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LARVICIDAL IMPACT OF SOME LOCAL MEDICINAL PLANT EXTRACTS AGAINST *AEDES AEGYPTI* (L.)

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Abstract

The larvicidal potential of different solvent (hexane, chloroform, ethyl acetate, acetone and methanol) crude leaf extracts of five plants (*Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus* and *Memecylon edule*) were tested against the fourth-instar larvae of *Aedes aegypti*. 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). The results revealed that larvicidal properties of the four selected plants and encourages further investigation for the bioactive compounds that might possess good larvicidal properties in pure form.

Key words: Larvicidal, Plant extract, Bioactive compounds, *Aedes aegypti* vector

Introduction

Mosquitoes are vector for various diseases including malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. Among these mosquito borne diseases dengue fever, dengue hemaorrhagic fever, yellow fever and chikungunya are prevalent in Southeast Asia and Africa (Maillard *et al*. 2013). It is transmitted by *Aedes aegypti* (Linn.). Synthetic insecticide is one of the methods available for controlling the mosquitoes. Mosquitoes develop resistance to synthetic insecticides (Wattal *et al*. 2011) and even to biopesticides (Tabashnik 2011). Synthetic insecticides adversely affect the environment also by contaminating air, water, and soil. There is an urgent need to find alternatives to the synthetic insecticides which are more potent and low-cost. Plants could be better rich source of alternative agents to control of mosquitoes, because they possess bioactive chemicals, which are specific to target-insects and are eco-friendly (Sukumar *et al*. 2012). 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 and may act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants. Plant based

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pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable (Markouk *et al*. 2010). Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities (Feinstein 2012). About 2000 species of terrestrial plants have been reported for their insecticidal properties (Wiseman and Chapagain 2011). 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. Recent research has proved that effectiveness of plant derived compounds, such as saponine (Chowdhury *et al.* 2008), steroids, isoflavonoids, essential oils, alkaloids and tannins (Ghosh *et al.* 2011) have potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitos (Joseph *et al*. 2009). The present investigation was carried out to validate the larvicidal potential of different solvent extracts of five medicinal plants (*Blepharis maderaspatensis* (L.) B. Heyne ex Roth., *Elaeagnus indica* Servett., *Maesa indica* (Roxb.) DC, *Phyllanthus wightianus* Müll.Arg. and *Memecylon edule* Roxb.) against fourth instar *Ae. aegypti* larvae. All the plants were selected based on their ethno botanical importances and least explored.

Materials and Methods

Healthy leaves of *B. maderaspatensis* (Acanthaceae), *E. indica* (Elaeagnaceae), *M. indica* (Myrsinaceae) *P. wightianus* (Phyllanthaceae) and *M. edule* (Melastomataceae) were collected from Botanical garden Mirpur, Carzon Hall Campus, University of Dhaka and farm of Sher-e-Bangla Agricultural University, Dhaka. The plants were identified with the references of standard books and herbariums from the Agronomy and Crop Botany Department of Sher-e-Bangla Agricultural University, Dhaka. 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 hrs and the extractives were filtered through Whatman filter paper No. 4 then the extracts were concentrated at 40ºC in vacuum and stored at 4ºC for this investigations.

Test insects: Ae. aegypti, larvae were obtained from Sher-e-Bangla Agricultural University campus, Dhaka. 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 larvae were used for larval bioassay which obtained from the stock culture maintained at Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka.

Larvicidal bioassay: The larvicidal activity of crude extracts of five selected plants were assessed by the protocol of WHO (2011) with some modifications and as per the method of Rahuman *et al*. (2010). Bioassay in a container where 25 fourth instar larvae were kept in 249 ml of distilled water with one 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 one ml of DMSO served as negative control. After 24 hur exposures the dead larvae were counted and corrected by using Abbott's (2007) formula and the percentage mortality was recorded from the average of six replicates.

Dose-response bioassay: Based on the preliminary screening, in which above 90% mortality of larvae occur 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 40 - 50 ppm 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 (2011). The average mortality percentages of six replicates were recorded and corrected by using Abbott's formula.

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.

Results and Discussion

The results of larvicidal efficacy of different solvent extracts of the selected plants was shown in Table 1. All the plant extracts showed good to moderate effect on fourth instar larvae of *Ae. aegypti* after 24 hrs of exposure at 400 ppm 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* exhibit considerable (42 - 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 four plants against 4th instar larvae of *Ae. aegypti* at 400 ppm (0.04%).

Name of plants	% mortality*				
	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
Blepharis maderaspatensis	8.0±1.0^{a}	48.0±6.0	90.6±0.5^{a}	26.6±2.3	10.6±1.1^{a}
Elaeagnus indica	22.6±2.0^{b}	100±0.0b	97.3±0.5^{ab}	21.3±0.5	34.6±1.1
Maesa indica	24.0±2.0^{ab}	100±0.0ab	14.6±1.5^{c}	85.3±1.5^{c}	6.6±3.0^{ab}
Memecylon edule	5.3±1.5^{ab}	4.00±0.0	10.6±0.5^{cd}	1.3±0.5	17.3±0.5
Phyllanthus wightianus	42.6±1.1	73.3±1.5	78.6±2.0	82.6±1.1^{c}	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 is given in Table 2. On the basis of preliminary screening, four extracts were subjected to dose-response larvicidal bioassay where it was 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 extract was found to be dose depended. *E. indica* ethyl acetate extract shows considerable 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.*

Name of plants	Extracts	Conc. (ppm)	% mortality*	LC_{50} ± SE (ppm) LCL-UCL	LC_{90}±SE(ppm) LCL-UCL	LC_{99} ± SE(ppm) LCL-UCL	$\chi^{2\,(df=4)}$
		100	18.6±0.5				
		150	30.6±0.5				
		200	42.6±2.0				
		250	62.6±1.5	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
B.maderaspatensis	Ethyle acetate	300	77.3±1.5				
		400	90.6±2.5				
		50	24.0±1.0				
		100	50.6±0.5				
		150	70.6±1.1				
	Acetone	200	89.3±3.7	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
E. indica		300	97.3±1.1				
		400	100.0±0.0				
		50	18.6±1.5				
		100	26.6±1.1				
		150	41.3±3.5				
		200	52.0±3.6	151.2±0.4 (93.3-224.2)	456.1±0.5 (284.6-2005.6)	1121.7±1.2 (527.0-1644.2)	20.0
E. indica	Ethyle acetate	300	80.0±4.0				
		400	97.3±0.5				
		100	16.0±1.0				
		150	30.6±1.5				
		200	52.0±2.6	173.2±0.7 (135.9-206.9)	189.8±0.9 (237.2-448.8)	441.0±2.2 (325.6-968.0)	17.8
	Acetone						
M. indica		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, χ^2 = Chisquare 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 to reduce the overall pesticide use to control of adults by aerial application of adulticidal chemicals. Bioactive crude extracts or isolated phyto-constituents could be used as alternative to the currently used synthetic insecticides. The bioactivity of plant extracts might be due to various compounds *viz.* phenolics, terpenoids, and alkaloids present in plants (Sakthivadivel and Daniel 2011). Among 20 different leaf extracts of five plants, four extracts gave high larvicidal potency with low lethal concentrations (LC_{50} <197 ppm) against 4th instar larvae of *Ae. aegypti*. Cavalcanti *et al.* (2011) reported that the larvicidal activity of essential oils of Brazilian plants against *Ae. aegypti* and observed the LC_{50} to range from 60 - 533 ppm. Similarly, Rahuman *et al.* (2010) 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 that screened larvicidal activity of petroleum ether extracts of sixty three plants against *Cu. quinquefasciatus, An. stephensi* and *Ae. aegypti* larvae of which six were found to be potential larvicides. Similarly, Pavela (2012) reported the larvicidal activity methanolic extracts of thirty one Euro-Asiatic plants against *Cu. quinquefasciatus*. Likewise, Nazar *et al.* (2013) investigated 100 coastal plant extracts including *B. maderaspatensis* against the *Cu. quinquefasciatus* larvae of which seventeen plants 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*. (2012) 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.* (2010) reported that eight plants showed 100% mortality against *Ae. aegypti* larvae at a concentration of 100 µg/ml with LC_{90} values range between 13.9 and 56.2 µg/ml to 100 µg/ml that supports present results. The present result was supported by Nazar *et al.* (2013) that the larvicidal activity of *Ocimum canum* oil tested against *Ae. aegypti* and *Cu. quinquefasciatus* (LC50 301 ppm) and *An. stephensi* (234 ppm). Similarly, Ansari *et al* (2009) reported 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 was found to be comparable with the findings of Nazar *et al.* (2013) who have reported that the effect of water extract of citrus seed extract showed LC_{50} values of 135, 319, 127 and 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 could 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 might be needed further purifications for their synthetic analogue.

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