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ORIGINAL RESEARCH ARTICLE

Evaluation of Larvicidal Activity of *Ageratina adinophora* (Spreng.) King & H.Rob against *Culex quinquefasciatus* (Say)

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ABSTRACT

Mosquito borne diseases are one of the most important health problems widespread in the developing and developed countries also. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. The present study was carried out on the larvicidal activity of three selective insidious medicinal plants – *Ageratina adinophora* (Spreng.) king & H.Rob against third instar larvae of *Culex quinquefasciatus*. Larvicidal activities were conducted at different concentration (50-250 ppm) of ethyl acetate, hexane, chloroform and acetone leaf extracts of these plants. The mortality was recorded after 24 hrs exposure and LC_{50} and LC_{90} were determined. The present investigation revealed that the LC_{50} and LC_{90} value of ethyl acetate, hexane, chloroform and acetone extract of *Ageratina adinophora* against *Cx. quinquefasciatus* I-instar larvae in 24 hrs were 136.75, 145.69, 139.49 and 143.64, 149.30, 158.24, 151.95 and 156.14 mg/L, respectively. The LC_{50} and LC_{90} value of II- instar larvae were 140.56, 148.75, 143.21 and 146.24, 153.14, 163.33, 155.71 and 159.23 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively. The LC_{50} and LC_{90} value of III- instar larvae were 144.90, 155.40, 146.80 and 150.77, 158.16, 175.18, 161.38 and 167.17 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively. For IV- instar larvae the calculated LC_{50} and LC_{90} values were 149.89, 165.55, 152.83 and 156.71, 164.57, 192.73, 170.07 and 176.35 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively. It is concluded that the highest larvicidal activity against *Cx. quinquefasciatus* was obtained with ethyl acetate extract of *Ageratina adinophora.*

Key words: *Ageratina adinophora. Culex quinquefasciatus*. Larvicidal activities.

1. INTRODUCTION

Prevention of mosquitoes from biting is one of the major problems in the world. Mosquitoes transmitting serious human diseases and causing millions of death every year [1]. Several mosquito species belonging to the genera *Anopheles*, *Culex*, *Aedes* are the vectors for many pathogens of various mosquito borne diseases like Malaria, Filarial, Japanese encephalitis, Dengue fever, Yellow fever etc. These Mosquitoes and mosquito-borne diseases spreads globally and cause high levels of human mortality, also act as impediment to the economic development of most of the developing countries across the world [2]. In 2010, WHO reported 216 million cases of malaria in the world with an estimated, 6, 55,000 malaria deaths. Out of 120 million actual cases of lymphatic filariasis in 83 countries, India alone contributes around 39% [3, 4]. Three billion people in the endemic areas are at risk of infection with Japanese encephalitis and incidence of the disease is 30,000–50,000 cases annually [5]. Over 40% of the world's population approximately (2.5 billion) is at risk from dengue, WHO estimated 50–100 million dengue infections worldwide, annually [6]. Moreover, there are an estimated 200,000 cases of yellow fever (causing 30,000 deaths) worldwide annually. India is afflicted with six major vector-borne diseases namely malaria, dengue, chikungunya, filariasis, Japanese encephalitis and leishmaniasis, causing millions of deaths every year [7]. *Cx. quinquefasciatus* say is widely dispersed domestic mosquito and principal vector of lymphatic filariasis in India [8]. Lymphatic filariasis affects at least 120 million people in 73 countries including India and in the remaining countries in Africa, Southeast Asia and pacific Islands [9]. *Cx. quinquefasciatus* is responsible for major public health problems in India with around

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31 million Microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection [10].

Plants are the nature's biochemical factories. they bio-synthesize a diverse array of different natural product, such as alkaloids, terpenes and terpenoids, phenolic compounds, flavonoids and coumarins through their structural mechanisms to reduce insect attacks, both constitutive and inducible, while insects have evolved strategies to overcome these plant defenses [11]. Antioxidant principles from natural sources possess multifacetedness in their multitude and magnitude of activities to provide enormous scope in correcting the imbalance [12]. Ancient literature mentions many herbal medicines for treating various diseases like diabetic mellitus, rheumatoid arthritis and cardiovascular diseases. Unfortunately in India many potential medicinal plants used as ancient folklore medicine, lack scientific documentation. The family Asteraceae comprises some 20,000 species and is the second largest family of higher plants. *Ageratina adenophora* is a perennial herbaceous shrub that may grow to 1 or 2 metres (3.3 or 6.6 ft) high. It has opposite trowel-shaped serrated leaves that are 6–10 cm (2.4–3.9 in) long by 3–6 cm (1.2–2.4 in) in width. The small compound flowers occur in late spring and summer, and are found in clusters at the end of branches. Each flowerhead is up to 0.5 cm in the diameter and creamy white in colour. They are followed by a small brown seed with a white feathery 'parachute' [13]. It is native to Mexico, but it is known in many other parts of the world as an introduced species and often a noxious weed. It has caused great economic loss in agriculture in southwestern China, and is threatening the native biodiversity there. It was first inadvertently introduced to Yunnan around 1940, and its rapid spread is due in part to its allelopathic competition with other plant species [14]. In this context, the purpose of the present investigation is to explore the larvicidal properties of *A. adenophora* leaf extract and against Chikungunya vector, *A. aegypti*, under the laboratory conditions.

2. MATERIALS AND METHODS

Collection and Identification of Plant material

The medicinal plants, *A. adenophora* were collected from Yercaudu in Tamilnadu, India. Bulk samples were air-dried in the shad. After drying, these were ground to fine powder. At the time of collection, voucher herbarium specimens were prepared and identified with the help of Plant Taxonomist, Department of Botany, Annamalai University, and Chidambaram.

Extraction method

The dried leaves (100 g) were powdered mechanically using commercial stainless steel Blender and extracted sequentially with ethyl acetate, hexane, chloroform and acetone (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26 mm Hg at 45^{o}C by 'Rotavapour' and the residue obtained was stored at 4^{o}C in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4^{o}C.

Larvicidal activity

The larvicidal activity of crude extract was evaluated as per the protocol previously described by WHO [15]. From the stock solution, six different test concentrations (50, 100, 150, 200, and 250 mg/l) were prepared and tested against the freshly moulted (0 – 6 hrs) III instar larvae of *Cx. quinquefasciatus*. The test medium (500 ml plastic cups) was prepared by adding 1 ml of appropriate dilution of test concentrations and mixed with 249 ml of dechlorinated water to make up 250 ml of test solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The control (without plant extracts) experiments were also run parallel with each replicate. For each experiment, six replicates were maintained at a time. A minimum of 25 larvae per concentration was used for all the experiments. The larval mortality was observed and recorded after 24 h post-treatment. Percent mortality was corrected for control mortality using Abbott's [16].

Statistical analysis

The larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} [17] and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit values were calculated using the [18] software package 12.0. Results with $p \leq 0.05$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Larvicidal activity of ethyl acetate, hexane, chloroform and acetone crude leaf extracts of *A. adenophora* are shown in Table 1 & 2. As evidenced from the table, generally increased larval mortality was observed with increased concentration of the extracts tested against *Cx.*

quinquefasciatus. The present investigation revealed that the LC_{50} value of ethyl acetate, chloroform, hexane and acetone extract of *A. adenophora* against *Cx. quinquefasciatus* I-instar larvae in 24 hrs were 136.75, 145.69, 139.49 and 143.64, 149.30, 158.24, 151.95 and 156.14 mg/L, respectively.

Table1: Larvicidal activity of different solvent leaf extracts *A. adenophora* against instar larvae of *Cx. quinquefasciatus*

Larval stage	Extract	LC_{50} (mg/L)	95%Confidence limits		LC_{90} (mg/L)	95%Confidence limits		Chi-squire	Regression
			LCL	UCL		LCL	UCL		
	Ethyl acetate	136.75	133.51	140.07	149.30	146.07	152.60	6052	Y=33.62x-66.82
I- instar larvae	Hexane	145.69	142.64	148.80	158.24	155.21	161.34	6105.2	Y=35.69x-72.21
	Chloroform	139.49	136.33	142.72	151.95	148.81	155.71	5988	Y=34.48x-68.94
	Acetone	143.64	140.57	146.78	156.14	153.07	159.27	6037.5	Y=35.37x-71.32
	Ethyl acetate	140.56	137.40	143.79	153.14	149.99	156.36	6098.5	Y=34.41x-68.92
II- instar larvae	Hexane	148.75	145.32	152.27	163.33	159.90	166.84	4735	Y=31.56x-63.58
	Chloroform	143.21	140.13	146.36	155.71	152.64	158.85	6035.1	Y=35.27x-71.04
	Acetone	146.24	143.07	149.48	159.40	156.23	162.63	5619.6	Y=34.25x-69.16
	Ethyl acetate	144.90	141.67	148.19	158.16	154.94	161.44	5691.2	Y=33.69x-67.81
III- instar larvae	Hexane	155.40	151.01	159.91	175.18	170.76	179.72	2818.7	Y=24.62x-48.95
	Chloroform	146.80	143.32	150.37	161.38	157.91	164.93	4722.9	Y=31.17x-62.54
	Acetone	150.77	146.99	154.66	167.17	163.38	171.05	3949.9	Y=28.58x-57.25
	Ethyl acetate	149.89	146.46	153.40	164.57	161.13	168.07	4803.1	Y=31.58x-63.72
IV- instar larvae	Hexane	165.55	160.00	171.30	192.73	186.98	198.64	1607.9	Y=19.41x-38.08
	Chloroform	152.83	148.91	156.85	170.07	166.14	174.10	3503	Y=27.60x-55.29
	Acetone	156.71	152.39	161.15	176.35	171.99	180.81	2789.9	Y=24.99x-49.87

Control no mortality - Mean values of five replicate

Table 2: Larvicidal activity of different solvent leaf extracts *A. adenophora* against instar larvae of *Cx. quinquefasciatus* at 250 mg/L

Mosquito larvae	Solvents	Concentration				
		50	100	150	200	250
	Ethyl acetate	40.4±2.64	61.4±3.42	84.2±3.78	100.00±0.00	100.00±0.00
I- instar larvae	Hexane	21.6±1.81	40.8±4.21	61.2±2.94	83.6±2.16	100.00±0.00
	Chloroform	32.6±3.56	56.8±3.64	81.8±4.24	100.00±0.00	100.00±0.00
	Acetone	22.8±1.94	42.6±2.54	67.6±3.27	92.2±1.81	100.00±0.00
	Ethyl acetate	31.8±2.58	52.4±2.96	72.6±2.86	91.6±2.44	100.00±0.00
II- instar larvae	Hexane	15.4±1.51	32.4±2.48	51.2±3.34	66.8±2.96	88.4±1.87
	Chloroform	24.6±2.48	47.8±2.30	68.6±2.12	91.6±1.51	100.00±0.00
	Acetone	20.8±2.12	34.6±2.16	60.4±1.81	81.2±2.58	95.87±1.64
	Ethyl acetate	22.6±2.16	40.8±2.96	60.8±2.88	75.8±2.50	96.4±2.60
III- instar larvae	Hexane	12.6±1.81	20.6±2.54	32.6±2.28	52.8±3.27	67.2±3.57
	Chloroform	20.4±1.78	35.6±2.20	56.6±2.96	71.2±3.24	88.2±2.19
	Acetone	16.4±2.77	27.8±1.51	41.6±2.50	60.8±3.08	80.4±2.58
	Ethyl acetate	15.6±1.87	28.2±1.41	43.8±2.28	63.4±2.30	88.2±2.19
IV- instar larvae	Hexane	4.4±1.14	12.4±1.81	24.6±2.60	35.4±2.64	53.4±1.73
	Chloroform	11.8±1.94	23.2±2.28	40.2±3.03	60.4±4.08	77.3±2.68
	Acetone	8.8±1.14	20.8±2.16	31.8±3.63	52.4±3.13	68.2±2.48

The LC_{50} and LC_{90} value of II- instar larvae were 140.56, 148.75, 143.21 and 146.24, 153.14, 163.33, 155.71 and 159.23 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively. The LC_{50} and LC_{90} value of III- instar larvae were 144.90, 155.40, 146.80 and 150.77, 158.16, 175.18, 161.38 and 167.17 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively. For IV- instar larvae the calculated LC_{50} and LC_{90} values were 149.89, 165.55, 152.83 and 156.71, 164.57, 192.73, 170.07 and 176.35 mg/L for ethyl acetate, chloroform, hexane and acetone, respectively (Table 1). It is concluded that the highest larvicidal activity against *Cx. quinquefasciatus* was obtained with ethyl acetate extract of *A. adenophora*.

DISCUSSION

Vector control is the most valuable method of reducing frequency of mosquito borne disease today. In this regards, herbal products are the greatest alternative due to their convenience, environmental protection and less toxic to humans. *A. adenophora* has been reported for its mosquito larvicidal activity from different country in the world. However, the current investigation revealed that *A. adenophora* are collected from Yercaudu in Tamilnadu, India. This result is also comparable to earlier reports of Elumalai [19] reported that the larvicidal activity of various extracts of *Gymnema sylvestre* against the Japanese Encephalitis vector, *Culex tritaeniorynchus.*

The LC_{50} values of 34.756 µg/mL (24.475-51.41), 31.351 µg/mLn (20.634-47.043) and 28.577 µg/mL (25.15932.308) were calculated for acetone, chloroform and methanol extract with the chi-square values of 10.301, 31.351 and 4.093 respectively. Krishnappa and Elumalai [20] reported that the methanol extract of *A. indicum*

exerted 100% mortality (zero hatchability) at 120, 150 and 180ppm for *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*, respectively. Similarly, the methanol extract of *D. palmatus* exerted 100% mortality (zero hatchability) at 200, 250 and 300ppm for *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*, respectively. Gokulakrishnan [21] reported that the larvicidal and Ovicidal efficacy of different solvent leaf extract of *Ariitolochia indica* against *Anopheles stephensi*. The hatch rates were assessed 48 h after treatment. The LC_{50} and LC_{90} values of Acetone, Benzene, Chloroform, Hexane and Methanol extracts of *A .indica*against *An. Stephensi* larvae in 24 h were 76.29, 58.82, 53.59, 65.84, 51.78 and 205.85, 193.23, 185.16, 196.72 and 181.00 ppm, respectively. Among five solvent extracts tested the maximum efficacy was observed in the methanol extract and followed by Chloroform, Benzene, Hexane and acetone extracts of *A. indica*. The Chi-square values were significant at $P< 0.05$ level. Among five solvent extracts tested methanol crude extract was found to be most effective for Ovicidal activity against *A.stephensi* mosquito. The extract of methanol exerted 100% mortality at 90 ppm against *A. stephensi.* Ovicidal activities of acetone, benzene, ethyl acetate, hexane and methanol extracts of *Melothria maderaspatana* against *Aedes aegypti*. The hatch rates were assessed 48 h post treatment. The crude extracts of acetone, benzene, ethyl acetate, hexane and methanol *M. maderaspatana* exerted 100% egg mortality (zero hatchability) at 240, 200, 160, 160 and 120 ppm for *Ae. aegypti,* Balu selvakumar [22]. Ilahi [23] studied the larvicidal activity of aqueous extracts of bark, fruits and leaves of *Melia azedarach* (Linn) against *Culex quinquefasciatus* at the concentrations of 50, 100, 500, 1000, 1500 and 2000 ppm. Among these extracts, the bark extract caused significantly higher mortality of 3rd and 4th instar larvae of Culex *quinquefasciatus*. Sakthivadivel [24] reported the larvicidal activity of *Argemone mexicana* (Linn), *Clausena dentate* (Wild) M. Roem, *Sepadessa baccifer* (Roth), *Dodonaea angustifolia* (Linn) and *Melia dubia* (Cav) against *Culex quinquefasciatus*. Elango [25] reported that the larvicidal activity of different crude extracts of *E. pedunculatum* was tested against fourth instar larvae *of An. stephens*i. The LC_{50} value of hexane extract was recorded to be 127.45ppm; for chloroform 127.39ppm; petroleum ether with 151.96ppm and the least LC_{50} value of 121.24ppm was recorded with ethanol extract. Likewise, the LC_{90} values and their LCL, UCL concentrations were determined as 231.20 (215.07252.44ppm), 249.13 (203.11-373.03ppm), 255.49 (234.22-271.74ppm) and 240.57 (194.81- 368.30pp) were noticed against hexane, chloroform, petroleum ether and ethanol extracts respectively towards the fourth instar larvae of *An. stephensi*. The data obtained in this experiment was pertinent to note that 200-300ppm concentration of the petroleum and ethanol extracts showed strong ovicidal activity with no hatchability in the experimental group eggs, contrarily 92.8 -100% egg hatchability was recorded with control. The percentage of egg hatchability was decreased with increasing concentration of the plant extracts. Mathivanan [26] reported that the LC50 and LC90 values of crude methanol extract of leaves of *E.coronaria* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 and 136.55, 127.24 and 120.86 mg/l, respectively. Significance level was set at $p <0.05$. The larvicidal and ovicidal, activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz.*, *Anopheles stephensi, Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Venkatachalam and Jebanesan [27] the methanolic extracts of few plants exhibited larvicidal activity against *Cx.quinquefasciatus.* Cavalcanti [28] reported that the larvicidal activity of essential oils of Brazilian plants against *Ae.aegypti* and observed the LC50 to range from 60 to 533 ppm.

CONCLUSION

During the present study, the Ethyl acetate and chloroform extract of leaves of *Ageratina adenophora* showed significantly higher larvicidal activity against I instar larvae of *Cx. quinquefasciatus*. The higher insecticidal potential of leaves extract presents the *Ageratina adenophora* leaves as the rich source of toxic metabolites.

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