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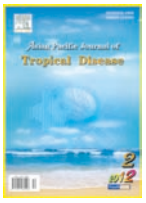

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Toxicity of *Aristolochia bracteata* methanol leaf extract against selected medically important vector mosquitoes (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the larvicidal, ovicidal and repellent activities of methanol extract of *Aristolochia bracteata* (*A. bracteata*) against *Aedes aegypti* (*Ae. aegypti*), *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Larvicidal efficacy of *A. bracteata* was tested at various concentrations against the early third instar larvae of *Ae. Aegypti*, *An. Stephensi* and *Cx. quinquefasciatus*. Bioassay test was carried out by WHO 2005; the 24h LC_{50} values of the *A. bracteata* leaf extract was determined by probit analysis. For ovicidal activity, slightly modified method of Su and Mulla was performed. Ovicidal activity was determined against selected mosquitoes to various concentrations ranging from 50-300 ppm under laboratory conditions. The hatch rates were assessed 48 h post treatment. The repellent efficacy was determined against selected mosquito species at three concentrations *viz.*, 1.0, 2.0 and 3.0 mg/cm^2 under laboratory conditions. **Results:** The LC_{50} and LC_{90} values of methanol leaf extract of *A. bracteata* against early third instar larvae of *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus* were 114.89, 120.82, 132.24 and 216.24, 230.31, and 238.22 ppm, respectively. The crude extract of *A. bracteata* exerted 100% egg mortality (zero hatchability) at 240, 300 and 360 ppm for *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. Similarly, a higher concentration of 6.0 mg/cm^2 provide 100% protection up to 210, 180 and 150 min against *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. **Conclusions:** The present results suggest that the *A. bracteata* methanol leaf extracts provided an excellent potential for controlling selected medically important vector mosquito.

1. Introduction

The mosquito is a common insect found around the world. There are about three thousand five hundred species of mosquitoes. Mosquitoes are the major vector of diseases, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, *etc*. causing millions of deaths every year[1-3]. *Anopheles* species are the most important species as they are capable vector for malaria parasites. Approximately half of the world's population is at risk of malaria, particularly those living in lower-income countries. It infects more than five hundred million people per year and kills more than one million. *Culex* mosquitoes are painful and persistent biters and are responsible for filariasis. These mosquitoes are very common in Indian sub-continent. *Aedes* mosquitoes on the other hand are also painful and persistent biters. *Ae. aegypti* is responsible for spreading Dengue and Chikungunya. Dengue is prevalent throughout the tropics and subtropics. The World Health Organization estimates that around 2.5 billion people are at risk of dengue[4]. Mosquitoes cause substantial mortality and morbidity among people living in tropical and sub tropical zones[5,6]. It is arguably one of most domestic mosquito vectors, feeding predominantly on man, mating and resting indoors and breeding in man-made containers in and around human habitations, especially in urban environments[7]. Chemical insecticides have been/ are being used to control these disease vectors. The greatest harm from chemical insecticides is that once introduced into the system, they may remain there forever or for a very long duration. Thus, they pose a threat to life and help insects to develop resistance against them. This is the reason that there has always been a need for such an insecticide which is more powerful, with lesser side effects and degrading after sometime, reducing the change to develop resistance against it. These problems have renewed interest in exploiting the pest control potential of plants. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for the control of

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mosquito and other pests. In addition to application as general toxicants against mosquitoes, phytochemicals may also have potential uses as larvicides, repellents, ovicides and oviposition deterrents, and growth and reproduction inhibitors[8-11].

A. bracteata (Aristolochiaceae) commonly called as Worm killer in English and aadutheendaapaalai in Tamil, wildly distributed in Deccan Gujarat, western and southern India, Bihar, Sindh, Bundelkhand and Bengal. *A. bracteata* is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, snake bites and insecticidal properties[12-14]. Furthermore, a mosquitocidal property of *A. bracteata* has not yet reported. Therefore, In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal, ovicidal and repellent efficacy of *A. bracteata* medicinal plant extracts against medically important selected vector mosquitoes.

2. Materials and methods

2.1. Plant material

Plant sampling was carried out during the growing season (March- April) of 2010 from different places of Koothur, Sirkali, Nagapattinam districts of the Tamilnadu. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany, Annamalai University, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

2.2. Extraction method

The dried leaf (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with methanol (500 mL, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45 ℃ by "Rotavapour" and the residue obtained was stored at 4 ℃.

2.3. Mosquito rearing

The mosquitoes, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, were reared in the Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast power in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2) ℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.4. Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO[15]. From the stock solution, six different test concentrations (*viz.* 40, 80, 120, 160, 200 and 240 ppm) were prepared and they were tested against the freshly moulted (0 - 6 h) third instar larvae of selected mosquitoes. The larvae of test species (25) were introduced in 500-mL plastic cups containing 250 mL of aqueous medium (249 mL of dechlorinated water + 1mL of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis[16].

2.5. Ovicidal activity

The method of Su and Mulla[17] was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of selected mosquitoes were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

2.6. Repellent activity

The repellent study was following the methods of WHO[18]. 3-4 days old blood-starved female selected mosquitoes (100) were kept in a net cage (45 cm×45 cm×40 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The *A. bracteata* leaf extract at 1.5, 3.0 and 6.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 30 min. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

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

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

2.7. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and chi-square value were calculated using the SPSS software package 12.0. Results with $P<0.05$ were considered to be statically significant.

3. Results

The toxicity of methanol crude leaf extract of *A. bracteata* was tested against larvae of *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. The data were recorded and statistical data ranging LC_{50}, LC_{90}, LCL, UCL and chi-square value

were calculated. Results on the larvicidal, ovicidal and repellent effects of leaf extract was reported in the present study, confirm their potential for control of the mosquito populations (Table 1–3). The LC_{50} and LC_{90} values of methanol leaf extract of *A. bracteata* against early third instar larvae of *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus* were 114.89, 120.82, 132.24 and 216.24, 230.31, and 238.22 ppm, respectively. The crude extract of *A. bracteata* exerted100% egg mortality (zero hatchability) at 240, 300 and 360 ppm for *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. Similarly, a higher concentration of 6.0 mg/cm^2 provide 100% protection up to 210, 180 and 150 min against *Ae. Aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*. This study showed that leaf extract of *A. bracteata* would be a potent source of natural larvicidal, ovicidal and repellent activities against selected medically important vector mosquito species.

Table 1
Larvicidal activity of crude methanol extract of *Aristolochia bracteata* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

Mosquitoes	Concentration (ppm)	24 h mortality (%)	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	χ^2
				LCL	UCL		
Ae. aegypti	40	29.6±2.6^b	114.89	89.22	140.82	216.24	21.341*
	80	38.0±1.8^c					
	120	54.0±1.4^d					
	160	72.0±1.5^e					
	200	86.6±1.2^f					
	240	99.0±1.6^g					
	Control	0.0±0.0^a					
An. stephensi	40	24.0±1.2^b	120.82	94.65	148.28	230.31	19.133*
	80	31.3±1.5^c					
	120	43.8±1.6^d					
	160	64.3±1.3^e					
	200	75.0±1.4^f					
	240	94.0±1.2^g					
	Control	0.0±0.0^a					
Cx. quinquefasciatus	40	18.4±1.6^b	132.24	112.84	159.27	238.22	14.643*
	80	28.6±1.4^c					
	120	41.2±1.5^d					
	160	58.7±1.2^e					
	200	71.0±1.4^f					
	240	92.9±1.8^g					
	Control	0.0±0.0^a					

Each value mean± SD represents mean of six values. Values in a column with a different superscript alphabet are *significantly different at $P < 0.05$ (MANOVA; LSD –Tukey's Test). LCL–Lower confidence limit; UCL–Upper confidence limit.

Table 2
Ovicidal activity of *Aristolochia bracteata* plant extracts against *Aedes aegypti*, *Anopheles stephensi* and *Cx. quinquefasciatus*.

Mosquitoes	Percentage of egg hatch ability, Concentration (ppm)						
	Control	60	120	180	240	300	360
Ae. aegypti	100.0±0.0	54.3±2.2	32.4±1.8	18.8±1.2	NH	NH	NH
An. stephensi	100.0±0.0	76.8±1.9	52.8±1.6	37.7±1.3	19.8±1.2	NH	NH
Cx. quinquefasciatus	100.0±0.0	84.3±1.7	65.4±1.4	48.6±1.4	35.8±1.5	17.7±1.2	NH

Each value mean± SD represents the mean of six values. NH – No hatchability (100% mortality)

Table 3
Repellent activity of crude methanol extract of *Aristolochia bracteata* against *Aedes aegypti*, *Anopheles stephensi* and *Cx. quinquefasciatus*.

Mosquitoes	Concentration (mg/cm^2)	% of repellency, Time post application of repellent(min)							
		30	60	90	120	150	180	210	240
Ae. aegypti	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.2±1.4	72.2±1.5	63.2±1.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.5±1.2	68.5±1.8
	6.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.3±1.6
An. stephensi	1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.2±1.6	76.3±1.2	63.7±1.3	48.4±1.7
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±1.4	75.5±1.8	62.2±1.5
	6.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.7±1.4	88.2±1.7
Cx. quinquefasciatus	1.5	100.0±0.0	100.0±0.0	100.0±0.0	91.4±1.7	83.4±1.3	68.3±3.2	52.8±1.2	38.1±1.2
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.1±1.2	76.1±1.7	63.7±1.5	44.4±1.8
	6.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.1±1.5	84.1±1.8	79.2±1.4

Each value mean± SD represents mean of six values.

4. Discussion

In our results showed that, crude extract of *A. bracteata* have significant larvicidal, ovicidal and repellent activities against selected medically important vector mosquito species. The results are comparable with an earlier report by Tripathy *et al*[19] the LC_{50} values of *Lantana cramera* (*L. cramera*) root extract for *An. stephensi*, *Ae. Aegypti* and *Cx. quinquefasciatus* were 132.55, 27.82, and 11.68 ppm, respectively, whereas those of *Anacardium occidentale* (A. occidentale) leaf extract were 56.81, 912, and 10.79 ppm, respectively. Screening of natural products for mosquito larvicidal activity against three major mosquito vectors *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi* resulted in the identification of three potential plant extracts *viz.*, *Saraca indica/asoca* (*S. indica/asoca*), *Nyctanthes arbor-tristis* (*N. arbor-tristis*), and *Clitoria ternatea* (*C. ternatea*) for mosquito larval control[20]. Kamaraj *et al*[21] they have been reported mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The acetone, chloroform, ethyl acetate, hexane, methanol and petroleum ether extracts of leaf, flower and seed of *Cassia auriculata (C.auriculata), Leucas aspera (L. aspera), Rhinacanthus nasutus (R. nasutus), Solanum torvum (S. torvum)* and *Vitex negundo (V. negundo)* were tested against fourth instar larvae of malaria vector, *Anopheles subpictus (An. subpictus)* and Japanese encephalitis vector, *Culex tritaeniorhynchus (Cx. tritaeniorhynchus).* Choochote *et al*[22] worked on the intrinsic toxicity of ethanolic extract of the whole plant of *Piper longum (P. longum), Piper ribesoides (P. ribesoides)* and *Piper sarmentosum (P. sarmentosum)* against *Ae. aegypti*. They reported the activity to be comparatively high in P. sarmentosum followed by *P. ribesoides* and *P. longum* with LD 50 values of 0.14, 0.15 and 0.26 μg/mg female adult mosquito. Murugesan and Muthusamy[23] reported that bioassays with an ethanolic extract of *Melia azedarach (M. azedarach)* were performed on the larval stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The root extract of *Valeriana jatamansi (V. jatamansi)* which exhibited adulticidal activity of 90% lethal concentration against adult *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *An. Albopictus* and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively[24]. Senthilkumar *et al*[25] have also reported that the larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globules (E. globules)*, *Cymbopogan citrates (C. citrates)*, *Artemisia annua (A. annua), Justicia gendarussa (J. gendarussa), Myristica fragrans (M. fragrans), Annona squamosa (A. squamosa)*, and *Centella asiatica (C. asiatica)* were tested against *An. stephensi*, and the most effective between 80% and 100% was observed in all extracts. The lethal concentration (LC_{50}) values of Ficus benghalensis (F. benghalensis) against early second, third and fourth larvae of *Cx. quinquefasciatus*, *Ae. Aegypti* and *An. Stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm, respectively[26]. Dua *et al*[27] determined LD_{50} values of the oil were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm^2 while LD_{90} values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm^2 against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluvialitis* and *An. stephensi* respectively. KDT_{50} of the oil were 20, 18, 15, 12, and 14 min and KDT_{90} values were 35, 28 25, 18, 23 min against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi*, respectively on 0.208 mg/cm^2 impregnated paper. Hossain *et al*[28] reported that the mortality rate was higher in 50 ppm doses of methanolic extracts of *Dregea volubilis (D. volubilis)* and *Bombax malabaricum (B. malabaricum)* both the plants against *Cx. quinquefaciatus*. The corresponding LC_{50}values were 56.97 ppm and 48.85 ppm. Abdalla *et al*[29] have also reported that the *A. arabiensis* extracts against *Cx. quinquefasciatus* that caused high, moderate and low larval mortality in the larvicidal experiment against 3rd instar larvae. It was found that, LC_{50}-LC_{90} values calculated were 273.53-783.43, 366.44-1018.59 and 454.99-1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively, of *An. Arabiensis* and 187.93-433.51, 218.27-538.27 and 264.85-769.13 ppm for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus*. Eliningaya *et al*[30] have been reported the mortality of *Cx. quinquefasciatus* ranged from 0.5 to 96.75% while for *A. gambiae* it was from 13.75% to 97.91%. The LC_{50} and LC_{95} value in the laboratory was similar for both species while in the semi- field they were different for each. Mullai and Jebanesan[31,32] who studied that the leaf extract of two cucurbitacious plants *Citrullus colocynthis* (*C. colocynthis*) and *Cucurbita maxima (C. maxima)* different solvents were tested for ovicidal and repellent activities against the mosquito *Cx. quinquefasciatus*. 100% mortality was observed at 450 ppm for *C. colocynthis* and 600 ppm for *C. maxima*. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *C. colocynthis* gives the complete protection time ranges from 107 to 271 min. *C. maxima* exerted the complete protection time of 78 to 215 min. Mullai *et al*[33] reported that the efficacies of the Cucurbitaceous plant *Citrullus vulgaris (C. vulgaris)* against the *An. stephensi* were tested for ovicidal and repellent activities against *An. stephensi*. For ovicidal activity, 100 per cent mortality was exerted at 250 ppm with benzene extract and the other extracts exerted 100 percent mortality at 300 ppm. Skin repellent test at 1.0, 2.5 and 5.0 mg per cm^2 concentration gave the mean complete protection time ranged from 119.17 to 387.83 min with the four different extracts tested. Samidurai *et al*[34] studied that crude leaf extracts of *Pemphis acidula (P. acidula)* were evaluated for larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} values of methanol, benzene, acetone were 10.81, 41.07, 53.22 ppm and 22.10, 43.99, 57.66 ppm, respectively. Hundred percent ovicidal activities were observed at 350 ppm and 450 ppm. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *P. acidula* gave 100% protection up to 2.30, 4.00 and 6.45 h and 2.45, 4.30 and 7.0 h respectively. The findings of the present investigation revealed that the leaf extract of *A. bracteata* possessed remarkable larvicidal, ovicidal and repellent activities against medically important selected vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] James AA. Mosquito molecular genetics: the hands that feed bite back. *Science* 1992; **257**: 37-38.

[2] Morisson AC, Zielinsky-Guitierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solution for control of the virus vector *Aedes aegypti*. *Plos Med* 2008; **5**: 362-366.

[3] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from O/CDS/WHOPES/GCDPP/2005.13.

[4] World Health Organisation. Malaria Factsheet No.94. Geneva: World Health Organisation. [Online] Available from http://www.who.int/mediacentre/factsheets/fs094/en/. [Accessed July 2010].

[5] Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vect Bor and Zoonot Dise* 2007; **7**: 76-85.

[6] Kroeger A, Nathan MB. Dengue: setting the global research agenda. *Lancet* 2006; **368**: 2193-2195.

[7] Nirmala A, Saroja S, Vasanthi HR, Lalitha G. Hypoglycemic effect of *Basella rubra* in streptozotocin - induced diabetic albino rats. *J Pharmac Phytoth* 2009; **1**: 25-30.

[8] Mathivanan T, Govindarajan M, Elumalai K, Krishnappa K, Ananthan A. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae). *J Vector Born Dis* 2010; **47**: 178-180.

[9] Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43-48.

[10] Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2011; **109**(2): 353-367.

[11] Krishnappa K, Elumalai K. Chemical composition, larvicidal and ovicidal activities of essential oil from *Clausena excavata* against armyworm, *Spodoptera litura* (Fab.) (Lepidoptera :Noctuidae). *Asian Pac J Trop Biomed* 2012; 1-3.

[12] Kavitha D, Nirmaladevi R. Assessment of *Aristolochia bracteolata* leaf extracts for its biotherapeutic potential. *Afr J Biotech* 2009; **8** (17): 4242-4244.

[13] Havagiray R, Chitme, Nitin P, Patel. Antiarthritis activity of *Aristolochia Bracteata* extract in experimental animals. *Open Natural Prod J* 2009; **2**: 6-15.

[14] Angalaparameswari S, Mohamed Saleem TS, Alagusundaram M, Ramkanth S, Thiruvengadarajan VS, Gnanaprakash K, et al. Anti-microbial activity of aristolochic acid from root of *Aristolochia bracteata* Retz. *Inter J Bio Life Sci* 2012; **8**: 4-2.

[15] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from WHO/CDS/WHOPES/GCDPP/2005.13.

[16] Finney DJ. *Probit analysis*. London: Cambridge University Press; 1979, p. 68-72.

[17] Su T, Mulla MS. Ovicidal activity of neem products (azadirachtin) against *Culex* tarsalis and *Culex quinquefasciatus* (Diptera:Culicidae). *J Am Mosq Cont Asso* 1998; **14**(2): 204-209.

[18] World Health Organization. Report of the WHO informal consultation on the evaluation and testing of insecticides. Geneva: World Health Organization; 1996. [Online] Available from CTD/WHOPES /IC /96.1.

[19] Tripathy A, Samanta L, Das S, Parida SK, Marai N, Hazra RK, et al. The mosquitocidal activity of methanolic extracts of *Lantana cramera* root and *Anacardium occidentale* leaf: role of glutathione S-transferase in insecticide resistance. *J Med Entom* 2011; **48**(2): 291-295.

[20] Mathew N, Anitha MG, Bala TS, Sivakumar SM, Narmadha R, Kalyanasundaram M. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitol Res* 2009; **104**(5): 1017-1025.

[21] Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus Grassi* and *Culex tritaeniorhynchus Giles* (Diptera: Culicidae). *Parasitol Res* 2009; **104**(5): 1163-1171.

[22] Choochote W, Chaithong U, Kamsuk K, Rattanachanpichai E, Jitpakdi A, Tippawangkosol P, et al. Adulticidal activity against *Stegonyia aegypti* (Diptera: Culicidae) of three *Piper* spp. *Rev Do Insti De Med Trop De Sao Pau* 2006; **48**(1): 33-37.

[23] Murugesan S, Muthusamy M. Efficacy of *Melia azedarach* on the larvae of three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Euro Mos Bulletin* 2011; **29**: 116-121.

[24] Dua VK, Alam MF, Pandey AC, Rai S, Chopra AK, Kaul VK. Insecticidal activity of *Valeriana jatamansi* (Valerianaceae) against mosquitoes. *J Am Mosq Control Assoc* 2008; **24**: 315-318.

[25] Senthilkumar N, Varma P, Gurusubramanian G. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* (Liston). *Parasitol Res* 2009; **104**: 237-244.

[26] Govindarajan M. Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extract against *Culex quinquefasciatus* Say. *Aedes aegypti* L. and *Anopheles stephensi* L. (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2010; **14**(2): 107-111.

[27] Dua VK, Pandey AC, Dash AP. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J Med Res* 2010; **131**: 434-439.

[28] Hossain E, Rawani A, Chandra G, Mandal S, Gupta C. Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. *Asian Pac J Trop Med* 2011; **4**(5): 436-441.

[29] Abdalla M, Elimam, Khitma H, Elmalik, Faysal S, Ali. Efficacy of leaves extract of *Calotropis procera* Ait. (Asclepiadaceae) in controlling *Anopheles* arabiensis and *Culex quinquefasciatus* mosquitoes. *Saudi J Bio Sci* 2009; **16**: 95-100.

[30] Eliningaya J, Kweka, Mramba Nyindo, Franklin Mosha, Ary G, Silva. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia* Raddi against African malaria vectors. *Parasites and vectors* 2011; **4**: 129-124.

[31] Mullai K, Jebanesan A. Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitacious plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera : Culicidae). *Trop Biomed* 2007; **24**(1): 1-6.

[32] Paramanik M, Chandra G. Studies on seasonal fluctuation of different indices related to filarial vector, *Culex quinquefasciatus* around foothills of Susunia of West Bengal, India. *Asian Pac J Trop Med* 2010; **3**(9): 727-730.

[33] Mullai K, Jebanesan A, Pushpanathan T. Mosquitocidal and repellent activity of the leaf extract of Citrullus vulgaris (cucurbitaceae) against the malarial vector, *Anopheles stephensi* liston (diptera culicidae). *Eur Rev Med Pharmacol Sci* 2008; **12**: 1-7.

[34] Samidurai K, Jebanesan A, Saravanakumar A, Govindarajan M, Pushpanathan T. Larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst. (Lythraceae) against filarial and dengue vector mosquitoes. *Aca J Entomol* 2009; **2**(2): 62-66.