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Contributed Paper

Mosquito Larvicidal Properties of *Anisomeles malabarica* (L.) Extracts Against the Malarial Vector, *Anopheles stephensi* (Liston)

Yuwalee Unpaprom [a], Rameshprabu Ramaraj*[b] and Murugan Kadarkarai [b]
[a] Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand.
[b] Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India.
* Author for correspondence; e-mail: rrameshprabu@gmail.com

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Abstract

Anopheles stephensi mosquitoes are the leading vector of malaria in Indian subcontinent, parts of Asia and the Middle East. Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Plants may be alternative sources of mosquito control agents. The present study assessed the role of larvicidal activities of methanolic leaf and inflorescence extract of *Anisomeles malabarica* (L.) against malarial vector *Anopheles stephensi* Liston (Insecta: Diptera : Culicidae). The larvicidal activity was assayed against the mosquito species at various concentrations ranging from (20 to 100 ppm) under the laboratory conditions. The LC_{50} and LC_{90} value of the methanolic leaf and inflorescence extract of *Anisomeles malabarica* (L.) was determined by Probit analysis. The percentage of mortality of *Anopheles stephensi* after the treatment of *Anisomeles malabarica* on I to IV instar larvae from 20,40,60,80 to100 ppm. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 50.24%, II instar was 54.70%, III instar was 59.03% and IV instar was 64.33%, respectively. LC_{90} value of I instar 106.99%, II instar was 113.33%, III instar was 118.04% and IV instar was 119.94%, respectively. The present results suggest that the effective *Anisomeles malabarica* leaf and inflorescence crude extracts have potential to be used as an ideal eco-friendly approach for the control of mosquito vectors.

Keywords: mosquitoe, *Anisomeles malabarica* (L.), *Anopheles steph*ensi Liston, larvicidal activity, eco-friendly

1. INTRODUCTION

The Mosquitoes constitute the most important single family "culicidae"of insects from the stand point of human health. Mosquitoes are vectors of a number of major diseases, including malaria, dengue fever, yellow fever and filariasis, chikungunya, schistosomiasis and Japanese encephalitis [1-2]. Malaria remains one of the most prevalent and deadly infectious diseases across Africa, Asia, and the Americas. Malaria is a parasitic disease. The World Health Organization (WHO) estimates 154–289 million malaria cases in 2010, with 660,000 associate deaths [3-5]. Malaria is caused by five species of Plasmodium that in-

fect humans (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* spp., *Plasmodium malariae* and *Plasmodium knowlesi*) and is transmitted by the bite of infected female Anopheline mosquitoes [7].

Anopheles stephensi (*An. stephensi*) has a very wide distribution, occurring from India and other countries in the middle east across Pakistan, Iran to Myanmar, Thailand and China. *An. stephensi* Liston is the main urban vector of malaria in the Indo-Pakistan sub-continent and Middle-East. Anopheles mosquitoes breed in fresh, shaded ponds, brackish or even polluted waters in man-made habitats such as water tanks, cisterns, wells, gutters, tyre tracks, water storage jars and containers [8]. Adult bite humans indoors or outdoors and rest mainly indoors afterwards. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds [9, 10].

At present, there are many insecticides available for control *An. stephensi*. Chemicals larvicides could be carcinogenic, mutagenic and teratogenic for humans. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on worldwide. Natural products, including plant extracts could be suitable candidates for such alternative approaches. Plants have been exposed to possess marked insecticidal properties and may act as suitable alternative product to fight against mosquito borne diseases [9]. Extracts from plant sources have demonstrated promising potential as insecticidal/larvicidal agents [10-13].

Medicinal plant extracts and their constituents have proved to be biodegradable and their activities were similar to those of the standard drugs, such as temephos and methoprene. Hence present study an attempt has been made to screen and evaluate the larvicidal properties of medicinal plant: *Anisomeles malabarica* (L). *Anisomeles malabarica* R. Br. Is an erect shrub commonly known as "Peimiratti or Malabar catmint", is a highly aromatic plant [14, 15]. *Anisomeles malabarica* (L.) (Family: Lamiaceae) is a medicinal plant, has been used as a folkloric medicine to treat amentia, anorexia, fevers, swellings, rheumatism, halitosis, epilypsy, hysteria, amentia, anorexia, dyspepsia colic, flatulence, intestinal worms, fever arising from teething in children, intermittent fevers, vitiated conditions of vata and kapha gout, swellings and diarrhea [15,16]. Citral, betulinic acid, â-sitosterol, ovatodiolide, anisomelic acid, anisomelolide, malabaric acid, 2-acetoxymalabaric acid, anisomelyl acetate, anisomelol and anisomelin were present in the leaves and inflorescence of this plant [17]. Consequently, in this study, we aim to explore the larvicidal properties of methanolic extract of *A. malabarica* plant leaf and inflorescence on the larvicidal effect on the malaria vector *An. stephensi*.

2. MATERIALS AND METHODS

2.1 Collection of Eggs and Maintenance of Larvae

The eggs of *An. stephensi* were collected from local (in and around Coimbatore, Tamil Nadu India) different breeding habitats with the help of a 'O' type brush. These eggs were brought to the laboratory and transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml water and kept for larval hatching. The larvae reared in plastic cups. They were daily

provided with commercial fish food [18] *ad libitum*. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through unidentified mosquitoes. The feeding was continued until the larvae transformed into the pupal stage.

2.2 Maintenance of Pupae and Adult

The pupae were collected from culture trays and were transferred to glass beakers containing 500 ml of water with help of a sucker. The glass beaker containing pupae was then kept in 90 x 90 x 90 cm mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four side (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained 27 ± 2°C, 75%-85% RH, under 14L:10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

2.3 Blood Feeding of Adult Mosquito Vector and Egg Laying

The adult mosquito was allowed to feed on the blood of a rat (shaved on the dorsal side) for the two days, to ensure adequate blood feeding, the fed was continued for five days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arranged made collection of eggs easier.

2.4 Plant Collection and Preparation of Extract

Anisomeles malabarica L. (Labiatae) were collected from in and around the Bharathiar University campus, Coimbatore, Tamil Nadu, India. *A. malabarica* leaves (AMLE) and inflorescence (AMIFE) was washed with tap water and shade dried at room temperature. The dried plant materials were powdered by an electrical blender. From the powder 200g of the plant material were extracted with 2.5 litres of methanol for 24 hrs in a Soxhlet apparatus [19]. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from 20 to 100 ppm.

2.5 Larval Toxicity Test of Plant Extract

A laboratory colony of *An. stephensi* larvae were used for the larvicidal activity. Twenty five number of first, second, third and fourth instar larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts. Larval food was given for the test larvae. At each tested concentration 2 to 5 trails were made and each trial consisted of three replicates. The control was set up by mixing 1ml of acetone with 249 ml of dechlorinated water.

2.6 Statistical Analysis

The average larval mortality data were subjected to Probit analysis for calculating LC_{50} (Lethal concentration that kills 50% of the exposed larvae), LC_{90} (Lethal concentration that kills 90% of the exposed larvae), and other statistics at 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated by using calculated using the SPSS 9.0 version

(software package) [20, 21]. The values were expressed as mean ± standard deviation of five replicates. Results with $p < 0.05$ were considered to be statistically significant. In addition, all data were subjected to analysis of variance (ANOVA), completely Randomised Design (CRD) and the means were separated using Duncan's multiple range test (DMRT) [22]. The control mortalities were corrected by using Abbott's formula [23].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment- Observed mortality in control}}{\text{100- Control mortality}} \text{ X } 100$$

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{ X } 100$$

3. RESULTS AND DISCUSSION

In the present study, *A. malabarica* extracts have been brought out toxicity on different larval instars of *An. stephensi* and moreover complete reduction of larval was noticed after combination of AMLE and AMIFE. *A. malabarica* leaves and inflorescence extracts treatment had detrimental effect upon *An. stephensi* larval growth and development. Larval toxicity effect of *A. malabarica* leaf extract (AMLE) on *An. stephensi* is shown in the table 1. The percentage of mortality of *An. stephensi* after the treatment of *A. malabarica* on I to IV instar larvae and pupae from 20,40,60,80 to100 ppm. 26% mortality was noted at I instar larvae by the treatment of AMLE at 20 ppm, whereas it has been increased to 88% at 100 ppm of AMLE treatment.

Table 1. Larval toxicity effect of AMLE (Methanolic extract) on the malarial vector, *An.stephensi.*

Larval stages	% of larval mortality					LC_{50}	LC_{90}	95% confidence Limit				χ^2 value
	Concentration (ppm)							LCL		UCL		
	20	40	60	80	100			LC_{50}	LC_{90}	LC_{50}	LC_{90}	
I	26e	43d	56e	71e	88e	50.24	(106.99)	44.40	(97.45)	55.51	(120.57)	0.907
II	24c	40c	54d	68c	87d	54.70	(113.33)	48.98	(102.89)	60.09	(128.41)	1.720
III	22b	37b	49b	63b	86c	59.03	(118.04)	53.50	(107.03)	64.53	(134.03)	3.444
IV	17a	34a	42a	59a	85b	64.33	(119.94)	52.55	(99.96)	77.60	(165.64)	5.436

χ^2 – Chi-square values are significant at P<0.05 levels.
Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT

There are several studies expressed the medicinal significant on *A. malabarica* [24-27]. Sharma et al. [28] reported that in India, the whole plant acetone extract of *A. malabarica* showed 100% ovipositional deterrence activity against the potato tuber moth, *Phthorimaea operculella*. Zahir et al. [29] have also communicated that the highest parasite mortality was found in leaf acetone and methanol extracts of *A. malabarica*; and leaf hexane and chloroform

extracts of *A. malabarica*. Kamaraj et al. [30] expressed *A. malabarica* (L.) R. Br., was tested against the parasitic nematode of small ruminants *H. contortus* using egg hatch assay and larval development assay. Methonolic extracts of *A. malabarica* were tested against the fourth instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* [29, 31]. Since the highest parasite mortality was reported in leaf acetone and methanol extracts of *A. malabarica*.

This study was agreed with the previous research reports related larvicidal control (different parasites and vectors) by *A. malabarica*. But our study was expressed detailed demonstration on different methanolic concentration effects of *A. malabarica* to control the larvae. Table 2 gives larval mortality (I to IV instar) mortality after the treatment of AMIFE. In I instar stage at 20 ppm concentration the larval mortality was 15% whereas at 100 ppm concentration it was increased to 84%. The LC 50 and LC 90 values were represented as follows: LC 50 value of I instar was 64.76%, II instar was 67.92%, III instar was 71.46% and IV instar was 74.55%, respectively. LC 90 value of I instar 118.22%, II instar was 121.60%, III instar was 123.65% and IV instar was 119.94%, respectively.

Table 2. Larval toxicity effect of AMIFE (Methanolic extract) on the malarial vector, *An.stephensi.*

Larval stages	% of larval mortality					LC_{50}	LC_{90}	95% confidence Limit				χ^2 value
	Concentration (ppm)							LCL		UCL		
	20	40	60	80	100			LC_{50}	LC_{90}	LC_{50}	LC_{90}	
I	16d	31d	44d	59d	84d	64.76	(118.22)	59.78	(108.09)	70.00	(132.53)	3.797
II	14c	29c	42c	55c	83d	67.92	(121.60)	62.91	(111.06)	73.36	(136.57)	4.340
III	12b	26b	38b	52b	81c	71.46	(124.13)	66.47	(113.46)	77.05	(139.27)	4.364
IV	9a	22a	35a	48a	80b	74.55	(12.61)	69.75	(113.61)	80.01	(137.73)	4.415

χ^2 – Chi-square values are significant at P<0.05 levels. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT

Table 3 gives the effect of AMLE and AMIFE on the larval duration of *An. stephensi*. Larval duration (I to IV instars) was extended after the treatment of (2.8 days at 20 ppm) AMIFE, whereas AMLE treatment alone reduces the larval duration at all concentrations. These growth regulatory property is may be due to the action of *A. malabarica* compounds (Anisomelic, Butulinic, Citral, Geranic and Ovatodilide) on the haemolymph, ecdysteriod and juvenile hormone titers significant reduction or delays) by inhibiting the release of morphogenetic peptides, prothorecicotropic hormone (PTTH) and allotropins from the brain corpus cardiacum complex.

Table 3. Effect of *Anisomeles malabarica* leaf and inflorescence Methanolic extract on larval duration of *An. stephensi*.

Treatment	Larval Duration (days)			
	I instar	II instar	III instar	IV instar
Control	**1.5cd**	**1.7 c**	**2.6 c**	**2.5 bc**
AMLE (ppm)				
10.0	1.4 cd	1.5 d	2.4 d	2.2 d
15.0	0.9 e	1.2 e	1.3 e	1.3 e
20.0	0.7 f	0.5 g	0.9 f	0.9 f
AMIFE (ppm)				
10.0	1.6 c	1.8 bc	2.7 c	2.4 c
15.0	1.8 b	1.9 b	2.9 b	2.6 b
20.0	2.1 a	2.2 a	3.1 a	2.8 a

Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT

The dried leaves of *A. malabarica* are frequently used in traditional Indonesian mixed herbal medicines. Investigations have tested chemical additives including inorganic salts, aromatic compounds [32]. Biological control with entomopathogenic bacteria has been increasingly used as a larvicidal to control populations of various medically important Diptera of the genera Culex and Anopheles. Like chemical larvicides, these agents can cause drastic density dependent mortality, killing all larvae within 24 - 48 hrs, after laboratory treatment. In the present study also, after the AMIE treatment, the percentage of larval reduction was noticed within 24 hours and 48 hours. Moreover, they are selective to insects and are consequently considered soft to no-target fauna commercial products. In the present study AMLE at different concentrations, brought out laboratory toxicity on the various larval instars of *An. stephensi*. *A. malabarica* leaf extract (AMLE), however, proved to be the most detrimental to the leaves at the concentrations tested. At the highest concentration tested (i.e. 100 ppm) the mortality (85%) was higher in AMIFE treated larvae when compared to other treatments.

4. Conclusions

Our investigation clearly demonstrates that methanolic of *A. malabarica* inflorescence and *A. malabarica* leaves possess significant larvicidal properties. This present study, the individual effect of *A. malabarica* inflorescence and leaf on the malarial vector, *An. stephensi* was observed with all levels of larvae. Mortality bioassay has been conducted to study the effect of AMIFE and AMLE extract on malarial vector, *An. stephensi* and higher larval mortality was occurred. Mortality of larval instars being higher after the treatment of AMIFE suggest that the leaves which contains Anisomelic and citral. AMIFE and AMLE treated larvae of *An. stephensi* showed prolonged development in this study. AMLE and AMIFE treated larvae was expressed growth inhibition, malformation and mortality. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. The present study plants are easily available, accessible, and affordable therefore the usage of traditional plants should be promoted among the local residents in order to reduce the man vector contact as well as vector-borne diseases.

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