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ORIGINAL RESEARCH ARTICLE

Studies on Combined Effect of Mangrove Plants against Three Dangerous Mosquitoes

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ABSTRACT

To investigate the Studies on combined effect of selected Mangrove plants against three dangerous mosquitoes. The Acetone and petroleum ether extracts of four Mangrove plant species *(Avicennia marina, Avicennia officinalis, Rhizophora apiculata* and *Rhizophora mucronata)*, were examined against the larvae of *A. aegypti, C. quinquefasciatus* and *A. stephensi* with gradually increasing concentration i.e. from 50 to 250 ppm of solvent extracts and to test their activity in combination with each other. In a 24-h bioassay experiment with plant extracts, highest mortalities were recorded at 250ppm of concentrations for leaves of combination of *A.marina* and *A. officinalis* its more effective to *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* with regards to 34.622 µg/ml, 87.681 µg/ml and 206.047 µg/ml LC_{50} values respectively with compared to other combination. For combination effect of four selected mangrove plants showed 75% reduction of LC_{50} values. The combined form of four plant extracts performed better than combined form of two plant extracts which could be revealed by the lower LC_{50} value exhibited by these four mangrove plant extracts against three life threating mosquitoes. From the results we obtained, the combination effects of ecofriendly responsible for better larvicidal activity compared to individual extracts. So, use of this combination in mosquito control can be of greater use.

Key words: Mangrove, Combined Effect, *Aedes aegypti*, Chikungunya fever. Larvicidal Activity.

INTRODUCTION

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries and have always been a challenge to the medical professionals struggling for the welfare of humanity. Mosquitoes are the most deadly vector for several of these disease-causing organisms like malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever [1]Mosquitoes also cause allergic responses on humans that include local skin and systemic reactions such as angioedema [2] *Aedes aegypti* (L.) is generally known as a vector for an arbovirus responsible for dengue and Chikungunya fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas [3]. Anopheles is an important vector for the transmission of malaria [4], *Culex quinquefasciatus* is important vector of *Brancraftian filariasis* in tropical and subtropical regions. [5] One of the methods to control the vectors in order to bring interruption in disease transmission, and the control of mosquitoes in larval stage has been efficient way in the integrated vector management [6] However, a high level of insecticide resistance has developed through chemical control of the vector and pests threatening the control strategies. To overcome these problems, it is necessary to search for alternative methods of vector control. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance [7]

Numerous plant products have been reported either as insecticides for killing larvae or adult mosquito control [7] [8]. Traditionally, plant parts have been used as an important source in the medicines against malaria. India has a rich and prestigious heritage of mangrove forest oriented medicines among the South Asian countries. Mangroves have great medicinal uses. Mangroves have mosquito larvicidal, antifungal, antiviral,

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anti-cancer and anti-diabetic compounds [9]. Present study was carried out on [10] reported that terminal portion of the root sample of *Rhizophora apiculata* extract in petroleum ether, exhibited the highest mosquito larvicidal activity against *Aedes aegypti.* [11] reported that the leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of Andrographis lineata were tested against fourth instar larvae of malaria vector, Anopheles subpictus and Japanese encephalitis vector, C. tritaeniorhynchus and the antifeedant activity of leaf aqueous extract was evaluated against the fourth instar larvae of gram pod borer, Helicoverpa armigera [12] [13] reported that the leaf methanol, benzene, and acetone extracts of Cassia fistula were studied for the repellent activity against Aedes aegypti. The ethyl acetate extracts of Hyptis suaveolens and Rhododendon tomentosum, Hyptis Harmaja, and Myrica gale significantly reduced biting activity of A. aegypti [14] As far as our literature survey could ascertain, no information was available on the larvicidal activities of the experimental plant species given here. Therefore, the aim of this study was to investigate the mosquito larvicidal activity of the combination of mangrove plant species from Tamil Nadu, India. The present study was an attempt to assess the larvicidal activity of leaf extracts against three dangerous mosquito larvae. This is the first report on the larvicidal activity of the combined extracts of selected Mangrove plants.

MATERIALS AND METHODS

Insect Rearing

The different types of mosquito larvae are collected from various parts of Parangipettai coastal environment, Tamil Nadu, India during April and July 2011 (**Fig 1**). The collected larvae maintained in mosquito cages half filled with tap water and fed with a mixture of dog biscuits and dried yeast powder in 3:1 ratio. Water in rearing container was changed daily for preventing scum from forming on the water surface.

Fig 1: Mosquito larvae

Plant materials

The leaves of selected mangrove plants species (*Avicennia marina, Avicennia officinalis, Rhizophora apiculata* and *Rhizophora mucronata*) were collected from Vellar estuary Parangipettai, Tamil Nadu, India and were botanically identified and authenticated by Herbal garden maintained in Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India.

Preparation of plant extracts in different solvent systems

A modified method of [15] was followed. The collected fresh leaves were washed with distilled water and were allowed for shade dried for a week, which was further finely grinded in a mortar and pestle. Petroleum ether and Aqueous was extracted in sox let apparatus. Each extract was concentrated by evaporation in rotary vacuum evaporator. The collected concentrated was evaporated to dryness. In addition to the Larvicidal activity of the plants *Avicennia marina, Avicennia officinalis, Rhizophora apiculata* and *Rhizophora mucronata* extracts are individually and all these extracts mixed in an equal proportion (1:1:1:1), and mixed well for both the extracts to interact well, and the larvicidal assay performed. The stock extracts mixed in 50ml: 50ml giving 100ml of 100% stock of combination extract. The residues of extracts dissolved in their respective solvents and made up to 2. % stock solutions and stored in deep freezer until they used.

Larvicidal Bioassay:

The larvicidal activity of mangrove extracts was assessed by following method [16] with suitable modification. Combined plant extracts tested for larvicidal activity against *An. stephensi, Ad. aegypti* and *Cx. quinquefasciatus*. From the stock solution five different test concentration 50, 100, 180 and 250 ppm prepared and tested against 4th instar of *An. stephensi, Ad.aegypti* and *Cx. quinquefasciatus* at different doses. DMSO in water served as a control. The larvae of these three mosquito species (20no) introduced in to small disposable test cups, each containing 200ml, of dechlorinated tap water in the cups and the required amount of respective plant extract added. (**Fig 2**) The larval mortality observed and recorded after 24hr of post treatment. The dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times on three different days and conducted under laboratory condition at (31±3°) and 87% relative humidity.

Fig 2: Bioassay (Larvicidal)

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and Chi-square values were calculated using the SPSS120 (Statistical package of social sciences software.Results with $p<0.05$ were considered to be statistically significant.

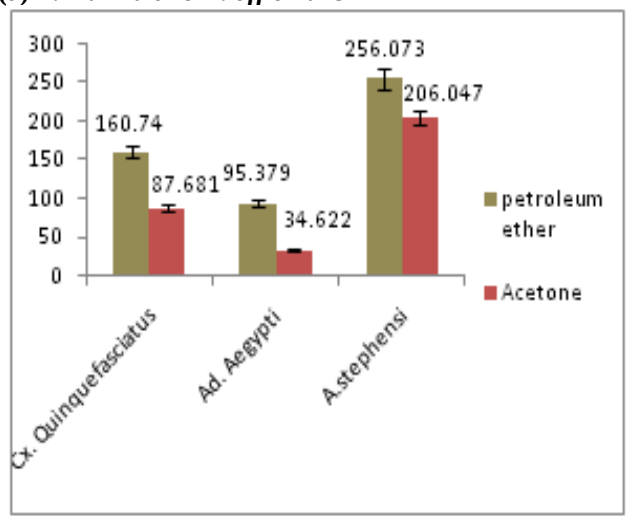
RESULTS

The mean mortality of combined larvicidal bioassay of *A.marina*, A. *officinalis*, *R.apiculata* and *R. mucronata* against three mosquito larvae presented in (**Table 1**). Results of log-probit analysis (at 95% confidence level) revealed that LC_{50} values gradually decreased with the exposure period having the lowest value at 200 ppm of combined extracts at 1:1 ratio to fourth instars larvae after 24-h exposure period. The rate of mortality (Y) was positively correlated with the concentration (X) of the plant extract as evident from established regression equations. From the obtained data, it is visible that, being a *A.marina* and A. *officinalis* combination its more effective to *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* with regards to 34.622 µg/ml, 87.681 µg/ml and 206.047 µg/ml LC_{50} values respectively with compared to others. The results revealed that as the concentration increased percentage of mortality also increased. At 50 ppm concentration the percentage mortality observed only 25%, whereas the concentration increased to 250 ppm the mortality was 95%.

Mean mortalities of Larvicidal activity of *Avicennia marina*, *Avicennia officinalis*, *Rhizophora apiculata* and *Rhizophora mucronata* (1:1:1:1), extracts of above mentioned four plant parts applied combinedly (1:1:1:1) against fourth instars larvae are presented in (**Table 2, 2.1, 2.2 & Fig. 3, 3.1**). From the obtained data, results revealed that as the combined effect of four plants showed 75% reduction of LC_{50} values. . The combined form of four plant extracts performed better than combined form of two plant extracts which could be revealed by the lower LC_{50} value exhibited by these four mangrove plant extracts against tested mosquitoes, *C. quinquefasciatus*, *A.aegypti* and *An. stephensi*.

Fig 3: Effect of larvicidal activity of (a) Avicennia marina and Avicennia officinalis and (b) *Rhizophora mucronata and Rhizophora apiculata* against three mosquito Larvae

(a) *A. marina* and *A. officinalis*



(b) *R.mucronata and R. apiculata*

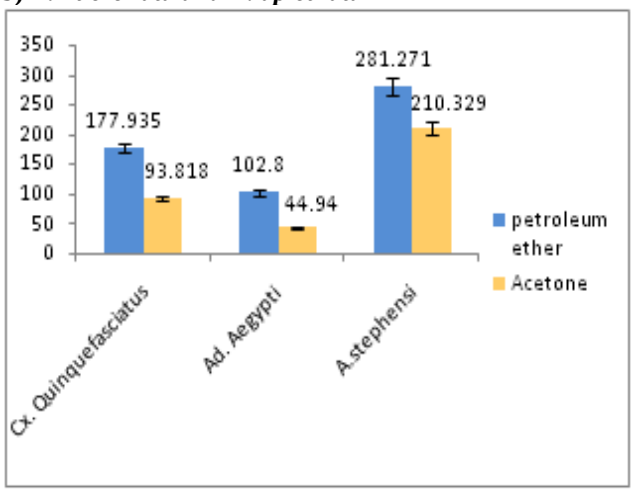


Fig 3.1: Effect of larvicidal activity of combined form of four mangrove plants against mosquito Larvae

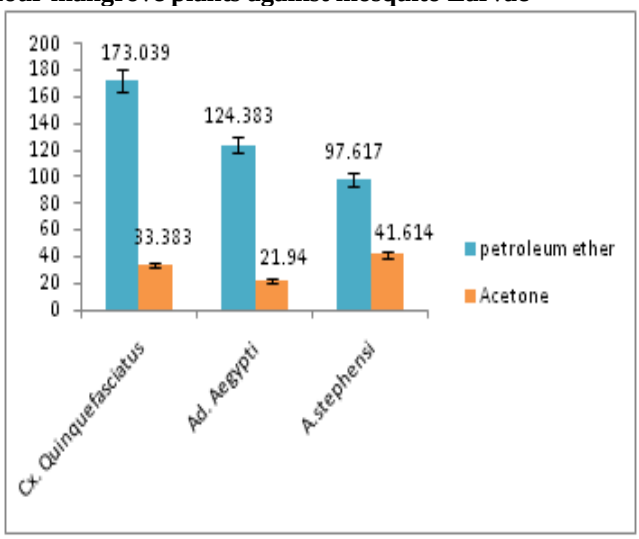


Table 1: Mean mortality of combined larvicidal activity of Mangrove plants against three mosquito larvae.

Plant species	Mosquito species	solvents	Concentrations			
			50	100	180	250
A. marina + A. officinalis	*Culex quinquefasciatus*	Petroleum ether	37±1.30	52±0.99	63±1.73	86±1.91
		Acetone	49±0.73	64±1.71	73±1.09	89±1.33
	Aedes aegypti	Petroleum ether	51±1.71	62±1.00	70±1.76	95±1.73
		Acetone	58±1.09	63±1.30	75±1.09	97±1.33
	Anopheles stephensi	Petroleum ether	31±0.09	46±1.07	52±1.73	61±1.30
		Acetone	35±1.01	43±1.33	51±0.73	57±1.03
R.mucronata + R.apiculata	*Culex quinquefasciatus*	Petroleum ether	45±1.30	57±1.00	68±1.33	79±1.21
		Acetone	53±0.73	67±1.09	77±0. 09	89±1.03
	Aedes aegypti	Petroleum ether	59±3.01	68±0.73	81±1.03	92±0.73
		Acetone	61±0.30	69±1.03	83±0.09	93±1.09
	Anopheles stephensi	Petroleum ether	21±0.99	35±1.73	41±0.33	54±0.03
		Acetone	24±2.09	39±1.03	51±1.73	63±0.09
A. marina +A.officinalis +R.mucronata + R. apiculata	*Culex quinquefasciatus*	Petroleum ether	65±1.99	76±1.03	89±1.73	100±0.00
		Acetone	73±1.03	81±0.33	92±0.73	100±0.00
	Aedes aegypti	Petroleum ether	67±1.09	73±1.33	81±1.09	95±0.33
		Acetone	76±0.30	82±1.03	90±0.31	100±0.00
	Anopheles stephensi	Petroleum ether	51±1.03	63±0.71	71±0.09	79±0.33
		Acetone	57±0.31	66±1.03	73±1.01	81±0.73

Table 2: Combined form of *A. marina* and *A. officinalis* against mosquito larvae

Mosquito species	Solvent	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	X^2
Culex quinquefasciatus	Petroleum ether	160.74	439.716	221.517+1.177	10.617
	Acetone	87.681	341.453	333.682+1.185	9.686
Aedes aegypti	Petroleum ether	95.379	290.937	335.608+1.182	11.688
	Acetone	34.622	175.767	452.670+2.103	14.180
Anopheles stephensi	Petroleum ether	256.073	755.048	224.802+3.098	12.461
	Acetone	206.047	626.735	228.070+4.091	13.466
Control	Petroleum ether	-	-	-	-
	Acetone	-	-	-	-

Table 2.1: Combined form of *R. mucronata* and *R. apiculata* against mosquito larvae

Mosquito species	Solvent	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	X^2
Culex quinquefasciatus	Petroleum ether	177.935	370.697	337.411+1.162	11.162
	Acetone	93.818	365.574	442.402+2.148	13.018
Aedes aegypti	Petroleum ether	62.800	266.540	448.640+2.164	14.245
	Acetone	44.940	272.486	356.011+3.117	11.259
Anopheles stephensi	Petroleum ether	281.271	564.940	512.912+3.118	10.660
	Acetone	210.329	515.770	117.963+3.156	11.248
Control	Petroleum ether	-	-	-	-
	Acetone	-	-	-	-

Table 2.2: Combined form of four mangrove plants against mosquito larvae

Mosquito species	Solvent	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	X^2
Culex quinquefasciatus	Petroleum ether	173.039	162.073	257.483+3.173	4.189
	Acetone	33.383	142.167	366.959+3.135	3.145
Aedes aegypti	Petroleum ether	124.383	237.097	349.031+2.175	1.062
	Acetone	21.940	178.472	354.019+2.181	3.383
Anopheles stephensi	Petroleum ether	97.617	462.159	336.725+2.136	3.438
	Acetone	41.614	343.286	345.131+3.123	4.745
Control	Petroleum ether	-	-		
	Acetone	-	-		

DISCUSSION

Mosquito borne diseases are one of the most public health problems in the developing countries and prevent the mosquito bite using repellent, causing larval mortality and killing mosquitoes. Coastal halophytes are widespread in tropical and subtropical regions and contain biologically active compounds for antifungal, antiviral, anti-cancer and anti-diabetic and mosquito larvicidal compounds [17] [18]

Our present study reports the assessment of larvicidal activity of coastal halophytes significantly higher against dangerous mosquitoes, *A. stephensi, A. aegypti* and *C. quinquefasciatus.* Among the plant extracts, the combined form of *Avicennia marina* and *Avicennia officinalis* were more susceptible to *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* with regards to 34.622 µg/ml, 87.681 µg/ml and 206.047 µg/ml LC_{50} values respectively with compared to others. Similar results are found in fifteen mangrove species extracted in acetone and petroleum ether separately, tested for their activity against the larvae of mosquito *Culex quinquefasciatus*. Petroleum ether extract of *R. apiculata* is most effective with LC_{50} of 25.7µg/L. The extract further shows synergistic larvicidal activity with pyrethrum. The synergism and the synergistic factor are 0.81 at 5 µg/L [19] The fruit and leaves of *Aegiceras corniculatum* and leaves of *Rhizophora mucronata* are more toxic to *C. costae* by showing the LC_{50} of 0.24, 0.26 and 0.28 mg/ml respectively [20]. Larval progress was affected showing several deformities, including dechitinized body wall, and body length of matured larvae was reduced as compared to controls. Deformities that developed in the body wall of larvae may be attributed to the dechitinizing effect of extract as reported by [15]

The combined larvicidal activity of four effective mangrove plants against three mosquito larvae was tested. The highest mortality was found in acetone extracts of combined plants against *Ad.aegypti, Cx. quinquefasciatus* and *An. stephensi* followed. The LC_{50} values are 21.940, 33.383 and 41.614 respectively. The combined effect of four plants showed 75% reduction of LC_{50} values. A similar result reported in the combined activity of both the *Nerium* and *Fenugreek* extracts is more effective than the individual activity. The mortality rate was steadily increasing along with the time of exposure and concentration [21]. [22] have reported highest mortality was found in crude extracts of C. caudatus fruits and T. acuminata flowers showed at 0.5% concentration when applied separately. 100% mortality was recorded when 0.2% crude mixed extract of C. caudatus fruits and T. acuminata flowers at 1:1 combination was applied and it was found to be the best combination when compared to other combinations and Second and third instars larvae of Cx. quinquefasciatus are more susceptible to crude extract mixture (1:1) of C. caudatus fruits and T. acuminata flowers. The performance of combined form of four plants was better than combined form of two plants which could be revealed by the lower LC_{50} value exhibited by these four mangrove plants against tested mosquitoes *A.aegypti, C. quinquefasciatus* and *A. stephensi*. Among the tested mosquitoes the larva of *A.aegypti* showed greater susceptibility than *C. quinquefasciatus* and *A. stephensi.* From the results we obtained, the combination effects of the extracts were much effective than the individual extracts. So, use of this combination in mosquito control can be of greater use.

CONCLUSION

In conclusion, an attempt has been made to evaluate the larvicidal effects of coastal halophytes against *A. stephensi, A. aegypti* and *C. quinquefasciatus.* The results reported here open the possibility of further investigations of efficacy on their field test and we hope that there will not be any eutropication of bacteria or fungi due to this extracts because, these plants are already proved to contain antibacterial and antifungal activities in many research articles. So these compounds can be used as a solution for the long lasting mosquito problem in the developing countries without damaging the environment, and if these are marketed for the public reach, the use of the insecticidal sprays and liquidators for the control of mosquitoes will be reduced and thereby we can prevent the environmental contamination and to an extent, prevent the ozone layer depletion

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