

Research Article

OVICIDAL AND LARVICIDAL EFFICACY OF CRATAEVA MAGNA (LOUR.) DC. (FAMILY: CAPPARIDACEAE) AGAINST THE *ANOPHELES STEPHENSI*, *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Plant crude extracts might be an alternative source for synthetic pesticides against clinically important vector mosquitoes. In this investigation we determined the ovicidal and larvicidal efficacy of five different solvent crude extract of *Crataeva magna* (Lour.) DC against the malarial, dengue and filarial vector mosquitoes. Larvicidal efficacy of *C. magna* was evaluated according to WHO protocol. Ovicidal efficacy of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* eggs/egg rafts, slightly modified method of Su and Mulla was performed. Ovicidal bioassay conducted with five different extracts confirmed the higher efficacy of methanol extracts exerted the zero hatchability at 240 ppm with *An. stephensi*, 320 ppm with *Ae. aegypti* and 400 ppm with methanol and ethyl acetate extract of *Cx. quinquefasciatus* after 48 h of exposure. Likewise, the methanol leaf extract of *C. magna* was found to be the most effective larvicide with LC₅₀ values of 121.69, 132.09 and 147.27 ppm after 24 h of exposure. The bioactive potential of *C. magna* as new ovicidal and larvicidal against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* vector mosquitoes are being discovered.

Keywords: *Crataeva magna*; Ovicidal; Larvicidal; Vector mosquitoes

INTRODUCTION

Mosquitoes transmit a variety of diseases, such as yellow fever, dengue fever, malaria, several forms of encephalitis and filariasis (WHO, 2014a). *Anopheles stephensi* transmits malaria in the plains of rural and urban areas of India and other parts of the world. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female mosquitoes. About 3.2 billion people almost half of the world's population are at risk of malaria. Young children, pregnant women and non-immune travellers from malaria free areas are particularly vulnerable to the disease when they become infected. Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places. Between 2000 and 2015, malaria incidence (the rate of new cases) fell by 37% globally. In that same period, malaria death rates fell by 60% globally among all age groups and by 65% among children under 5. Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 89% of malaria cases and 91% of malaria deaths. (WHO, 2015a).

Dengue and chikungunya is caused by Flavivirus and Alphavirus, transmitted by Aedes mosquitoes, are a cause of great concern to public health in India. Recently, dengue transmission has strongly increased in urban and semi-urban tropical areas worldwide, becoming a major international public health concern. According to the latest estimation,

there were about 50-100 millions of dengue infections worldwide every year (WHO, 2012). Over 2.5 billion people are now at risk from dengue. Currently, there is no specific treatment for dengue, even if the development of a vaccine is in progress (Murrell *et al.* 2011). Its prevention and control solely depends on effective vector control measures (Suresh *et al.* 2015; WHO, 2015b).

The *Culex quinquefasciatus* is an important vector of lymphatic filariasis and Japanese encephalitis in India. Lymphatic filariasis is caused by Filarididae nematodes, namely *Wuchereria bancrofti*, which is responsible for 90% of cases, *Brugia malayi* and *B. timori*. Microfilariae are vectored through the bites of infected *Cx. quinquefasciatus* to humans, are the most common vectors across urban and semi-urban areas of Asia. (Chadee *et al.*, 2002). Furthermore, these mosquitoes also transmit key pathogens and parasites that dogs and horses are very susceptible to, including dog heartworm, West Nile virus, and Eastern equine encephalitis (WHO, 2012). The World Health Organization (2014b) estimated that there may be 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema. Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty.

In this scenario, mosquito vector control is crucial (Benelli, 2015a). However, synthetic chemicals have strong negative effects on human health and the environment and induce

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resistance in a number of mosquito strains (Hemingway and Ranson 2000). These problems have warranted the need for developing alternative strategies using eco-friendly products (Benelli, 2015b; Pavela, 2015). Plants offer an alternative source of insect control agents because they contain a range of bioactive chemicals (Govindarajan *et al.*, 2013; Benelli *et al.*, 2015a,b), many of which are selective and have little or no harmful effect on non-target organisms and the environment (Benelli *et al.*, 2015a).

Crataeva magna (Lour) DC belonging to family Capparaceae is a high value medium sized deciduous medicinal tree of tropical climate found in tropical regions of the world and also grows almost all over India, especially in the semiarid regions. Medicinal usage has been reported in traditional systems of medicine, such as Ayurveda and Unani (Kirtikar and Basu, 1995; Bopana *et al.*, 2008). This plant is known to possess immense pharmacological activity-nephrotoxicity, arthritis (Geetha *et al.*, 1998), urinary disorders (Deshpanda *et al.*, 1982). In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation (Gagandeep *et al.*, 2006; Kirtikar and Basu, 2005). Considering the biological potential of plant origin, here we have investigated mosquito ovicidal and larvicidal activity of extracts from plant family Capparidaceae. To the best of our knowledge, nothing has been reported about the activity of the plant extracts from *C. magna* leaves against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Materials and methods

Collection, extraction and preparation of plant specimens

The fresh and fully developed leaves of *Crataeva magna* (Lour.) DC were collected from foot hills of courtallam, Tirunelveli District, Tamil Nadu, India, and the taxonomic identification was made by Dr.V.Chelladurai, Retired Research Officer-Botany, Central Council for Research in Ayurvedha and Sida, Tirunelveli, Government of India. After collection, the plant sample (*Crataeva magna*) were washed with fresh water, shade dried and powdered. The dried powder was then subjected to extraction in various solvents viz, hexane, benzene, chloroform, ethyl acetate, and methanol using soxhlet apparatus and solvent evaporation by vacuum evaporator. The plant material was reduced to a viscous dark brown residue and crude extracts were further concentrated to paste and they were covered by aluminium foil sheet and stored in a freezer until assayed. One g of plant crude was dissolved in acetone solvent and 1.0% stock solution was prepared. From this stock solution, different concentrations were prepared and these solutions were used for ovicidal and larvicidal bioassays.

Maintenance of mosquito culture

The eggs of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were collected from the Field station, Centre for Research in Medical Entomology (ICMR-Government of India), Madurai, Tamil Nadu, and India. These eggs were brought

to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 ml of water for hatching. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 12:00 noon to 4:00 p.m., *An. stephensi* and *Cx. quinquefasciatus* were fed during 6:00 to 10:00 p.m. A membrane feeder with the bottom end fitted with parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4°C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37°C was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2°C, 70–85% relative humidity, with a photoperiod of 12 h light and 12 h dark.

Ovicidal bioassay

For ovicidal efficacy of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* eggs/egg rafts, slightly modified method of Su and Mulla (1998) was performed. The different leaf extracts diluted in the appropriate solvent to achieve various concentrations ranging from 80 to 480 ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs/egg rafts under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula

$$\% \text{ of mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

Larvicidal bioassay

Larvicidal activity of *C. magna* was evaluated according to WHO (2005). Based on the wide range and narrow range tests, crude extract was tested at 60, 120, 180, 240 and 300 ppm concentrations. Twenty numbers of early third instar larvae were introduced into a 500 ml glass beaker containing 249 ml of dechlorinated water, and 1 ml of desired concentrations of crude extracts was added. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set of control groups (1 ml of acetone and 249 ml distilled water) with five replicates for each individual concentration.

Statistical analysis

The average larval mortality data were subjected to profit analysis for calculating LC₃₀, LC₅₀, LC₉₀, LC₉₉ and other statistics at 95% confidence limits of upper confidence limit, lower confidence limit, and Chi-square values were calculated using the Statistical Package of Social Sciences 20.0 software. Results with *P*<0.05 were considered to be statistically significant.

RESULTS

The percentage of egg hatchability of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were treated with five different solvents at different concentrations of *C. magna* plant extracts and the results are listed in Table 1. The toxicity of leaf extracts was dependent on its concentration against *An. stephensi*. There was zero hatchability (100% mortality) was attained at the concentration of 240 ppm with methanol. The ethyl acetate extract attained the zero hatchability at the concentration of 320 ppm. The chloroform, benzene and hexane extracts exerted the zero hatchability at 400 ppm. At 480 ppm all the five extracts exerted the zero hatchability. Control eggs exerted the hatchability rate ranged from 96.8 to 100%. The *C. magna* extracts against *Ae. aegypti* exerted the zero hatchability (100% mortality) at 480 ppm with benzene and hexane extracts, while the ethyl acetate and chloroform exerted its complete ovicidal activity at 400 ppm and the methanol extract exerted its complete ovicidal activity at 320 ppm. The hatchability rate of control group with methanol, ethyl acetate, chloroform, benzene and hexane extracts were 99.0, 98.2, 100.0, 99.4 and 97.3%. The hatchability rate of benzene and hexane extracts were 12.4 and 26.5% at 400 ppm, whereas ethyl acetate and chloroform extracts were 20.5 and 29.1% at 320 ppm and methanol extract was 28.9% at 240 ppm. The ovicidal activity of *C. magna* against *Cx. quinquefasciatus* revealed that the hatchability rate of control

egg rafts was ranged from 96.8% to 100%. The methanol and ethyl acetate extracts was notable, which attained the 100% mortality at 400 ppm and the hatchability rate ranged from 58.2% to 16.2%. The chloroform, benzene and hexane extracts attained the 100% mortality at a slightly higher concentration of 480 ppm they exerted the hatchability rate of 10.2, 18.9 and 29.3%.

Larvicidal activity of *C. magna* plant (leaf) extract with five different solvents was tested against the larvae of three important vector mosquitoes viz., *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and the results are presented in Tables 2-4. The leaf extract of *C. magna* screened for larvicidal activity, revealed highest activity in methanol extract followed by ethyl acetate, chloroform, benzene and hexane extracts against *An. stephensi*. The LC₃₀, LC₅₀, LC₉₀, LC₉₉ and regression equation of methanol extract was 76.90, 121.69, 231.16 and 320.40 ppm, and Y=17.7+0.275x respectively and the chi-square values were significant at P<0.05 level. The LC₃₀, LC₅₀, LC₉₀, LC₉₉ values of ethyl acetate, chloroform and benzene extracts were 81.35, 128.32, 243.10 and 336.67 ppm; 85.44, 133.83, 252.09 and 348.49 ppm; 92.96, 143.94, 268.54 and 310.13 ppm respectively. The hexane extract showed lowest activity on *An. stephensi* with LC₃₀, LC₅₀, LC₉₀, LC₉₉ values of 113.59, 165.32, 291.74 and 394.81 ppm.

Among the five extracts tested for larvicidal activity of *C.*

Table 1: Ovicidal activity of *Crataeva magna* leaf extracts against *An. stephensi*, *Ae. aegypti* and *Cx. Cx. Quinquefasciatus*

Mosquito species	Name of the solvent	Percentage of egg hatchability						
		Concentration (ppm)						
		Control	80	160	240	320	400	480
<i>An. stephensi</i>	Hexane	100 ± 0.0	78.2 ± 0.4	70.6 ± 0.8	63.8 ± 0.5	48.5 ± 0.4	22.3 ± 0.8	NH
	Benzene	100 ± 0.0	62.4 ± 0.2	54.9 ± 0.2	46.0 ± 0.2	31.2 ± 0.6	NH	NH
	Chloroform	96.8 ± 0.6	57.5 ± 0.8	50.2 ± 0.4	38.3 ± 0.8	27.4 ± 0.2	NH	NH
	Ethyl acetate	99.8 ± 0.2	49.8 ± 0.6	40.5 ± 0.7	27.5 ± 0.9	NH	NH	NH
	Methanol	98.2 ± 0.4	38.1 ± 0.5	26.3 ± 0.5	NH	NH	NH	NH
<i>Ae. aegypti</i>	Hexane	97.3 ± 0.7	80.5 ± 0.9	74.8 ± 0.6	68.3 ± 0.2	52.3 ± 0.3	26.5 ± 0.9	NH
	Benzene	99.4 ± 0.3	72.9 ± 0.3	68.3 ± 0.3	57.5 ± 0.5	35.4 ± 0.6	12.4 ± 0.5	NH
	Chloroform	100 ± 0.0	68.6 ± 0.7	54.8 ± 0.9	48.0 ± 0.3	29.1 ± 0.8	NH	NH
	Ethyl acetate	98.2 ± 0.4	57.4 ± 0.6	48.5 ± 0.7	35.6 ± 0.9	20.5 ±	NH	NH
	Methanol	99.0 ± 0.6	43.8 ± 0.3	36.7 ± 0.2	28.9 ± 0.3	NH	NH	NH
<i>Cx. quinquefasciatus</i>	Hexane	99.6 ± 0.5	85.1 ± 0.9	78.4 ± 0.2	72.3 ± 0.4	55.1 ± 0.2	29.3 ± 0.6	NH
	Benzene	100 ± 0.0	80.4 ± 0.5	69.2 ± 0.5	63.1 ± 0.6	40.6 ± 0.4	18.9 ± 0.3	NH
	Chloroform	98.2 ± 0.8	71.3 ± 0.2	59.8 ± 0.6	52.0 ± 0.9	33.8 ± 0.5	10.2 ± 0.5	NH
	Ethyl acetate	96.7 ± 0.6	64.5 ± 0.7	56.9 ± 0.8	40.3 ± 0.7	24.3 ± 0.6	NH	NH
	Methanol	99.1 ± 0.3	58.2 ± 0.3	42.7 ± 0.1	33.5 ± 0.2	16.2 ± 0.8	NH	NH

Each value (Mean ± S.D) represents the mean of five replicates. NH: No Hatchability

Table 2: Profit analysis for larvicidal activity of *Crataeva magna* against *Anopheles stephensi*

Solvent used	LC ₃₀ (ppm) (LCL-UCL)	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	LC ₉₉ (ppm) (LCL-UCL)	Regression equation	χ ² (df=4)
Hexane	113.59 (99.11-126.28)	165.32 (153.50-177.34)	291.74 (272.34-316.21)	394.81 (364.37-434.29)	Y=0.8+0.303x	9.020 ^a
Benzene	92.96 (43.61-125.28)	143.94 (109.71-176.95)	268.54 (226.15-348.31)	370.13 (304.74-504.35)	Y=11.5+0.282x	14.336 ^a
Chloroform	85.44 (26.10-120.83)	133.83 (94.43-170.38)	252.09 (207.82-342.63)	348.49 (280.92-502.39)	Y=15.3+0.278x	18.628 ^a
Ethyl acetate	81.351 (19.02-117.81)	128.32 (86.66-166.12)	243.10 (198.47-337.67)	336.67 (268.92-49.82)	Y=20.5+0.272x	20.598 ^a
Methanol	76.90 (10.93-114.04)	121.69 (178.00-160.45)	231.16 (186.73-329.04)	320.40 (253.47-488.38)	Y=17.7+0.275x	22.771 ^a

LCL: Lower Confidence Limits; UCL: Upper Confidence Limits; χ²: Chi Square; df: Degrees of Freedom; ^a Significant at P<0.05

Table 3: Profit analysis for larvicidal activity of *Crataeva magna* against *Aedes aegypti*

Solvent used	LC ₃₀ (ppm) (LCL-UCL)	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	LC ₉₉ (ppm) (LCL-UCL)	Regression equation	χ ² (df=4)
Hexane	124.48 (110.09-137.19)	177.17 (165.7-331.79)	305.91 (285.52-331.79)	410.87 (379.00-452.39)	Y=-4.9+0.312x	6.221 ^a
Benzene	100.47 (53.75-132.07)	155.00 (123.38-187.68)	288.25 (244.45-369.24)	396.89 (328.79-532.44)	Y=9+0.277x	12.498 ^a
Chloroform	93.19 (42.91-126.03)	145.96 (111.32-179.48)	274.91 (231.32-357.28)	380.03 (312.49-518.90)	Y=12.1+0.275x	14.052 ^a
Ethyl acetate	87.48 (34.38-121.16)	138.42 (102.12-172.53)	262.88 (219.71-346.03)	364.36 (297.74-505.31)	Y=14.8+0.273x	15.457 ^a
Methanol	83.71 (24.48-119.33)	132.09 (92.22-168.72)	250.34 (206.08-341.09)	346.75 (279.15-501.39)	Y=16.5+0.275x	18.788 ^a

LCL: Lower Confidence Limits; UCL: Upper Confidence Limits; χ²: Chi Square; df: Degrees of Freedom;

Table 4: Profit analysis for larvicidal activity of *Crataeva magna* against *Culex quinquefasciatus*.

Solvent used	LC ₃₀ (ppm) (LCL-UCL)	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	LC ₉₉ (ppm) (LCL-UCL)	Regression equation	χ ² (df=4)
Hexane	140.57 (126.41-153.23)	193.82 (181.60-206.75)	323.94 (302.24-351.79)	430.03 (396.35-474.28)	Y=-8.7+0.308x	4.234 ^a
Benzene	124.92 (81.33-156.24)	183.88 (152.34-219.75)	327.99 (278.45-422.43)	445.47 (368.63-600.28)	Y=-3.1+0.295x	11.270 ^a
Chloroform	111.27 (49.14-156.27)	175.25 (134.75-220.69)	331.59 (271.78-464.97)	459.05 (365.34-682.28)	Y=6.2+0.27x	15.488 ^a
Ethyl acetate	100.62 (42.95-136.86)	156.36 (117.77-195.29)	292.57 (242.59-396.12)	403.62 (326.07-578.13)	Y=9.8+0.27x	16.274 ^a
Methanol	93.52 (34.71-129.78)	147.27 (108.00-185.27)	278.64 (230.63-377.36)	385.74 (311.43-553.14)	Y=12.6+0.27x	16.975 ^a

LCL: Lower Confidence Limits; UCL: Upper Confidence Limits; χ²: Chi Square; df: Degrees of Freedom; ^a Significant at P<0.05

magna against *Ae. aegypti* the methanol extract showed more potent larvicidal activity than the other four extracts. The LC₃₀, LC₅₀, LC₉₀, LC₉₉ and the regression equation of methanol extract were 83.71, 132.09, 250.34 and 346.75 ppm and Y=16.5+0.275x respectively and the chi-square value was significant at P<0.05 level. The LC₅₀ and LC₉₉ value of ethyl acetate, chloroform, benzene and hexane extract were 138.42 and 364.36 ppm; 145.96 and 380.03 ppm; 155.0 and 396.89 ppm, 177.17 and 410.87 ppm respectively. The methanol extract of *C. magna* registered as most potent larvicide among the five extracts evaluated with the LC₅₀ value of 147.27 ppm, the other extracts showed with the LC₅₀ values of 156.36 ppm (ethyl acetate), 175.25 ppm (chloroform), 183.88 ppm (benzene) and 194.82 ppm (hexane) respectively. The LC₉₀ values were 278.64 ppm (methanol), 292.57 ppm (ethyl acetate), 331.59 ppm (chloroform), 327.99 ppm (benzene) and 323.94 ppm (hexane) against *Cx. quinquefasciatus*. The chi-square values were 16.975, 16.274, 15.488, 11.270 and 4.234 significant at P<0.05 level with these extracts. There was no mortality observed in control group.

DISCUSSION

The ovicidal activity of *C. magna* plant leaf extracts were tested against three clinically important mosquito species namely *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The concentration of the leaf extracts vary with the different crude extracts used. *C. magna* showed ovicidal efficacy with the concentration of 80-480 ppm, among the three mosquitoes tested with *C. magna* the methanol extract was more effective with *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus*. 240 ppm was the effective concentration for *An. stephensi*, whereas *Ae. aegypti* and *Cx. quinquefasciatus* were required 320 and 400 ppm with methanol extracts and at this concentration 100% mortality (zero hatchability) were recorded. The hexane extract of *C. magna* are less effective than other extracts against all the tested mosquito species. The current observations were similar to those of Cheah et al. (2013). In their ovicidal assay, 500 ppm of *Artemisia annua* extract was found to have a severe ovicidal effect. The hatchability percentages in the 500 ppm group were 48.84 ±

4.08, 38.42 ± 3.67, and 79.35 ± 2.09% for *Ae. aegypti*, *An. sinensis*, and *Cx. quinquefasciatus*, respectively. Another study reported that zero hatchability was observed in

An. stephensi and *Ae. aegypti* exposed to 300 ppm of *Delonix elata* leaf methanol extract and 500 ppm of *D. elata* seed methanol extract, respectively (Govindarajan et al. 2012).

Considering the larvicidal efficacy of *C. magna* plant extract results showed that this crude extracts exerted insecticidal effect against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* third instar larvae. The bioactivity of phytochemical against mosquito larvae can vary significantly depending upon the plant species, plant parts, age of plant parts, solvent used in extraction and mosquito species. Concentrations can occur when bioactive chemicals accumulate in the various parts of the plant, such as leaves, stems, bark, flowers, fruits, seeds and roots. Mosquito larvae of different species display different susceptibilities to the same phytochemicals (Shallan et al., 2005).

In the present investigation the *An. stephensi* was more susceptible than the other two species tested (*Ae. aegypti* and *Cx. quinquefasciatus*). Out of the five organic solvent extracts the methanol extract from leaves of *C. magna* significant larvicidal activity against *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus* were recorded (LC₅₀=121.69, 132.09 and 147.27 ppm), the methanol extract followed by ethyl acetate (LC₅₀=128.32, 138.11 and 156.36 ppm), chloroform (LC₅₀=133.83, 145.96 and 175.25 ppm), benzene (LC₅₀=143.94, 155.0 and 183.88 ppm) and hexane extracts (LC₅₀=165.32, 177.17 and 193.82 ppm), respectively. The *Cx. quinquefasciatus* was more tolerant towards the crude extracts to all the bioactivity evaluated. Similar results were observed by Kamaraj et al. (2011). They revealed methanol and ethyl acetate extracts of coriander seeds were toxic against larvae of *An. stephensi* and *Cx. quinquefasciatus*.

The results of the present study are comparable with earlier studies. The toxicity of the late third instar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* to hexane

extracts of *Artemisia annua* was tested and the LC₅₀ values were 244.55, 276.14 and 374.99 ppm Cheah *et al.* (2013). Kannathasan *et al.* (2007) determined the larvicidal assay with methanol leaf extracts of *Vitex negundo*, *V. trifolia*, *V. peduncularis*, and *V. altissima* with LC₅₀ values of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth instar larvae of *Cx. quinquefasciatus*. In addition to that, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Govindarajan, 2011). Therefore, it could be an alternative to synthetic insecticides because they are effective, eco-friendly, easily biodegradable, and inexpensive. Whereas, synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals.

CONCLUSION

The result of the present investigation indicates that the leaf extract of *C. magna* exhibits both ovicidal and larvicidal efficacy against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Further analysis to isolation, purification and characterization of bioactive components is underway in our laboratory, which will be reported in the near future.

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