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Larvicidal activity of *Lantana camara aculeata* against three important mosquito species

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Abstract
The resistance to chemical insecticides among mosquito species has been considered as a setback in vector control. The present study is focused on natural products of plant origin with insecticidal properties for control of insect vectors. Aqueous, ethanol, methanol, acetone and chloroform extracts of *Lantana camara aculeata* were evaluated against the fourth instar larvae of three important medically significant mosquito species namely *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.* Phytochemical screening of the leaves showed the presence of phytocompounds such as tannins, alkaloids, flavonoids, anthocyanin, quinines, triterpenoids, flavonoids, saponin and steroids. The leaf extract was also subjected to GC-MS analysis. The percentage mortality of the different mosquito species was tested after 24 hrs of exposure to different concentration of the leaf extract. The extracts of this plant showed potent larvicidal efficacy and can be considered for further investigation**.**

Keywords: *Lantana camara aculeata*, Phytochemicals, Larvicidal activity, *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus.*

1. Introduction
Mosquitoes are the major public health problem throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates [1]. Mosquitoes transmit malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis and chikungunya to humans [2].
Mosquito-borne diseases contribute significantly to disease burden, death, poverty and social debility all over the world, particularly in tropical countries. Among these diseases, malaria remains the most serious vector-borne disease affecting some 300-500 million people and 1.4 to 2.6 million deaths annually throughout the world. More than 40% of the world population lives in areas prone to malaria [3]. Dengue fever can manifest as the classic form of the diseases, which debilitates the patient for a week or more, or as the haemorrhagic form which, in many cases leads to death [4]. Chikungunya virus, a member of alpha virus genus is of considerable public health concern in Southeast Asian and African countries [5].
Mosquitoes in the larval stage are attractive target for control operation due to their low mobility in the breeding habitats and the ease to control in these habitats [6]. Measures to control the mosquito form an essential component of diseases prevention programs in developing countries. Due to increasing resistance of mosquitoes to the insecticides Lima *et al.,* [7] has focussed interest on alternative compounds for mosquito control.
Plants, being a natural source of various compounds are known to contain larvicidal agents, which may act in combination or independently. According to Ghayal *et al* [8] phytochemicals act as general toxicants both against the adult as well as larval stages of mosquitoes, while others interfere with the growth and development, reproduction, produce olfactory stimuli action as a repellent or attractant.
Natural products are best option because they are less harmful to environment and non-target organisms. Several extracts and compounds from different plants families have been evaluated for new and promising larvicides [9]. In recent years, the top priority in finding a new insecticide is that, they must be of plant origin and should not have any ill effects on the ecosystem. Researches have proved the effectiveness of plant derived secondary compounds, such as saponin [10], steroids [11], isoflavonoids [12], essential oil [13], alkaloids and tannins [14], as mosquito larvicides. Plant compounds and their essential oils provide alternative source of mosquito repellents agents [15]. *L. camara aculeata* an important medicinal plant with several medicinal uses in traditional medication system belongs to the family Verbenacea.

It has been used to cure many health problems in different parts of the world. The whole plant and its infusion are also considered to be antipyretic, diaphoretic and antimalarial [16]. The leaves and flowering tops are given as a febrifuge and diaphoretic, it is used to treat yellow fever and has been reported to contain quinine like alkaloid, lantamine [17]. The extract of *L. camara aculeata* roots has been reported to exhibit antimalarial activity against *Plasmodium falciparum* [18] and as a potent source of oleanolic acid a hepatopancreatic agent [19]. Essential oils of different parts of *L. camara aculeata* are reported to possess phenolic compounds; flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoides and tannin are the major phytochemical groups [20]. Essential oil from the leaves of *L. camara* was reported to possess adulticidal activity against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles fluvialitis* and *Anopheles stephensi* [21].

In the present study the larvicidal activity of *L. camara aculeata* plant extracts was investigated against the fourth instar larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.*

2. Materials and Methods

2.1. Selection of plant

L. camara aculeata were collected in the month of May-2014 from the natural population in and around Ranipet, Vellore District, TamilNadu, India and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre (Voucher No: 2158), West Tamabaram, Chennai-45. The whole plant was dried under shade at room temperature for about 20 days.

2.2. Preparation of plant Extract

The dried plant was powdered and sieved to get fine powder using an electric blender. 70g of the powder was filled in the thimble and extracted successively with chloroform, acetone, ethanol, methanol and aqueous using soxhlet extractor for 10 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5 ºC in an airtight bottle until further use.

2.3. Phytochemical screening

Phytochemical screening was carried out using standard procedure [22] and the presence of several phytochemicals listed in Table 1 was tested.

2.4. Separation of bioactive compounds using TLC

Preparation of extract

10 mg / ml of the extract in ethanol solvent was used for TLC examination. The same procedure was followed for methanol extract preparation [23]. The silica gel 60 F_{254} coated aluminum sheets were cut in size 1.5X5.5cm. Prepared methanol extract was loaded on silica plate and air dried. The extracts were standardized in ethyl acetate with acetone and finally chloroform: ethyl acetate: methanol (3; 1.5; 0.5) ratio showed separated bands.

2.5. GC–MS analysis

GC–M S analysis was carried out an SHIMADZU QP 2010T which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column −624 ms (30 m × 0.32 mm × 1.8 m) operating in an electron mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature was 140°C; ion source temperature of 200°C. The oven temperature was programmed from 45°C. Mass spectra were taken at 70 eV.

2.6. GC-MS Identification of compounds

Interpretation of mass spectrum GC–MS was conducted using database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the unknown compounds stored in the NIST library. The compound prediction was based on Dr. Duke's Phytochemical and Ethnobotanical Database by Dr. Jim Duke of the agricultural research service/USDA. The names of the components of the test material were ascertained.

2.7. Selection of Mosquito species

The mosquito species selected for the present study were *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.*

Ae. aegypti (Linnaeus), the yellow fever mosquito spreads dengue fever, chikungunya, yellow fever and other diseases. *Ae. aegypti* is a vector for transmitting several trophical fevers only the female bites for blood which she needs to mature her eggs.

An. stephensi is the vector of malaria in India and larvae of these species are generally found in distinctly different habitat. These are nocturnal and crepuscular in nature and also transmit the filarial worm causing filariasis [24].

Cx. Quinquefasciatus is the vector of West Nile which causes encephalitis or meningitis affecting the brain tissue, resulting in permanent neurological damage [25].

2.8. Mosquito culture

All tests were carried out against laboratory reared *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes was maintained at 25-29 ºC in the insectariums. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 percent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production [26].

2.9. Larvicidal Bioassay

A total of three trials were carried out with five replicates per trial against vector mosquitoes for the following bioassays. Toxicity assays of the crude extract were conducted separately using the fourth instar larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.* Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml acetone and volume raised to 100 ml with distilled water. From this different dilutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were prepared in 200 ml deionised water in 250 ml beaker and 25 fourth instar larvae were released in it and mortality as scored after 24 h. The beakers were kept in a temperature control room at 28 ºC ± 2 ºC and the larvae were exposed to 200 ml water containing 0.1ml of acetone served as control. Each treatment was replicated five times [27].

2.10. Larval susceptibility tests

The larval susceptibility tests were carried according to standard WHO procedure [28]. The extract solutions of different concentrations were prepared and larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus,* were placed in each test solution to observe the larvicidal property as per the following procedure.

Groups of 25 larvae were placed in 200 ml of the extract solution. Control experiments without extract were run in

parallel. The larvae in each solution were then left for 24 h and the numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates. Mortality was recorded when control mortality ranged from 5 – 20 per cent, it was corrected by Abbott's [29] formula.

2.11. Statistical analysis

The average larval mortality data was subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit, lower confidence limit and chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P< 0.05$ were considered to be statistically significant [30].

3. Results

The results of phytochemical characterization of *L. camara aculeata* are presented in Table-1. The preliminary phytochemical screening revealed the strong presence of triterpenoids, saponins, flavonoids, alkaloids and terpenoids in aqueous, methanol and ethanol extracts. The other phytocompounds present were phenols, coumarins, glycosides, tannins and steroids.

GC-MS characterizations of methanolic extracts of *L. camara aculeata* are presented in Table-2. Major compounds observed were caryophyllene, lycoxanthin, pentol, cholestan, octadecanoic acid, cyclopropyl, hexadecanoic acid etc (Fig. 2).

The phytocompounds present in the plant extracts were further analysed by TLC (Fig.1). Methanolic extract of *L. camara* showed seven bands under iodine visualization. The compounds detected in band 1 to 7 with R_f values of 0.96, 0.91, 0.78, 0.68, 0.46, 0.23 and 0.19 showed the presence of steroids, catechin and tannins respectively.

Based on the probit analysis between the concentrations of plant extract against fourth instar larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* after 24 hrs exposure are represented in Table 3, 4 and 5. The results clearly indicate that the plant extract of *L. camara aculeata* at very low concentrations was toxic against all the three mosquito species tested. The methanolic plant extract was found to be more potent against *Cx. quinquefasciatus* and *An. stephensi* with LC_{50} and LC_{90} value of 35.36 ppm and 107.42 ppm and 35.65 ppm and 106.95 ppm when compared to *Ae. aegypti* with LC_{50} and LC_{90} of 39.54 ppm and 118.62 ppm respectively Fig 3.

Methanolic extract of *L. camara aculeata* showed 100% mortality at 150 ppm against the fourth instar larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus.* Ethanolic whole plant extracts were found to be equally effective against the fourth instar larvae of all the three mosquito species. LC_{50} and LC_{90} values were 50.17 ppm and 155.64 ppm against *Cx. quinquefasciatus* when compared to *Ae. aegypti* (60.93 ppm and 181.99 ppm) and *An. stephensi* (79.03 ppm and 237.19 ppm) respectively. All other tested extracts also showed mosquito larvicidal activity at a relatively high concentration when compared to methanol and ethanol plant extracts.

4. Discussion

Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level.

A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control [31]. In the present study methanolic extract of *L. camara aculeata* showed 100% larvicidal activity against the fourth instar larvae of *Cx. quinquefasciatus* when compared to *Ae. aegypti* and *An. stephensi.*

Triterpenoids are generally credited with mosquito larvicidal activities [32]. Thus, high mortality rate recorded in the present study could be due to the presence of terpenoids and triterpenoids which are hydrocarbons present in the extract that inhibits the developmental stages of insects [33, 34, 35].

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities which have been observed by many researches [20]. Similarly, the presence of lantadene triterpenoids and furanonaphtha quinones in *Lantana* sp., have been reported to have mosquito larvicidal properties [9]. DEKA [36], has reported the antifeeding and repellent effect of *L. camara aculeata* on a mosquito. The terpenic compounds, mainly precocenes, with their anti-juvenile hormonal activity are probably responsible for the insecticidal properties.

Table 1: Phytochemical screening of plant extracts of *L. camara aculeata*

S.no	Phytochemicals	Aqueous	Acetone	Chloroform	Ethanol	Methanol
1	Carbohydrates	+++	++	++	+++	+
2	Tannins	+++	–	–	–	+++
3	Saponins	+++	++	++	+++	+++
4	Flavonoids	+++	+++	+++	+++	+++
5	Alkaloids	+++	+++	+++	+++	+++
6	Betacyanin	+++	++	+++	+++	+++
7	Quinones	+++	+	++	++	+++
8	Glycosides	-	–	++	–	–
9	Cardio glycosides	-	–	–	–	–
10	Terpenoids	+++	+	-	+++	+++
11	Triterpenoids	-	++	+++	+++	+++
12	Phenols	+++	–	-	–	+
13	Coumarins	+++	+	+	–	++
14	Acids	++	+	+	+	+
15	Protein	-	-	–	–	–
16	Steroids	++	+++	–	+++	+++

+++ Strongly positive ++ Positive + Trace - Not detected

Table 2: GC-MS analysis of methanol whole plant extracts of *L. camara aculeata.*

S. No	Retention Time	Compounds	Molecular Formula	Molecular Weight
1	11.95	a-Caryophyllene	$C_{15}H_{24}$	204.36
2	13.72	2-[4-Methyl-6-[2,6,6-trimethylcyclohex-1-enyl]hexa-1,3,5- trinet] cyclohex-1-en-1-carboxaldehyded	$C_{23}H_{32}O$	324.49
3	14.05	17-[1,5-Dimethylhexyl]-10,13-dimethyl-3-styrylhexadeahydrocylopenta[a]phenanthren-2-one	$C_{35}H_{52}O$	488.78
4	16.00	Lycoxanthin	$C_{40}H_{56}O$	552.87
5	17.43	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
6	17.93	Ethaneperoxoic acid, 1-cyano-1-[2-[2-phenyl-1,3-dioxolan-2-yl]ethyl]pentyl ester	$C_{19}H_{25}NO_5$	347.17
7	19.17	9-Octadecenoic acid [z]-, methyl esters	$C_{19}H_{36}O_2$	296.48
8	19.35	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254.40
9	23.82	Dasycarpidan-1-methanol, acetate [ester]	$C_{20}H_{26}N_2O_2$	326.43
10	27.82	1H-Cyclopropa [3,4] benz [1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate,[1aR-(1aà,1bá,4aá,5á,7aá,7bà,8à,9á,9aà)]-	$C_{28}H_{83}O_{10}$	534
11	28.72	Cholestan-3-ol, 2-methylene-[3á,5 à]-	$C_{27}H_{48}O$	388.66
12	17.63	Benzenepropanoic acid, 3,5-bis [1,1,-dimethylethyl]-4-hydroxy-,methyl ester	$C_{18}H_{28}O_3$	292.41
13	23.22	9-Octadecenoic acid (Z), hexyl ester	$C_{24}H_{46}O_6$	366.63
14	12.88	1H-Cyclopropa[3,4] benz [1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate,[1aR-(1aà,1bá,4aá,5á,7aá,7bà,8à,9á,9aà)]-	$C_{28}H_{38}O_{10}$	534
15	11.05	1,6,10-Dodecatriene,7,11,-dimethyl-3-methyene-,[Z]-	$C_{15}H_{24}$	204.35

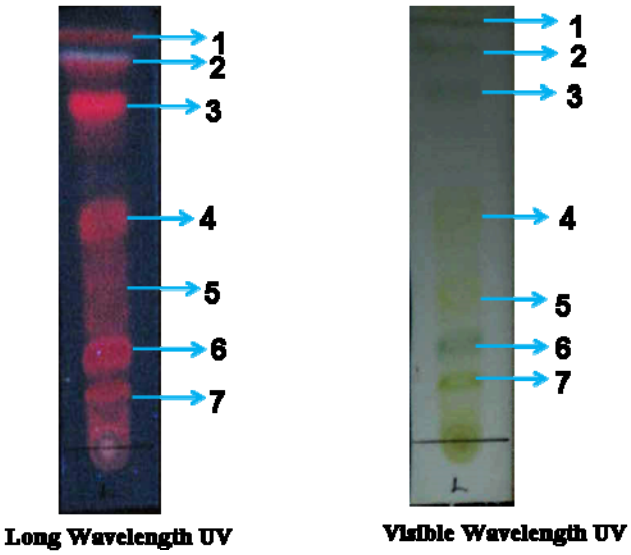

Fig 1: Separation of bioactive fraction of whole plant extracts of *L. camara aculeata* by TLC

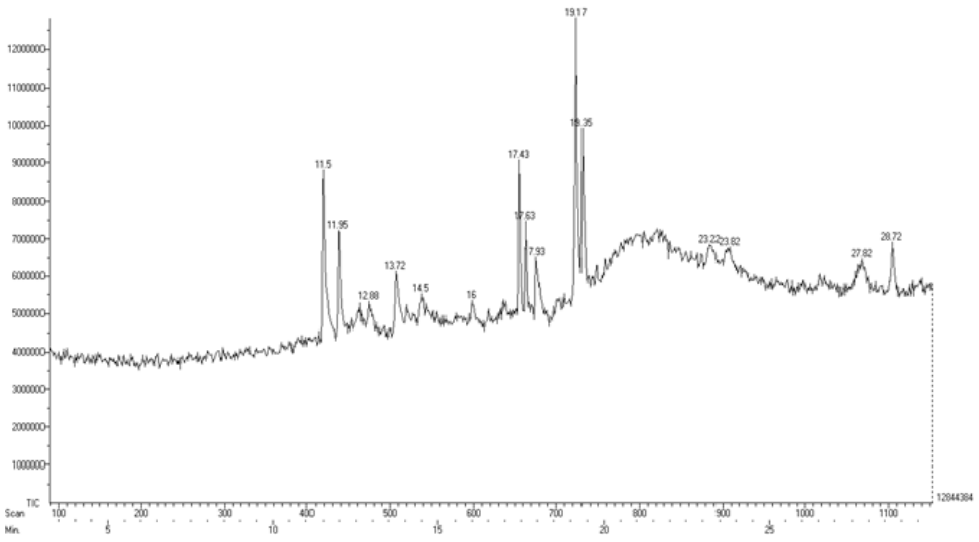

Fig 2: GC-MS analysis of methanol plant extract *L. camara aculeata*

Table 3: Larvicidal activity of plant extracts of *L. camara aculeata* against fourth instar larvae of *Ae. Aegypti*

Extract	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	150 100 75 50 25	60 50 45 25 20	98.10 (123.58-77.87)	294.30 (319.42-276.29.)	0.918
Acetone	150 100 75 50 25	85 65 50 45 35	60.64 (77.59-47.39)	184.72 (201.92-168.42)	0.987
Chloroform	150 100 75 50 25	70 55 40 35 20	86.91 (102.03-74.03)	260.73 (279.44-242.30)	0.980
Ethanol	150 100 75 50 25	85 70 55 40 25	60.93 (70.71-52.50)	181.99 (198.41-169.72)	0.973
Methanol	150 100 75 50 25	100 85 65 55 40	39.54 (53.46-29.25)	118.62 (127.07-104.39)	0.975

Table 4: Larvicidal activity of plant extracts of *L. camara aculeata* against fourth instar larvae of *An. Stephensi*

Extract	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	150 100 75 50 25	56 36 30 25 15	132.82 (154.70-114.30)	344.01 (361.19-327.88.)	0.984
Acetone	150 100 75 50 25	70 55 45 35 30	80.86 (98.80-66.12)	242.04 (261.42-227.09)	0.786
Chloroform	150 100 75 50 25	65 50 40 30 20	98.86 (116.55-83.86)	294.44 (311.42-274.09)	0.992
Ethanol	150 100 75 50 25	80 60 45 30 20	79.03 (89.86-69.50)	237.19 (246.07-214.59)	0.994
Methanol	150 100 75 50 25	100 90 70 55 45	35.65 (57.58-21.71)	106.95 (121.05-92.42)	0.949

Table 5: Larvicidal activity of plant extracts of *L. camara aculeata* against fourth instar larvae of *Cx. Quinquefasciatus*

Extract	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	150 100 75 50 25	65 50 45 20 15	95.55 (112.20-81.37)	244.11 (258.77-231.09)	0.929
Acetone	150 100 75 50 25	90 70 55 35 20	64.24 (72.66-56.81)	192.74 (209.45-166.33)	0.980
Chloroform	150 100 75 50 25	80 65 55 35 25	66.54 (77.65-57.19)	198.72 (211.04-179.59)	0.963
Ethanol	150 100 75 50 25	95 75 60 45 35	50.17 (67.06-42.15)	155.64 (172.22-132.74)	0.992
Methanol	150 100 75 50 25	100 90 80 60 40	35.36 (44.95- 27.81)	107.42 (120.09-91.47)	0.902

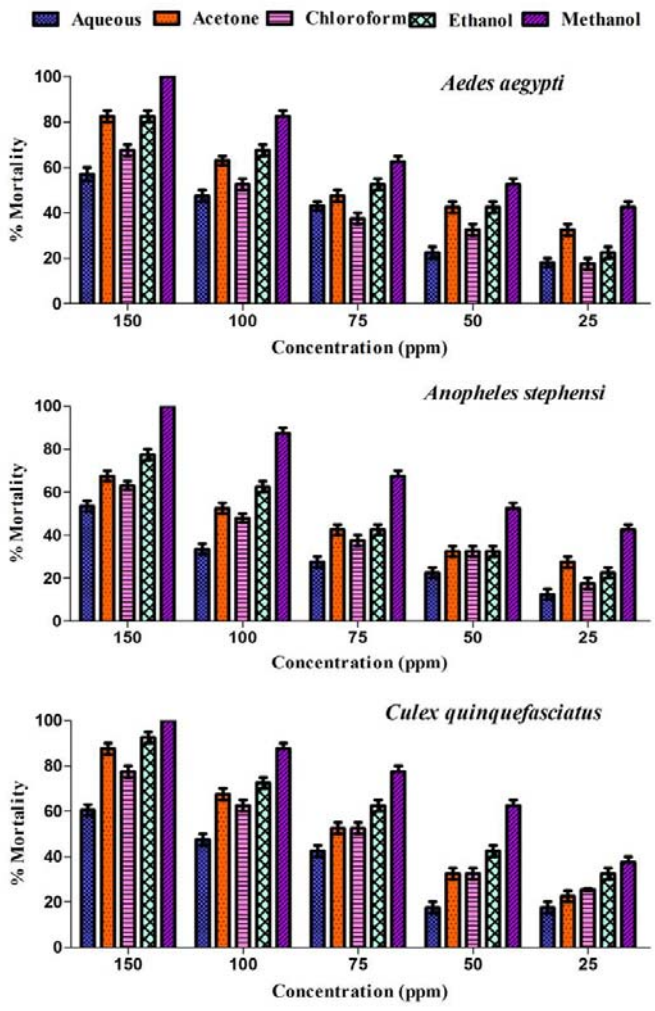

Fig 3: Larvicidal activity of plant extract of *L. camara aculeata* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*

5. Conclusion

It is evident that the plant products are emerging as a potential source of mosquito control. Crude extract or isolated bioactive compounds from the plant *L. camara aculeata* could be used in stagnant water bodies which are known to be the breeding grounds for the mosquitoes. The weed *L. camara aculeata* extracts showed promising activity in mosquito control and its commercial utilization is very much feasible.

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