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Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae)

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Abstract. Mosquitoes are the vectors of several life threatening diseases like dengue, malaria, Japanese encephalitis and lymphatic filariasis, which are widely present in the north-eastern states of India. Investigations on five local plants of north-east India, selected on the basis of their use by indigenous communities as fish poison, were carried out to study their mosquito larvicidal potential against *Anopheles stephensi* (malaria vector), *Stegomyia aegypti* (dengue vector) and *Culex quinquefasciatus* (lymphatic filariasis vector) mosquitoes. Crude Petroleum ether extracts of the roots of three plants *viz*. *Derris elliptica*, *Linostoma decandrum* and *Croton tiglium* were found to have remarkable larvicidal activity; *D. elliptica* extract was the most effective and with LC_{50} value of 0.307 µg/ml its activity was superior to propoxur, the standard synthetic larvicide. Half-life of larvicidal activity of *D. elliptica* and *L. decandrum* extracts ranged from 2-4 days.

INTRODUCTION

Mosquitoes transmitting a number of diseases, such as malaria, lymphatic filariasis, dengue and Japanese encephalitis, are the most important single group of insects in terms of public health importance (Rahuman *et al.*, 2009). Synthetic insecticides have been in use as mosquito larvicide worldwide for the last several decades (Chavasse & Yap, 1997). Though highly effective, their use is beset with several drawbacks like non-selectiveness, harmful effect on beneficial organisms, poor biodegradability and bio-magnification of pesticidal residue in the food chain (Evans & Raj, 1988). This has prompted the search for biologically active, safe and environment friendly plant based insecticides as an alternative green control measure of arthropods of public health importance (Nathan *et al.*, 2005). Plants are a rich source of bioactive organic chemicals and synthesize a number of secondary metabolites to serve as defense chemicals against insect attack (Pushpatha & Muthukrishnan, 1995). These chemicals may serve as insecticides, antifeedents, oviposition deterrents, repellents and insect growth regulators (Sukumar *et al.*, 1991; Pavela *et al.*, 2005; Rajkumar & Jebanesan, 2005; Kostic *et al.*, 2008; Elango *et al.*, 2009). They offer an advantage over synthetic pesticides as they are less toxic, easily biodegradable and less prone to development of resistance. North-eastern region of

India (NE India), comprising 8 states *viz.* Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, is inhabited by people of varying ethnicity. Various mosquito borne diseases, topped by malaria, are widely present in this region that is endowed with a rich flora. The geo-climatic conditions of NE India support fast proliferation and growth of natural fauna and flora (Nath *et al.*, 2006). There is immense potential for medicinal plants in this region as many communities in their indigenous system of medicine are known to use not only various locally available plants to treat several diseases (Majumdar *et al.*, 1978; Tiwari *et al.*, 1980) but also smoke of some plants/leaves to drive away mosquitoes. The field of traditional knowledge of herbal wealth in NE India is vast and needs exploration and documentation. In this context, the present study was taken up to investigate mosquito larvicidal activity of a few selected locally available plants based on the folklore medicine and literature with the hope that it may provide a lead to the development of anti-mosquito agent through tapping of local plant resources.

MATERIAL AND METHODS

Selection of plants

A total of five indigenous plants were chosen for the study. The selection of plants was based on their use by local communities as fish poison and plants known to be not attacked by insect pests. The selected plants (Table 1), after identification by a qualified plant taxonomist, were collected from various areas of Assam state and brought to the laboratory for processing.

Extraction and fractionation of plant material

The plants were shade dried, cut into small pieces and part(s) of interest were grounded in a metal mortar. The homogenized material was further grounded in a high speed waring blender to make a fine powder. Following the method of O'Neill *et al.* (1987) the plant material was soxhlet extracted using 3 solvents *viz.* Petroleum ether (non polar), methanol (moderately polar) and water (highly polar) to ensure that all possible active compounds were extracted in solvents of different polarities. The methanol extract was further fractionated through partition chromatography, using chloroform and water, into methanolic-chloroform and methanolic-aqueous fractions. Thus, for each plant, a total of 4 'crude extracts' *viz.* Petroleum ether (PE), Methanolic chloroform (MC), Methanolic aqueous (MA) and Water (W) were obtained.

Test mosquito species

For assessment of larvicidal activity, bioassays were carried out against 3 laboratory bred mosquito species *viz.* *Anopheles stephensi*, *Stegomyia aegypti* and *Culex quinquefasciatus*. Cyclical colonies of these mosquitoes were maintained in the insectary at controlled temperature (27±2°C) and relative humidity (70±10%) following standard procedures.

Mosquito larvicidal activity detection bioassays

Initial toxicity of various extracts was assessed at a single dose of 500 µg/ml against the test mosquito species. It was seen that PE and MC extracts exhibited higher larvicidal activity compared to MA and Water

Table 1. Plants evaluated for mosquito larvicidal activity

Sl. no	Plant name	Family	Collection place	Plant part used
1.	*Derris elliptica*	Fabaceae	Nambor Forest (Golaghat, Assam)	Root, Shoot
2.	*Linostoma decandrum*	Thymeleaceae	Nambor Forest (Golaghat, Assam)	Root, Shoot
3.	*Croton tiglium*	Euphorbiaceae	Hozai (Nagaon, Assam)	Seeds
4.	*Litsea salicifolia*	Lauraceae	Titabor (Jorhat, Assam)	Arial parts
5.	*Croton caudatus*	Euphorbiaceae	Mariani (Jorhat, Assam)	Root

extracts. Hence, further evaluation was done only for PE and MC extracts.

Quantification of larvicidal activity

For quantification of larvicidal activity, LC_{50} and LC_{90} values of the PE and MC extracts of the test plants were determined. Briefly, different dosages of the test extract(s) were prepared through serial dilution in 250 ml distilled water. Each dose was replicated thrice and 20 third instar larvae per replicate of the test mosquito species were exposed for 48 hours at a constant temperature of 27±2°C. For comparison, a standard synthetic insecticide (Propoxur) at its LC_{50} dosage as the positive control was run concurrently along with untreated control. Larval mortality counts were taken after 48 hours of exposure. Corrected mortality was worked out for each dose using formula (Abbott, 1925) for accounting any mortality in Control. Log-concentration-response probit analysis programme (Chi, 1997) was used to determine LC_{50} and LC_{90} values expressed in µg/ml.

Residual larvicidal activity assessment

Half-life of the most active extract(s) was assessed by determining its LC_{50} values on different days after preparing formulation. Half-life of an extract was the time taken in days for doubling of its LC_{50} value.

RESULTS

LC_{50} values of the crude PE and MC extracts of the 5 plants against the three vector species of mosquitoes are shown in Tables 2, 3 and 4. *Derris elliptica* root extracts possessed the highest larvicidal activities against all species of mosquitoes followed by *Croton tiglium* seed extracts and *Linostoma decandrum* root extracts. *Litsea salicifolia* extracts exhibited the weakest larvicidal activities. In general, PE extract of these plants showed higher larvicidal activity than the MC extract.

PE extract of the roots of *D. elliptica* was most effective against *An. stephensi* (LC_{50} 0.31 µg/ml), and superior to standard propoxur

Table 2. Larvicidal activity of the test plants against *Anopheles stephensi*

Plant code /crude extract		LC_{50} (µg/ml)	LC_{90} (µg/ml)	Regression equation
*Derris elliptica**	PE	0.307	0.58	Y = 7.35 + 4.59 x
(Root)	MC	2.3	5.6	Y = 3.72 + 3.41 x
*Derris elliptica**	PE	112.2	333.5	Y = -0.55 + 2.70 x
(Shoot)	MC	44.9	69.5	Y = -0.54 + 3.35 x
Linostoma decandrum#	PE	11.2	57.0	Y = 3.08 + 1.82 x
(Root)	MC	12.0	48.5	Y = 2.71 + 2.11 x
Linostoma decandrum#	PE	15.7	148.9	Y = 3.42 + 1.31 x
(Shoot)	MC	186.7	590	Y = 1.38 + 1.59 x
Croton tiglium	PE	20.8	52.0	Y = -0.78 + 4.39 x
(Seeds)	MC	18.2	34.5	Y = -0.81 + 4.61 x
Litsea salicifolia	PE	467.4	–	Y = -0.27 + 2.90 x
(Aerial parts)	MC	320	1935	Y = 0.88 + 1.64 x
Croton caudatus	PE	13.2	73.9	Y = 3.07 + 1.71 x
(Roots)	MC	407.4	2792.6	Y = 0.99 + 1.53 x
Propoxur (Standard)		0.41	0.65	Y = 7.51 + 6.58 x

* Same plant. # Same plant. – Indeterminate.
PE: Petroleum ether extract.
MC: Methanolic-chloroform extract.

Table 3. Larvicidal activity of the test plants against *Culex quinquefasciatus*

Plant code /crude extract		LC_{50} (µg/ml^{-1})	LC_{90} (µg/ml^{-1})	Regression equation
Derris elliptica[*]	PE	0.616	1.44	Y = 5.72 ± 3.45 x
(Root)	MC	4.21	12.4	Y = 3.29 + 2.73 x
Derris elliptica[*]	PE	170.9	669.9	Y = 0.17 + 2.16 x
(Shoot)	MC	148.7	596.8	Y = 0.38 + 2.12 x
Linostoma decandrum[#]	PE	4.1	28.2	Y = 4.05 + 1.53 x
(Root)	MC	45.3	572.2	Y = 3.07 + 1.16 x
Linostoma decandrum[#]	PE	16.3	91.8	Y = 2.92 + 1.70 x
(Shoot)	MC	256.1	2098	Y = 1.62 + 1.40 x
Croton tiglium	PE	19.9	55.4	Y = 1.26 + 2.87 x
(Root)	MC	47.0	82.0	Y = -4.18 + 5.49 x
Litsea salicifolia	PE	294.3	889.0	Y = -1.59 + 2.66 x
(Aerial parts)	MC	101.9	668.1	Y = 1.84 + 1.56 x
Croton caudatus	PE	69.2	504.4	Y = 2.26 + 1.48 x
(Root)	MC	220.2	2974	Y = 2.34 + 1.13 x
Propoxur (Standard)		0.41	0.72	Y = 6.53 + 5.93 x

* Same plant. # Same plant.
PE : Petroleum ether extract.
MC: Methanolic-chloroform extract.

Table 4. Larvicidal activity of the test plants against *Stegomyia aegypti*

Plant code /crude extract		LC_{50} (µg/ml)	LC_{90} (µg/ml)	Regression equation
Derris elliptica[*]	PE	0.616	1.44	Y = 5.72 + 3.45 x
(Root)	MC	4.21	12.4	Y = 3.29 + 2.73 x
Derris elliptica[*]	PE	170.9	669.9	Y = 0.17 + 2.16 x
(Shoot)	MC	148.7	596.8	Y = 0.38 + 2.12 x
Linostoma decandrum[#]	PE	4.1	28.2	Y = 4.05 + 1.53 x
(Root)	MC	45.3	572.2	Y = 3.07 + 1.16 x
Linostoma decandrum[#]	PE	16.3	91.8	Y = 2.92 + 1.70 x
(Shoot)	MC	256.1	2098	Y = 1.62 + 1.40 x
Croton tiglium	PE	19.9	55.4	Y = 1.26 + 2.87 x
(Root)	MC	47.0	82.0	Y = -4.18 + 5.49 x
Litsea salicifolia	PE	294.3	889.0	Y = -1.59 + 2.66 x
(Aerial parts)	MC	101.9	668.1	Y = 1.84 + 1.56 x
Croton caudatus	PE	69.2	504.4	Y = 2.26 + 1.48 x
(Root)	MC	220.2	2974	Y = 2.34 + 1.13 x
Propoxur (Standard)		0.41	0.72	Y = 6.53 + 5.93 x

* Same plant. # Same plant.
PE: Petroleum ether extract.
MC: Methanolic-chloroform extract.

Table 5. Residual larvicidal activity of *Derris elliptica* and *Linostoma decandrum* roots

Sl No	Extract	Mosquito species	LC_{50} (µg/ml) on day				Half life (in days)
			0	3	5	8	
1.	*Derris elliptica* (PE extract)	*An. stephensi*	0.55	1.52	1.87	3.14	<3
		St. aegypti	1.18	2.32	2.81	4.49	~ 3
			0	2	4	8	
2.	*Linostoma decandrum* (PE extract)	*An. stephensi*	13.0	30.0	85.7	82.1	<2
		Cx. quinquefasciatus	5.1	7.9	11.2	17.3	>3 & <4

(LC_{50} 0.44 µg/ml). While PE extract of *L. decandrum* roots was found most effective against *Cx. quinquefasciatus* (LC_{50} 4.13 µg/ml), *C. tiglium* PE extract was most toxic against *St. aegypti* (LC_{50} 13.3 µg/ml). Shoot extracts of *D. elliptica* and *L. decandrum*, however, recorded relatively weaker larvicidal activity, suggesting that the larvicidal activity was concentrated mainly in the roots of both these plants.

Residual life of PE extract of *D. elliptica* root was determined against *An. stephensi* and *St. aegypti* and that of *L. decandrum* root against *An. stephensi* and *Cx. quinquefasciatus* mosquitoes (Table 5). The larvicidal activity of both the extracts did not persist much with half-life (time taken for doubling of LC_{50} value in days) ranging between 2-4 days.

DISCUSSION

Plants are a rich source of biologically active chemicals and can be considered a potential source of mosquito control agents (Wink, 1993). NE India is extremely rich in flora and fauna. Nearly 40% of the NE India is forested with many geographically isolated areas inhabited by people of varying ethnicity with little access to modern health care facilities. Such people generally rely on traditional system of medicine, the knowledge which is passed on from generation to generation. Plants used by the indigenous communities not only serve as rich source of medicine for many ailments but also as poison to kill insect and pests. Most of the plants evaluated for mosquito larvicidal activity in this study are used locally as fish poison by various communities (Lalthanzara & Lalthanpuii, 2009). We found excellent activity in petroleum-ether extract of *Derris elliptica* roots especially against *An. stephensi* larvae. Mosquito larvicidal activity of *D. elliptica* has also been reported by other workers (Prempree & Sukhapanth, 1990). Rotenone is a natural plant toxin used for centuries by indigenous peoples of Southeast Asia and South America for the harvesting of fish for human consumption. Dried *Derris* roots contain approximately 5% of rotenone (Ling, 2003). In our study the PE extract of *D. elliptica* roots recorded higher activity (LC_{50} values ranging from 0.307 to 1.05 µg/ml against different species of mosquitoes) compared to its MC extracts. Komalamisra *et al.* (2005) in Thailand reported similar results but with higher LC_{50} (ranging between 11.2 and 18.8 µg/ml). Perhaps the rotenone content in the roots of *D. elliptica* found in the north-east India is relatively higher than *D. elliptica* found in Thailand. *Litsea salicifolia* has been reported to be an effective mosquito repellant (Phukan & Kalita, 2005). However, it recorded only a moderate mosquito larvicidal activity in our study. Singha *et al.* (2011) reported that *Croton caudatus* extract, possessing a weak mosquito larvicidal activity, resulted in higher synergistic activity in combination with *Tiliacora acuminate* extract. We also noticed only a mild larvicidal activity of *C. caudatus*. Larvicidal action of *Croton tiglium* plant extract was demonstrated against *Cx. quinquefasciatus* mosquito (Deshmukh *et al.*, 1982) and we recorded its potential against the larvae of *St. aegypti* and

An. stephensi mosquitoes. *Linostoma decandrum* plant exhibited very good larvicidal activity against all the three species of mosquitoes, especially *An. stephensi* and *Cx. quinquefasciatus*, with higher activity shown by the root extracts than the shoot extracts. This plant is endemic in Assam and is known as 'Deobih' in local language.

Plant based chemicals have been reported to possess larvicidal, insect growth regulator, repellent and oviposition attractant activities (Venketachalam & Jebasam, 2001; Dwivedi & Karwasara, 2003) and these have been used extensively on agricultural pests but on limited scale against insect vectors of public health importance. In our study, crude petroleum ether extracts of the three plants *viz. D. elliptica*, *L. decandrum* and *C. tiglium* exhibited remarkable mosquito larvicidal activity. Since crude extracts are generally a mixture of several compounds, it is logical to further fractionate these active crude extracts to characterize the active principle(s) responsible for the larvicidal activity through structural chemistry work like gas chromatography and mass spectrometry (GCMS), infrared spectroscopy (IR) and nuclear magnetic resonance (NMR). This may open up the possibility of developing a potential eco-friendly phytopesticide.

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