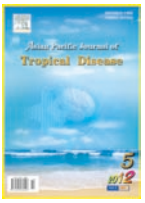
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(12)60087-5

Spatial and temporal distribution of mosquito larvicidal compounds in mangroves

M Syed Ali[1], S Ravikumar[2*], J Margaret Beula[3]

[1]*PG Department of Biotechnology, Mohammed sathak college of Arts and Science, Sohlinganallur, Chennai, Tamil Nadu, India*
[2]*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi, Tamil Nadu, India*
[3]*Department of Chemistry, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 20 September 2012
Received in revised form 25 October 2012
Accepted 28 October 2012
Available online 28 October 2012

Keywords:
Aedes aegypti
Avicennia marina
Insecticides
Larvicidal activity
Mosquito control

ABSTRACT

Objective: To identify the larvicidal activity of the mangrove plant extracts. **Methods:** Parts (bark, root, leaf and flower) of mangrove plants, *Avicennia marina* (*A. marina*), *Acanthus ilicifolius*, *Bruguiera cylindrica* and *Excoecaria agallocha* (*E. agallocha*), were dissolved in DMSO to prepare a graded series of concentration. Batches of 25 early 4th instars larvae of *Aedes aegypti* (*Ae. aegypti*) were transferred to a 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (0.01-0.10 mg). Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of 1 mL of DMSO and 199 mL of distilled water only. After 24 h, the percentage of mortality was determined. **Results:** The bark extract of *A. marina* showed maximum larvicidal activity against the 4th instars larvae of *Ae. aegypti*, followed by the leaf extract of *E. agallocha*. The presence of flavonoids, terpinoids and sponins in the ethanolic extracts of *A. marina* might be responsible for the larvicidal activity. **Conclusions:** It is concluded from the present study that the mosquitocidal toxins of *A. marina* might be a prospective alternative in mosquito control programme involving mangrove biopesticides.

1. Introduction

Mosquitoes are responsible for more diseases than any other groups of arthropods[1]. Interest in *Aedes aegypti* (*Ae. aegypti*) lies in the fact that it acts as a vector for an arbovirus responsible for yellow fever in Central and South America and in West Africa. This mosquito is also the vector of dengue hemorrhagic fever, which is endemic to South East Asia, the Pacific islands area, Africa and the Americas[2]. Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and to the increasing resistance of mosquitoes to current commercial insecticides. Although yellow fever has been reasonably brought under control with its vaccine, no vaccine is available for dengue. The only way of decreasing the incidence of this disease is thus the eradication of *Ae. aegypti*. Experience has shown that aerial toxicants for the eradication of this mosquito are not effective, since it is highly domesticated and many adults rest indoors in hidden places like closets. The only successful way of reducing mosquito densities to a level where dengue or yellow fever epidemics do not occur is by attacking the larval breeding places[3]. The ideal control method is thus the systematic treatment of their breeding places through larvicides[4]. Researchers are now looking for natural insecticides which do not have any ill effects on non-target population and are easily degradable. The search is underway to find out newer insecticides which will be effective and safe, and also easily available at low cost. Due to spiraling costs of insecticides and labour, paucity of funds and resistance developed by plasmodia or anophelines to chemicals, diseases carried by mosquitoes are back since 1980. Hence, there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products are generally preferred because of their less harmful nature to non-target

*Corresponding author: S Ravikumar, School of Marine Sciences, Department of Oceanography and CAS, Alagappa University, Thondi, Tamil Nadu-623409, India.
Mobile: +0091-456-1243470
Fax: +0091-456-1243470
E-mail: ravibiotech201321@gmail.com
Foundation Project: Supported by Ministry of Environment and Forest with grant number D.O.NO.3/2/2004 CS(M)-11.12.2006.

organisms and due to their innate biodegradability[5].

Marine halophytes are a salt tolerant plant having enormous diversity[6]. Most of the plants have special adaptation to accumulate salts and some have the property to excrete salt through the leaves. The former are collectively called as salt intruders and the later are known as salt extruders. Previous studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial[7-11], antifungal[7], antiplasmodial[12-15], hepatoprotective[16-18], ichtyotoxic[10], free radical scavenging activities[11] and antifertility[19]. Mangrove plant extracts have been used as a popular method to treat several health disorders for centuries. Plant-derived substances have recently become of great interest owing to their versatile applications. The present study was made an attempt to assess the larvicidal properties of four mangrove plant extracts against *Ae. aegypti* mosquito larvae.

2. Materials and methods

2.1. Plant materials

Different plant parts (leaf, bark, root and flower) of mangrove samples from *Avicennia marina* (Avicenniaceae) (*A. marina*), *Acanthus ilicifolius* (Acanthacae) (*A. ilicifolius*), *Bruguiera cylindrica* (Rhizophoraceae) (*B. cylindrica*) and *Excoecaria agallocha* (Euphorbiaceae) (*E. agallocha*) were collected from Pichavaram mangrove forest (latitude 11°27′ N and longitude 79°47′ E) of South East coast of India. The identified mangrove plants were authenticated by Prof. K. Kathiresan, Faculty of Marine Science, Annamalai University, Porto Novo, India. Voucher specimens were deposited in the herbarium cabinet facility (Sponsored by Indian Council of Medical Research, New Delhi, India) and maintained in the School of Marine Science, Alagappa University, Thondi campus, Thondi, Ramanathapuram Dist, Tamil Nadu, India. All the collected samples were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals.

2.2. Extract preparation

Shade dried mangrove plant samples were subjected to percolation by soaking in ethanol and water mixture (3:1, v/v). After 21 d of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (>45 °C) and then freeze-dried (-80 °C) to obtain solid residue. The percentage of extraction was calculated by using the following formula:

Percentage of extraction (%) = Weight of the extract (g)/Weight of the plant material (g)×100%. (1)

The extracts of mangrove plant were screened for the presence of phytochemical constituents by following the method of Ravikumar *et al*[13].

2.3. Mosquito larval culture

To satisfy the need of enormous number of mosquitoes for the day to day bioassays, a colony is essential. The eggs and egg rafts of *Ae. aegypti* were procured from Vector Control Research Centre, Puducherry, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30-40 min, time enough to allow eggs to hatch into larvae. They were reared indoors at (28±2) °C and 14 h/10 h light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and yeast powder at a 3:1 weight ratio. After 5 d post emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with 100 g/L sucrose solution and allowed to blood feed from white mice for 2-3 h. A few days after having a blood meal, the gravid mosquito laid their eggs.

2.4. Larvicidal activity

The test for the larvicidal effect of ethanolic extract derived from mangrove plants against *Ae. aegypti* was conducted in accordance with the WHO standard method[20]. Each extract of mangrove plant was dissolved in DMSO to prepare a graded series of concentration. Batches of 25 early 4th instar larvae of *Ae. aegypti* were transferred to a 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (0.01-0.10 mg). Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of 1 mL of DMSO and 199 mL of distilled water only. After treatment, symptoms of the treated larvae were observed and recorded immediately without time intervals and no food was offered to the larvae. The larvae were considered dead if, at the end of 24 h, they showed no sign of swimmimg movements even after gentle touching with a glass rod, as described in the WHO's technical report series. Subsequently, the lower concentration of crude extract that had successfully produced more than 50% larval mortality rate was used in a toxicity test on a non-target organism. The corrected mortality was calculated by with Abbott's formula:

Corrected mortality (%) = [Test mortality (%)-Control mortality (%)]/[1-Control mortality (%)]×100. (2)

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis to calculate LC_{50}, LC_{90} and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL) and regression equation. *Chi*-squre and analysis variation values were calculated using the Stat plus 2009 software. Results with $P<0.05$ were considered to be statistically significantly different.

3. Results

The yield rate of extracts ranged from 6.12% to 14.79% and the values are represented in Table 1. The results revealed that *A. marina* (14.79%) showed maximum yield,

followed by *B. cylindrica* (13.84%). The LC_{50} and LC_{90} values of the mangrove plant extracts against *Ae. aegypti* are listed in Table 2. The bark extract of *A. marina* showed maximum larvicidal activity as supported by its minimum concentration of LC_{50} value, followed by the leaf extract of *E. agallocha*. The regression equations of the mangrove plant extracts are also shown in Table 2. The chi-square and analysis of variance between the concentration and time of exposure was significant at $P<0.05$ level (Table 2). The preliminary phytochemical studies revealed that the extracts from mangrove plants had a variety of phytochemical constituents, namely, alkaloids, carboxylic acid, coumarins, flavonoids, phenols, saponins, xanthoproteins, protein, steroids, tannins, and sugars (Table 3).

Table 1
Parentage of ethanolic extracts from mangrove plant species.

Mangrove plant	Plant part	Parentage of extract (%)
A. marina	Bark	12.09
	Leaf	14.79
	Root	11.71
A. ilicifolius	Leaf	7.86
B. cylindrica	Bark	9.81
	Leaf	13.84
	Root	6.12
E. agallocha	Leaf	8.53

4. Discussion

Plants and plant parts have been provided as a good source of inspiration for novel drug compounds, as plant derived drugs have made large contribution to human health. The use of plant extracts, as well as other alternative forms of medical treatment, is enjoying great popularity in the late 1990s. Mangroves are widespread in tropical and subtropical regions and grow in the saline intertidal zones of sheltered coast lines and antifungal compounds[21]. The activity of crude plant extracts is often attributed to the complex mixture of active compounds[22-23]. The preliminary screening is a good mean to evaluate the potential larvicidal activity of plants popularly used for this purpose. The evidence for the use of marine flora to be precise in treatment of human aliments is extensive. We found out that the *A. marina* showed maximum larvicidal activity against *Ae. aegypti*. Previous studies reported that the mangrove extracts are proved to have potential larvicidal activities against several mosquito larvae such as *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*[24]. The mangrove plants are well known in the scientific literature for its other biological activities. Traditionally, many of the mangrove plants such as *Clerodendron inerme*, *Derris araripensis*, *Halophila ovalis*, *Excoecaria agallocha*, *Heritiera littoralis*, *Morinda citrifolia*, *Rhizophora apiculata*, *Salvadora persica* and *Sonneratia caseolaris*, are proved to have potential cytotoxic and larvicidal activities,

Table 2
Larvicidal activity of mangrove plants against *Ae. aegypti* larvae, dengue vector.

Mangrove plant	Part	LC_{50} (mg/mL)		LC_{90} (mg/mL)	Regression equation	*r*	Chi-square	*P* value
		Mean ± SE	LCL-UCL					
A. marina	Bark	55.1±7.7	51.8-82.5	135.8	$Y = 1.866+1.193X$	0.981*	4.852 0	0.028 4*
	Leaf	77.4±17.0	63.4-91.4	140.8	$Y = 3.400+1.323X$	0.801	10.341 0	0.210 0
	Root	79.1±12.0	58.0-98.4	189.1	$Y = 4.000+1.400X$	0.612	9.181 0	1.011 0
A. ilicifolius	Leaf	74.7±7.3	60.1-89.3	125.6	$Y = 2.000+0.727X$	0.627	4.934 3	0.821 0
B. cylindrica	Hypocotyl	82.0±19.0	61.0-92.0	121.0	$Y = 3.000+0.712X$	0.501	9.110 0	0.109 0
	Leaf	91.0±78.0	73.0-97.0	110.9	$Y = 3.000+0.621X$	0.711	4.911 0	0.971 0
E. agallocha	Leaf	67.1±17.7	51.8-82.5	130.3	$Y = 1.675+0.909X$	0.901*	7.413 8	0.033 1*

LCL means lower confidence level and UCL upper confidence level. *Significant at $P<0.05$ level. The flower of *A. marina* and root of *B. cylindrica* had no arvicidal activity agaisnt *Ae. aegypti* larvae.

Table 3
Phytochemical constituents in mangrove plant species.

Mangrove species	Plant part	Alkaloid	Carboxylic acid	Coumarin	Flavanoid	Terpenoid	Phenol	Saponin	Xantho-protein	Protein	Resin	Steroid	Tannin	Sugar
A. marina	Bark	++	+	-	-	++	+	-	-	-	-	+	++	-
	Flower	-	-	-	-	-	-	-	-	+	-	-	-	+
	Leaf	+	-	-	+	-	-	-	-	+	-	+	-	+
	Root	+	+	-	-	-	+	-	-	-	-	-	+	-
A. licifolius	Leaf	+	-	-	-	+	-	-	-	-	-	+	-	+
B. cylindrica	Hypocotyl	+	-	-	-	-	+	+	-	+	-	-	-	-
	Leaf	+	-	-	-	-	-	-	-	-	-	-	+	-
	Root	-	+	+	+	-	+	-	-	-	-	+	+	+
E. agallocha	Leaf	++	-	-	-	++	++	+	-	+	-	+	+	+

-: Absent; +: Medium; ++: Rich.

and the larvicidal properties of the mangrove plants might be the presence of unique chemical constituents such as flavonoids, hydrocarbons, iridoid bigylcoside, neolignans, phenols, protein, steroids, alkaloids, saponins and triterpenes. In addition, *Rhizophora mucronata* also showed higher inhibitory activity against bacterial, viral and fungal pathogens[8]. The larvicidal activity of the *A. marina* bark extract might due to the presence of major chemical classes such as saponins, flavonoids, alkaloids and triterpenoids. Triterpenoids are proved to have strong larvicidal compounds[3]. It is concluded from the present findings that the bark extract of *A. marina* have potential larvicidal activity against *Ae. aegypti* larvae. Further studies are in progress to isolate the active principles for the development of a new biopesticides.

Acknowledgments

This work was supported by Ministry of Environment and Forest with grant number D.O.NO.3/2/2004 CS(M)-11.12.2006.

References

[1] Samidurai K, Jebanesan A, Saravanakumar A, Goviindarajan M, Pushpanathan T. Larvicidal, ovicidal and repellent activities of *Pemphis acidula* forest. (Lythraceae) against filarial and dengue vector mosquitoes. *Acad J Ento* 2009; **2**(2): 62-66.

[2] Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*. *Parasitol Res* 2008; **103**(2): 959-962.

[3] Ravikumar S, Syed Ali M, Margaret Beula J. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asain Paci J Tro Biomed* 2011; 1(suppl. 2): S141-S144.

[4] Knio KM, Usta J, Dagher S, Zournajian H, Kreydiyyeh S. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. *Biores Tech* 2008; **99**(4): 763-768.

[5] Kamaraj C, Rahuman AA, Bagavan A. Antifeedant and larvicidal effects of plant extracts against *Spodoptera litura* (F.), *Aedes aegypti* L. and *Culex quinquefasciatus* Say. *Parasitol Res* 2008; **103**(2): 325-331.

[6] Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Adv Mar Biol 2001; **40**: 81-251.

[7] Ravikumar S, Ramanathan G, Subhakaran M, Jacob Inbaneson S. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int J Med Sci* 2009; **1**(5): 184-191.

[8] Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi A. Antibacterial potential of chosen mangrove plants against isolated urinary tract infections bacterial pathogens. *Int J Med Sci* 2010; **2**(3):94-99.

[9] Raja M, Ravikumar S, Gnanadesigan M, Vijayakumar V. *In vitro* antibacterial activity of diterpene and benzoxazole derivatives from *Excoecaria agallocha* L. *Int J Biol Chem Sci* 2010; **4**(3): 692-701.

[10] Manilal A, Sujith S, Seghal Kiran G, Selvin J, Shakir C. Biopotentials of mangroves collected from the southwest coast of India. *Glo J Biotechnol Biochem* 2009; **4**(1): 59-65.

[11] Agoramoorthy G, Chen FA, Venkatesalu V, Kuo DH, Shea PC. Evaluation of antioxidant polyphenols from selected mangrove plants of India. *Asian J Chem* 2008; **20**(2):1311-1322.

[12] Ravikumar S, Ramanathan G, Jacob Inbaneson S, Ramu A. Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. *Parasit Res* 2011; **108**(1): 107-113.

[13] Ravikumar S, Jacob Inbaneson S, Suganthi P, Gnanadesigan M. *In vitro* antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive *Plasmodium falciparum*. *Parasitol Res* 2011; **108**(4): 873-878.

[14] Ravikumar S, Jacob Inbaneson S, Suganthi P, Venkatesan M, Ramu A. Mangrove plants as a source of lead compounds for the development of new antiplasmodial drugs from South East coast of India. *Parasitol Res* 2011; **108**(6): 1405-1410.

[15] Ravikumar S, Jacob Inbaneson S, Suganthi P, Gokulakrishnan R, Venkatesan M. *In vitro* antiplasmodial activity of ethanolic extracts of seaweed macroalgae against *Plasmodium falciparum*. *Parasitol Res* 2011; **108**(6): 1411-1416.

[16] Ravikumar S, Gnanadesigan M, Jacobinbanesan S, Kalaiarasi A. Hepatoprotective and antioxidant properties of *Suaeda maritima* (L.) Dumort ethanolic extract on concanavalin-A induced heaptotoxicity in rats. *Indian J Exp Biol* 2011; **49**(6): 455-460.

[17] Ravikumar S, Gnanadesigan M. Hepatoprtective and antioxidant properties of marine halophyte *Luminetzera racemosa* bark extract in CCL_4 induced heaptotoxicity. *Asian Pacific J Trop Biomed* 2011; **1**(4): 348-335.

[18] Gnanadesigan M, Ravikumar S, Jacob Inbanesan S. Hepatoprtective and antioxidant properties of marine halophytes Luminetzera racemosa bark extract in CCL4 induced heaptotoxicity. *Asian Pacific J Trop Med* 2011; **1**(5): 462-465.

[19] Ravikumar S, Abideen S, Syed Ali M, Babuselvam M. *In vitro* human sperm immobilizing activity of marine halophytes. *J Pharma Res* 2011; **4**(4): 1291-1293.

[20] WHO. Report of the WHO informal consultation on the evaluationon the testing of insecticides. Geneva: WHO; 2009; p. 69.

[21] Kathiresan K. Globally threatened mangrove species in India. *Curr Sci* 2010; **98**(12): 1551.

[22] Gayathri S, Saravanan D, Radhakrishnan M, Balagurunathan R, Kathiresan K. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt-marsh plant species. *Ind J Biotech* 2010; **9**(4): 397-402.

[23] Nabeel MK, Kathiresan K, Manivannan S. Antidiabetic activity of the mangrove species *Ceriops decandra* in alloxan-induced diabetic rats. *J Diabetes* 2010; **2**(2): 97-103.

[24] Thangam TS, Kathiresan K. Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of *Rhizophora apiculata* with pyrethrum against *Culex quinquefasciatum*. *Int J Pharmacognosy* 1997; **35**(1): 69-71.