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Insecticidal compounds from Rhizophoraceae mangrove plants for the management of dengue vector *Aedes aegypti*

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ABSTRACT

Background & objectives: Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin could serve as potential alternatives in future. Larvicidal efficacies of different parts of mangrove plants belonging to Rhizophoraceae family were tested against the late IV instar larvae of dengue vector, *Aedes aegypti*.

Methods: Different plant parts (leaf, bark, root, stilt root, hypocotyl and flower) of Rhizophoraceae family mangrove plants (*Bruguiera cylindrica, Ceriops decandra, Rhizophora mucronata* and *R. apiculata*) were collected from Karangadu southeast coast of India. The larval mortality was observed after 24 h exposure. Repellency bioassays were carried out in a 10 × 10 × 3 m room at 27–35°C and 60–80% RH. The bark (A3 and E1) and stilt root (A3 and E4) fractions of *R. mucronata* with different concentrations (0.25, 0.50, 0.75, 1, 2 and 4 mg/cm) were applied on one arm.

Results: The stilt root crude extract of *R. mucronata* showed maximum larvicidal activity (LC_{50} value 0.0275 ± 0.0066 µg/ml and LC_{90} = 0.0695 ± 0.156 µg/ml) followed by the bark extract (LC_{50} value of 0.03 ± 0.0076 µg/ml and LC_{90} = 0.0915 ± 0.156 µg/ml). Column chromatographic fractions of *R. mucronata* bark extracts (E1) showed maximum larvicidal activity (LC_{50} = 0.0496 ± 0.0085 µg/ml and LC_{90} = 0.1264 ± 0.052 µg/ml) followed by the acetone extract (LC_{50} = 0.0564 ± 0.0069 µg/ml and LC_{90} = 0.1187 ± 0.05 µg/ml). Ethanolic fraction (E4) of *R. mucronata* stilt root extracts showed maximum larvicidal activity (LC_{50} = 0.0484 ± 0.0078 µg/ml and LC_{90} = 0.1191 ± 0.025 µg/ml) followed by acetone fraction (A3) (LC_{50} = 0.0419 ± 0.0059 µg/ml and LC_{90} = 0.0955 ± 0.069 µg/ml). Repellent activity of *R. mucronata* stilt root and bark extracts (A3) showed maximum percentage of protection (97.5%) with 9.1 h protection time at 4 mg concentration of the stilt root extract. Moreover, ethanolic fraction of the stilt root (E4) extract showed maximum percentage of protection (100%) with 10 h protection time at 4 mg concentration. GC-MS analysis revealed that *R. mucronata* possesses variety of biopesticidal compounds.

Interpretation & conclusion: The results as well as the significance of this preliminary investigation highlight the importance of *R. mucronata* as a novel source for natural insecticidal products.

Key words Biopesticides; dengue; mangrove repellent activity; mosquito larvicidal; *R. mucronata*

INTRODUCTION

Blood feeding female mosquitoes are responsible for the intolerable biting nuisance and transmission of a large number of diseases, such as dengue, malaria, yellow fever, chikungunya and encephalitis. They cause serious health problems to humans and present obstacles to the socioeconomic progress in developing countries, particularly in the tropical region[1]. *Aedes aegypti* (Diptera: Culicidae) is present in Asia, Africa, Central and South America and transmits etiologic agents of human diseases like dengue and yellow fever causing by flavivirus[2]. *Aedes aegypti* population from different geographical areas may differ in behaviour[3]. The environment for *Ae. aegypti* proliferation must include water-filled containers for immature[4] nectar and blood as engery source for adults[5]; blood preferentially human, for egg development[6] and shady habitat for resting and oviposition[7]. These requirements are fulfilled by some of the areas in Thondi coastal thereby acting as important foci for vector proliferation. Marine halophytes are salt-tolerant plants having enormous diversity[6]. Most of the plants have special adaptation potential to accumulate salts and some to excrete through the leaves. Previously, mangrove plants have displayed various levels of biological activities including antibacterial[7], antifungal[8], antimicrobial[9], antiplasmodial[10–11], hepatoprotective[12–13], larvicidal[2] and antifertility[14].

Rhizophoraceae are medicinal plants of east and southeast Asia. The most common representatives are *Rhizophora mucronata*, *R. mangle* and *R. apiculata. Rhizophora mucronata* is an astringent, a folk remedy for angina and hemorrhage; its old leaves or roots are

used for childbirth[15]. Although information has been available for hundreds of years, there is no scientific investigation on the mode of action of the active substances to determine the various plant parts of the mangrove from biological activity against insects. Based on the foregoing, the present study was sort to scientifically evaluate the insecticidal activity of Rhizophoraceae mangrove plants.

MATERIAL & METHODS

Plant materials

Different plant parts (leaf, bark, root, stilt root, hypocotyl and flower) of Rhizophoraceae mangrove plants (*Bruguieracylindrica, Ceriops decandra, R. mucronata* and *R. apiculata*) were collected from Karangadu mangrove forest (latitude 09° 36′ N and longitude 78° 83′ E) of southeast coast of India (Table 1). The identified mangrove plants were authenticated by Prof. K. Kathiresan, Faculty of Marine Sciences, Annamalai University, Porto Novo, India. Voucher specimens were deposited in the herbarium cabinet facility (Sponsored by ICMR, New Delhi) maintained in the School of Marine Sciences, Alagappa University, Thondi Campus, Thondi, Ramanathapuram district, Tamil Nadu, India.

Preparation of crude extract

All the collected samples were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals. Shade-dried mangrove plant samples were subjected for percolation by soaking in ethanol and water mixture (3:1). After 21 days 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 using the following formula:

$$\text{Percent of extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100$$

Insect rearing

Aedes aegypti eggs maintained in the School of Ma-

Table 1. Mosquito larvicidal activity of ethanolic crude extracts of Rhizophoraceae plant parts against *Aedes aegypti*

Name of the species	Parts	LC_{50} ± SE (LCL–UCL)	LC_{90} ± SE (LCL–UCL)	R^2	χ^2	*p*-value
Bruguiera cylindrica	Leaf	0.091 ± 0.078 (0.073 – 0.097)	0.1109 ± 0.069 (0.91 – 1.26)	0.711	4.911	0.971
	Root	No mortality				
	Hypocotyl	0.082 ± 0.119 (0.981 – 0.012)	0.121 ± 0.256 (0.116 – 0.13)	0.501	9.11	0.109
Ceriops decandra	Leaf	0.0892 ± 0.0063 (0.0767 – 0.1018)	0.129 ± 0.006 (0.098 – 0.136)	0.829	12.458	0.734
	Collar	0.082 ± 0.0064 (0.0692 – 0.0948)	0.130 ± 0.025 (0.119 – 0.1561)	0.272	65.141	1.25
	Hypocotyl	No mortality				
Rhizophora apiculata	Bark	0.0943 ± 0.0077 (0.0789 – 0.1096)	0.148 ± 0.091 (0.132 – 0.1569)	0.542	4.326	1.128
	Leaf	0.085 ± 0.009 (0.081 – 0.958)	0.198 ± 0.095 (0.189 – 0.099)	0.569	3.269	1.126
	Hypocotyl	0.083 ± 0.007 (0.0689 – 0.0962)	0.1303 ± 0.08 (0.112 – 0.145)	0.762	8.003	1.659
	Collar	0.0846 ± 0.0068 (0.0709 – 0.0983)	0.1283 ± 0.065 (0.114 – 0.139)	0.802	54.45	2.238
	Flower	No mortality				
Rhizophora mucronata	Bark	0.03 ± 0.0076 (0.014 – 0.0451)	0.0915 ± 0.156 (0.089 – 0.159)	0.982**	1.736*	0.049*
	Leaf	0.078 ± 0.006 (0.069 – 0.042)	0.087 ± 0.09 (0.068 – 0.121)	0.859	9.365	0.123
	Hypocotyl	0.053 ± 0.0063 (0.04 – 0.07)	0.1037 ± 0.015 (0.069 – 0.112)	0.916	4.8492	0.079
	Stilt root	0.0275 ± 0.0066 (0.014 – 0.0407)	0.0695 ± 0.156 (0.015 – 0.078)	0.996**	4.189*	0.036*
	Collar	0.0673 ± 0.0052 (0.056 – 0.0777)	0.1097 ± 0.015 (0.094 – 0.112)	0.962	13.899	2.28

*Significant at $p < 0.05$ level; ** Significant value of regression analysis; SE—Standard error; LCL – Lower confidence level; UCL – Upper confidence level.

rine Sciences, Division of Marine Pharmacology, Thondi (latitude 9° 44′ 01.78″ N and longitude 79° 01′ 00.98″ E) was used to start the colony and the larvae were reared in plastic and enamel trays containing tap water. Experiments were carried out, at 27 ± 2°C and 75–58% relative humidity under 14:10 light and dark cycles. Larvae were fed with brewers yeast and dog biscuits in 3:1 ratio. Pupae were transferred from the trays to a cup containing tap water and were maintained in our cages (45 × 45 × 40 cm) where adults emerged. Adults were maintained in cage and were continuously provided with 10% sucrose solution in air-tight cylindrical glass container with a cotton wick. On Day 5, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. Glass petri dishes with 50 ml of tap water lined with filter paper were kept inside the cage for oviposition.

Mosquito larvicidal activity

The test for the larvicidal effect of ethanolic crude extracts and chromatographic fractions (the colloidal form of crude ethanolic extract fraction of bark and stilt root of *R. mucronata* was subjected to column chromatography packed with 500 g of silica gel (60–120 mesh) (MERCK) with minimum height of 50 cm and eluted successively with 30 ml of hexane, benzene, chloroform, acetone, ethanol and water. The fractions were labeled (E1–E5, A1–A5, C1–C5, W1–W5, B1–B5 and H1–H5) corresponding to the solvent used and tested against *Ae. aegypti* using standard WHO test[16]. Each extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a graded series of concentrations. Batches of 25 early IV instar larvae of *Ae. aegypti* were transferred to 250 ml enamel bowl containing 199 ml of distilled water and different concentrations of plant extracts (0.01–0.1 mg). Each experiment was conducted with three replicates. Concurrent control group was maintained with 1 ml of DMSO and 199 ml of distilled water. After treatment, symptoms in treated larvae were observed and recorded immediately after 24 h and no food was offered to the larvae. The larvae were considered dead if any at the end of 24 h, showed no sign of swimming movements even after gentle touching with a glass rod as described in the World Health Organization's technical report series.

Mortality was corrected by applying Abbott's formula given below, whenever found necessary.

$$\text{Corrected percent mortality} = \frac{\text{(Percent of test mortality– Percent of control mortality)}}{\text{100 – Percent of control mortality}} \times 100$$

Insect repellent bioassay

The repellent activity was determined by the percentage protection time in relation to dose method[17]. Repellency bioassays were carried out in a 10 × 10 × 3 m room at 27–35°C and 60–80% RH. Because the target *Ae. aegypti*, is usually a day-biting mosquito, the testing period was run between 0–10 h. Three-to-four days old blood-starved 100 adult female *Ae. aegypti* mosquitoes were randomly selected and placed in an experimental cage (30 × 30 × 30 cm) and left to acclimatize for 1 h. The arms of tested person were cleaned with ethanol and air-dried, after that 25 cm^2 dorsal side of the skin of both the arms was exposed and the remaining area being covered by rubber gloves. The bark (A3 and E1) and stilt root (A3 and E4) fractions of *R. mucronata* with different concentrations (0.25, 0.50, 0.75, 1, 2 and 4 mg/cm^2) were applied on one arm and another arm was maintained without the extract (control). The control and treated arms were introduced simultaneously into the cage. First bite by the *Ae. aegypti* was noted to 5 min for every 1 h from the 10 h. Subsequently, the test arm was introduced into the cage for the same period of time and the numbers of mosquitoes that landed and attempted to feed were recorded. The experiment was conducted for three times. It was observed that there was no skin irritation by the bark and stilt root extracts of *R. mucronata*. The percentage protection was calculated using the following formula:

$$\text{Percent protection} = \frac{\text{(No. of bites received by control – No. of bites received by treated)}}{\text{No. of bites received by control}} \times 100$$

GC-MS analysis

GC-MS analysis of active ethanolic (E4) and acetone (A3) fraction extracts from stilt root of *R. mucronata* was done individually using Agilent GC-MS 5975 Inert XL MSD (United States) gas chromatography equipped with J&W 122-5532G DB-5mm 30 × 0.25 mm × 0.25 µm and mass detector (EM with replaceable horn) was operated in EMV mode. Helium was used as carrier gas with the flow rate of 1 ml/min. The column oven temperature was kept at 80°C for 2 min then programmed at 10–250°C/min, which was held for zero min, and 5–280°C/min which was held at 9 min. Electron impact spectra in positive ionization mode were acquired between m/z 40 and 450.

Ethical clearance

Ethical clearance of this research study was obtained from the Ethics Review Committee of Mohamed Sathak AJ College of Pharmacy, Chennai, India (Ref. No. 991/C/06/CPCSEA).

Statistical analysis

The larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% Fiducial limits of upper confidence limit and lower confidence limit and chi-square values were calculated by using the software developed by Statplus 2009. The result with $p < 0.05$ was considered to be statistically significant.

RESULTS & DISCUSSION

The LC_{50} and LC_{90} values of different mangrove plant part extracts against *Ae. aegypti* are listed in Table 1. The stilt root extract of *R. mucronata* showed maximum larvicidal activity (LC_{50} = 0.0275 ± 0.0066 µg/ml and LC_{90}= 0.0695 ± 0.156 µg/ml) followed by the bark extract of *R. mucronata* (LC_{50} = 0.03 ± 0.0076 µg/ml and LC_{90} = 0.0915 ± 0.156 µg/ml). Similarly, no mortality was found in the extracts of *B. cylindrica* (root) and *C. decandra* (hypocotyl). Ethanolic fraction of *R. mucronata* bark extracts (E1) showed maximum larvicidal activity (LC_{50} = 0.0496 ± 0.0085 µg/ml and LC_{90} = 0.1264 ± 0.052 µg/ml) followed by the acetone fraction (A3) extract (LC_{50} = 0.0564 ± 0.0069 µg/ml and LC_{90} = 0.1187 ± 0.05 µg/ml) (Table 2). However, the ethanolic fraction (E4) of the stilt root extract showed maximum larvicidal activity (LC_{50} = 0.0484 ± 0.0078 µg/ml and LC_{90} = 0.1191 ± 0.025 µg/ml) followed by the acetone fraction (A3) extract (LC_{50}= 0.0419 ± 0.0059 µg/ml and LC_{90} = 0.0955 ± 0.069 µg/ml) (Table 3). The regression analysis reveals that the

Table 2. Effect of different chromatographic fractions of *R. mucronata* bark extract on larvicidal activity of *Ae. aegypti*

Solvent fraction	LC_{50} ± SE (LCL–UCL)	LC_{90} ± SE (LCL–UCL)	R^2	χ^2	*p*-value
Ethanol (E1)	0.0496 ± 0.0085 (0.0328 – 0.0664)	0.1264 ± 0.052 (0.113 – 0.139)	0.976**	0.500*	0.039*
Ethanol (E2)	0.0805 ± 0.0065 (0.0676 – 0.0934)	0.1268 ± 0.036 (0.106 – 0.138)	0.907	4.351	0.8241
Ethanol (E3)	0.0883 ± 0.0063 (0.0457 – 0.0709)	0.1327 ± 0.025 (0.126 – 0.1336)	0.909	0.697	0.9995
Ethanol (E4–E5)	No mortality				
Acetone (A1)	0.0897 ± 0.0072 (0.0753 – 0.104)	0.1405 ± 0.012 (0.136 – 0.156)	0.908	3.817	0.875
Acetone (A2)	0.0981 ± 0.0076 (0.0829 – 0.1133)	0.1467 ± 0.012 (0.139 – 0.156)	0.856	4.403	1.92
Acetone (A3)	0.0564 ± 0.0069 (0.0428 – 0.0701)	0.1187 ± 0.05 (0.105 – 0.136)	0.937**	0.569*	0.023*
Acetone (A4)	0.1006 ± 0.0079 (0.0848 – 0.1165)	0.1562 ± 0.016 (0.142 – 0.166)	0.904	7.034	0.9677
Acetone (A5)	0.0976 ± 0.008	0.1548 ± 0.069 (0.150 – 0.169)	0.909	2.365	0.9677
Chloroform (C1)	0.08 ± 0.0068 (0.0813 – 0.1139)	0.1315 ± 0.025 (0.129 – 0.1401)	0.948	21.272	0.875
Chloroform (C2)	0.0959 ± 0.0084 (0.079 – 0.1128)	0.1602 ± 0.0129 (0.151 – 0.169)	0.947	7.752	0.458
Chloroform (C3–C5)	No mortality				
Water (W1)	0.098 ± 0.0079 (0.0822 – 0.1138)	0.1581 ± 0.056 (0.149 – 0.162)	0.946	22.857	0.309
Water (W2)	0.0963 ± 0.0065 (0.0833 – 0.1093)	0.1457 ± 0.012 (0.136 – 0.152)	0.871	7.034	0.9677
Water (W3–W5)	No mortality				
Benzene (B1)	0.0943 ± 0.0077 (0.0789 – 0.1096)	0.1481 ± 0.126 (0.136 – 0.159)	0.905	2.3655	0.9677
Benzene (B2–B5)	No mortality				
Hexane (H1)	0.1016 ± 0.0075 (0.0865 – 0.1166)	0.149 ± 0.069 (0.125 – 0.156)	0.841	22.8577	0.309
Hexane (H2)	0.0992 ± 0.0077 (0.0838 – 0.1146)	0.1578 ± 0.025 (0.143 – 0.163)	0.982**	36.925	0.5711
Hexane (H3–H5)	No mortality				

*Significant at $p < 0.05$ level; ** Significant value of regression analysis; SE–Standard error; LCL – Lower confidence level; UCL – Upper confidence level.

Table 3. Effect of different column chromatographic fractions of solvent of *R. mucronata* stilt root extract on larvicidal activity of *Ae. ageypti*

Solvent fraction	LC_{50} ± SE (LCL–UCL)	LC_{90} ± SE (LCL–UCL)	R^2	χ^2	*p*-value
Ethanol (E1)	0.076 ± 0.007 (0.061 – 0.091)	0.137 ± 0.025 (0.126 – 0.145)	0.931	1.6425	0.990
Ethanol (E2)	0.084 ± 0.006 (0.071 – 0.096)	0.127 ± 0.009 (0.115 – 0.132)	0.916	2.365	0.967
Ethanol (E3)	0.103 ± 0.008 (0.085 – 0.121)	0.153 ± 0.069 (0.142 – 0.165)	0.757	13.899	2.28
Ethanol (E4)	0.048 ± 0.007 (0.032 – 0.063)	0.119 ± 0.025 (0.101 – 0.126)	0.996**	0.528*	0.049*
Ethanol (E5)	No mortality				
Acetone (A1)	0.092 ± 0.007 (0.077 – 0.107)	0.140 ± 0.025 (0.136 – 0.156)	0.905	3.817	0.875
Acetone (A2)	0.094 ± 0.007 (0.079 – 0.109)	0.150 ± 0.009 (0.145 – 0.163)	0.918	3.103	0.992
Acetone (A3)	0.041 ± 0.005 (0.030 – 0.053)	0.095 ± 0.069 (0.075 – 0.125)	0.938**	1.203*	0.041*
Acetone (A4)	0.095 ± 0.007 0.095 ± 0.007	0.141 ± 0.026 (0.125 – 0.159)	0.849	0.697	0.999
Acetone (A5)	No mortality				
Chloroform (C1)	0.086 ± 0.007 (0.072 – 0.101)	0.140 ± 0.098 (0.139 – 0.141)	0.918	3.817	0.875
Chloroform (C2)	0.104 ± 0.007 (0.088 – 0.119)	0.158 ± 0.29 (0.149 – 0.159)	0.888	4.403	1.92
Chloroform (C3)	0.092 ± 0.007 (0.078 – 0.106)	0.149 ± 0.25 (0.135 – 0.169)	0.902	6.683	0.571
Chloroform (C4–C5)	No Mortality				
Water (W1)	0.088 ± 0.006 (0.076 – 0.100)	0.133 ± 0.0269 (0.126 – 0.145)	0.922	0.697	0.999
Water (W2)	0.098 ± 0.007 (0.082 – 0.113)	0.158 ± 0.0248 (0.136 – 0.159)	0.906	22.857	0.309
Water (W3)	0.092 ± 0.008 (0.043 – 0.208)	0.160 ± 0.256 (0.156 – 0.169)	0.803	0.694	0.9995
Water (W4-W5)	No mortality				
Benzene (B1-B5)	No mortality				
Hexane (H1-H5)	No mortality				

*Significant at $p < 0.05$ level; ** Significant value of regression analysis; SE—Standard error; LCL – Lower confidence level; UCL – Upper confidence level.

higher significant slope for the *R. mucronata* crude extract of stilt root and bark extract on IV instar larvae ($R^2 = 0.996^{**}$ and $R^2 = 0.982^{**}$, respectively). The chi-square and analysis of variance between the concentration and time of exposure was significant at $p < 0.05$ level (Table 1). Whereas, the ethanolic (E1) and acetone (A3) fractions from bark extract showed higher significant slope by $Y = 2.8 + 0.636x$ ($R^2 = 0.976$) and $Y = 2.9 + 0.745x$ ($R^2 = 0.937$). Ethanolic (E4) and acetone (A3) fraction from stilt root extract showed higher significant slope by $Y = 2.666 + 0.678x$ ($R^2 = 0.996$) and $Y = 3.533 + 0.812x$ ($R^2 = 0.938$), respectively. Repellent activity of acetone fraction (A3) of *R. mucronata* stilt root extract showed maximum percentage of protection (97.5%) and protection time (9.1 h) at 4 mg concentration. The acetone fraction (A3) of the bark extract showed maximum percentage of protection (88.6%) and protection time (8.2 h) was observed at 4 mg of concentration of bark extract (Table 4). The ethanolic fraction of bark (E1) extract showed maximum percentage of protection (97.7%) and protection time (9.3 h) at 4 mg concentration. Moreover, the ethanolic fraction of the stilt root (E4) extract showed maximum percentage of protection (100%) and protection time (10 h) at the 4 mg concentration (Table 5).

GC-MS analysis of acetone (A3) fraction from 11 compounds, viz. 2-hydroxy-1-ethyl acetate (22.15%), mono (2-ethylhexyl) ester (2.33%), hexanedioic acid (2.11%), phthalic acid (1.78%), cyclopentane (1.77%), benzamide (1.76%), propanoic acid (1.16%), hydrazine carboxamide (0.93%), pentadecanoic acid (0.91%),

Table. 4. *In vitro* repellent activity of acetone fraction of *R. mucronata* bark and stilt root (A3 & E1) against *Ae. aegypti*

Concentration (mg/cm^2)	Percent protection	Protection time (h)
Acetone fraction of stilt root (A3)		
0.25	73.2	7.2
0.50	77.1	7.6
0.75	86.6	7.8
1	89.6	8.2
2	92.4	8.5
4	97.5	9.1
Acetone fraction of bark (A3)		
0.25	70.27	6.4
0.50	71.7	6.8
0.75	79.2	7.4
1	86.6	7.6
2	83.3	7.8
4	88.6	8.2

Table 5. *In vitro* repellent activity of ethanolic fraction of *R. mucronata* bark and stilt root (A3 & E1) against *Ae. aegypti*

Concentration (mg/cm^2)	Percent protection	Protection time (h)
Ethanolic fraction of bark (E1)		
0.25	79.2	6.5
0.50	84.8	6.8
0.75	86.6	7.4
1	87.5	7.7
2	92.5	8.2
4	97.7	9.3
Ethanolic fraction of stilt root (E4)		
0.25	78.08	6.1
0.50	84.8	7.3
0.75	88.8	7.4
1	92.4	7.8
2	97.5	9.2
4	100	10

hexadecyl acetate (0.81%) and oxalic acid (0.80%) (Fig. 1 and Table 6) and ethanolic fraction (E4) extract of *R. mucronata* stilt root posses eight compounds, viz. 1,2, benzendicarboxylic acid (5.39%), phthalic acid (1.99%), butanoic acid (0.35%), hexadecanoic acid (0.29%), propanoic acid (0.15%), 9-octadecenamide (0.13%), benzene acetic acid (0.09%) and 4-(1-1-dimethyl-ethyl)-methyl acetate (0.08%) (Fig. 2 and Table 7). The control substance caused no mortality for the larvae.

In view of the residue problems in the environment

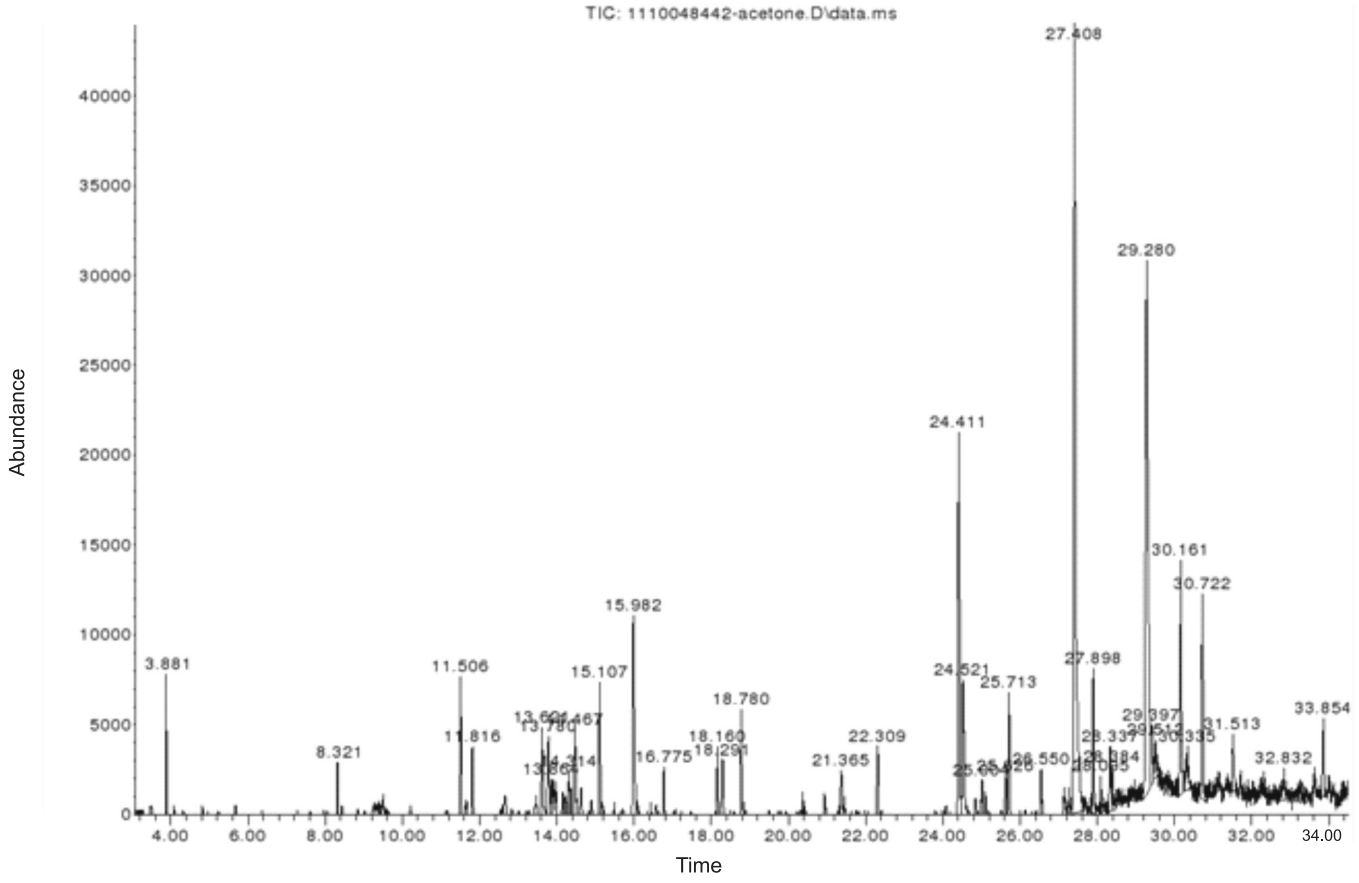

Fig. 1: GC-MS data of acetone fraction (A3) of *R. mucronata* stilt root.

Table 6. Composition of acetone fraction extract (A3) from stilt root of *R. mucronata*

Retention time	Components	Area (%)	Quality
3.882	Propanoic acid	1.16	85
11.505	Cyclopentane	1.77	88
11.818	Hydrazine carboxamide	0.93	80
13.778	Benzamide	1.76	82
18.159	Pentadecanoic acid	0.91	87
18.780	Phthalic acid	1.78	88
25.712	Hexanedioic acid	2.11	93
27.407	2-hydroxy-1-ethyl acetate	22.15	97
27.898	Mono (2-ethylhexyl) ester	2.33	81
28.385	Oxalic acid	0.80	88
29.280	Hexadecyl acetate	0.81	87

Table 7. Composition of ethanolic fraction (E4) from stilt root of *R. mucronata*

Retention time	Components	Area (%)	Quality
3.882	Propanoic acid	0.15	74
10.205	Butanoic acid	0.355	81
11.594	Benzene acetic acid	0.09	80
15.977	4-(1-1-dimethyl)-methyl acetone	0.08	81
16.577	Phthalic acid	1.99	88
17.554	Hexadecanoic acid	0.29	87
18.302	1,2-benzendicarboxylic acid	5.39	91
30.062	9-octadecenamide	0.13	81

and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to search and find plant extracts that are safe for non-target animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as having insecticidal-larvicidal activity, the need of the hour is more effective plant products. Further research in this direction will undoubtedly lead to environmentally viable and cost-effective alternatives to the harmful conventional insecticides for mosquito control, with improved formulations and enhanced activity. Different parts of plants contain a complex of chemicals with unique biological activity[18] which is thought to be due to toxins and secondary metabolites which act as attractants or deterrents[19]. This study reveals that the ethanolic extracts of the bark and stilt root of *R. mucronata* have significant larvicidal as well as repellent activities. The variations in lethal concentrations are probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant and the season[20–21]. Thangam and Kathiresan[22] investigated the effectiveness

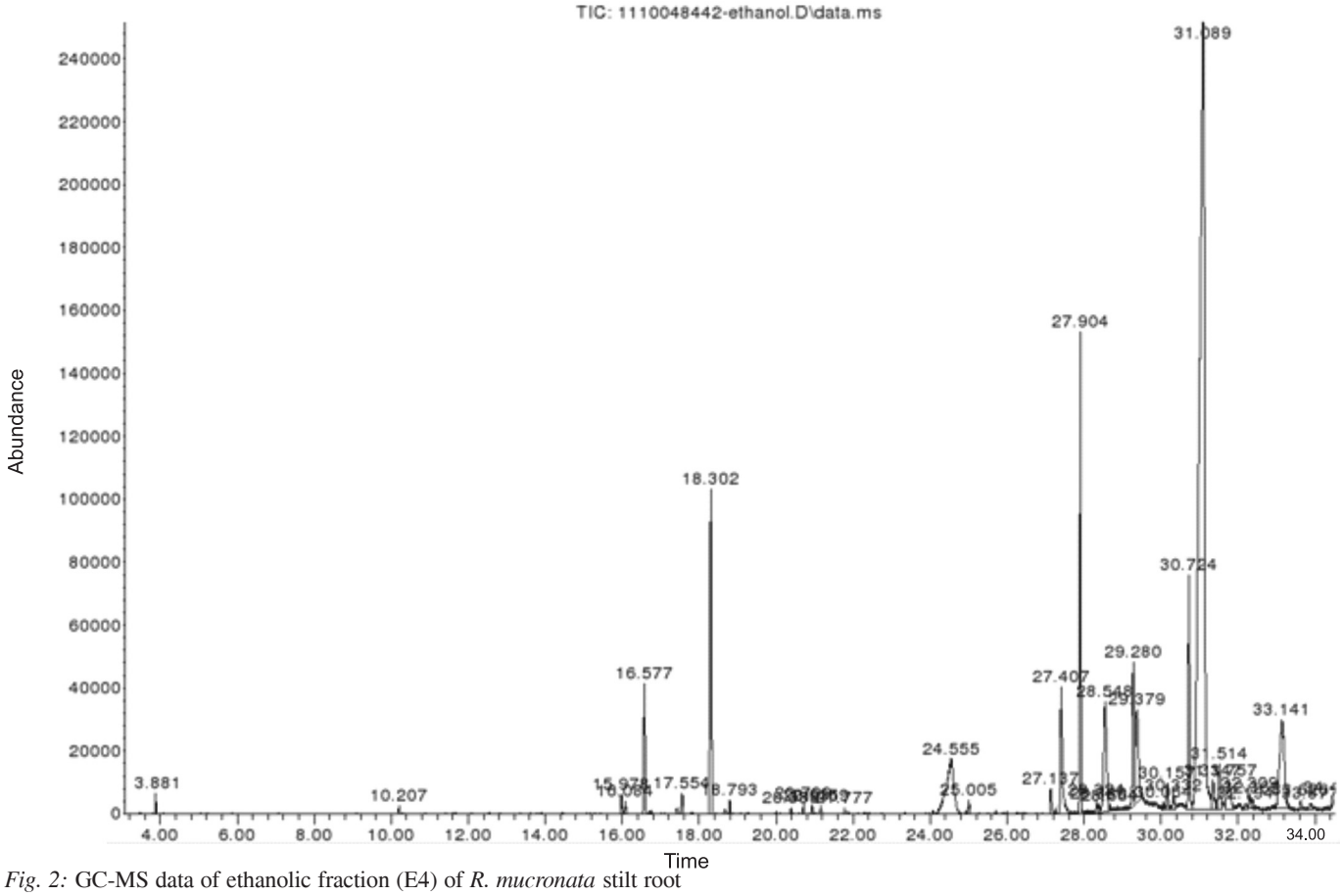

Fig. 2: GC-MS data of ethanolic fraction (E4) of *R. mucronata* stilt root

of mangrove plants in killing the larvae or repelling adult female mosquitoes. In this study, it has been found that all the extracts showed moderate larvicidal activity and the highest larval mortality was found in acetone and ethanolic fraction of the stilt root and bark extracts of *R. mucronata*. The ethanolic extract of bark and stilt root extract of *R. mucronata* possessed higher activity than the other plant parts of Rhizophoraceae. The extracts of bark and stilt root of *R. mucronata* were highly effective against the larvae of *Ae. aegypti* (Table 1). Fractionation extracts of acetone and ethanol of bark and stilt root showed maximum larvicidal activity (Tables 2 and 3) against the larvae of *Ae. aegypti.* The ethanolic fraction (E4) of *R. mucronata* stilt root showed maximum protection for 10 h followed by acetone fraction of stilt root (A3) with 9.1 h (Tables 4 and 5). The essential oil of *Tagetes minuta,* providing a repellency of 90% protection for 2 h was observed by Tyagi *et al*[23]. Essential oil obtained from *Vitex negundo* leaves showed repellency ranging from 1 to 3 h[24]. The leaf of *Excoecaria agallocha* was found most effective against *Cx. quinquefasciatus* by giving 56% of protection while *Acanthus ilicifolius* was most effective against *Ae. aegypti* by giving 74% protection[25]. The present investigation reveals that the repellent activity of stilt root extract of A3 and E4 fractions are comparable with previously screened plants in the laboratory by using different species of mosquitoes[26]. Natural products are the best option because of their less harmful nature to environment and non-targeted organisms. Several plant extracts, viz. *Annona muricata, Solanum xanthocarpum*, and *Curcuma zedoaria* have been previously proved to have potential mosquito larvicidal activity. Much efforts have also been focused on the phytochemical and their essential oils as potential sources of mosquito control agents as they are relatively safer, easily degradable, cost-effective and readily available with no or least mammalian toxicity[27].

The present study has further shown that the mosquito larvicidal and repellent activity might be due to the presence of various phytochemical constituents such as propanoic acid, cyclopentane, hydrazinecarboxamide, benzamide, pentadecanoic acid, cyclopentanone, hexanedioic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and mono (2-ethylhexyl) ester which may cause alterations in the spiracular valves of the siphon and anal papillae[28], degeneration of the pupals[29], toxicity of prothoracic glands in instar larvae[30] and inhibition of poly (ADP-ribose) polymerase enzyme which is involved in the DNA repair in adult mosquito[29], alterations in the respiratory system of mosquito larvae[28], alterations in the siphon[29], induction of carcinogenicity, reproductive and developmental toxicity[31], alterations with antennal olfactory receptors[32], and toxic effect of prothoracic glands in instar larvae[33]. Similarly, larvicidal activity of the fraction (E4) of *R. mucronata* stilt root against the larvae of *Ae. aegypti* showed potential larvicidal property which might be due to the presence of various phytochemical constituents such as propanoic acid, butanoic acid, benzene acetic acid, 4-(1,1-dimethylethyl)-methyl ester, phthalic acid, hexadecanoic acid, 1,2-benzendicarboxylic acid and 9-octadecenamide which may cause degeneration of the pupal alterations in the respiratory system of mosquito larvae, and reproductive system[28–29, 31]. Earlier report showed that the compound like diterpeniodfurna 6 alpha-hydroxyvouacapan-7-beta, 17 beta, 17 beta-lactone, 7 beta-dihydroxyvoucapan-17 betaoic acid and methyl 6 alpha, 7 beta-dihydroxyvouacapan-17 beta-oate from seeds of *Pterodns polygalaeflorus* exhibited LC_{50} values of 50.08, 14.69 and 21.76 µg/ml, respectively against IV instar larvae of *Ae. aegypti*[34]. Most of the studies in this direction have reported the bioactive of only crude extracts. However, if we could isolate and chemically characterize the active compounds, then probably it will be easy to plan a cost-effective synthesis of these molecules. As it is not wise to disturb ecological balance by collecting *R. mucronata*, in large quantities, synthesis of active molecules in laboratory is the best option. The results as well as the significance of this preliminary investigation highlight the importance of *R. mucronata* as a novel source for natural insecticidal products.

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