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Studies on effects of indigenous plant extracts on malarial vector, *Anopheles subpictus* Grassi (Diptera:Culicidae)

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Abstract. Mosquitoes transmit serious human diseases, causing millions of deaths every year. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The present investigations were made to evaluate the repellent, ovicidal and oviposition-deterrent potential of leaf hexane and chloroform extracts of *Aegle marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrata* and *Tagetes erecta* against *Anopheles subpictus* Grassi (Diptera:Culicidae). The hexane extract of *A. lineata* was more effective in exhibiting the repellent action against the mosquito as compared with *A. marmelos* extract. Complete protections for 150 min were found in hexane extract of *A. lineata* at 500 ppm against *An. subpictus* bites. Mean percent hatchability of the ovicidal activity was observed 24 h after treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. No hatchability was observed with hexane and chloroform extracts of *A. lineata*, *A. paniculata* and hexane extract of *T. erecta* were exerted at 1,000 ppm. The percentage of effective oviposition repellency of 93.07, 93.95, 98.03, 90.43, 92.63, 81.53, 94.81, 97.50, 97.26, 92.22, 82.85 and 72.77 at 500 ppm and the lowest repellency of 62.03, 53.64, 73.47, 59.05, 57.95, 48.17, 62.22, 72.99, 75.48, 67.77, 40.57 and 52.11 at 31.25 ppm in hexane and chloroform extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata* and *T. erecta*, respectively. The oviposition activity index (OAI) values revealed that the solvent plant extracts have deterrent effect, and they caused a remarkable negative response resulting in oviposition of very few eggs. These results clearly reveal that the hexane extract of *A. lineata*, served as a potential repellent, ovicidal and oviposition- deterrent against *An. subpictus*.

INTRODUCTION

Malaria remains one of the most serious world health problems and the major cause of mortality and morbidity in the endemic regions. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar *et al*., 2007). *Anopheles subpictus* is known to transmit malaria and filariasis in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local "frequent feeding strategy" in this primarily zoophagic and endophilic malaria vector (Amerasinghe & Amerasinghe, 1999). The search for new strategies or natural products to control destructive insects and

vectors of diseases is desirable due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic nonbiodegradable residues contaminating the environment and undesirable effects on nontarget organisms (Jantan *et al*., 2005).

The present study was carried out on *Aegle marmelos*, an Indian plant with religious and medicinal importance. The ethyl acetate extracts of the stem bark exhibited moderate insecticidal activity against *Phaedon cochleariae* and *Musca domestica* (Samarasekera *et al*., 2004). The methanol and aqueous extracts of *Andrographis lineata* established pharmacological evidence to support the folklore claim that it is used traditionally as a hepatoprotective agent (Sangameswaran *et al*., 2008). The methanol and ethyl acetate extracts of *Andrographis paniculata* were tested on *Callosobruchus chinensis* (Bright *et al*., 2001). *Cocculus hirsutus* is a widely growing plant found in the plains of India and the aerial parts aqueous extract showed significant diuretic activity and laxative effect in rats (Ganapaty *et al*., 2002). *Tagetes erecta* methanol and dichloromethane extracts showed a significant activity against *Sitophilus oryzae* (Broussalis *et al*., 1999).

As far as our literature survey could ascertain, no information was available on the repellent, ovicidal and oviposition - deterrent activities of the experimental plant species given here against *An. subpictus*. Therefore, the aim of this study was to investigate the mosquito repellent, ovicidal and oviposition - deterrent activities of hexane and chloroform extracts of six plant species from Tamil Nadu, India.

MATERIALS AND METHODS

Plant collection

The leaves of *A. marmelos* (Linn.) Correa ex Roxb (Rutaceae), *A. lineata* Wallich ex Nees. (Acanthaceae), *A. paniculata* (Burm.f.) Wall. ex Nees. (Acanthaceae), *C. hirsutus* (L.) Diels (Menispermaceae), *Eclipta prostrata* L. (Asteraceae), and *T. erecta* L. (Compositae) were selected on the basis of aromatic smell, bitter taste, resistance to damage by insect pests, ethnopharmacological, traditionally used medicinal value and ethnobotanical literature survey. The plant materials were collected from the Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13º13'4 N, 79º59'7E; Altitude 118 feet), Chennai, Tamil Nadu, and the taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

Mosquito culture

Anopheles subpictus larvae were collected from stagnant water area of Melvisharam (12º56'23"N, 79º14'23"E) and identified in Zonal Entomological Research Centre, Vellore (12º552 483N, 79º72483E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out, at 27±2ºC and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary (45×45×40 cm) where adults emerged. Adults were maintained in glass cages and were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day five, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. Glass petri dishes with 50 ml of tap water lined with filter paper was kept inside the cage for oviposition. They were maintained and reared in the laboratory as per the method of Rahuman *et al*. (2008).

Preparation of plant extracts

The leaves were dried for 7-14 days in the

shade at the environmental temperatures (27-37ºC day time). The dried leaves (1240 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (2,800 ml, Qualigens) chloroform (2,200 ml, Qualigens) in a Soxhlet apparatus (boiling point range 60–80ºC) for 8 h. The yield of extracts was hexane (7.04g) and chloroform (10.74g). The extract was concentrated under reduced pressure 22–26 mm Hg at 45ºC, and the residue obtained was stored at 4ºC. One gram of crude extract was first dissolved in 100 ml of acetone (stock solution). From the stock solution, 1,000 and 500 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution.

Repellency activity

The stock solutions of the extracts were diluted with acetone, polysorbate 80 and distilled water to obtain test solutions of 31.25, 62.50, 125.00, 250.00, and 500.00 ppm. For repellent experiment, 50 laboratory reared blood-starved adult female mosquitoes that were between 3 and 10 days old were placed into separate laboratory cages (45×45×40 cm). Before each test, the forearm and hand of a human subject were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry 10 min before extracts application. The different plant extracts being tested (31.25–500 ppm) were applied from the elbow to the fingertips. The arm was left undisturbed. An arm treated with acetone and polysorbate 80 served as control. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min, every 30 min, from 18:00 h to 06:00 h. Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. If no bites were confirmed at 150 min, tests were discontinued and protection time was recorded as 150 min. An attempt of the mosquito to insert its stylets was considered a bite. No mosquito attempted to bite the control arm during the observation period; that trial was discarded, and the test was repeated with a new batch of mosquitoes to ensure that lack of bites was due to repellence and not to mosquitoes not being predisposed to get a blood meal at the time. The experiments were conducted five times in separate cages and in five replicates. Different volunteer were used to nullify any effect of skin differences on repellency. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula (Venkatachalam & Jebanesan, 2001; Fradin & Day, 2002). Protection = ({No. of bites received by control arm} {No. of bites received by treated arm}) (No. of bites received by control arm) X 100.

Ovicidal assay

For ovicidal activity, the freshly laid eggs were collected by providing ovitraps in mosquito cages. Ovitraps were kept in the cages 2 days after the female mosquitoes were given a blood meal. The eggs were laid on filter paper lining provided in the ovitrap. After scoring, 100 gravids were placed in a screen cage (45×45×40 cm) where ten oviposition cups (350 ml plastic cups, 91 mm in height and 75mm in diameter, covered with black paper on the outside) were introduced for oviposition 30 min before the start of the dusk period. Of these ten cups, nine were each filled with test solution of 15.62, 31.25, 62.50, 125.0, 250.0, 500.0 and 1000 ppm, and one was filled with 100 ml of water containing acetone and polysorbate 80 that served as a control. A minimum of 100 eggs was used for each treatment, and the experiment was replicated five times. After 3 h of treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cubs filled with dechlorinated water for hatching assessment after counting the eggs under microscope (Su & Mulla, 1998). The percent egg mortality was calculated on the basis of nonhatchability of eggs with unopened

opercula (Chenniappan & Kadarkarai, 2008). The hatching rate of eggs was assessed after 98 h post treatment as per the method of Rajkumar & Jebanesan (2009).

Oviposition - deterrence assay

To study the oviposition deterrence effect and the number of eggs deposited in the presence of different solvent extracts of experimental plants, a multiple concentration test was carried out. For bioassay test, 20 males and 20 females were separated in the pupal stage and were introduced into screen cages (45×45×40 cm) in a room at 27±2°C and 75–85% relative humidity with a photoperiod of 14:10 h light and dark cycles. The sex of individual pupae can be determined by looking at the ninth segment of the abdomen and by the size of the pupae. The ninth segment on male mosquitoes is more prominent during the pupal stage, while the female pupa is usually larger in size than the male (male, 1.28–1.60mm and female, 2.11–2.43mm). The pupae were allowed to emerge into adults in the test cages. Adults were provided continuously with 10% sucrose solution in a plastic cup with a cotton wick. They were blood fed (from pigeon) on day five after emergence. In the multiple concentration test, five cups, each containing 100 ml distilled water with a 9-cm piece of white filter paper for oviposition as well as solvent extracts at a concentration of 31.25, 62.50, 125.0, 250.0, and 500.0 ppm were placed in each cage. A sixth cup without extract served as a control. The control was set up with acetone, water and polysorbate 80. The positions of the plastic cups were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in treated and control cups were counted under a stereomicroscope. The percent effective repellency for each concentration was calculated using the following formula.

$$ER\% = \frac{NC - NT}{NC} \times 100$$

where ER=effective repellency, NC=number of eggs in control, and NT=number of eggs in treatment (Rajkumar & Jebanesan, 2009). The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula.

$$OAI = \frac{NT - NS}{NT + NS}$$

where NT = total number of eggs in the test solution and NS = total number of eggs in the control solution. Oviposition active index of +0.3 and above are considered as attractants, while those with – 0.3 and below are considered as repellents (Kramer & Mulla, 1979). 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 and that the test solutions were a deterrent.

Statistical analysis

One-way analysis of variance (ANOVA) was used for the multiple concentration tests and for percent mortality to determine significant treatment differences (Sokal & Rohlf, 1981). Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSIONS

Mosquitoes are the most deadly vector for several of these disease causing organisms. The tested solvent plant extracts have exerted promising repellent, ovicidal and oviposition - deterrent activities against *An. subpictus*. The results from the skin repellent activity of leaf hexane and chloroform extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E.*

prostrata and *T. erecta* against blood-starved adult female of *An. subpictus* are given Table 1. The results clearly show that repellent activity was dose dependent. In the present study, we observed 150 min protection at 500 ppm in hexane extracts of *A. lineata*, *E. prostrata* and chloroform extract of *C. hirsutus* against *An. subpictus*. The control provided only 3.2±0.68 min of protection.

The results are comparable with a earlier report by Pandey *et al.* (2009), who reported that the thymol compound isolated from *Trachyspermum ammi* provided complete repellency toward *Anopheles stephensi* adults at the dose of 25.0 mg/mat after 1 h duration, whereas same degree of repellency was obtained by the essential oil of seeds at the dose of 55.0 mg/mat, indicating its double-fold activity than the oil. Earlier scientists have reported that the essential oils were extracted from 18 plant species, belonging to 11 families, and the oils were then prepared as 10% solution in absolute ethanol with additives evaluated the repellent effects and the result showed that the night-biting mosquitoes, *Anopheles dirus* and *Culex quinquefasciatus* and *Aedes albopictus* were more sensitive to all the essential oils (repellency 4.5–8 h) than was *Aedes aegypti* (repellency 0.3–2.8 h), whereas deet and IR3535 provided excellent repellency against *Ae. aegypti*, *Ae. albopictus*, *An. dirus* and *Cx. quinquefasciatus* (repellency 6.7–8 h) (Tawatsin *et al.*, 2006). In a similar study

Table 1. Repellent activity of different plant extracts against malaria vector, *Anopheles subpictus*

Botanical name/ Family (Herbarium numbers) Vernacular names	Concentrations (ppm)	% of repellency* (±SE)							
		Time post application of repellent (min)							
		60 mins		90 mins		120 mins		150 mins	
		Hexane	Chloroform	Hexane	Chloroform	Hexane	Chloroform	Hexane	Chloroform
Aegle marmelos (Linn)	31.25	100±0.0	70±1.83	66±1.84	52±1.36	42±1.34	36±1.24	22±1.74	20±1.18
Correa ex Roxb /	62.50	100±0.0	84±1.33	74±2.63	62±1.74	54±2.71	48±1.22	34±1.72	36±1.78
Rutaceae	125.00	100±0.0	89±1.48	80±2.49	84±2.76	66±2.49	68±1.32	52±1.84	41±1.34
(LE/ZB/067-07) Vilvam	250.00	100±0.0	96±1.94	86±1.78	92±2.18	78±2.98	89±1.48	62±1.34	56±1.14
	500.00	100±0.0	100±0.0	94±1.41	100±0.0	84±1.89	100±0.0	71±1.42	84±1.56
Andrographis lineata	31.25	100±0.0	70±2.38	90±2.42	51±1.68	84±1.46	38±2.74	54±1.21	16±3.11
Wallich ex Nees.	62.50	100±0.0	81±2.76	100±0.0	72±1.84	90±1.74	54±1.86	68±1.36	34±2.18
(AL/ZB/024-07)	125.00	100±0.0	100±0.0	100±0.0	86±2.73	96±2.80	64±2.34	74±1.38	49±1.76
Siriyanangai /	250.00	100±0.0	100±0.0	100±0.0	92±3.00	100±0.0	72±3.00	90±1.74	60±1.38
Acanthceae	500.00	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89±1.94	100±0.0	74±1.46
Andrographis paricutata	31.25	60±1.33	72±2.71	52±2.14	61±1.86	42±1.72	54±3.11	31±2.73	21±1.72
(Burm.f.) Wall. ex Nees.	62.50	74±1.72	84±2.16	64±2.16	78±1.76	54±1.33	67±2.93	46±1.32	35±1.74
(AP/ZB/065-07)	125.00	86±1.48	96±1.84	72±1.34	84±1.82	68±1.49	74±2.86	51±1.34	42±1.83
Periyanangai	250.00	100±0.0	100±0.0	89±1.22	96±1.89	78±2.33	86±2.17	68±1.33	56±1.56
Acanthaceae	500.00	100±0.0	100±0.0	94±1.34	100±0.0	94±3.10	92±2.14	76±1.68	69±1.89
Cocculus hirustus(L.)	31.25	56±1.70	75±1.76	45±1.11	63±1.56	32±1.33	39±1.76	20±2.33	21±1.12
Diels/ (Menispermaceae)	62.50	68±2.34	86±1.36	51±1.28	78±2.52	41±1.18	46±1.86	34±1.43	38±1.89
(CH/ZB/098-07)	125.00	72±3.10	100±0.0	62±1.36	88±2.14	52±1.69	78±2.34	40±1.62	66±1.10
Vellakattukkodi	250.00	83±3.24	100±0.0	68±1.39	100±0.0	60±1.59	90±1.86	56±1.72	89±1.16
	500.00	96±2.84	100±0.0	78±1.48	100±0.0	70±1.84	100±0.0	64±1.84	100±0.0
Eclipta prostata L./	31.25	80±2.33	51±1.06	68±1.11	42±1.33	42±1.72	30±1.46	24±2.11	16±2.17
Asteraceae	62.50	94±1.10	73±1.88	76±1.36	65±1.48	56±1.64	46±1.58	32±2.34	34±2.84
(EP/ZB/146-07)	125.00	100±0.0	86±1.73	100±0.0	72±1.39	72±1.38	52±1.68	66±1.86	43±3.11
Manjal Karisallankannai	250.00	100±0.0	92±1.52	100±0.0	86±1.28	100±0.0	76±1.70	82±1.30	58±2.16
	500.00	100±0.0	100±0.0	100±0.0	92±2.08	100±0.0	84±1.62	100±0.0	70±2.96
Tagetes erceta L.	31.25	62±1.89	44±1.13	33±1.99	36±2.36	24±1.63	28±1.63	18±1.76	14±2.96
Compositae	62.50	74±1.84	62±1.06	46±1.96	55±3.15	38±1.28	46±1.53	26±2.88	39±2.91
(TE/ZB/122-07)	125.00	86±1.21	70±1.09	58±1.86	61±2.00	46±1.31	56±1.48	38±1.78	43±2.37
Tulukkaccevvanti	250.00	100±0.0	86±1.11	64±1.30	74±1.14	57±1.47	63±1.36	46±1.65	56±1.89
	500.00	100±0.0	94±2.66	72±2.45	85±1.65	65±1.71	78±1.74	54±1.60	66±2.90

* Mean value of five replicates ±SE

Table 2. Ovicidal activity of different plant extracts against eggs of *Anopheles subpictus*

Plants	Solvents	Percentage of egg hatching±SD							
		Concentrations (ppm)							
		15.62	31.25	62.50	125	250	500	1000	Control
A.marmelos	Hexane	92±1.24	82±1.41	71±1.76	56±1.62	42±1.74	20±1.17	15±1.76	98±1.70
	Chloroform	86±1.76	74±1.72	67±1.12	60±1.20	43±1.32	30±1.70	22±1.76	94±1.16
A.lineata	Hexane	75±1.23	65±1.64	54±2.76	31±2.11	23±1.78	12±1.84	NH	96±1.16
	Chloroform	88±2.17	62±1.32	50±1.76	41±1.21	23±1.10	16±1.62	NH	100±0.00
A. paniculata	Hexane	96±1.75	89±1.00	65±1.70	43±1.86	38±1.71	16±1.33	NH	94±1.33
	Chloroform	71±2.41	62±2.11	56±2.17	42±2.76	33±1.92	25±1.76	NH	94±1.33
C. hirsutus	Hexane	86±2.15	70±1.78	54±2.74	48±2.94	32±1.86	26±1.90	16±2.11	96±1.34
	Chloroform	96±1.31	84±1.69	65±2.16	54±1.11	42±1.33	32±1.94	24±1.74	94±2.74
E. prostrata	Hexane	98±1.36	82±3.00	74±1.89	66±1.19	36±1.11	28±1.40	20±1.20	100±0.00
	Chloroform	98±2.14	74±2.16	64±2.16	52±1.78	46±1.84	28±1.66	10±1.86	96±1.71
T. erceta	Hexane	84±1.33	74±1.11	56±1.65	45±1.56	35±1.81	18±1.67	NH	98±1.70
	Chloroform	70±1.64	64±1.24	54±1.34	45±1.62	39±1.80	26±1.36	19±1.39	100±0.00

NH = No hatchability (100% mortality).

the repellent activity against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* which is due to 5% vanillin which has been added to the essential oil of *Curcuma longa* (Tawatsin *et al.*, 2001). The seed acetone extract of *Tribulus terrestris* showed 100% repellency in 0, 4, 6 h; in 1, 6 h and in 0, 2, 4 h, at 10% concentration against *Anopheles culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* respectively (Singh *et al.*, 2008). Venkatachalam & Jebanesan (2001) have also reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. aegypti* activity at 1.0 and 2:5 mg/cm^2 concentrations gave 100% protection up to 2:14±0:16 h and 4:00±0:24 h, respectively, and the total percentage protection was 45.8% at 1: 0 mg/cm^2 and 59.0% at 2:5 mg/cm^2 for 10 h. The essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2 provided 100% protection up to 120 min against *Cx. quinquefasciatus* (Pushpanathan *et al.*, 2008). The essential oils of *Ipomoea cairica*, *Momordica charantia* and *Tridax procumbens* exhibited relatively high repellency effect (>300 minutes at 6% concentration), followed by *Centella asiatica* and *Psidium guajava* which showed less effective (<150 minutes at 6% concentration) against malarial vector, *An. stephensi* (Rajkumar & Jebanesan, 2007). Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *Citrullus colocynthis* gave complete protection time ranging from 107 to 271 minutes and *Cucurbita maxima* exerted the complete protection time of 78 to 215 minutes against *C. quinquefasciatus* (Mullai & Jebanesan, 2007). Compared with earlier reports the present study revealed that the hexane extracts of *A. lineata*, *E. prostrata* and chloroform extract of *C. hirsutus* showed more repellent activity against *An. subpictus*.

The percentages of egg hatchability of *An. subpictus* with the leaf hexane and chloroform extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata* and *T. erecta* are presented in Table 2. In the present investigation, the ovicidal activity of hexane, chloroform extracts of *A. lineata*, *A. paniculata* and hexane extract of *T. erecta* proved to have good ovicidal activity against *An. subpictus*. The hexane and chloroform extracts of *A. lineata* and *A. paniculata* and hexane extract of *T. erceta* exerted 100% mortality (no hatchability) at 1,000 ppm and at 250 ppm a very low hatchability

(31±2.11,41±1.21, 43±1.86, 42±2.76 and 35±1.81) was recorded against *An. subpictus* respectively. Almost 100% hatchability was obtained in the control experiments. 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 *Cx. quinquefasciatus* (Miura *et al*., 1976).

Earlier authors Mullai & Jebanesan (2006) reported complete ovicidal activity (100 % mortality) was attained at 300 ppm for methanol, benzene, petroleum ether and ethyl acetate extracts of *Citrullus pubescens* against *Cx. quinquefasciatus*. In a similar study Govindarajan *et al*. (2008a) reported that the younger age groups of egg rafts or eggs showed poor hatchability rate when exposed to higher concentrations of extract and older age groups of egg rafts or eggs showed high hatchability rate when exposed to lower concentrations of extract. The benzene extracts of *Citrullus vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm and at 200 ppm a very low hatchability (11.8%), complete ovicidal activity at 300 ppm and the fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), fraction III, and IV afforded 4.9 and 5.3% hatchability recorded against *An. stephensi* and *Ae. aegypti* respectively (Mullai *et al*., 2008). The mean percent hatchability of the egg rafts were observed after 48 h treatment. 100% mortality was observed at 450 ppm for *C. colocynthis* and 600 ppm for *Cucurbita maxima* against *Cx. quinquefasciatus* (Mullai & Jebanesan, 2007). The seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *C. quinquefasciatus* (Ouda *et al*., 1998). The ovicidal effect of *Solenostemma argel* was low however, concentrations of 0.05% and 0.1% exhibited significant effects ($p<0.05$), producing 65 and 75%; 62.9 and 62.9%, respectively, on the 1st and 2nd day after treatment, respectively, and the 0.1% concentration reduced egg hatch by 33.7%, compared with the control and 100% mortality values were evident in concentrations as low as 0.025% at 2 days post hatching against *Culex pipiens* (Al-Doghairi *et al*., 2004). Azadirachtin isolated from *Azadirachta indica* has been shown to exert complete ovicidal action in the eggs of *Culex tarsalis* and *Culex tritaeniorhynchus* at 10ppm concentration (Su & Mulla, 1998). The methanol containing water that served as a control showed 94% hatchability in 0–3-h-old egg rafts/eggs, but the 100% hatchability was noted in egg rafts/eggs beyond the age of 0–3 h old in leaf methanol (90%) extract of *Cassia fistula* against egg raft of *Cx. quinquefasciatus* (Govindarajan *et al*., 2008b). In the present investigation, the ovicidal activity of hexane, chloroform extracts of *A. lineata*, *A. paniculata* and hexane extract of *T. erecta* proved to be effective and promising against *A. subpictus* compared with earlier reports.

The present results showed that in the oviposition - deterrence assay, gravid *An. subpictus* preferred to lay eggs in the distilled water control cups than in the cups treated with solvent extracts of six plants Table 3. There was also a marked difference in the number of eggs laid. The present results showed that the 500ppm treated cups tested with leaf hexane and chloroform extracts of *E. prostrata* received a mean number of 14±1.62and 28±1.12 eggs per cup while the control cups received a mean number of 512±2.69 and 360±3.11 eggs per cup, respectively. The mean number of eggs laid in hexane leaf extracts of *A. lineata* and *C. hirsutus* showed12±1.23 and 20±1.70, respectively, compared with the controls. The present results indicated that the oviposition - deterrence was concentration dependent, as 500 ppm of hexane and chloroform of leaf extracts of experimental plant exhibits strong deterrent effect when compared with 31.25 ppm against oviposition. The solvent leaf extracts strongly deterred oviposition by gravid *An. subpictus*, with a significantly lower proportion of eggs being laid on ovitraps containing extracts in comparison with control solutions

Table 3. Oviposition response of *Anopheles subpictus* to different plant extracts

Plants	Concentrations	Hexane				Chloroform			
		Number of eggs ± SE				Number of eggs ± SE			
		Treated	Control	ER%	OAI	Treated	Control	ER%	OAI
A. marmelos	500.00	36±1.11	520±1.00	93.07	-0.87	29±1.71	480±1.27	93.95	-0.88
	250.00	42±1.23	492±1.50	91.46	-0.84	33±2.17	460±1.33	92.82	-0.86
	125.00	62±1.46	412±1.21	84.95	-0.73	68±1.36	320±1.47	78.87	-0.64
	62.50	77±1.72	375±1.23	79.46	-0.65	73±1.42	280±1.68	73.92	-0.58
	31.25	82±1.88	216±1.77	62.03	-0.44	89±1.82	192±1.52	53.64	-0.36
A.lineata	500.00	12±123	612±1.34	98.03	-0.96	44±1.76	460±2.30	90.43	-0.82
	250.00	33±1.11	510±1.86	93.41	-0.87	56±1.82	420±1.73	86.66	-0.76
	125.00	48±1.76	420±1.74	88.57	-0.79	62±1.14	360±1.34	82.27	-0.70
	62.50	52±1.84	360±1.26	85.55	-0.74	72±1.82	280±1.12	74.28	-0.59
	31.25	61±1.13	230±1.30	73.47	-0.58	86±1.63	210±1.10	59.04	-0.41
A.paricutata	500.00	28±1.24	380±1.00	92.63	-0.86	48±1.40	260±1.69	81.53	-0.68
	250.00	34±1.26	360±1.28	90.55	-0.82	62±1.11	220±1.32	71.81	-0.56
	125.00	43±1.74	284±1.74	84.85	-0.73	72±1.62	190±1.43	62.10	-0.45
	62.50	62±1.14	260±1.66	76.15	-0.61	80±1.10	170±1.15	52.94	-0.36
	31.25	74±1.84	176±1.17	57.95	-0.48	85±1.70	164±1.16	48.17	-0.32
C. hirsutus	500.00	20±1.74	386±1.70	94.81	-0.90	12±1.36	480±2.31	97.50	-0.95
	250.00	26±1.25	312±2.62	91.66	-0.84	22±1.21	376±3.00	94.14	-0.88
	125.00	32±1.53	275±1.33	88.36	-0.79	32±1.69	352±1.76	90.17	-0.83
	62.50	48±1.84	220±1.36	78.18	-0.64	48±1.74	311±1.68	84.56	-0.73
	31.25	68±.11	180±2.16	62.22	-0.45	74±1.10	274±1.70	72.99	-0.57
E. prostrata	500.00	14±1.62	512±2.69	97.26	-0.94	28±1.12	360±3.11	92.22	-0.85
	250.00	41±1.61	468±1.45	91.23	-0.85	34±1.76	312±2.21	89.91	-0.80
	125.00	56±1.43	368±1.36	88.44	-0.73	46±2.16	284±1.46	83.38	-0.72
	62.50	74±1.21	314±1.40	80.86	-0.61	52±3.00	264±1.69	80.30	-0.67
	31.25	84±1.0	290±1.60	75.48	-0.55	68±1.72	211±1.66	67.77	-0.51
T. erceta	500.00	36±2.69	210±3.11	82.85	-0.70	49±3.45	180±1.16	72.77	-0.57
	250.00	44±3.17	196±2.17	77.55	-0.63	59±2.34	175±1.26	66.28	-0.49
	125.00	58±1.98	172±2.71	66.27	-0.49	66±1.68	164±1.76	59.75	-0.42
	62.50	71±3.16	150±6.90	52.66	-0.35	78±2.16	146±2.66	46.57	-0.30
	31.25	82±3.60	138±1.98	40.57	-0.25	87±2.67	136±1.40	52.11	-0.21

Mean value of five replicates ±SE.
ER = Effective repellency.
OAI = Oviposition active index.

($p<0.05$). The maximum percentage of effective repellency against oviposition was 98.03 noted in 500 ppm followed by 93.41, 88.57, 85.55 and 73.47 that were noted in 250, 125, 62.50, and 31.25 ppm in hexane extracts of *A. lineata*, respectively. The oviposition activity index (OAI) value of hexane, chloroform extracts of *A. marmelos*, *A. lineata*, *A. paniculata*, *C. hirsutus*, *E. prostrata* and *T. erecta* at 500 ppm were -0.87, -0.88, -0.96, -0.82, -0.86, -0.68, -0.90, -0.95, -0.94, -0.85, 0.70 and -0.57 respectively (Table 3).

In a similar study Xue *et al.* (2006) pointed out the oviposition - deterrent effectiveness (76–100% repellency) against *Ae. albopictus* of 21 commercial insect repellent products (at 0.1% concentration), including 12 botanical, six deet-based, and three synthetic organics. Xue *et al.* (2001; 2003) have reported that the ovipositional deterrent effects of deet and several repellent compounds, such as AI3-37220, AI3-35765, AI3-54995, AI3-55051 against *Ae. albopictus* under laboratory and field conditions. Mehra & Hiradhar (2002)

revealed that the crude acetone extract of *Cuscuta hyaline* was an effective oviposition deterrent against *Cx. quinquefasciatus* at a concentration of 80 ppm. Coria *et al.* (2008) have reported that the full oviposition deterrency was obtained with *Melia azedarach* leaf extract at 1 g/L against *Ae. aegypti*. The benzene, chloroform, ethyl acetate, and methanol *A. indica* showed the highest effective attractancy of 90.09%, 94.20%, 85.43%, and 95.75% were observed at 100 ppm and the lowest effective attractancy of 47.17%, 61.94%, 49.28%, and 68.12% were observed at 25 ppm against *An. stephensi*, respectively (Govindarajan *et al.*, 2008a). Rajkumar & Jebanesan (2009) have reported that the oviposition deterrence effects of ethanolic leaf extract of *Cassia obtusifolia* at higher concentration (400 mg/l) showed 92.5% effective repellency against oviposition, followed by 300, 200, and 100 mg/l showed 87.2%, 83.0%, and 75.5%, respectively. The leaf extract of *Solanum trilobatum* reduced egg laying by gravid females of *An. stephensi* from 18% to 99% compared with ethanol-treated controls at 0.01, 0.025, 0.05, 0.075, and 0.1% (Rajkumar & Jebanesan, 2005).

The oviposition deterrent properties against *An. stephensi* have been observed for various plant extracts including the methanol extract of *Pelargonium citrosa*, which exhibited 56% and 92% inhibition of oviposition at 1 and 4 ppm, respectively (Jeyabalan *et al.*, 2003). There were no significant differences in the numbers of eggs laid on distilled water or on distilled water containing Tween 20 and acetone for day 3 (P=0.6412) or day 5 (P=0.1344) and the numbers of eggs laid on terpineol or its control on day 3 (P=0.939) and day 5 (P=0.857) against *Ae. aegypti* (Waliwitiya *et al.*, 2009). The crude aqueous extract of *Ricinus communis* at different concentrations (1200, 600 and 200ppm) showed (90–100%) oviposition deterrence and effective repellence against *Anopheles arabiensis* when the extract was used as material of choice (Abdalla *et al.*, 2009). Autran *et al.* (2009) have reported that the essential oil from leaves and stems of *Piper marginatum* exhibited an oviposition deterrent effect against *Ae. aegypti* at 50 and 100 ppm in that significantly lower numbers of eggs (<50%) were laid in glass vessels containing the test solutions compared with the control solution. Tawatsin *et al.* (2006) have reported that the relatively high oviposition deterrencies were obtained from essential oils of *C. longa* (94.7%), *Schefflera leucantha* (91.6%), and *Z. officinale* (90.1%), *Vitex trifolia* (89.1%), *Melaleuca cajuputi* (87.9%), *Hedychium coronarium* (87.5%), *P. guajava* (87.1%), *Manglietia garrettii* (86.1%), and *Houttuynia cordata* (85%) and moderate degrees of deterrency were obtained from *Piper nigrum* (82%), *Litsea cubeba* (80.6%), and *Eleutherococcus trifoliatus* (80.2%) against *Ae. aegypti*. The essential oil of *Cinnamomum zeylanicum* resulted into highest repellent (RD95) values of 49.6, 53.9, and 44.2 mg/mat against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively, apart from oviposition-deterrent potential (Prajapati *et al.*, 2005). The present study revealed that the leaf hexane and chloroform extracts of *E. prostrata* and hexane leaf extracts of *A. lineata* and *C. hirsutus* showed more oviposition - deterrent activity against *An. subpictus* compared with the similar earlier authors reports. The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn will increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme, they could lead to development of new classes of possible safer insect control agents.

The present study revealed the repellent, ovicidal and oviposition-deterrent activities at low concentrations and short exposure time of some Indian medicinal plants. As naturally occurring insecticides, these plant-derived materials

could be useful as an alternative for synthetic insecticides in controlling field populations of *An. subpictus*. The present study plants are easily available, accessible and affordable therefore the usage of traditional repellent plants should be promoted among the local residents in order to reduce the man–vector contact as well as vector-borne diseases. The screening results suggest that the hexane extract of *A. lineata* are promising in mosquito control. Further studies on isolation of bioactive fraction / constituent may provide futuristic lead products for field application of mosquito control.

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