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RESEARCH ARTICLE

Larvicidal, ovicidal, and oviposition-deterrent activities of four plant extracts against three mosquito species

K. P. Prathibha · B. S. Raghavendra · V. A. Vijayan

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Abstract In mosquito control programs, insecticides of botanical origin have the potential to eliminate eggs, larvae, and adults. So, the larvicidal, ovicidal, and oviposition-deterrent activities of petroleum ether and ethyl acetate extracts of the leaves of Eugenia jambolana, Solidago canadensis, Euodia ridleyi, and Spilanthes mauritiana were assayed against the three vector mosquito species, namely Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. The larval bioassay was conducted following the World Health Organization method. The maximum larval mortality was found with ethyl acetate extract of S. mauritiana against the larvae of A. stephensi, A. aegypti, and C. quinquefasciatus with LC₅₀ values of 11.51, 28.1, 14.10 ppm, respectively. The mean percent hatchability of the ovicidal activity was observed at 48-h post-treatment. The percent hatchability was found to be inversely proportional to the concentration of the extract and directly proportional to the number of eggs. The flower head extract of S. mauritiana gave 100 % mortality followed by E. ridleyi, S. canadensis, and E. jambolana against the eggs of the three mosquito vectors. For oviposition-deterrent effect, out of the five concentrations tested (20, 40, 60, 80, and 100 ppm), the concentration of 100 ppm showed a significant egg laying-deterrent capacity. The oviposition activity index value of E. jambolana, E. ridlevi, S. canadensis, and S. mauritiana against A. aegypti, A. stephensi, C. quinquefasciatus at 100 ppm were -0.71, -0.71, -0.90,-0.93, -0.85, -0.91, -1, -1, -0.71, -0.85, -1, and -1, respectively. These results suggest that the leaf/flower extracts of certain local plants have the potential to be developed as possible eco-friendly means for the control of mosquitoes.

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Keywords Eugenia jambolana · Solidago canadensis · Euodia ridleyi · Spilanthes mauritiana · Anopheles stephensi · Aedes aegypti · Culex quinquefasciatus · Larvicidal · Ovicidal · Oviposition deterrent

Introduction

Insect-borne diseases remain to this day a major source of illness and death worldwide (Pavela 2009). Mosquitoes alone transmit such diseases in more than 700 million people annually worldwide (Aregawi et al. 2008). They are the vectors for the transmission of malaria, dengue fever, chikungunya, yellow fever, lymphatic filariasis, Japanese encephalitis, etc., causing millions of deaths every year (Rahuman et al. 2008). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng et al. 1999). Malaria, vectored by species of the Anopheles mosquitoes, remains one of the most prevalent mosquito-borne diseases in the tropical world with 200-450 million infections and up to 2.7 million deaths annually (WHO 2010). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. Aedes aegypti is another important vector, transmitting the arbovirus causing dengue and dengue hemorrhagic fever (DHF) in humans (Rigua-Perez et al. 1997). Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease-such as dengue hemorrhagic fever and dengue shock syndrome or the usual manifestations involving the central nervous system (Pancharoen et al. 2002). In recent years, A. aegypti has been responsible for the spread of the chikungunya virus which affected the south west Indian ocean islands in 2005, spreading to India and resulted in an outbreak that has involved >1.5 million patients (Taubitz et al. 2007).

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Culex quinquefasciatus, the vector of lymphatic filariasis, is widely distributed in tropical countries. Around 120 million people are infected worldwide, and 44 million people have the common chronic manifestation of bancroftian filariasis (Bernhard et al. 2003). The pathogen *Wuchereria bancrofti* is the most predominant filarial nematode usually characterized by progressive debilitating swelling at the extremities, scrotum, or breast (elephantiasis) in an infected individual (Myung et al. 1998).

The search for new strategies with natural products to control destructive insects and vectors of diseases is desirable due to the prevalence of vector resistance to synthetic insecticides, problem of toxicity, non-biodegradable effects, and effects on non-target organisms (Jantan et al. 2005). Extracts from plants may be alternative sources of mosquito control agents since they constitute rich sources of bioactive compounds that are biodegradable into non-toxic products and potentially stable (Amer and Melhorn 2006).

Further, phytochemicals are advantageous due to their ecosafety, target specificity, higher acceptability, and suitability for rural areas. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Markouk et al. 2001). In this regard, the chemicals derived from plants have been projected as weapons in future mosquito control programs. According to Jayaraj (1993), more than 1,000 plant species in India are found to possess insecticidal properties, 384 contain antifeedants, 297 contain repellents, and 27 contain attractants and possess growth inhibitors. For example, Govindarajan (2009) has reported that the leaf extracts of Cassia fistula possess larvicidal, ovicidal, and repellent activities against A. aegypti. In Mysore, Madhu et al. (2009) and Aivazi and Vijayan (2010) have reported that *Curcuma aromatica*, *Piper* longum, and Ruta graveolens extracts have larvicidal activity against mosquito vectors.

In the light of the said information, the present study was carried out to analyze the ovicidal, larvicidal, and ovipositiondeterrent activities in the larvae of three important vectors, *Anopheles stephensi*, *A. aegypti*, and *C. quinquefasciatus*, with four local plant species namely *Eugenia jambolana*, *Solidago canadensis*, and *Euodia ridleyi*, *Spilanthes mauritiana* using petroleum ether and ethyl acetate as a solvents.

E. jambolana commonly known as Jambul belongs to the family Myrtaceae. The seeds of this plant are used for ulcer healing and have gastro-protective property (Chaturvedi et al. 2009). The leaves of this plant have been employed for the inhibition of buffalo pox (Bhanuprakash et al. 2007). *S. canadensis* is commonly known as the golden rod and belongs to the family Asteraceae. This rod has been used topically for wound healing and for the treatment of

tuberculosis, diabetes, and enlargement of liver (Apati et al. 2003). On the other hand Euodia is purely an ornamental plant. It is a native of tropical forests and is very much at home in partially shaded garden areas, where its yellow-green foliage forms a bright-colored accent. Some contain aromatic oils that act as coolant for fever, as lotions for the improvement of complexions, and tonics in the treatment of stomach complaints (Horace et al. 1977). Similarly, Spilanthes species was found to have many medicinal uses. The whole plant of Spilanthes acmella can be used in the treatment of dysentery and rheumatism (Baruah and Leclercq 1993). Likewise, the flower heads of S. mauritiana can be chewed to relieve toothache and has haemostatic and analgesic properties (Oliver-Bever 1986). It is also used as a preventive medicine for scurvy and stimulates digestion (Burkill 1966). However, there have been no reports on the insecticidal property of these plants from India.

Materials and methods

Plant materials

The leaves of *E. jambolana* and *S. canadensis* were collected from in and around Mysore, Karnataka. The leaves of *E. ridleyi* and the flowers of *S. mauritiana* were procured from in and around the Wayanad district, Kerala, India. These were shade-dried, powdered by using hand mixer grinder, and subjected to extraction with petroleum ether (60–80 °C) and ethyl acetate in a Soxhlet apparatus. The solvents from the plant extracts were removed using a vacuum rotary evaporator under reduced pressure at 40 °C. The extracts so obtained as thick viscous paste were completely dried by evaporation at room temperature and stored in brown bottles at 4 °C, and the known amount of these were dissolved in a solvent (acetone) to obtain stock solutions.

Mosquito colony

The egg, larvae, and adult stages of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* were obtained from the colony maintained in the Vector Biology Research Laboratory of the Zoology Department at Mysore University. They were maintained at 27 ± 2 °C, 75–85 % RH with 14:10 L/D photo period cycle. The pupae were transferred from a tray ($41 \times 23 \times 5$ cm) to a cup containing tap water and placed in cages ($45 \times 45 \times 45$ cm) where the adult emerged. The adult males were continuously provided with soaked dried grapes, and the females were fed on mouse placed in resting cage for blood meal for which ethical clearance was obtained. After 3 days, ovitrap was kept in the cages, and the eggs collected were transferred under the same condition for further experiments.

Larval bioassay

Larval bioassay was conducted as per WHO standard procedure (WHO 2005) to determine the efficacy of the plant extracts against the larvae of the three species of mosquitoes. Test concentrations were prepared by adding 1 ml of known concentration of phytoextract to 249 ml of dechlorinated tap water in a 500-ml-capacity beaker. Parallel control tests were also maintained by adding 1 ml of acetone to 249 ml of dechlorinated water. Twenty-five early fourth instar larvae of each species were released into each beaker, and the mortality was recorded after 24 h. No food was provided either to the test or control during the experiment. Three replicates were maintained for both test and control. The moribund and dead larvae in replicates were combined and expressed as percentage mortality at each concentration. The larvae were considered as dead or moribund, if they were not responsive to a gentle prodding with a fine needle. If the mortality was observed in the control, it will be corrected using Abbott's formula (Abbott 1925) and will be expressed as corrected mortality. All the bioassays were carried out at room temperature of 27±2 °C and 75-85 % humidity.

Ovicidal assay

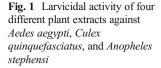
The ovicidal activity was assessed by following the method of Su and Mulla (1998). The egg raft/eggs of the three mosquito species were collected from the Vector Biology Research Lab. A minimum of 100 eggs of each mosquito species were taken in individual ovicidal cups of different concentrations (20, 40, 60, 80, and 100 ppm) of plant extracts. Simultaneously, one cup was kept with normal water containing acetone that served as a control. The experiment was repeated three times. After the treatment, the counted eggs from each concentration were individually transferred to stored water cups for the assessment of hatchability. The percent egg mortality was calculated on the basis of unhatched eggs after 98-h post-treatment by employing the following calculation (Elango et al. 2009).

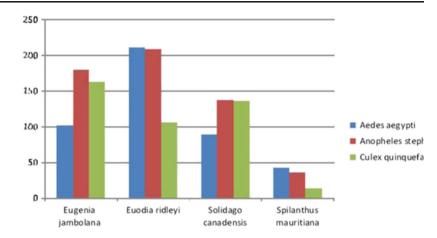
% of egg mortality =
$$\frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100.$$

Mosquito oviposition deterrence test

The effect of *E. jambolana*, *S. canadensis*, *E. ridleyi*, and *S. mauritiana* extracts on egg laying capacity of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* was studied by introducing 20 gravid females and an equal number of males into the cages $(45 \times 45 \times 40 \text{ cm})$ at $27 \pm 2 \text{ °C}$ and 75-85 % relative humidity with a photoperiod of 14:10-h light and dark cycles.

Table 1 Larvicidal activity	/ of four different plant extra	acts against <i>Aedes ae</i> g	Table 1 Larvicidal activity of four different plant extracts against Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi	Anopheles stephensi			
Mosquito species Aedes aegypti	Name of the plants Eugenia jambolana Solidago canadensis	Solvents Petroleum ether	LC50±SE (ppm), LCL–UCL 40.80±0.387 (37.94-43.63) 15.98±0.430 (8.67–26.8)	LC90±SE (ppm), LCL-UCL 83.28±0.38 (74.14-97.78) 61.16±0.430 (33.4-563.8)	Regression equation $Y=4.18X\pm12.59$ $Y=3.00X\pm9.60$	X (dx)=3 6.64 13.80	Significance -
	Euodia ridleyi Spilanthus mauritiana	Ethyl acetate	$149.4\pm2.199 (126.50-167.94)$ $14.10\pm0.284 (12.94-15.32)$	195.6 ± 2.199 (172.55–306.6) 33.08±0.284 (28.91–39.4)	$Y=3.91X\pm11.9$ $Y=2.25X\pm8.51$	17.89 7.26	
Anopheles* stephensi	Eugenia jambolana Solidago canadensis	Petroleum ether	96.0 ± 0.535 (91.06–100.6) 35.31 ± 0.581 (20.19–50.44)	$156.4\pm0.535 (145.1-172.8) \\ 101.3\pm0.581 (62.05-763.1)$	$Y=4.37X\pm12.69$ $Y=3.50X\pm12.01$	1.46 15.59	0.001
	Euodia ridleyi Snilanthus mauritiana	Ethyl acetate	119.9 ± 0.748 ($114.4-124.9$) 11.51 ± 0.43 ($8.65-14.6$)	$184.8\pm0.74 (171.7-205.9)$ $28.12\pm0.436 (20.62-53.93)$	$Y=3.13X\pm10.57$ $Y=3.57X\pm0.91$	0.06 7.93	
Culex* quinquefasciatus	Eugenia jambolana Solidago canadensis	Petroleum ether	53.84±0.316 (48.97–58.58) 28.30±0.555 (11.76–50.84)	127.4 ± 0.310 (111.8-152.1) 96.47\pm0.555 (52.81-2,351.2)	$Y=3.75X\pm 11.70$ $Y=3.65X\pm 10.33$	1.58 19.35	0.001
	Euodia ridleyi Spilanthus mauritiana	Ethyl acetate	75.31±1.829 (66.01–81.5) 5.103±0.510 (3.22–7.82)	98.3±1.82 (89.4−125.8) 11.0±0.150 (7.42−37.3)	$Y=3.78X\pm11.67$ $Y=3.60X\pm10.05$	10.33 6.91	
*Significant between A. ae	gypti, A. stephensi, and C. q	uinquefasciatus basec	*Significant between A. aegypti, A. stephensi, and C. quinquefasciatus based on the non-overlapping of 95 % fiducial limits (P<0.05)	iducial limits (P<0.05)			





The cages contained six bowls each with 100 ml of dechlorinated water mixed with concentrations of 20, 40, 60, 80, and 100 ppm of respective plant extracts, the sixth bowl being a control containing water mixed with acetone; the experiment was replicated three times. The number of eggs/egg rafts was counted after 48 h in the treated and control cups under a stereomicroscope. The effective repellency for each concentration was calculated using the following formula, following the method of Rajkumar and Jebasan (2009).

$$\mathrm{ER\%} = \frac{N_{\mathrm{C}} - N_{\mathrm{T}}}{N_{\mathrm{C}}} \times 100,$$

where ER=effective repellency, $N_{\rm C}$ =number of eggs in control, and $N_{\rm T}$ =number of eggs in treatment. The oviposition results were expressed as mean number of eggs and oviposition activity index (OAI) which was calculated using the following formula:

$$OAI = \frac{N_t - N_s}{N_t + N_s},$$

where N_t =total number of eggs in the test solution, and N_s = total number of eggs in the control solution (Kramer and Mulla 1979). The oviposition active indices of +0.3 and above are considered as attractants, while those with -0.3 and below are considered as repellents (Govindarajan et al. 2011). Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups. Thus, the test solutions were considered as deterrents.

Result and discussion

The results of the larvicidal activity of petroleum ether and ethyl acetate extracts of *E. jambolana*, *S. canadensis*, *E. ridleyi*, and *S. mauritiana* against the larvae of the three vector mosquitoes, i.e., *A. aegypti*, *A. stephensi*, and *C. quinquifasciatus* are presented in Table 1 and Fig. 1. Among the four plants tested, the maximum larvicidal activity was observed in the ethyl acetate extracts of *S. mauritiana* against *A. stephensi* than that of *C quinquefasciatus* and *A. aegypti* with LC₅₀ values of 11.51, 5.103, and 14.10 ppm, respectively. The chi-square values are significant (P<0.05).

The result of the egg hatchability of the three mosquito species tested against the two different solvents of different concentration of *E. jambolana*, *S. canadensis*, *E. ridleyi*, and *S. mauritiana* extracts is listed in Table 2 and Fig. 2. The percent hatchability was found to be inversely proportional to the concentration of the extracts. Among the four plant extracts tested for ovicidal activity against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*, the ethyl acetate extracts of *S. mauritiana* exerted 100 % mortality (zero hatchability) at 100 ppm against all the three mosquito species, followed by *S. canadensis*, *E. ridleyi*, and *E. jambolana*.

In the oviposition deterrence assay, it was found that the gravid female *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* preferred to lay eggs in the control cups rather than in the test cups. Out of the five concentrations of plant extracts tested, the higher concentration of 100 ppm was found to influence the egg-laying capacity to a large extent. The present results indicated that the oviposition deterrence was concentration dependent as 100 ppm of the plant extracts exhibited the strongest deterrent effect. From Table 3 and Fig. 3, it is clear that among the four plant extracts tested, *S. mauritiana* extract exhibited a higher repellency with a value of -0.20 followed by *S. canadensis*, *E. ridleyi*, and *E. jambolana* with a value of -0.15, -0.01, and -0.01, respectively. *A. stephensi* showed a significant activity (P < 0.05) followed by *C. quinquefasciatus* and *A. aegypti*.

Mosquito species	Eugenia jambolana			Solidago canadensis			Euodia ridleyi			Spilanthes mauritiana		
	Concentration	ER%	OAI	Concentration	ER%	OAI	Concentration	ER%	OAI	Concentration	ER%	OAI
Aedes aegypti	20	18.8	-0.10	20	26.0	-0.01	20	27.08	-0.15	20	33.68	-0.20
	40	15.5	-0.08	40	46.8	-0.09	40	52.08	-0.35	40	62.10	-0.45
	60	27.7	-0.16	60	47.3	-0.31	60	84.37	-0.72	60	83.15	-0.71
	80	61.0	-0.40	80	62.1	-0.45	80	91.66	-0.84	80	94.17	-0.90
	100	83.3	-0.71	100	83.15	-0.71	100	94.79	-0.90	100	96.80	-0.93
Anopheles stephensi	20	8.60	-0.04	20	2.10	-0.01	20	39.58	-0.24	20	45.83	-0.29
	40	25.0	-0.14	40	23.07	-0.13	40	66.6	-0.50	40	78.12	-0.64
	60	44.5	-0.20	60	43.95	-0.26	60	94.6	-0.84	60	93.5	-0.88
	80	68.5	-0.52	80	72.52	-0.56	80	100	-1.00	80	100	-1.0
	100	92.3	-0.85	100	95.60	-0.91	100	100	-1.00	100	100	-1.0
Culex quinquefasciatus	20	1.10	-0.005	20	1.08	-0.005	20	35.1	-0.21	20	41.05	-0.25
	40	21.1	-0.11	40	17.39	-0.09	40	59.37	-0.42	40	69.4	-0.53
	60	40.0	-0.25	60	42.39	-0.26	60	89.3	-0.80	60	91.5	-0.84
	80	68.8	-0.52	80	70.69	-0.54	80	98.7	-0.97	80	100	-1.00
	100	90.0	-0.81	100	92.39	-0.84	100	100	-1.00	100	100	-1.00

Table 2 Ovicidal activity of four different plant extracts against Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi

The widespread use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and development of resistance in the majority of vector species. This has necessitated the search and development of environmentally safe, biodegradable, low-cost, and indigenous methods for vector control which can be used with minimum care by individuals and communities in specific situations (Mittal and Subbarao 2003). Mosquito-borne diseases are one of the most serious public health problems in developing countries. It could be controlled by preventing mosquito bites using repellents, larvicides, and eliminating the mosquitoes. In this regard, a number of plant extracts have been reported to have mosquitocidal or repellent activities, but very few plant products have shown practical utility for mosquito control (Sukumar et al. 1991; Kalyanasundaram and Das 1985; Kalyanasundaram and Babu 1982). Our results showed that the crude petroleum ether and ethyl acetate extracts of *E. jambolana*, *S. canadensis*, *E. ridleyi*, and *S. mauritiana* have significant larvicidal, ovicidal, and oviposition-deterrent properties (P<0.05) against the three important vector mosquito species tested. Raghavendra et al. (2011) in an earlier study have reported the larvicidal activity of *E. jambolana* leaf extract against *A. aegypti* in Mysore. Further, Prathibha et al. (2011) have reported the larvicidal efficacy of *E. ridleyi* against *C. quinquefasciatus*. Our results are also comparable to that of Vahitha et al. (2002) who have tested *Pavonia zeylanica* and *Acacia ferruginea* for their larval efficacy against *C. quinquefasciatus*. Singh et al. (2003) also observed the larvicidal activity of *Ocimum canum* oil against

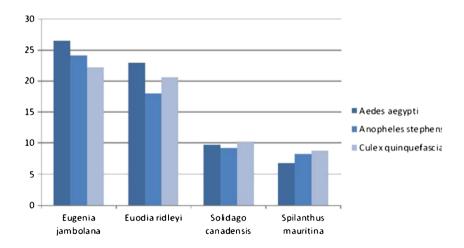


Fig. 2 Ovicidal activity of four different plant extracts against *Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi*



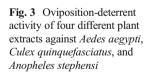
Table 3 Oviposition-deterrent activity of Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi to different plant extracts

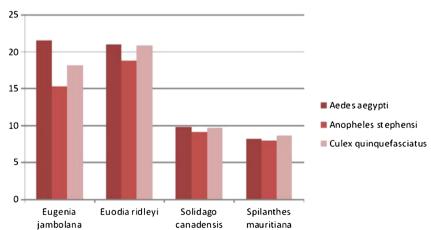
Mosquito species	uito species Eugenia jambolana		Euodia ridleyi		Solidago cana	densis	Spilanthus mauritiana		Significance
	Concentration	% of egg hachability	Concentration	% of egg hachability	Concentration	% of egg hachability	Concentration	% of egg hachability	
	_	_	_	_	-	_	-	65	
	_	-	-	-	_	-	-	42	
	_	-	—	-	_	-	_	23 12	
	_	-	_	-	_	-	_	05	
Aedes aegypti	Control	100	Control	100	Control	100	Control	100	_
6.7	20	96	20	95	20	72	20	65	
	40	84	40	70	40	52	40	42	
	60	61	60	59	60	20	60	23	
	80	40	80	34	80	12	80	12	
	100	14	100	12	100	08	100	05	
Anopheles*	Control	100	Control	100	Control	100	Control	100	0.001
stephensi	20	92	20	87	20	61	20	56	
	40	70	40	79	40	39	40	28	
	60	53	60	61	60	12	60	09	
	80	31	80	35	80	03	80	01	
	100	09	100	14	100	00	100	00	
Culex*	Control	100	Control	100	Control	100	Control	100	0.001
quinquefasciatus	20	94	20	93	20	65	20	59	
	40	76	40	71	40	42	40	31	
	60	59	60	56	60	13	60	11	
	80	37	80	29	80	03	80	00	
	100	12	100	10	100	00	100	00	

*Significant between A. aegypti, A. stephensi, and C. quinquefasciatus based on the non-overlapping of 95 % fiducial limits (P<0.05)

A. aegypti, C. quinquefasciatus, and A. stephensi. Pushpalatha and Muthukrishnan (1995) have showed that methanol leaf extracts of Vitex negundo, Vitex trifolia, Vitex peduncularis, and Vitex altissima had larvicidal activity against C. quinquefasciatus and A. stephensi. The present results agree with the earlier reports of Govindarajan (2009) on the leaf extract of C. fistula with different solvents, viz., methanol, benzene, and acetone regarding the larvicidal, ovicidal, and repellent activities against A. aegypti. Govindarajan et al. (2008) have also observed that the leaf extract of *Azadirachta indica* with different solvents, viz., benzene, chloroform, ethyl acetate, and methanol, had larvicidal activity, ovicidal activity, and oviposition attractancy against *A. stephensi*.

Oviposition deterrency may be due to the changes induced in the physiology and behavior of the adult mosquito species reflected by their egg-laying capacity. Some phytochemicals act as general toxicants against both adult and larval stages of mosquitoes, while others interfere with growth and development (growth inhibitors) or with reproduction





(chemosterilent) or produce olfactory stimuli acting as repellent or attractant. In this regard, Rajkumar and Jebasan (2009) have reported the oviposition deterrence effects of the extracts of Cassia obtusifolia with repellency at higher concentration (400 mg/L). Mehra and Hiradhar (2002) revealed that the crude acetone extract of Cuscuta hyalina was an effective oviposition deterrent against C. quinquefasciatus at a concentration of 80 ppm. Further, Coria et al. (2008) reported the oviposition deterrency of Melia azadarach leaf extract against A. aegypti. In the present investigation too, oviposition deterrent test indicated that S. mauritiana extract has good repellency followed by S. canadensis, E. ridlevi, and E. jambolana against three mosquito species. This result also agrees with that of Govindarajan et al. (2011) who has shown that the larvicidal and ovicidal activities of crude leaf extracts of Ervatamia coronaria and Caesalpinia pulcherrima against A. stephensi, A. aegypti, and C. quinquefasciatus. Our result also agrees with that of Elango et al. (2009) who showed that indigenous plant extracts such as Aegle marmelos, Andrographis lineate, Andrographis paniculata, Cocculus hirsutus, Eclipta prostrata, and Tagetes erecta have oviposition-deterrent, ovicidal, and repellent activity against Anopheles subpictus in Vellore District, Tamil Nadu, India. Similarly, Tawatsin et al. (2006) too have reported that the relatively high oviposition-deterrent activity was obtained by essential oils of Curcuma longa, Zingiber officinale, V. trifolia, Melaleuca cajuputi, Manglietia garrettii, and Houttuynia cordata. Thus, it is obvious that with our vast biodiversity, many bioactive compounds could be isolated and utilized for mosquito control.

Conclusion

The results of the present investigation revealed that the experimental plant extracts were effective in reducing the various life stages of A. stephensi, A. aegypti, and C. quinquefasciatus. Thus, the plants S. mauritiana, E. ridleyi, E. jambolana, and S. canadensis exhibit not only medicinal property but also larvicidal, ovicidal, and repellent activities against the three important vector mosquitoes in Mysore, India. Further, analysis to isolate the active compound for larval control is underway in our laboratory. More studies are needed to elucidate the ovicidal activity against a wide range of mosquito species and the active compound responsible for repellent activity. The flora of India is rich with aromatic plant diversity and has many potential for development of natural insecticides for control of vector and pest species. The present results could encourage the search for new active natural compounds from the Indian flora, offering an alternative to synthetic repellents and insecticides.

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References

- Abbott WS (1925) A method of computing of the effectiveness of an insecticide. J Econ Entomol 18:265–267
- Aivazi AA, Vijayan VA (2010) Efficacy of *Ruta graveolens* extract and its synergistic effect with cypermethrin against *Anopheles stephensi* Liston. J Toxicol Environ Chem 92:893–901
- Amer A, Melhorn H (2006) Repellency effect of forty-one essential oils against Aedes, Anopheles and Culex mosquitoes. Parasitol Res 99: 478–490
- Apati P, Szentmihalyi K, Kristo ST, Papp I, Vinkler P, Szake E, Kery A (2003) Herbal remedies of *Solidago*—correlation of phytochemical characteristics and antioxidative properties. J Pharm Biomed Anal 32(4–5):1045–1053
- Aregawi M, Cibulskis R, Otten M, Williams R, Dye C (2008). World malaria report. Geneva: World Health Organization (WHO). 190
- Baruah RN, Leclercq PA (1993) Characterization of the essential oil from flower heads of *Spilanthes acmella*. J Essent Oil Res 5:693–695
- Bernhard L, Bernhard P, Magnussen P (2003) Management of patients with lymphoedema caused by filariasis in north-eastern Tanzania: alternative approaches. Physiotherapy 89:743–749
- Bhanuprakash VM, Hosmani V, Balamurugan RK, Singh SD (2007) In vitro antiviral activity of Eugenia jambolana plant extract on buffalopox virus: conventional and QPCR methods. Int J Trop Med 2:3–9
- Burkill IH (1966) A dictionary of the economic products of the Malay peninsula, vol 2. Governments of Malaysia and Singapore by the Ministry of Agriculture and Co-operatives, Kuala Lumpur
- Chaturvedi AG, Bhawani PK, Agarwal S, Goel A, Singh GRK (2009) Ulcer healing properties of ethanolic extract of *Eugenia jambolana* seed in diabetic rats: study on gastric mucosal defensive factors. J Physiol Pharmacol 53:16–24
- Coria C, Almison W, Valladares G, Caspinella C, Luduena F, Defago M, Palacios S (2008) Larvicide and oviposition deterrent effects of fruit and leaf extracts from *Melia azadirachta* L. on *Aedes aegypti* (L.) (Diptera Culicidae). Bioresour Technol 99(8):3066–3070
- Elango G, Rahuman AA, Bagavan A, Kamaraj C, Zahir AA, Venkatesan C (2009) Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Parasitol Res 104(6):1381–1388
- Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K (2008) Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae) Parasitol Res 103(3):691–695.
- Govindarajan M (2009) Bioefficacy of Cassia fistula Linn. (Leguminosae) leaf extract against chikungunya vector, Aedes aegypti (Diptera: Culicidae). Eur Rev Med Pharmacol Sci 13(2): 99–103
- Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. (2011). Ovicidal and repellent activity of botanical extracts against Culex quinquefasiatus, Aedes aegypti and Anopheles stephensi, (Diptera: Culicidae). Asian. Pac. J. Trop. Med 1(1):43–48
- Horace F, Clay JC, Hubbard (1977) The Hawaii garden tropical shrubs. The University Press of Hawaii, Singapore, 287
- Jantan I, Yalvema MF, Ahmad NW, Jamal JA (2005) Insecticidal activities of the leaf oils of eight *Cinnamomum* species against *Aedes aegypti* and *Aedes albopictus*. Pharm Biol 43:526–532
- Jayaraj S (1993) Neem in pest control. Paper presented at the world neem conference, 24–28 Feb 1993. Bangalore, India, pp 37–43

Kalyanasundaram M, Babu CJ (1982) Biologically active plant extracts as mosquito larvicides. Ind J Med Res 76:102–106

- Kalyanasundaram M, Das PK (1985) Larvicidal and synergistic activity of plant extracts for mosquito control. Ind J Med Res 82:19–23
- Kramer WL, Mulla S (1979) Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. Environ Entomol 8:1111–1117
- Madhu SK, Shoukath AK, Vijayan VA (2009) Efficacy of bioactive compounds from *Curcuma aromatica* against mosquito larvae. Acta Trop 113:7–11
- Markouk M, Bekkouche K, Larhsini M, Bausaid H, Lazrek HB, Jana M (2001) Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. J Ethano Pharmacol 73:293–297
- Mehra BK, Hiradhar PK (2002) *Cuscuta hyalina* Roth. and insect development inhibitor against common house mosquito *Culex quinquefasciatus* Say. J Environ Biol 23:335–339
- Mittal PK, Subbarao SK (2003) Prospectus of using herbal products in the control of mosquito vector. ICMR Bull 33:1–10
- Myung K, Massaugbodji A, Ekone S, Atchade P, Kiki-Fagla V, Kilon AD (1998) Lymphatic filariasis in a hyperendemic region: ten year follow up survey. Am J Trop Med Hyg 59(2):222–226
- Oliver-Bever B (1986) Medicinal plants in Tropical West Africa. Cambridge University Press, Cambridge, pp 80–81
- Pancharoen C, Kulwichit W, Tantawichien T, Thisyakom U, Thisyakam C (2002) Dengue infection a global concern. J Med Assoc Thail 85: 25–33
- Pavela R (2009) Larvicidal effects of some Euroasiatic plants against *Culex quinquefasciatus* Say. Larvae (Diptera: Culicidae). Parasitol Res 105:887–892
- Peng Z, Yamng J, Wang H, Simons (1999) Production and characterization of monoclonal antibodies to two new mosquito Aedes aegypti salivary properties. Insect Biochem Med Biol 29:909– 914
- Prathibha KP, Raghavendra BS, Vijayan VA (2011) Evaluation of larvicidal effect of *Euodia ridleyi*, Hoch, leaf extract against three mosquito species at Mysore. Int J Biol Sci Eng 02(01):106–109
- Pushpalatha E, Muthukrishnan J (1995) Larvicidal activity of a few plant extracts against *Culex quinquefasciatus* and *Anopheles stephensi*. Ind J Malar Res 32:14–23

- Raghavendra BS, Prathibha KP, Vijayan VA (2011) Larvicidal efficacy of Eugenia jambolana Linn, extract in three mosquito species at Mysore. J Entomol 8(5):491–496
- Rahuman AA, Venkatesan P, Gopala Krishnan G (2008) Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.). Schrad Parasitol Res 103:1383–1390
- Rajkumar S, Jebasan A (2009) Larvicidal and oviposition activity of *Cassia obtusifolia* Linn. (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Cuoicidae). Parasitol Res 104(2):337–340
- Rigua-Perez JG, Clark GG, Gubler DJ (1997) Dengue and dengue hemorrhagic fever. Lancet 352:971–977
- Singh NP, Kumari V, Chauhan D (2003) Mosquito larvicidal properties of the leaf extract of a herbaceous plant, *Ocimum canum* (Family: Labiatae). J Commun Dis 35(1):43–45
- Su T, Mulla S (1998) Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc 14(2):204–209
- Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 7:210–237
- Taubitz W, Cramer TP, Kapaun A, Pefeffer M, Drasten C, Dobler G, Burchard GD, Loscher T (2007) Chikungunya fever in travelers: clinical presentation and course. Clin Infect Dis 45(1):e1–e4
- Tawatsin A, Asavadachanukorn P, Thavara U, Wonngiskongman P, Bansidhi J, Boonruad T, Chavalittumrong P, Soonthomchareonnon N, Komalamisra N, Mulla MS (2006) Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). Southeast Asia J Trop Med Public Health 37(5):915–931
- Vahitha R, Venkatachalam MR, Murugan K, Jebanesan A (2002) Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. Bioresour Technol 82(2): 203–204
- World Health Organisation (2005).Guideline for laboratory in field testing of mosquito larvicidesWHO/CDS/WHOPES/GCDPP/2005.13.
- World Health Organization (2010) Malaria. Factsheet No. 94 Geneva. http://www.who.int/mediacentre/factsheets/fso94/en/. Accessed July 2010