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Effects of *Leucas aspera* (Willd.) Spreng (Lamiaceae) Leaf Extracts Against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract: The mosquitocidal activity of hexane, diethyl ether, dichloromethane and ethyl acetate leaf extracts of *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* was studied. The ethyl acetate extract was found to be the effective larvicide with LC_{50} value of 352.84 ppm against *An. stephensi* and 483.21 ppm against *Ae. aegypti*. *Ae. aegypti* was found to be more susceptible to the aqueous extracts of *L. aspera* in the case of ovicidal and adult emergence inhibition activity followed by *An. stephensi* and *Cx. quinquefasciatus*. The results show promising adult emergence inhibition activity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed.

Key words: *Leucas aspera* · Leaf Extracts · Mosquitocidal Activity · Vector Mosquitoes

INTRODUCTION

Mosquitoes constitute a major public health problem as vectors and WHO has declared them "public enemy number one" as they are responsible for the transmission of various dreadful diseases [1]. One of the approaches for control of mosquito-borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of vectors. In recent years, mosquito control programmes have failed because of the ever increasing insecticide resistance [2]. Synthetic insecticides have created a number of ecological problems, ecological imbalance, harm to human and animals, environment ill effect, non-target organisms being affected in addition to the physiological resistance of vectors to synthetic insecticides [3]. Development of resistance by pests and vectors against the botanicals has not been reported [4]. Botanical insecticides are generally pest specific, readily biodegradable, target specificity, lower bioaccumulation and lack toxicity to higher animals [5].

L. aspera (Willd.) Spreng belonging to Lamiaceae family is known for its medicinal properties and the leaves are used in traditional medicine for treating dyspepsia, cough, colds, painful swellings, intermittent fevers, ulcers and chronic skin eruptions [6]. Further the plant is used as an insecticide [6] and is shown to exhibit larvicidal activity against *Cx. quinuqefasciatus* [7]. Therefore the present study was carried out to evaluate *L. aspera* leaf extracts for mosquitocidal properties against vector mosquitoes *viz*., *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Preparation of Plant Extract: L. *aspera* leaves collected in and around Kancheepuram district Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered plant parts (1 kg) was subjected to sequential extraction using 3 L of hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 h to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 1,00,000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4°C until testing for bioassays.

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Test Mosquitoes: All tests were carried out against laboratory reared vector mosquitoes *viz.*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes were maintained at 25-29°C and 80-90 per cent R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Bioassays: A total of three trials were carried out with five replicates per trial against vector mosquitoes for the following bioassays.

Larvicidal Assay: Standard WHO [8] protocol with slight modifications was adopted for the study. From the stock solution of each crude solvent extract, concentration of 250, 500, 750 and 1000 ppm was prepared. Twenty five early third instar larvae were introduced in 250 ml beaker containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 h. When the control mortality ranged from 5-20 per cent, it was corrected by Abbott's [9] formula.

Ovicidal Assay: For ovicidal assay, the method of Su and Mulla [10] was performed. Freshly laid mosquito eggs were exposed for 12 h in concentrations of 500 and 1000 ppm. Hundred eggs collected on a filter paper for *Ae. aegypti* and *An. stephensi* and an egg raft containing approximately 100 eggs in the case of *Cx. quinquefasciatus* were immersed in aqueous extract. After the exposure period, the eggs were carefully removed and thoroughly washed/rinsed in distilled water and were left separately on enamel trays containing distilled water for hatchability. Control experiments were performed using distilled water. The number of eggs hatched was counted and the per cent hatchability was calculated.

Adult Emergence Inhibition Assay: This test was performed according to the standard protocol described by WHO [11]. The powdered plant material were put in cotton gauze sachets and immersed (for 6 h) in 250 ml beaker containing 200 ml water. Hundred early third instar larvae were exposed for 12 h to the aqueous extracts at concentration of 500 and 1000 ppm. A beaker containing only water (200 ml) served as control. Dead larvae and pupae were removed and counted after 24 h. Observation on larval, pupal mortality and adult emergence was recorded. The number of adults that failed to emerge from the pupae was counted in order to calculate the per cent inhibition.

Statistical Analysis: Probit analysis [12] was used for determination of LC_{50} and LC_{90}. Data from mortality and effect of concentrations was subjected to Chi-square analysis to test differences in mortality among vector species in different concentrations.

RESULTS AND DISCUSSION

The ethyl acetate extract of *L. aspera* was found to be the most effective larvicide against *An. stephensi*, the LC_{50} being 352.84 ppm followed by the same extract with LC_{50} value of 483.21 ppm against *Ae. aegypti* after 24 h of exposure. The hexane extract was next in potency with LC_{50} of 652.52 against *Cx. quinquefasciatus* (Table 1). The results of present study are comparable with earlier reports. The toxicity to the late third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina* and *Luffa acutangula* showed the LC_{50} values of 465.85, 567.81 and 839.81ppm, respectively [13]. Arivoli *et al.* [7] reported that the hexane, diethyl ether, dichloromethane, ethyl acetate and methanol leaf extracts of *L. aspera* and *Vitex negundo* when tested against the larvae of *Cx. quinquefasciatus*, ethyl acetate and hexane extract of both plants provided maximum mortality. Aqueous extract of *L. aspera* was found to be ovicidal against all three mosquito species and the ovicidal response was in the decreasing order, *Ae. aegypti* followed by *An. stephensi* and *Cx. quinquefasciatus* with hatchability values of 39.4 and 21.2; 42.4 and 27.8; 50.6 and 30.2 per cent at 500 and 1000 ppm, respectively (Table 2). The seed extract of *Atriplex canescens* showed complete ovicidal activity at 1,000 ppm concentration in eggs of *Cx. quinquefasciatus* [14]. Saxena *et al.* [15] also found a significant reduction of hatchability in *An. stephensi* treated with *Annona squamosa*. The finding of the present investigation was comparable with the above ovicidal studies and reveals that the *L. aspera* leaf extracts possesses ovicidal activity against mosquitoes.

L. aspera aqueous extracts tested against the three vector mosquitoes at 500 and 1000 ppm were found to be effective showing adult emergence inhibition percentage against *Ae. aegypti* (62.8 and 37.6), followed by *An. stephensi* (62.2 and 23.2) and *Cx. quinquefascaitus*

Table 1: Probit analysis of larvicidal efficacy of *L. aspera* leaf extracts against vector mosquito species

Vector mosquito species	Extracts	LC_{50}	LC_{90}	Chi-square value	Regression value
Ae. aegypti	Hexane	1359.25	6751.97	0.05*	1.84
	Diethyl ether	927.62	4308.47	0.21*	1.23
	Dichloromethane	844.63	4251.98	3.53*	1.83
	Ethyl acetate	483.21	3195.91	7.19	1.56
An. stephensi	Hexane	1592.05	8501.06	0.54*	1.76
	Diethyl ether	2262.20	5547.41	0.65*	1.07
	Dichloromethane	1077.84	6384.63	0.04*	1.25
	Ethyl acetate	352.84	1033.60	3.26	2.75
Cx. quinquefasciatus	Hexane	652.52	2805.78	1.12*	1.87
	Diethyl ether	1934.67	2996.86	0.42*	1.07
	Dichloromethane	704.32	1163.21	0.50*	1.54
	Ethyl acetate	1003.16	9937.84	9.80	1.29

* Significant at $P < 0.05$ level

Table 2: Effect of *L. aspera* aqueous leaf extracts on vector mosquito species

Vector mosquito species	Concen-tration (ppm)	Larval mortality (%)*	Total larval period in days	Pupal mortality (%)*	Total pupal period in days	Adult emergence (%) (a)	Total developmental period in days (b)	Growth index (a/b)	Hatchability (%)
Ae. aegypti	500	28.8±2.17	11	8.4±0.89	4	62.8±2.39	15	4.2	39.4±2.41
	1000	52.2±1.64	11	10.2±1.30	4	37.6±1.21	15	2.5	21.2±3.27
	Control	8.2±0.84	8	1.2±0.84	2	90.6±0.89	10	9.1	90.2±2.17
An. stephensi	500	31.2±1.64	9	6.6±1.14	3	62.2±2.59	12	5.2	42.4±2.61
	1000	62.8±2.28	9	14.0±1.58	3	23.2±2.68	12	1.9	27.8±3.19
	Control	9.2±1.48	8	1.4±0.89	2	89.4±2.30	10	8.9	90.8±3.83
Cx. quinquefasciatus	500	24.4±2.30	9	9.2±1.10	3	66.4±2.88	12	5.5	50.6±2.70
	1000	47.2±2.49	9	10.4±1.67	3	42.4±1.67	12	3.5	30.2±2.77
	Control	4.4±1.82	8	1.8±0.84	2	93.8±2.28	10	9.4	91.2±3.70

* Significant at $P < 0.001$ level

(66.4 and 42.4) (Table 2). The larvae showed restless movement for some time and then settled at the bottom of the beaker with abnormal wagging of the tail and died slowly. Prolonged larval and pupal periods were observed among the test organisms. In the control it took 8 days for all the larvae to become pupae and 2 days for the pupae to develop into adults; whereas in the aqueous extract it took 11 days for *Ae. aegypti* and 9 days in the case of *An. stephensi* and *Cx. quinquefasciatus* larvae to develop into pupae and 4 days for the pupae to develop into adults. Such abnormalities in the metamorphosis could be due to an imbalance in hormones. Saxena and Saxena [16] have also observed such prolonged larval and pupal periods while using plant extracts for the control of mosquito larvae. Sujatha *et al.* [17] reported on the morphogenetic abnormalities among the developmental stages of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* at lower concentrations of plant extracts, *Madhuca longifolia*, *Acorus calumus* and *Ageratum conyzoides*. Lengthening the larval period in *Ae. aegypti* by the influence of botanicals has been reported by Supavarn *et al.* [18]. Such lengthening of larval and pupal periods and formation of larval-pupal and pupal-adult intermediates indicates the interference of the bio-active compounds with the normal hormonal activity coordination of the metabolic processes of the developing stages. Likewise Arivoli and Samuel [19] reported on the adult emergence inhibition activity in *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* when treated with the whole plant extracts of *Citrullus colocynthis*.

It may concluded that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. The crude leaf extracts of *L. aspera* showed mosquitocidal properties particularly adult emergence inhibition activity against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*. Further studies on the screening, isolation and purification of bioactive

phytochemical constituents/ compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *L. aspera* to control the immature stages of vector mosquitoes.

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