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Research Article

LARVICIDAL POTENTIAL OF SOME INDIAN MEDICINAL PLANT EXTRACTS AGAINST AEDES AEGYPTI (L.)

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ABSTRACT

The larvicidal potential of different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) leaf extracts of four plants (*Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus* and *Memecylon edule*) was tested against the fourth-instar larvae of *Aedes aegypti*. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24-h exposure. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was detected in acetone extract of *E. indica* (LC_{50} 90.89, LC_{90} 217.21 and LC_{99} 441.88 ppm) followed by *M. indica* acetone extract (LC_{50} 173.21, LC_{90} 289.86 and LC_{99} 441.04 ppm). These results revealed that larvicidal properties of the selected plants and encourages further effort to investigate the bioactive compounds in those extracts that might possess good larvicidal properties when it will be isolated in pure form.

Keywords: *Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus, Memecylon edule,* dengue vector mosquito.

INTRODUCTION

Mosquitoes are vector for various disease including malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. Among these mosquito borne diseases dengue fever dengue hemaorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asia and Africa[1]. It is transmitted by *Aedes aegypti* (Linn.). One of the methods available for controlling the mosquitoes is use of synthetic insecticides. Mosquitoes develop genetic resistance to synthetic insecticides[2] and even to biopesticides such as *Bacillus sphaericus*[3]. Also synthetic insecticides adversely affect the environment by contaminating air, water, and soil. There is a urgent need to find alternatives to the synthetic insecticides which is more potent and low-cost.

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly[4]. Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemical may act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable[5].

Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities[6]. About 2000 species of terrestrial plants have been reported for their insecticidal properties [7]. Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities.

Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponine[8], steroids[9][10], isoflavonoids[11], essential oils[12], alkaloids and tannins[13] has potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitos[14].

The present investigation was carried out to validate the larvicidal potential of different solvent extracts of four (*Blepharis maderaspatensis* (L.) B. Heyne ex Roth., *Elaeagnus indica* Servett., *Maesa indica* (Roxb.) DC, *Phyllanthus wightianus* Müll.Arg. and *Memecylon edule* Roxb.) medicinal plants against fourth instar *Ae. aegypti* larvae. All the plants were selected based on their ethnobotanical literatures and least explored. This is first hand report on larvicidal activity of all the selected plants against *Ae. aegypti* larvae.

MATERIALS AND METHODS

Plant material

Healthy leaves of *B. maderaspatensis* (Acanthaceae), *E. indica* (Elaeagnaceae), *M. indica* (Myrsinaceae) *P. wightianus* (Phyllanthaceae) and *M. edule* (Melastomataceae) were collected from various regions of Eastern Ghats of Tamil Nadu, India. The plants were identified with the references of standard books and herbariums from the Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, India. The plant materials were cleaned, air-dried at room temperature for two weeks and coarsely powdered.

Preparation of extracts

Powdered plant materials were extracted successively by using different solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone and methanol) in soxhlet apparatus for 18 h and the extractives were filtered through Whatman filter paper No. 4 then the extracts were concentrated at 40º C in vacuum and stored at 4º C for this investigations.

Test insects

Ae. aegypti, larvae was obtained from National Centre for Disease Control (NCDC) Coonoor, Tamil Nadu and maintained at Department of Biotechnology, Periyar University Salem. Larvae were fed a diet of Brewer's yeast and powdered dog biscuits in the ratio of 3:1, kept at 27 ± 2º C and 75% - 85% relative humidity (RH), with a photoperiod of 14:10 LD for the larval growth. Late third instars to early fourth instars larva were used for larval bioassay which obtained from the stock culture maintained at Department of Biotechnology, Periyar University, Salem.

Larvicidal bioassay

The larvicidal activity of crude extracts of the selected plants was assessed by the protocol of WHO[15] with some modifications and as per the method of Rahuman *et a*[16]. For the bioassay in a container 25 fourth instar larvae were kept in 249 ml of distilled water with 1 ml of extracts (400 ppm) in DMSO. Tween-80 was used as an emulsifier at concentration of 0.02% (v/v). The chamber containing the control larvae received 1 ml of DMSO served as negative control. After 24 hours exposures the dead larvae were counted and corrected by using Abbott's[17] formula and the percentage mortality was recorded from the average of six replicates.

Dose-response bioassay

Based on the preliminary screening results, in which above 90% mortality of larvae occurs alone, were subjected to dose–response larvicidal bioassay. The desired mortality percentage was observed in acetone and ethyl acetate extracts of *E. indica,* ethyl acetate extract of *B. maderaspatensis* and acetone extract of *M. indica* at 400 ppm concentration were subjected to dose dependent bioassay. Different concentrations (50-400 ppm) of the above mentioned crude extracts were tested for larvicidal activity described by WHO[15]. The average mortality percentages of six replicates were recorded and corrected by using Abbott's formula.

Statistical analysis

Data were analyzed using one-way ANOVA. Significant differences between treatments were determined using Tukey's multiple range tests ($P \leq 0.05$). LC_{50}, LC_{90} and LC_{99} values were calculated using probit analysis[18].

RESULTS AND DISCUSSION

The results of larvicidal efficacy of different solvent extracts of the selected plants were shown in Table 1. All the plant extracts showed good to moderate effect on fourth instar larvae of *Ae. aegypti* after 24 h of exposure at 400 ppm (0.04%) concentration. The highest mortality (100%) was observed in acetone extracts of *E. indica* and *M. indica.* Significant ($p>0.05$) activity was detected in ethyl acetate extracts of *E. indica1* (97%) and *B. maderaspatensis* (90%) followed by *M. indica* chloroform extract (85%). Most of the extracts of *P. wightianus* exhibits considerable (45-82%) larvicidal activity and the remaining extracts of the selected plants showed least larvicidal activity. The least activity was detected in *M. edule* chloroform extract (1%).

Table 1: Larvicidal activity of different solvent leaf extracts of selected plants against 4th instar larvae of *Ae. aegypti* at 400 ppm (0.04%)

Plant names	% Mortality*				
	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
B. maderaspatensis	8.0±1.0 [a]	48.0±6.0	90.6±0.5 [a]	26.6±2.3	10.6±1.1 [a]
E. indica	22.6±2.0 [b]	100±0.0 [b]	97.3±0.5 [ba]	21.3±0.5	34.6±1.1
M. indica	24.0±2.0 [cb]	100±0.0 [cb]	14.6±1.5 [c]	85.3±1.5 [c]	6.6±3.0 [ca]
M. edule	5.3±1.5 [da]	4.00±0.0	10.6±0.5 [cd]	1.3±0.5	17.3±0.5
P. wightianus	42.6±1.1	73.3±1.5	78.6±2.0	82.6±1.1 [ec]	70.6±2.0

Control—Nil mortality, Total no of larvae =25, *-Mean value of six replicates ± SD. Significant at $p>0.05$ level.

The toxicity of dose–response larvicidal bioassay was given in Table 2. According to preliminary screening results, four extracts were subjected to dose–response larvicidal bioassay which has above 90% larval mortality. Among them significant mortality rate was observed in acetone extract of *E. indica* with LC_{50} LC_{90} and LC_{99} values of 90, 217 and 441 ppm respectively followed by acetone extract of *M. indica* with LC_{50} LC_{90} and LC_{99} values of 173, 289 and 441 ppm respectively. The larvicidal activity of the different selected plant extracts were found to be dose depended. *E. indica* ethyl acetate extract shows considered mortality with LC_{50} LC_{90} and LC_{99} values of 151, 456 and 1121 ppm respectively.

Table 2: Dose–response larvicidal bioassay of different solvent leaf extracts against 4th instar larvae of A. aegypti

Plant names	Extracts	Conc. (ppm)	% Mortality*	LC_{50} ± SE (ppm) (LCL-UCL)	LC_{90} ± SE (ppm) (LCL-UCL)	LC_{99} ± SE (ppm) (LCL-UCL)	$\chi^{2\,(df=4)}$
B. maderaspatensis	Ethyl acetate	100	18.6 ± 0.5	197.6 ± 0.2 (181.6-213.8)	438.0 ± 0.3 (381.6-531.3)	838.3 ± 0.8 (664.3-1174.3)	4.9
		150	30.6 ± 0.5				
		200	42.6 ± 2.0				
		250	62.6 ± 1.5				
		300	77.3 ± 1.5				
		400	90.6 ± 2.5				
E. indica	Acetone	50	24.0 ± 1.0	90.8 ± 0.1 (80.1-101.1)	217.2 ± 0.2 (191.5-254.8)	441.8 ± 0.6 (358.7-587.5)	5.2
		100	50.6 ± 0.5				
		150	70.6 ± 1.1				
		200	89.3 ± 3.7				
		300	97.3 ± 1.1				
		400	100.0 ± 0.0				
E. indica	Ethyl acetate	50	18.6 ± 1.5	151.2 ± 0.4 (93.3-224.2)	456.1 ± 0.5 (284.6-2005.6)	1121.7 ± 1.2 (527.0-16044.2)	20.0
		100	26.6 ± 1.1				
		150	41.3 ± 3.5				
		200	52.0 ± 3.6				
		300	80.0 ± 4.0				
		400	97.3 ± 0.5				
M. indica	Acetone	100	16.0 ± 1.0	173.2 ± 0.7 (135.9-206.9)	289.8 ± 0.9 (237.2-448.8)	441.0 ± 2.2 (325.6-968.0)	17.8
		150	30.6 ± 1.5				
		200	52.0 ± 2.6				
		250	78.6 ± 3.5				
		300	98.6 ± 0.5				
		400	100.0 ± 0.0				

Control- Nil mortality, Significant at $p<0.001$ level, *-Mean value of six replicates ± SD, LC= Lethal Concentration, LCL=Lower Confidence Limit, UCL=Upper Confidence Limit, SE=Standard Error, $\chi 2$=chi-square and *df*=degree of freedom.

Nowadays, the control of mosquitoes at larval stage is focused with plant extracts. The advantage of targeting mosquito at the larval stage is they cannot escape from their breeding sites until the adult emergences and also to reduce the overall pesticide use to control of

adults by aerial application of adulticidal chemicals. Bioactive crude extracts or isolated phyto-constituents from the plant could be used as alternative to the currently used synthetic insecticides. The biological activity of plant extracts might be due to various compounds, including phenolics, terpenoids, and alkaloids present in plants[19].

Our preliminary screening for larvicidal properties of different solvent leaf extracts of four plants among 20 extracts, 4 extracts gave high larvicidal potency with low lethal concentrations (LC_{50} <197 ppm) against 4th instar larvae of *Ae. aegypti*. Likewise, Cavalcanti *et al*[12] reported that the larvicidal activity of essential oils of Brazilian plants against *Ae. aegypti* and observed the LC_{50} to range from 60 to 533 ppm. Similarly, Rahuman and Venkatesan[20] screened the petroleum ether extracts of *Citrullus colocynthis*; methanol extracts of *Cannabis sativus, Cannabis indica* and *Momordica charantia*; and acetone extract of *Trichosanthes anguina* against the larvae of *Ae. aegypti* the LC_{50} values are 74.57, 309.46, 492.73, 199.14, and 554.20 ppm respectively which supports the present results were comparably good.

The findings of present results, among the 25 tested extracts only 4 extracts has potential larvicidal activity which are comparable to earlier reports of Sakthivadivel and Daniel[21] that screened larvicidal activity of petroleum ether extracts of sixty three plants against *Cu. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* larvae of which six found to be potential larvicides. Similarly, Pavela[22] reported the larvicidal activity methanolic extracts of thirty one Euro-Asiatic plants against *Cu. quinquefasciatus*. Likewise, Nazar *et al*[23] investigated 100 coastal plant extracts including *B. maderaspatensis* against the *Cu. quinquefasciatus* larvae of which seventeen plants were possessed larvicidal properties and also the whole plant extract of *B. maderaspatensis* showed no activity but, the present investigation revealed that larvicidal properties of *B. maderaspatensis* against *Ae. aegypti*.

The findings of present study are quite comparable with previous reports of Vinayaka *et al*[24] who have reported the larvicidal activities of different solvent leaf extracts of *Elaeagnus kologa* in which methanol, ethyl acetate and acetone extracts showed 100% in 15 and 20 mg/ml concentrations against *Ae. aegypti.* Suwanneepromsiri *et al*[25] investigated fourteen plant extracts, among those, only eight plants were showed 100% mortality against *Ae. aegypti* larvae at a concentration of 100 µg/ml with LC_{90} values range between 13.9-56.2 µg/ml to 100 µg/ml that supports present results.

The present result was supported by earlier reports of Singh *et al*[26] that the larvicidal activity of *Ocimum canum* oil tested against *Ae. aegypti* and *Cu. quinquefasciatus* (LC_{50} 301 ppm) and *An. stephensi* (234 ppm). Similarly, Ansari *et al*[27] was observed the larvicidal activity of *Pinus longifolia* oil against *An. stephensi* (LC_{50} 112.6 ppm), *Ae. aegypti* (82.1 ppm) and *Cu. quinquefasciatus* (85.7 ppm). The results of our study is compared with the findings of Sumroiphon *et al*[28] who have reported that the effect of water extract of citrus seed extract showed LC_{50} values of 135, 319 and 127, 411 ppm against the larvae of *Ae. aegypti* and *Cu. quinquefasciatus* respectively.

CONCLUSION

All the tested plants possessed different range of larvicidal property which may be used as a traditional mosquito control agent. On the basis of the present investigation results we conclude that acetone, ethyl acetate extract of *E. indica,* acetone extract *M. indica* and ethyl acetate extract of *B. maderaspatensis* contains potent larvicidal bioactive principles which may be needed further purifications to have its synthetic analogues which will be carry out in future.

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