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Research Article

OVICIDAL, PUPICIDAL AND REPELLENT ACTIVITY OF *AGERATINA ADENOPHORA* EXTRACT FRACTIONS: A MOST POTENT POWER FOR MALARIAL VECTOR CONTROL

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A B S T R A C T

The mosquitocidal activity of *Ageratina adenophora* fractions was controlled on malarial vector, *Anopheles stephensi*. In the ovicidal experiment, 100% mortality was exerted by important fraction 6, 5 and 4 tested at 40, 50 and 60 ppm. Among this fraction 6 was found to be most effective for pupicidal activity provided 23.73, 27.92 and 30.00 at 25, 50 and 75 ppm. Furthermore, maximum repellency of fraction 6 and 5 tested were 0.25, 0.50 and 0.75 mg/cm2 at 40, 80, 120, 160, 200 and 240 minutes. We hypothesized that fraction 6 was provides malarial control for the mosquitocidal activity of *A. adenophora* compounds against *An. stephensi*.

INTRODUCTION

Mosquitoes are disturbance bothers and a noteworthy vector for the transmission of a few life debilitating illnesses. Mosquitoes are the real vectors for the transmission of malaria, dengue fever, yellow fever, filariasis, and a few different illnesses (Dhanasekaran *et al.* 2013; Baranitharan and Dhanasekaran, 2014; Benelli, 2015; Baranitharan *et al.* 2017). *Anopheles stephensi* is the most important vector of malaria fever in the urban districts of India and other West Asian countries. Malaria remains one of the most widespread diseases in the tropical world (Baranitharan *et al.* 2016; Jayaprasad *et al.* 2015). Malaria afflicts 36% of the world people i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions (Panneerselvam *et al.* 2013; Gokulakrishnan *et al.* 2016; Dhanasekaran *et al.* 2018). However, malaria death rates among children in Africa have been decreased by an expected 58% since 2000 (WHO, 2014). Malaria slaughtered an expected 306 000 under-fives comprehensively, incorporating 292 000 kids in the African Region (WHO, 2015). Death rates has fallen by 61 per cent for 2000 and 2015, with a more 13 countries "approaching elimination" (WHO, 2016). Presence of the report, India statement for 6 per cent of all malaria cases in the world, 6 per cent deaths, and 51 per cent of the cases in world.

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The statement estimates the total cases in India found in 1.31 million and deaths at 194 (WHO, 2017). Medicinal plant products have been making use of conventionally by human being population in variety part rural areas global against vector-borne diseases and parasitology diseases (Pavela and Benelli, 2016). Biological activity components produced by overall medicinal plants can act as larvicides, and toxin against insects (Baranitharan *et al*. 2014).

A. mexicana, known as Mexican poppy or Mexican prickly poppy, is a species of poppy found in Mexico and now in the United States, India and Ethiopia. *A. mexicana* is reported to have antimicrobial activity, wound healing capacity in rat, larvicidal and chemosterilant activity, nematicidal and allelopathic potential. In Mexico infusion of aerial part of the plant is used as hypoglycemic (Dash and Murthy, 2011). Chemical investigations of this plant have revealed the presence of alkaloids, amino acids, phenolics and fatty acids. *A. mexicana* has been investigated in terms of modern pharmacology for its anti-malarial activity (Bertrand *et al.* 2010), molluscicidal and nematicidal activity, anticancer activity, antimicrobial activity, hepatoprotective activity, anti-HIV activity and neuropharmacological activity (Capasso *et al.* 2002). In this investigate, *A. adenophora* leaf extract fractions using mosquitocidal activity. The fractions were tested as ovicidal, pupicidal and repellent activity against *An. stephensi*.

MATERIALS AND METHODS

Plant material

Plant sampling was carried out during the growing season (March- April) of 2015 from different places of Yerkadu, Salem District of the Tamilnadu. The samples were authenticated at the Department of Botany of Annamalai University. The leaves were washed many times with water to remove all the unwanted impurities. Then, the leaves were shade-dried under room temperature and kept in a hot air oven for 50 ºC for half an hour. After that, the material was ground by using electric blender. 500 g of powdered plant material was packed inside a Soxhlet apparatus, and successive extraction was carried out using as solvent methanol for 72 h. The solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany), and the dried extract was stored at 4 ºC until further bioassay.

Ovicidal activity

Following the method is Su and Mulla (1998). After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a photomicroscope (Leica, Germany). Each experiment was replicated 5 times (n=100 per replicate), along with controls. The hatch rates were assessed 48 h post-treatment by following formula.

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

Pupicidal activity

Groups of ten pupae were initiated into 500 ml of the check medium containing specific concentrations of the crude extract in a plastic cups in 5 replications. In control, the same number of pupae was maintained in 500 ml of dechlorinated water containing acceptable volume of DMSO. All containers were maintained at room temperature (28±2°C) with naturally prevailing photoperiod (12:12h/L:D) within the laboratory. Any pupa was thought-about to be dead if did not move once poked repeatedly with a mushy brush. Mortality of each pupa was recorded when 24 h of exposure to the extract.

Repellent activity

Following the method by WHO (2009). Three day-old blood-starved female *An. stephensi* mosquito (n=100) were stored in a net cage (45cm × 30cm × 45cm). The volunteer had no contact with lotion, perfumed soaps on the day of the assay. A dorsal facet skin portion on the arms of a volunteer (25 cm^2) was exposed. Extract fractions were applied at 0.25, 0.50 and 0.75 mg/cm^2 on the forearm exposed area. *An. stephensi* were tested from 16:00 to 20:00. Every check concentration was continual five times. The proportion of repellent was calculated by the subsequent formula:

$$\text{Repellency (\%)} = [(T_a\text{-}T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control and T_b is that the number of mosquitoes in the treated group.

Data analysis

Larvicidal data were investigated to probit analysis (Finney, 1971) to calculate the LC_{50}, LC_{90} utilizing statistical package of social science (SPSS) rendition 16.0 for Windows. Ovicidal and pupicidal data were analyzed using two-way ANOVA (factors: the tested fraction and the tested dose) followed by Tukey's HSD test. The significance level was set at $P<0.05$.

RESULTS

A. adenophora extract consist of six compounds were checked for their bio-efficacy against the selected mosquito species, which have been tested for their ovicidal activity of *An. stephensi*. Furthermore, there were no hatchability was recorded above 10ppm (29.17±1.6), 20ppm (20.54±1.8), 30ppm (15.82±2.4), 40 and 50 ppm (100% egg mortality) in the experimental group for represented by table 1.

Table 1 Ovicidal activity of *Ageratina adenophora* selected compounds tested against eggs (0-6h old) of *An. stephensi.*

Fractions	Percentage of egg hatch ability, concentration (ppm), 48 hrs post treatment						
	control	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm	60 ppm
Fraction 1	100.0±0.0^{a}	84.81±2.2^{b}	72.65±2.8^{c}	60.83±2.4^{d}	49.67±2.6^{e}	36.21±1.7^{f}	24.43±2.1^{g}
Fraction 2	100.0±0.0^{a}	78.62±2.8^{b}	69.54±1.6^{c}	57.95±2.8^{d}	50.81±1.6^{e}	38.49±1.8^{f}	22.54±1.5^{g}
Fraction 3	100.0±0.0^{a}	72.56±2.4^{b}	64.91±2.7^{c}	53.22±2.3^{d}	45.76±1.3^{e}	32.26±1.2^{f}	18.67±1.1^{g}
Fraction 4	100.0±0.0^{a}	53.22±1.6^{b}	44.61±1.7^{c}	37.53±2.8^{d}	25.81±2.9^{e}	13.94±2.7^{f}	NH
Fraction 5	100.0±0.0^{a}	47.81±2.6^{b}	38.59±2.4^{c}	22.64±1.7^{d}	17.54±1.6^{e}	NH	NH
Fraction 6	100.0±0.0^{a}	29.17±1.6^{b}	20.54±1.8^{c}	15.82±2.4^{d}	NH	NH	NH

Each values represent mean ± S.D. of five replications; NH - No hatchability (100% mortality); Within each row, different letters indicate significant differences (ANOVA, Tukey's LSD test, P<0.05)

Statistically significant pupicidal activity was tested with six fractions of *A. adenophora*. The fraction 6 was found to be most effective for this activity provided 23.73, 27.92 and 30.00 at 25, 50 and 75 ppm concentration and 79.10, 93.06 and 100% mortality. Moreover, pupicidal activity of fraction 5 (21.76, 25.54 and 29.28 mortality), fraction 4 (19.38, 23.49 and 27.62 mortality), fraction 3 (18.69, 21.43 and 24.55 mortality), fraction 2 (16.62, 19.84 and 22.56 mortality) and fraction 1 (13.56, 16.82 and 20.76 mortality) of *A. adenophora* were tested against *An. stephensi*, respectively (Table 2).

Table 2 Pupicidal activity of *Ageratina adenophora* selected compounds tested against pupae of *An. stephensi.*

Compounds	Concentrations	24 h of exposure period			
		Mortality*		Adult emergence	
		Pupal mortality	% Mortality	Adult	% Emergence
Fraction 1	25 ppm	13.56±1.48^{q}	54.86	11.44±1.48^{c}	44.13
	50 ppm	16.82±1.98^{m}	65.06	7.18±1.65^{g}	23.93
	75 ppm	20.76±1.57^{d}	76.86	1.24±1.36^{p}	4.13
Fraction 2	25 ppm	16.62±1.82^{r}	55.40	13.38±1.62^{b}	38.6
	50 ppm	19.84±1.54^{j}	74.46	6.16±1.86^{j}	20.53
	75 ppm	22.56±1.25^{e}	81.20	1.44±1.95^{o}	4.80
Fraction 3	25 ppm	18.69±1.82^{p}	62.30	11.31±1.48^{d}	31.70
	50 ppm	21.43±1.57^{l}	71.10	6.57±1.53^{h}	21.90
	75 ppm	24.55±1.98^{g}	88.50	3.45±1.28^{m}	11.50
Fraction 4	25 ppm	19.38±1.66^{o}	64.60	10.62±1.75^{e}	27.40
	50 ppm	23.49±1.57^{h}	83.30	3.51±1.68^{l}	11.70
	75 ppm	27.62±1.34^{b}	92.73	0.38±1.66^{r}	1.26
Fraction 5	25 ppm	21.76±1.82^{n}	72.53	8.24±1.39^{f}	21.46
	50 ppm	25.54±1.22^{i}	85.13	4.46±1.67^{k}	14.86
	75 ppm	29.28±1.54^{c}	97.60	0.72±1.85^{q}	2.40
Fraction 6	25 ppm	23.73±1.29^{k}	79.10	6.27±1.62^{i}	17.90
	50 ppm	27.92±1.57^{f}	93.06	2.08±1.58^{n}	6.93
	75 ppm	30.00±0.00^{a}	100.00	0.00±0.00^{s}	0.00
Control	0 ppm	0.0±0.0^{s}	0.0	30.0±0.0^{a}	100.0

Value represents mean ± S.D of five replications; *Mortality of the pupae observed after 24 h of exposure period; Within each row, different letters indicate significant differences (MANOVA, Tukey's LSD test, P<0.05)

The repellent activity of fraction 6 and 5 was the highest, showing 100% protection up to 40, 80, 120, 160, 200, 240 minutes against *An. stephensi,* with fraction 4 (91.7, 96.5 and 99.9 at 240ppm), fraction 3 (80.3, 85.5 and 90.3% protection at

240ppm), fraction 2 (83.6, 89.9 and 94.2% protection at 240ppm) and fraction 1 (75.8, 81.4 and 87.7% protection at 240ppm) (Table 3).

Also, the ethanol extract of whole plant parts of *Leucas aspera* tested against the *An. stephensi* I-IV instar larvae and pupae showed LC_{50} of 9.695, 10.272, 10.823, 11.303 and 12.732%, respectively (Kovendan *et al.* 2012).

Table 3 Repellent activity of *Ageratina adenophora* selected compounds tested against *An. stephensi* vector mosquito.

Compounds	Concentration (mg/cm^2)	% of repellency					
		Time post application of repellent (min)					
		40 min	80 min	120 min	160 min	200 min	240 min
Fraction 1	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	86.6±2.6^a	75.8±2.2^a
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	88.4±2.8^a	81.4±2.4^a
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	91.2±2.7^a	87.7±2.5^a
Fraction 2	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	83.6±2.2^a
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	89.9±1.6^a
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	94.2±1.4^a
Fraction 3	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	92.6±2.7^a	80.3±2.6^a
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	94.4±2.9^a	85.5±2.2^a
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	98.8±2.5^b	90.3±2.9^a
Fraction 4	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	91.7±2.4^{ab}
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	96.5±2.2^b
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	99.9±2.8^b
Fraction 5	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
Fraction 6	0.25	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
	0.50	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
	0.75	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c	100±0.0^c
Control	0.00	0.0±0.0a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a

Each value mean ± S.D represents mean of six values. Within each row, different letters indicate significant differences (ANOVA, Tukey's HSD test, P<0.05).

DISCUSSION

The results of our study focus on the larvicidal, ovicidal and pupicidal activity of the fractions of *A. adenophora* leaf extract against larvae of *An. stephensi*. In a previous report, as compliments to other plant-borne compounds from *Coleus aromaticus*, the fractions were tested as larvicides, ovicides and repellency against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. Ovicides activity investigation, 100% mortality was exerted by main fraction 6 and 5 tested at 40, 50 and 60 ppm. Pupicidal activity of fraction 6 is 100% mortality present at 75ppm. Furthermore, high repel activity of compound tested at 0.75 mg/cm^2 was observed at least 240 minutes (Baranitharan *et al.* 2017). LC-MS analysis of the chloroform extract gave a tentative identification of 13 compounds; Bis-(3-oxaundecyl) tetrasulfide was identified as the major compound in A7 fraction. Methanol extract showed strong repellent action against oviposition, along with weak adulticidal action against mosquito (Ke-Xin *et al.* 2015). The ovicidal activity was *Melissa officinalis* citronellal component exerted 45 mg/L, and repellent activity was observed at 0.75 and 1.50 mg/cm^2 concentrations gave 100% protection up to 210 min against *An. stephensi* (Baranitharan *et al.* 2016). Moreover, eucalyptol showed the lowest action value (1,419 mg L^{-1}) among 21 tested compounds against *Ae. aegypti* larvae. Ethanol fractions of *Eichhornia crassipes* displayed the larvicidal and pupicidal activity against *Cx. quinquefasciatus* with LC_{50} values of 71.43, 94.68, 120.42, 152.15 and 173.35 ppm for first, second, third, fourth instar and pupae, respectively (Santos *et al.* 2010).

The presence of metabolites like flavonoids, alkaloids, anthroquinones and anthocyanins to be proved in the extracts might be the reasoning for the larvicidal and pupicidal action of the plant extracts. Repellent action was not exhibited by these extracts at the tested concentrations (Jayanthi *et al.* 2012).

The eggs of mosquito vector, *Cx. quinquefasciatus* was most affected by methanol extract of *A. adenophora* showed highest ovicidal activity (Rajeshwary *et al.* 2014).

CONCLUSION

In this present investigate, fraction 6 of *Ageratina adenophora* leaf extract was found to be the most control of mosquito live cycle some stages were eggs, pupae and adults in malarial vector, *An. stephensi*.

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