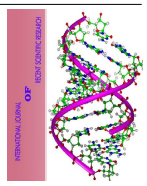
ISSN: **0976-3031**

Available at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 2, Issue, 5, pp. 143 -149, May, 2011



MOSQUITOCIDAL ACTIVITY OF *Hyptis suaveolens* (L.) POIT (LAMIACEAE) EXTRACTS AGAINST *Aedes aegypti, Anopheles stephensi* AND *Culex quinquefasciatus* (DIPTERA: CULICIDAE)

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Received 7th February, 2011; Accepted 20th March, 2011; Published online 28th May, 2011

ABSTRACT

Hyptis suaveolens extracts were evaluated for larvicidal, adult emergence inhibition and ovicidal activity against vector mosquitoes *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Among the vector mosquito species, *Culex quinquefasciatus* was found to be the most susceptible with a LC_{50} value of 203.37 ppm in hexane extract. Significant adult emergence inhibition was noted when larval and pupal development was arrested resulting in decreased pupal transformation. Larval and pupal periods were prolonged with appearance of larval–pupal and pupal–adult intermediates, with an overall increase in the developmental period and ovicidal activity was more pronounced in the egg rafts of *Culex quinquefasciatus*.

Key words: *Hyptis suaveolens* extracts, mosquitocidal activity, *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are the oldest human enemy and represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO, 1992, 1998). Mosquitoes constitute a major public health problem as vectors of serious human diseases (Hag *et al*., 1999). WHO has declared the mosquito "Public enemy number one" as they are responsible for the transmission of various dreadful diseases (WHO, 1996a). Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encepahalitis and Filariasis (Service, 1983; Gubler, 1998). Plant products have been used traditionally by human communities (Jacobson, 1958). The plant world comprises a rich storehouse of phytochemicals, which are widely used in the place of synthetic insecticides. The continuous use of synthetic insecticides causes side effects to non-target organisms and insecticide resistance against mosquitoes (Kelm *et al*., 1997). Phytoproducts on account of minimal hazardous effect on the environment and wide range of availability offer promises in future mosquito control programmes. They have revolutionized the fields of vector control as they possess different bioactive components and can be used as general toxicants against various larval stages of the mosquito (Sharma *et al*., 2004; Mohan *et al*., 2005). Botanical photochemical with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent mosquitocidal properties and the chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors (Sukumar *et al*., 1991). *Hyptis suaveolens* (L.) belonging to the family Lamiaceae is a potent medicinal herb used as a stimulant, carminative, wound healing agent and as a relief for stomachache (Chitra *et al*., 2009). The leaves are used to treat skin diseases and bronchial disorders (Koche *et al*., 2010). The plant also possesses antispasmodic, antirheumatic, anti-inflammatory, antifertility, antiseptic (Chitra *et al*., 2009), antihelminthic (Oliver-Bever, 1960), antiparasitic (Dalziel, 1937), antifungal (Pandey *et al*., 1982; Singh *et al*., 1992), anticonvulsant (Akah and Nwambie, 1993), anticancer (Kingston *et al*., 1979), antibacterial, antioxidant and antimicrobial properties (Iwu *et al*., 1990; Asekun *et al*., 1999; Asekuu and Ekundaya, 2000; Annie *et al*., 2003; Nantitanon *et al*., 2007). The plant gained increasing attention when it exhibited insecticidal properties (Peerzada, 1997; Facey *et al*., 2005; Othira *et al*., 2009). The plant showed feeding deterrent (Simmond and Blaney, 1992) and insect repellent activity (Obeng-Ofori *et al*., 1996; Oparacke *et al*., 2002). *Hyptis* extracts were used effectively to control cow pea borer, *Maruca testulalis* (Gbehounou, 2007) and *Trogoderma granarium* (Musa *et al*., 2009). The fumes of *Hyptis suaveolens* dried leaves are used as an insectifuge to repel mosquitoes and control insect pests of stored grains (ICMR, 2003; Ijeh *et*

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al., 2007; Alok *et al*., 2010) and possesses larvicidal activity against *Aedes aegypti* (Cavalcanti *et al*., 2004) and *Culex quinquefasciatus* (Okigbo *et al*., 2010). An attempt has, therefore, been made in the present study to observe the impact of *Hyptis suaveolens* extracts on the larvicidal, adult emergence inhibition and ovicidal activity of vector mosquitoes *viz*., *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant extracts: *Hyptis suaveolens* plants collected in and around Tamilnadu were brought to the laboratory and shade dried under room temperature. The aerial parts of the plant taken up for the study were powdered using an electric blender. Dried and powdered aerial 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 ^{0}C until testing for bioassays.

Test mosquitoes: All tests were carried out against laboratory reared vector mosquitoes *viz.*, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes were maintained at 25-29 ^{0}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 activity: Standard WHO (1996b) protocol with slight modifications was adopted for the study. From the stock solution, 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, 48 and 72 h. However, when the control mortality ranged from 5-20 per cent, the observed percentage mortality was corrected by Abbott's (1925) formula,

$$\text{Per cent mortality} = \frac{\%\text{ Mortality in treated} - \%\text{ Mortality in control}}{100 - \%\text{ Mortality in control}} \times 100$$

Adult emergence inhibition test: This test was performed according to the standard protocol described by WHO (1975). 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 crude 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.

Ovicidal activity: For ovicidal activity, the method of Su and Mulla (1998) was performed. Freshly laid mosquito eggs were used for the treatments and were exposed for 12 h in desired concentrations of 500 and 1000 ppm. Hundred eggs collected on a filter paper for *Aedes aegypti* and *Anopheles stephensi* and an egg raft containing approximately 100 eggs in the case of *Culex quinquefasciatus* were immersed in water treated with 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 untreated water. The number of eggs hatched was counted and the per cent hatchability was calculated using the following formula,

$$\text{Per cent hatchability} = \frac{\text{Total number of hatched larvae}}{\text{Total number of eggs/egg raft}} \times 100$$

Statistical analysis: Probit analysis (Finney, 1971) was used for determination of LC_{50} and LC_{90}. Data from mortality and effect of concentrations were subjected to analysis of variance. The percentage data obtained was angular transformed. Difference between the treatments was determined by Tukey's test ($P < 0.05$). Students 't' test was also performed. The highest different values from average detected by statistical testing are marked with letter "a" the next text lower with "b" and continued accordingly (Snedecor and Cochran, 1989).

RESULTS

Larvicidal activity

The results of the larvicidal activity are presented in Table 1 and 2. Among the vector mosquito species, *Culex quinquefasciatus* larvae were found to be more susceptible followed by *Aedes aegypti* and *Anopheles stephensi*. The hexane extract was found to be the most effective with LC_{50} value of 203.37 ppm and providing 100 per cent mortality at 1000 ppm (24 h) and 750 ppm (48 h) against the larvae of *Culex quinquefasciatus* followed by *Aedes aegypti* with a LC_{50} value of 543.66 ppm.

Adult emergence inhibition activity

Effective larval and pupal period lasted 11 and 4 in *Culex quinquefasciatus* followed by 10 and 3 in *Anopheles stephensi*; 9 and 3 days in *Aedes aegypti* treated individuals. In the case of control it took 8 and 2 days.

Table 1. Per cent larvicidal activity of *Hyptis suaveolens* extracts against vector mosquitoes

Solvents	Concentration (ppm) 250			500			750			1000		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
						Aedes aegypti						
Hexane	34.4 ±3.58 (35.9)^{c}	39.2 ±4.38 (38.8)^{d}	47.2 ±4.38 (43.4)^{c}	35.2 ±4.38 (36.4)^{c}	42.4 ±4.56 (40.6)^{c}	49.6 ±7.26 (44.8)^{c}	54.4 ±7.27 (47.5)^{c}	59.2 ±10.35 (50.3)^{c}	70.4 ±8.29 (57.0)^{c}	72.8 ±10.35 (58.6)^{d}	81.6 ±6.07 (64.6)^{d}	85.6 ±6.69 (67.7)^{d}
Diethyl ether	11.2 ±3.35 (19.6)^{b}	19.2 ±3.35 (26.0)^{bc}	25.6 ±3.58 (30.4)^{b}	16.8 ±3.35 (24.2)^{b}	22.4 ±4.56 (28.3)^{b}	28.8 ±3.35 (32.5)^{b}	32.8 ±3.35 (34.9)^{b}	40.8 ±3.35 (39.7)^{b}	49.6 ±5.35 (44.8)^{b}	40.8 ±4.38 (39.7)^{b}	43.2 ±3.35 (41.1)^{b}	47.2 ±3.35 (43.4)^{b}
Dichloro-methane	8.8 ±3.35 (17.3)^{b}	16.8 ±5.21 (24.2)^{b}	23.2 ±4.38 (28.8)^{b}	20.8 ±4.38 (27.1)^{b}	32.8 ±9.42 (34.9)^{bc}	37.6 ±10.8 (37.8)^{bc}	30.4 ±4.56 (33.5)^{b}	47.2 ±5.93 (43.4)^{bc}	55.2 ±7.69 (48.0)^{b}	42.4 ±10.8 (40.6)^{b}	63.2 ±7.15 (52.7)^{c}	71.2 ±9.12 (57.5)^{c}
Ethyl acetate	13.6 ±3.58 (21.6)^{b}	24.8 ±3.35 (28.4)^{c}	30.4 ±4.56 (33.5)^{b}	32.8 ±5.93 (34.9)^{c}	42.4 ±6.01 (40.6)^{c}	49.6 ±5.37 (44.8)^{c}	38.4 ±3.58 (38.3)^{b}	59.2 ±7.15 (50.3)^{c}	60.8 ±7.15 (51.2)^{bc}	59.2 ±3.35 (50.3)^{c}	70.4 ±5.37 (57.0)^{c}	78.4 ±3.36 (62.3)^{cd}
Control	0.8 ±1.79 (5.1)^{a}	0.8 ±1.79 (5.1)^{a}	0.8 ±1.79 (5.1)^{a}	0.8 ±1.79 (5.1)^{a}	1.6 ±2.19 (7.3)^{a}	2.4 ±2.19 (8.9)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	1.6 ±2.19 (7.3)^{a}	0.8 ±1.79 (5.1)^{a}	3.2 ±1.79 (10.3)^{a}	5.6 ±2.19 (13.7)^{a}
						Anopheles stephensi						
Hexane	15.2 ±5.93 (22.9)^{b}	24.8 ±3.35 (29.9)^{c}	32.8 ±7.69 (34.9)^{d}	33.6 ±8.29 (35.6)^{d}	34.4 ±6.07 (35.9)^{c}	44.8 ±3.35 (42.0)^{d}	29.6 ±4.56 (32.9)^{c}	41.6 ±8.29 (40.2)^{c}	55.2 ±5.93 (48.0)^{c}	40.8 ±3.35 (39.7)^{b}	59.2 ±9.12 (50.3)^{c}	68.8 ±7.69 (56.0)^{c}
Diethyl ether	6.4 ±4.56 (14.7)^{a}	13.6 ±3.35 (21.6)^{b}	22.4 ±4.56 (28.3)^{cd}	19.2 ±3.35 (26.0)^{c}	28.8 ±3.35 (32.5)^{bc}	34.4 ±4.56 (35.9)^{c}	21.6 ±3.58 (27.7)^{b}	30.4 ±3.58 (33.5)^{b}	36.8 ±7.16 (37.4)^{b}	33.6 ±8.29 (35.4)^{b}	40.8 ±8.67 (39.7)^{b}	52.8 ±7.69 (46.6)^{b}
Dichloro-methane	5.6 ±5.37 (13.7)^{a}	6.4 ±6.69 (14.7)^{ab}	10.4 ±9.63 (18.8)^{ab}	6.4 ±6.07 (14.7)^{ab}	8.8 ±7.69 (17.3)^{a}	14.4 ±3.58 (22.3)^{b}	31.2 ±3.35 (33.9)^{c}	36.8 ±5.93 (37.4)^{bc}	43.2 ±4.38 (41.1)^{b}	34.4 ±5.56 (35.9)^{b}	37.6 ±6.07 (37.8)^{b}	53.6 ±3.58 (47.1)^{b}
Ethyl acetate	1.6 ±3.58 (7.3)^{a}	10.4 ±3.58 (18.8)^{b}	16.8 ±1.79 (24.2)^{bc}	15.2 ±5.93 (22.9)^{bc}	24.8 ±3.35 (29.9)^{b}	30.4 ±4.56 (33.5)^{c}	33.6 ±3.58 (35.4)^{c}	40.8 ±3.35 (39.7)^{c}	45.6 ±2.19 (42.5)^{b}	55.2 ±12.78 (47.9)^{c}	60.8 ±13.08 (51.2)^{c}	69.6 ±10.81 (56.5)^{c}
Control	0^{a}	0^{a}	0^{a}	0^{a}	0.8 ±1.79 (5.1)^{a}	1.6 ±2.19 (7.3)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	1.6 ±2.19 (7.3)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	3.2 ±3.35 (10.3)^{a}
						Culex quinquefasciatus						
Hexane	60.8 ±11.10 (51.2)^{d}	74.4 ±8.76 (59.6)^{d}	100 0 (90.0)^{d}	77.6 ±3.58 (61.8)^{d}	96.8 ±4.38 (79.7)^{d}	100 0 (90.0)^{d}	82.4 ±3.58 (65.2)^{d}	100 0 (90.0)^{c}	100 0 (90.0)^{d}	100 0 (90.0)^{d}	100 0 (90.0)^{c}	100 0 (90.0)^{d}
Diethyl ether	13.6 ±6.69 (21.6)^{bc}	22.4 ±3.58 (28.3)^{bc}	32 ±5.66 (34.1)^{c}	35.2 ±8.67 (36.4)^{c}	42.4 ±8.29 (40.6)^{c}	50.4 ±4.56 (45.2)^{c}	40.8 ±5.22 (39.7)^{c}	45.6 ±2.19 (42.5)^{b}	57.6 ±3.58 (49.4)^{c}	54.4 ±16.40 (47.5)^{b}	57.6 ±8.72 (49.4)^{b}	67.2 ±7.69 (55.1)^{c}
Dichloro-methane	4.8 ±3.35 (12.7)^{ab}	14.4 ±4.56 (22.3)^{b}	20.8 ±3.35 (27.1)^{b}	18.4 ±3.58 (25.4)^{b}	27.2 ±3.35 (31.4)^{b}	35.2 ±5.22 (36.4)^{b}	28.8 ±5.22 (32.5)^{b}	41.6 ±8.29 (40.2)^{b}	49.6 ±6.69 (44.8)^{bc}	39.2 ±4.38 (38.8)^{b}	51.2 ±8.67 (45.7)^{b}	55.2 ±7.16 (48.0)^{b}
Ethyl acetate	18.4 ±3.57 (25.4)^{c}	26.4 ±2.19 (30.9)^{c}	31.2 ±3.35 (33.9)^{c}	29.6 ±4.56 (32.9)^{c}	34.4 ±6.07 (35.1)^{bc}	40.8 ±5.22 (39.7)^{b}	32.8 ±5.22 (34.9)^{bc}	40.8 ±5.93 (39.7)^{b}	48.8 ±5.22 (44.3)^{b}	73.6 ±6.07 (59.1)^{c}	88.8 ±9.12 (70.5)^{c}	100 0 (90.0)^{d}
Control	0^{a}	0^{a}	0.8 ±1.79 (5.1)^{a}	0^{a}	0.8 ±1.79 (5.1)^{a}	1.6 ±2.19 (7.3)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	1.6 ±2.19 (7.3)^{a}	0^{a}	1.6 ±2.19 (7.3)^{a}	4.0 ±2.83 (11.5)^{a}

Values are mean (%) of the five-replicates of three trials ±standard deviation and figures in parentheses are angular transformed. ANOVA followed by TUKEY test performed. Different superscripts in the column indicate significance difference at P <0.05 levels

Table 2. Probit analysis of larvicidal efficacy of *Hyptis suaveolens* extracts against vector mosquitoes

Vector mosquito species	Extracts	LC_{50}	LC_{90}	Chi-square value	Regression value
Aedes aegypti	Hexane	543.66	3546.69	7.49	1.57
	Diethyl ether	1443.53	8362.41	2.05*	1.68
	Dichloromethane	1292.36	6016.58	0.23*	1.92
	Ethyl acetate	853.04	3549.90	2.52*	2.07
Anopheles stephensi	Hexane	1523.19	6964.42	0.39*	1.22
	Diethyl ether	1490.78	6908.41	2.93*	1.92
	Dichloromethane	1396.41	4854.16	7.63	2.37
	Ethyl acetate	944.08	2129.29	0.25*	3.63
Culex quinquefasciatus	Hexane	203.37	778.81	13.36	2.20
	Diethyl ether	888.00	4204.99	1.06*	1.90
	Dichloromethane	1321.05	5030.90	0.21*	2.21
	Ethyl acetate	774.16	3020.83	21.18	2.17

*Significant at P < 0.05 level

Table 3. Effect of *Hyptis suaveolens* aqueous extracts on the growth and metamorphosis of vector mosquitoes

Vector Mosquito species	Concentration (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)	Egg mortality (%)
Aedes aegypti	500	30.8± 1.48	9	7.4±1.14	3	61.8±1.30	12	5.2	41.0 ±5.34
	1000	55.6± 1.82	9	14.0±1.58	3	30.4±2.70	12	2.5	21.8 ±2.17
	Control	8.2± 0.84	8	1.2±0.84	2	90.6±0.89	10	9.1	90.2 ±2.17
Anopheles stephensi	500	42.4± 2.41	10	8.4± 0.88	3	49.2± 2.17	13	3.8	46.8 ±3.42
	1000	73.4± 3.71	10	16.0± 1.58	3	10.6± 3.78	13	0.8	26.8 ±2.68
	Control	9.2±1.48	8	1.4±0.89	2	89.4±2.30	10	8.9	90.8 ±3.83
Culex quinquefasciatus	500	26.2±2.39	11	6.4±1.52	4	67.4±1.82	15	4.5	52.6 ±2.88
	1000	50.4±2.19	11	11.6±1.82	4	38.0±3.81	15	2.5	31.6±3.05
	Control	4.4±1.82	8	1.8±0.84	2	93.8±2.28	10	9.4	91.2±3.70

*****Students 't' test performed showing statistically significance difference at P <0.001 levels

Larval duration significantly increased in treated individuals and total developmental period (larval and pupal development) took 15, 13 and 12 days in *Culex quinuefasciatus*, *Anopheles stephensi* and *Aedes aegypti* whereas in control it took 10 days. The data also revealed gradual increase in pupal duration. Adult emergence against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* recorded at 500 and 1000 ppm was 61.8 and 31.4; 49.2 and 10.6; 67.0 and 38.4 respectively. Among the three species of vector mosquito, *Culex quinquefasciatus* was most susceptible followed by *Aedes aegypti* and *Anopheles stephensi*. Student t-test analysis showed significant difference at $P < 0.001$ level on all three mosquito larval and pupal mortality treated with aqueous extracts and control (Table 3). Adult emergence inhibition suggested a general toxic effect of the extract, which was found to be dose dependent. The metamorphic abnormalities like larval inability to moult to next stage and larval pupal intermediates noticed were higher when compared to control (untreated) groups. Inability of adults to shed completely its exuvia, which remained attached to its appendages, was also noticed. The treated adult could not fly above normal level and rested for longer period on the water surface when compared to untreated adult mosquitoes. In this context of observation, exposure of third instar larvae (all three vector mosquito species) to aqueous extract, resulted in death at larval-pupal moult and pupal-adult eclosion suggesting inhibition of moulting process.

Ovicidal activity

Ovicidal activity of *Hyptis suaveolens* aqueous extract against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at 500 and 1000 ppm was 41.0 and 21.8; 46.8 and 26.8; 52.6 and 31.6 respectively. The decrease in hatchability was found to be dose dependent. Among the three species of vector mosquito, *Culex quinquefasciatus* was most susceptible followed by *Anopheles stephensi* and *Aedes aegypti* (Table 3).

DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides (Chandre *et al*., 1998). Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment and due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought (Hag *et al*., 1999). Natural pesticides, especially those derived from plants, are more promising in this aspect (Amer and Mehlhorn, 2006). A considerable number of plant products/derivatives have shown to be effective against mosquitoes with a safe manner. The screening of plants for mosquitocidal property may eventually lead to their use in natural product-based mosquito abatement practices (Bowers *et al*., 1995). Larvae from the three medically important mosquito genera *Aedes*, *Anopheles* and *Culex* are all susceptible to a greater or lesser extent to some phytochemicals (Shaalan *et al*., 2005). Plants belonging to the family Lamiaceae have been screened/studied for their larvicidal activity against mosquitoes. Plants that showed promising larvicidal activity are methanolic stem extracts of *Satureja hortensis* (LC_{50} 28.0ppm), *Ocimum basilicum* (LC_{50} 32.0ppm), *Thymus vulgaris* (LC_{50} 48.0ppm), flower extracts of *Lavandula officinalis* (LC_{50} 59.0ppm) and stem extracts of *Stachys byzantica* (LC_{50} 65.0ppm). Plants that showed moderate larvicidal activity (LC_{50} values ranging from 100-250ppm) are methanlolic extracts of *Salvia virdis* flower (LC_{50} 110ppm), *Hysopus officinalis* stem (LC_{50} 150ppm), *Salvia officinalis* stem (LC_{50} 159ppm), *Salvia farinaceae* (LC_{50} 195ppm) and *Sideritis euxina* (LC_{50} 250.0ppm). Plants possessing LC_{50} values less than 500ppm include methanolic stem extracts of *Origanum vulgare* (LC_{50} 256.0ppm), *Stachys cretica* (LC_{50} 292.0ppm), *Salvia verbenaca* (LC_{50} 311.0ppm), *Teucrium hircanicum* (LC_{50} 316.0ppm) and *Salvia verticillata* (LC_{50} 410.0ppm) against the larvae of *Culex quinquefasciatus* (Pavela, 2008, 2009). Further Sakthivadivel and Daniel (2008) also revealed that the petroleum ether leaf extracts of *Leucas aspera* and *Ocimum sanctum* also possessed LC_{50} ranging between 100-200ppm against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The results of the above mentioned

reports were comparable with the LC_{50} value of the present study thus exhibiting moderate larvicidal activity.

Insect growth regulators have a pronounced effect on the developmental period, growth, adult emergence and egg hatching resulting in effective control. The small dose response slope observed for most phytochemicals over 24 h renders most of them unusable despite them having reasonable or excellent LC_{50} values. Thus the growth inhibiting activity of a phytochemical may be essential to its uptake by mosquito control industries (Shaalan *et al.*, 2005). Arivoli and Samuel (2011) reported on the adult emergence inhibition activity in *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* when treated against the whole plant extracts of *Citrullus colocynthis*. Adult emergence inhibition was also observed in *Calophyllum inophyllum*, *Solanum suratense*, *Samadera indica* and *Rhinocanthus nasutus* leaf extracts against vector mosquitoes (Muthukrishnan and Pushpalatha, 2001). Exposure of *Anopheles stephensi* larvae to sub-lethal doses of neem leaves extract prolonged larval development (Murugan *et al*., 1996; Su and Mulla, 1999). The failure in adult emergence could be due to insufficient availability of chitin during metamorphosis resulting in death of larvae and pupae entangled in the weak integument. Similar phytoextract induced deformities (Saxena and Sumithra, 1985) and degenerative effects (Dhar *et al*., 1996) were noted also in *Anopheles stephensi.*

In the case of ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs. It has been shown that the age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin, to *Culex quinquefasciatus* (Miura *et al.*, 1976). Mullai and Jebanesan (2006) reported complete ovicidal activity (100 per cent mortality) at 300ppm for methanol, benzene, petroleum ether and ethyl acetate extracts of *Citrullus pubescens* against *Culex quinquefasciatus*. The benzene extracts of *Citrullus vulgaris* also exerted 100 per cent mortality (no hatchability) at 250 ppm and (11.8%) at 200 ppm against *Anopheles stephensi* and *Aedes aegypti* respectively (Mullai *et al.*, 2008). The seed extract of *Atriplex canescens* showed complete ovicidal at 1000 ppm concentration in eggs of *Culex quinquefasciatus* (Ouda *et al*., 1998). The ethyl acetate extract of *Aegle marmelos* and the methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* exerted 100 per cent mortality (no hatchability) at 1000 ppm against *Anopheles subpictus* (Elango *et al*., 2009). The hexane extract of *Andrographis lineata, Coccilus hirsutus,* and *Tagetes erecta* exerted 100 per cent mortality (no hatchability) at 1000 ppm against *Culex tritaeniorhynchus* (Elango *et al*., 2010). The aqueous extracts of *Citrullus colocynthis* showed ovicidal activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Arivoli and Samuel, 2011). The results reported in the present study open the possibility of further investigations on evaluation, identification and isolation of the bioactive component(s) of *Hyptis suaveolens* extracts and its systemic effects on target mosquitoes, which would eventually enable the application of the extract as larvicidal, adult emergence inhibition and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings for the effective control of vector mosquitoes.

Acknowledgement

Authors are thankful to the Department of Science and Technology, New Delhi for financial assistance.

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