is the major option for mosquito-borne disease control. Technically, the problems associated with resistance to synthetic insecticides in the case of vector mosquitoes have been the foremost cause for recurrence and resurgence in mosquito-borne diseases. It is important to test the susceptibility status of mosquito vectors in different areas to stop the resurgence of communicable diseases. Mosquito control programmes largely target the larval stage at their breeding sites with larvicides. Larviciding is a successful method of reducing mosquito population in their breeding places before they emerge as adult. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Plant extracts are reported to be eco-friendly mosquito control agents. Botanical insecticides and phytotoxic compounds have received much attention. Many plant species have been screened for anti-mosquito activities and their active phytochemical compounds have also been studied. Numerous works have been carried out for testing the efficacy of several plant extracts against different mosquito species.

The present study provides information about the larvicidal efficacy of leaf extracts of nine medicinal plants. Each plant extract showed a dose response action. The results of larval mortality of *Aedes aegypti* tested against methanol and aqueous extracts of selected medicinal plants exposed for three days are presented below (Table 1).

Plants selected	Extracts		500ppm			1000ppm			2000ppm	
		24	48	72	24	48	72	24	48	72
Azadirachta indica	methanol	3.33%	10.00%	23.33%	6.66%	16.66%	56.66%	16.66%	23.33%	76.66%
	aqueous	0.00%	6.66%	26.66%	3.33%	16.66%	46.66%	6.66%	26.66%	70.00%
Eupatorium perfoliatum	methanol	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%	6.66%	10.00%	13.33%
	aqueous	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%
Glyricidia sepium	methanol	0.00%	3.33%	10.00%	0.00%	3.33%	26.66%	3.33%	23.33%	66.66%
	aqueous	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%	10.00%	20.00%
Justicia adhatoda	methanol	0.00%	6.66%	20.00%	3.33%	13.33%	30.00%	20.00%	36.66%	43.33%
	aqueous	0.00%	0.00%	0.00%	0.00%	3.33%	6.66%	3.33%	6.66%	16.66%
Lantana camara	methanol	36.66%	53.33%	86.66%	43.33%	63.33%	93.33%	56.66%	70.00%	100%
	aqueous	16.66%	36.66%	63.33%	26.66%	43.33%	90.00%	30.00%	66.66%	96.66%
Mimosa pudica	methanol	0.00%	0.00%	0.00%	0.00%	3.33%	6.66%	10.00%	16.66%	20.00%
	aqueous	0.00%	0.00%	0.00%	0.00%	3.33%	3.33%	3.33%	3.33%	6.66%
Ocimum sanctum	methanol	0.00%	0.00%	3.33%	3.33%	3.33%	6.66%	6.66%	13.33%	36.66%
	aqueous	0.00%	0.00%	0.00%	0.00%	0.00%	3.33%	3.33%	6.66%	13.33%
Phyllanthus amarus	methanol	3.33%	6.66%	13.33%	6.66%	10.00%	30.00%	10.00%	20.00%	43.33%
	aqueous	0.00%	0.00%	3.33%	3.33%	10.00%	13.33%	3.33%	6.66%	36.66%
Strychnos nuxvomica	methanol	0.00%	0.00%	3.33%	3.33%	6.66%	13.33%	6.66%	10.00%	30.00%
	aqueous	0.00%	0.00%	0.00%	0.00%	3.33%	6.66%	3.33%	10.00%	13.33%

 Table 1: Results of larval mortality tested against nine medicinal plants for both extracts at three different concentrations for three days expressed as mean mortality percentage

The results of the present study revealed that among the extracts tested, methanol extracts exhibited highest larvicidal activity. Significant differences were observed in the toxicity of methanol and aqueous extracts of plants against the third instar larvae of mosquitoes (p value- 6.299E-24). Out of the 9 plants attempted, *Lantana camara* found to be capable of inducing high mortality against mosquito larva at varying concentration and retention time. 100% mortality was observed at 2000 ppm concentration methanol extract after72 hours of exposure.

The effect of methanolic and aqueous extracts of the selected plants at 24, 48 and72 hrs are graphically represented (Fig: 3-8). After 24 hours of exposure among the 9 plants selected, methanol extract of *Lantana camara* showed a larvicidal activity of 36.66% at 500 ppm concentration. The second highest mortality was observed in methanolic leaf extract of *Azadirachta indica* and *Phyllanthus amarus*. At this concentration 0% mortality was showed by all other plants. Larvicidal activity was found to be increasing at 1000 ppm and 2000 ppm concentrations for every plants selected.

After 48 hours of exposure, majority of plants started showing larvicidal activity except aqueous extracts of *Eupatorium perfoliatum*. Highest larvicidal activity was showed by 2000 ppm methanol and aqueous extracts of *Lantana camara* (70% and 66.66% respectively). Aqueous extracts of *Azadirachta indica* showed the second highest larvicidal activity (26.66%) at 2000 ppm.

After 72 hours of exposure, 500 ppm concentration aqueous extracts of *Eupatorium perfoliatum, Glyricidia sepium, Justicia adhatoda, Mimosa pudica, Ocimum sanctum* and *Strychnos nuxvomica* did not show any larvicidal activity against the vector larvae. *Lantana camara* showed 100% larvicidal activity at 2000 ppm methanolic extract concentration and 96.66% mortality at 2000 ppm aqueous extract concentration. 70% mortality rate was observed for the aqueous extracts and 76.66% was observed for methanolic extracts of *Azadirachta indica*.

The results of the present study are comparable with earlier reports. Biological activity of plants is due to the presence of secondary metabolites including alkaloids, terpenoids present in them (Rafael *et al.*, 2009). The types of solvents used for the extraction affect the efficacy between volatile phytochemicals (Shaalan *et al.*, 2005).

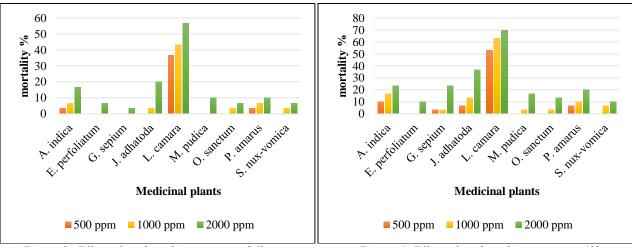
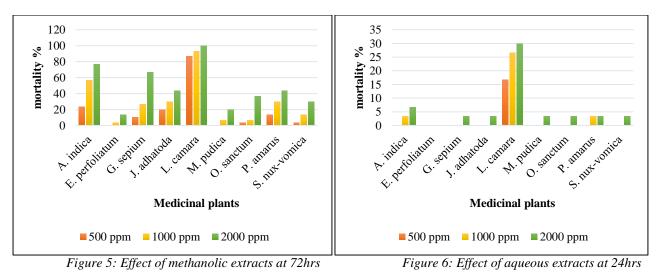
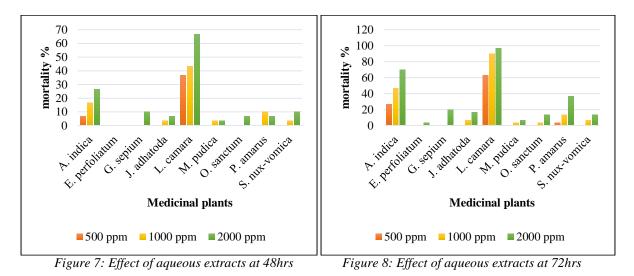




Figure 4: Effect of methanolic extracts at 48hrs





A comparison of larvicidal activity of methanolic and aqueous extracts of each plants at 3 different concentrations for 3 days were observed. The results of medicinal plants showing higher mortality percentages are graphically noted as dose-response lines (Figure 9-12). Methanol versus aqueous extracts of the selected medicinal plants for larvicidal activity at different concentrations showed different mortality rates. When compared the results of each plant, methanolic extracts showed more activity than aqueous extracts against the larva of selected vector, *Aedes aegypti*. Mortality rates were observed to be increasing from 24hrs to 72 hrs.

In the case of *Azadirachta indica*, above 50% (56.66%) mortality was observed at 1000 ppm methanol extract concentration after a retention time of 72hrs. This above 50% mortality was observed in aqueous extract only at 2000 ppm concentration after 72hrs. *Azadirachta indica* leaf extracts showed the second highest larvicidal efficiency. This might be due to the presence of Azadirachtin which is reported to be eco-friendly and not toxic to vertebrates (Al- Sharook *et al.*, 1991). It is clearly proved that crude or partially-purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang *et al.*, 2002).

Eupatorium perfoliatum leaf extracts did not manifested any significant larvicidal activity. Maximum activity was observed at 2000 ppm methanolic extract concentration (13.33%). *E. perfoliatum* has long had a reputation for treating febrile disorders, and was widely used by Indians for this purpose. Chloroform extract of *E. perfoliatum* aerial parts actively inhibited the malarial vector *Plasmodium falciparum* in vitro. The cytotoxicty of this compound was relatively weak. Using a rodent malaria, Lira-Salazar and co-workers in 2006 found that homeopathic potencies of *E. perfoliatum* tincture demonstrated significant – though not completely inhibitory – effects against *Plasmodium berghei*, which were stable over several days. No larval mortality observed in the project done by Latha *et al.*, 1999 and this justifies with the results obtained in this work.

Aqueous leaf extracts of *Glyricidia sepium* presented no larvicidal activity at 500 and 1000ppm concentrations for three days. 66.66% mortality was observed at 2000ppm methanolic leaf extract concentration. Krishnaveni *et al.* (2015) studied the effect of *Gliricidia sepium* leaves extracts on *Aedes aegypti*. It was found that, the methanolic extract of *G. sepium* leaves had an inhibitory effect on the growth of larvae than other solvent extracts. The methanolic extract of *G. sepium* leaves constitutes flavonoids, steroids, glycoside, carbohydrate, and saponins compound.

Justicia adhatoda did not displayed a mortality percentage above 50. Methanolic extracts showed a value equivalent to 43.33% at the highest. The larvicidal activity of leaf extract of *J. adhatoda* might be due to the presence of phytochemical constituent like vasicine (alkaloid) as reported by Rashmi Pa and Linu Mathew in 2012. It was reported that *J. adhatoda* extract possesses potential antibacterial activity and anti-fungal activity. Although the genus *Justicia* contains only a few species that have been chemically and biologically studied, a broad range of biological applications was observed. Lignans are the major components of the active extracts of the species of *Justicia*, exhibiting important pharmacological properties, such as antiviral, antitumoral, anti-inflammatory, and antiplatelet aggregations activities. (Geone M. Corrêa *et al.*, 2011). Here *J. adhatoda* leaf extracts showed no significant larvicidal activity.

Both extracts of *Lantana camara* exhibited high larvicidal activity even at 24hrs of exposure when compared to other plant extracts. The work demonstrates the potency of *Lantana camara* extract, especially methanol extract in the control of *Aedes aegypti* mosquito larvae. Previous studies have shown that the methanol and ethanol flower extract of *Lantana camara* was found to have higher rate of larvicidal rate against *Aedes aegypti*, (Saxena *et al.*, 2012). Essential oil obtained from the leaves of *Lantana camara* showed adulticidal activity against important vectors of malaria, dengue, dengue hemorrhagic fever, yellow fever and chikungunya (VK. Dua, 2010).

Mimosa pudica leaf extracts showed not much larvicidal potential, the maximum being 20% at 2000ppm methanolic extract concentration and only 6.66% for aqueous extract. The leaf extracts of *M. pudica* showed only weak larvicidal activity against the vector. No significant works were done before using this plant for mosquito larvicidal activity. The ethanolic extract of *M. pudica* leaves demonstrated and antimalarial activity and significant antiplasmodial activity against *Plasmodium berghei*. Phytochemical screening revealed the presence of some vital antiplasmodial constituents such as terpenoids, flavonoids and alkaloids. (Baby Joseph *et al.*, 2013).

Ocimum sanctum methanolic leaf extracts exhibited comparatively better effects than that of aqueous extracts. The acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* were studied against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The highest larval mortality was found in leaf extract of *O. sanctum* against the larvae of *A. aegypti* and *C. quinquefasciatus*. (Anees, 2008). In this study, *Ocimum sanctum* showed only slight mortality (less than 50% mean mortality).

Phyllanthus amarus showed highest larvicidal effect of 43.33% for methanolic extract after 72hrs and 36.66% for aqueous extracts at 2000 ppm concentration. *Phyllanthus amarus* exhibited the third highest meant mortality percentage when compared to other 6 plant extracts screened. Similar results were obtained from the study of Oyewole. Here essential oil from *P. amarus* showed larvicidal activity even at low concentration. This has implication on the use of the oil as larvicide with characteristic less toxicity and minimal environmental pollution. There is also a likelihood of low resistance developed to the oil by the mosquitoes. Linalool constitutes the major monoterpenoid identified in a significant amount in this report. (Oyewole, 2010). Leaf ethyl acetate and methanol extract of *P. amarus* showed 100% mortality against *An. stephensi* and *Cx. quinquefasciatus* after 48 h exposure as reported by (Kamaraj *et al.*, 2011).

Strychnos nuxvomica leaf extracts did not manifested a larvicidal potential above 30%. Both extracts showed weak response against the larvae. Results of the larvicidal effects of leaf extracts of *Strychnos nuxvomica* against *Culex quinquefasciatus* was reported and the study exhibited the presence of larvicidal properties in the plant suggesting their use in larval mosquito population control. *Strychnos nuxvomica* ethyl acetate leaf extract was found to be more effective than hexane and chloroform by producing 100 per cent mortality in 500 ppm at 48 hours. (Arivoli and Tennyson, 2012). From the present study methanol and aqueous leaf extracts of *S. nuxvomica* didn't showed any significant larvicidal activity against *Aedes aegypti* larvae. The present study indicates that leaf extracts of *L. camara* had biocontrol activity against *Aedes aegypti*.

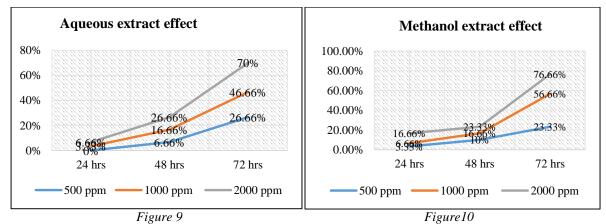


Figure 9 and 10 shows the dose-response line of larvicidal activity of methanol and aqueous leaf extracts of A. indica for three days.

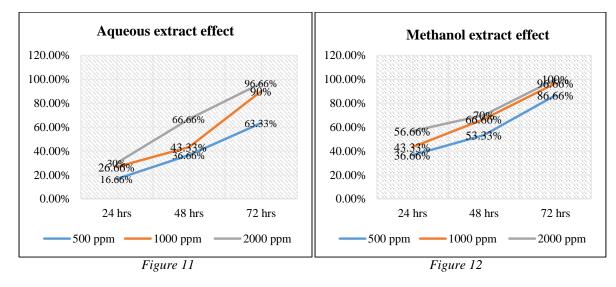


Figure 11 and 12shows the dose-response line of larvicidal activity of methanol and aqueous leaf extracts of L. camara for three days. From above results, it is clear that methanolic extracts showed more larvicidal activity than those of aqueous extracts. At 72hrs methanolic extract of *Lantana camara* showed 100% mortality rate. Above 50% larvicidal activity was observed at the very first day itself at 2000ppm concentration.

Statistical analysis was carried out for calculating the LC_{50} values of methanolic and aqueous extracts of *Lantana camara* (Table: 2 and 3).

	LC50 values (ppm)	χ2	df	
24hrs	1363.274	0.083	1	
48hrs	340.211	0.191	1	
72hrs	148.466	0.227	1	

*Table 2: LC*₅₀ values of methanolic leaf extracts of Lantana camara on Aedes aegypti mosquito larvae after 24, 48 and 72 hrs of treatment

	LC50 values (ppm)	χ2	df
24hrs	9506.536	0.163	1
48hrs	1052.205	0.563	1
72hrs	360.722	0.237	1

 Table 3: LC₅₀ values of aqueous leaf extracts of Lantana camara on Aedes aegypti mosquito larvae after 24, 48 and 72 hrs of treatment

Lantana camara methanolic leaf extract reveals the lowest LC_{50} value of 148.466 ppm after 72 hours of incubation and 340.211 ppm after 48 hours of incubation. It shows that *Lantana camara* methanolic leaf extract is the most effective in terms of larvicidal activity compared to that of aqueous leaf extract of the same plant. On the other hand, aqueous extract of the plant showed high LC_{50} value of 9506.536 ppm after 24hours of incubation. Results show that leaf extract of *Lantana camara* is highly lethal to *Aedes aegypti* larvae.

Flavonoids and cardiac glycosides were present in methanol extract sample of both leaf and flower whereas saponin was present in leaf and terpenoid was found to be present in the methanol extract of flower. Phytol is a diterpene which is present in higher concentration in the methanol leaf extract of *Lantana camara*. The phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells. 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) was found to be present in both methanol leaf and flower extract of *Lantana camara*. These results conclude that the presence of these phytochemicals in *Lantana camara* might be reason for its larvicidal activity. (Sathish and Maneemegalai, 2008).

Jarque- Bera normality test showed that the data was normal, since parametric tests were opted for further statistical analysis. The results of one-way ANOVA showed that there is no significance between *M. pudica* and *O. sanctum*, *J. adhatoda* and *P. amarus*, *O. sanctum* and *S. nuxvomica* with a p value of 1. *A. indica* when compared with all other plants showed significant differences (p values = 0.00001028^* , 0.00001041^* , 0.0003649^* , 0.0008262^*). *L. camara* showed the lowest p value (0.00001028^*) and this makes the significant difference when compared with other plants (F value is 81.23 and p value is 4.051E-84).

Larvicidal mortality data at different concentrations were subjected to one-way ANOVA and the results obtained statistically implies significant difference between 500ppm/2000ppm and 1000ppm/2000ppm (F value = 12.18, p value = 0.000009188).

Paired t test showed significant difference between methanol and aqueous extracts with a p value of 6.299E-24 (< 0.05) and t test value of 11.26.

In the present study, the methanolic extract of *Lantana camara* leaves was found to have an inhibitory effect on the growth of larvae of *A. aegypti*. It was concluded that the methanolic extract of *Lantana camara* was the most effective when compared to aqueous extracts by the mortality rate of *A. aegypti*. As the leaf extract of *L. camara* and *A. indica* are highly toxic even at low doses, these plants may eventually prove to be useful larvicide. The products of these plants can be well utilized for preparing biocides or phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be eco-friendly and may serve as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

4. References

- i. Al-Sharook, Z., Balan, K., Jiang, Y., Rembold, H. (1991). Insect growth inhibitors from two tropical meliaceae: Effect of crude seed extracts on mosquito larvae. J ApplEnt, 111, 425–430.
- ii. Anees, A.M. (2008). Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Parasitol Res, 103(6), 1451–1453.
- iii. Arivoli, S., and Samuel Tennyson. (2012). Larvicidal Efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) Leaf extracts against the Filarial Vector *Culex quinquefasciatus* Say (Diptera: Culicidae). World Journal of Zoology, 7 (1), 06-11.

- Baby Joseph, Jency George, and Jeevitha Mohan. (2013). Pharmacology and Traditional Uses of *Mimosa pudica*. International Journal of Pharmaceutical Sciences and DrugResearch, 5(2), 41-44.
- v. Dr. Peter Jupp. (2014). Arthropod vectors: Mosquitoes. AfriVIP, 9-12.
- vi. Dua, V.K., Pandey, A.C., and Dash, A.P. (2010). Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. Indian. Journal of Medical Research, 131, 434-439.
- vii. Geone M. Corrêa, Antônio, F., and Alcântara, C. (2012). Chemical constituents and biological activities of species of *Justicia* a review. Brazilian Journal of Pharmacognosy, 22(1), 220-238.
- viii. Hammer, O., Harper, D.A.T., and Ryan, P.D. (2001). PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica, 4(1), 9.
- ix. Jang, Y.S., Kim, M.K., Ahn, Y.J., and Lee, H.S. (2002). Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiens pallens* (Diptera: Culicidae). AgricChemBiotechnol, 44, 23–26.
- x. Kamaraj, C., Bagavan, A., Elango, G., AbduzZahir, A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T., and Abdul Rahuman, A. (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus&Culex tritaeniorhynchus*. Indian J Med Res, 134, 101-106.
- xi. Kamaraj, C., Rahuman, A.A., Bagavan, A., Elango, G., Zahir, A.A., and Santhoshkumar, T. (2011). Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors. Asian Pacific Journal of Tropical Medicine,4(9),698-705.
- xii. Krishnaveni, K.V., ThaiyalNayaki, R., and Balasubramanian, M. (2015). Effect of *Gliricidia sepium* leaves extracts on *Aedes aegypti*: Larvicidal activity. Journal of Phytology, 7, 26-31.
- xiii. Latha, C., Vijayakumar, P.D., SaleenaVelayudhan and Ammini Joseph. (1999). Biological activity of indigenous plant extracts as potential larvicides. International Journal of Experimental Biology, 37, 206-208.
- xiv. Lira- Salazar, G., Marines- Montiel, E., Torres- Monzon, J., Hernandez, F., and Salas- Benito, J.S. (2006). Effects of homeopathic medications *Eupatorium perfoliatum* and *Arsenicum album* on parasitemia of *Plasmodium berghei*- infected mice. Homeopathy, 95(4), 223-228.
- xv. Mamta Saxena, Jyoti Saxena and Sarita Khare. (2012). A brief review on: Therapeutical values of *Lantana camara* plant. International Journal of Pharmacy & Life Sciences, 3(3), 1551-1554.
- xvi. Minitab. (2010). MINITAB Statistical Software, Release 16 for Windows. Minitab, Inc.
- xvii. Nandita Chowdhury, Anupam Ghosh and Goutam Chandra. (2008). Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*.BMC Complementary and Alternative Medicine, 8, 10.
- xviii. Oyewole, I. O. (2010). Larvicidal activity of the essential oil from *Phyllanthus amarus* against three species of mosquitoes. Scholars research, 2(6), 136-141.
- xix. Rafael, L., Teresinha, N., Moritz, J.C., Maria, I.G., Eduardo, M.D., and Tania, S.F. (2009). Evaluation of antimicrobial and antiplatelet aggregation effect of *Solidagochilensis*eyen. Int J Green Pharm, 3, 35-39.
- xx. Ramanibai Ravichandran, Ezhumalai Parthiban, Alwarsamy Madhavarani, and Madakkannu Boothapandi. (2015). Larvicidal activity of Selected Medicinal Plant extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quiquefasciatus* (Diptera: Culicidae). International Journal of Multidisciplinary Research and Development, 2 (6), 375-378.
- xxi. RamanibhaiRavichandran, and KanayairamVelayutham. (2014). Larvicidal efficacy of medicinal plant extracts for the control of mosquito vectors. International Journal ofPharma and Bio Sciences, 5(4), 707 715.
- xxii. Rashmi, Pa., and Linu Mathew. (2012). The effect of elicitors on the production of vasicine from *Justicia adhatoda* cultures. IJPSR, 3(10), 3923-3926.
- xxiii. Samuel Tennyson, Arivoli, S., Raveen, R., Bobby, M., and Dhinamala, K. (2012). Larvicidal activity of Areca catechu, Nicotiana tabacum and Piper betle leaf extracts against the dengue vector Aedes aegypti (L.) (Diptera: Culicidae). International Journal ofResearch in Biological Sciences, 2(4), 157-160.
- xxiv. Samuel Tennyson, John Ravindran, K., and Arivoli, S. (2012). Screening of twenty-five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, S1130-S1134.
- xxv. Sanjeeb Kalita., Gaurav Kumar., Loganathan Karthik., and Kokati VenkataBhaskara Rao. (2012). A Review on Medicinal Properties of *Lantana camara* Linn. Research JournalPharm. and Tech, 5(6), 711-715.
- xxvi. Sathish Kumar, M. and Maneemegalai, S. (2008). Evaluation of Larvicidal Effect of *Lantana Camara* Linn. against Mosquito Species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biological Research, 2 (3-4), 39-43.
- xxvii. Shaalan, E.A.S., Canyonb, D.V., Younes, M.W.F., Wahab, H.A., and Mansour, A.H. (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31(8), 1149-66.
- xxviii. Srinivasan, R., Shivakumar, S.M., and Natarajan. (2013). Larvicidal Potential of Some Indian Medicinal Plant Extracts Against *Aedes aegypti*. Asian journal of pharmecutical andclinical research, 77-80.
- xxix. World Health Organization. (2005). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/ 2005.13, 9.