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Screening of a few chosen ascidians of Tuticorin coast for larvicidal activity

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ABSTRACT

The larvicidal activity of two species of ascidians- Aplidium indicum and Phallusia nigra were investigated against the larvae of Anopheles stephensi and Culex quinquefasciatus. The extracts were more toxic to Culex quinquefasciatus than to Anopheles stephensi. This investigation explores the importance of marine organisms as a valuable resource for the discovery of novel larvicidal molecules.

Key words: *Aplidium indicum*, *Phallusia nigra*, *Anopheles stephensi*, *Culex quinquefasciatus*, larvicidal activity.

INTRODUCTION

Arthropod borne diseases constitute a major health problem in India. Among the arthropod vectors, mosquitoes are important as they are responsible for diseases like Malaria, Filaria, Yellow fever, Dengue, Chickungunya etc. In India of all vector borne diseases, Malaria and Filaria cause most concern to public health [1,2]. The use of chemicals hazardous to human and wild life has not only developed resistance in insects, but also in the outbreak of pests. These factors have evoked considerable interest in the adoption of biological control in recent years [3]. Eradicating mosquitoes in larval stages is more effective than that of adults. In the absence of vaccine, vector control is the only practical approach. Larvicidal activity of more than hundreds of plant species have been tested against mosquito vectors [4,5,6,7,8]. *Bacillus sphaericus*, a mosquito control agent, originates from the presence of binary toxin composed of two proteins that work together to lyse gut cells of susceptible larvae [9]. Oceans have a rich wealth of bioactive compounds secreted by animals as chemical defence. Ascidians commonly called 'sea squirts' are an interesting group of marine, sedentary organisms found to occur in abundance in Tuticorin coast. They are considered as a nuisance as they grow on all underwater marine structures and are usually thrown away. Such discards may have a wealth of natural products. Hence in the search for a new compound with larvicidal activity, ascidians may prove to be a timely alternative. In the present study, two species of ascidians collected from Tuticorin coast has been screened for larvicidal properties against the larvae of *Anopheles stephensi* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Animal material: Two species of ascidians – *Aplidium indicum*, a colonial ascidian (Family - Polyclinidae) and *Phallusia nigra*, a simple ascidian (Family - Ascidiidae) were selected for the present study. Identification of the ascidians to the species level was carried out by using the key to identification of Indian ascidians [10].

Collection and extraction: colonies of *Aplidium indicum* and individuals of *Phallusia nigra* were collected, washed well, dried in hot air oven at 50^{o}C for 48 hours and then powdered using mortar and pestle. The dry powder was soaked in methanol followed by methanol: water and methylene chloride for 24 hours. The supernatant was filtered with whatman No.1 filter paper in separate bottles. The crude extracts were concentrated with rotary evaporator. The resultant extracts were then mixed with required amount of universal solvent dimethyl sulphaoxide or ethyl acetate and stored at 8^{o}C until use.

Preparation of the stock solution: 5 ml of the concentrated methanol and methanol : water extract of *Aplidium indicum*, *Phallusia nigra* was dissolved separately in 50ml of Dimethyl sulphaoxide. 5 ml of concentrated methylene chloride extract of *Aplidium indicum* and *Phallusia nigra* was dissolved separately in 50 ml of Ethyl acetate. These were used as the stock solution.

Collection of larvae: The second instar larvae of *Culex quinquefasciatus* were collected from the Centre for Research in Medical Entomology, Madurai. *Anopheles stephensi* larvae were collected from stagnant water pools in and around Tuticorin.

Experimental set up: Six sets of four bowls each with 25ml of water were arranged. 25 larvae of *Anopheles stephensi* were transferred from the stock plastic bowl to each of the 24 bowls. The bowls were labelled as a, b,c, and d. Bowls labelled 'd' acted as the control without any ascidian extract.1ml, 2ml and 2.5 ml of Methanol extract of *Aplidium indicum* was added to the first set of bowls. 1ml, 2ml and 3ml of methanol: water extract to the second set of bowls and 0.2ml, 0.4ml and 0.6ml of methylene chloride extract to the third set of bowls. 0.5ml, 1ml and 1.5ml of methanol extract of *Phallusia nigra* was added to the fourth set of bowls, 1 ml, 1.5 ml and 2 ml of methanol: water extract to the fifth set of bowls and 0.3ml, 0.4ml and 0.5ml of methylene chloride extract to the sixth set of bowls. The same set up was followed for assessing the larvicidal activity to *Culex quinquefasciatus* also. The experiment was conducted in triplicate and the mean taken. Larvicidal effect of the extract was monitored by counting the number of dead larvae at one hour interval for 24 hours. The data obtained were tabulated.

RESULTS

Table 1 shows the percent mortality of larvae to the extracts of ascidians

Effect of Aplidium indicum extract to Anopheles stephensi: 2.5ml, 2ml and 1ml methanol extract showed 100 percent mortality with in 7 hours, 10 hours and 21 hours respectively. 3ml, 2ml, and 1ml of methanol: water extract showed 100 percent mortality in 14 hours and 20 hours. 76 percent mortality was observed with 1 ml extract. 0.6ml methylene chloride extract showed 100 percent mortality with in 20 hours. With 0.4ml and 0.2ml the percentage of mortality was 76 and 60.

Effect of Phallusia nigra extract to Anopheles stephensi: 1.5 ml, 1ml and 0.5ml methanol extract showed 100 percent mortality within 14 hours, 20 hours and 24 hours respectively. 92 percent mortality was recorded with 2ml methanol: water extract. The mortality rate showed a decreasing trend with the decrease in the quantity of extract used. 100 percent mortality was observed with 0.5ml methylene chloride extract. The percent mortality was 82 and 72 with 0.4ml and 0.3ml extract.

Table 1: 24 hour mortality studies with extracts of *Aplidium indicum* and *Phallusia nigra* to larvae of *Anopheles stephensi* and *Culex quinquefasciatus*

	Aplidium indicum						***Phallusia nigra***					
	A. stephensi			***C. quinquefasciatus***			***A. stephensi***			***C. quinquefasciatus***		
	Con (ml)	**Hrs**	**%**	**Con (ml)**	**Hrs**	**%**	**Con (ml)**	**Hrs**	**%**	**Con (ml)**	**Hrs**	**%**
Methanol	1.0	21	100	1.0	18	100	0.5	24	100	0.5	22	100
	2.0	10	100	2.0	9	100	1.0	20	100	1.0	17	100
	2.5	7	100	2.5	5	100	1.5	14	100	1.5	10	100
Methanol: water	1.0	24	76	1.0	24	96	1.0	24	40	1.0	24	40
	2.0	20	100	2.0	13	100	1.5	24	60	1.5	24	68
	3.0	14	100	3.0	9	100	2.0	24	92	2.0	18	100
Methylene chloride	0.2	24	60	0.2	24	6	0.3	24	72	0.3	24	84
	0.4	24	76	0.4	24	76	0.4	24	82	0.4	21	100
	0.6	20	100	0.6	22	100	0.5	24	100	0.5	16	100

Effect of Aplidium indicum extract to Culex quiquefasciatus: 2.5ml, 2 ml and 1ml methanol extract showed 100 percent mortality with in 5 hours, 9 hours and 18 hours respectively. 100 percent mortality was observed with 3ml and 2ml methanol: water extract with in 9 and 13 hours. With 1ml extract 96 percent mortality was observed. Within 22 hours 100, 76 and 6 percent mortality was recorded with 0.6ml, 0.4ml and 0.2ml methylene chloride extracts.

Effect of Phallusia nigra extract to Culex quiquefasciatus: 100 percent mortality was observed with 1.5ml, 1ml and 0.5ml methanol extract within 10 hours, 17 hours and 22 hours. 2 ml methonal: water extract was 100 percent larvicidal with in 18 hours. With 1.5ml and 1ml, the percentage mortality was 68 and 40. 0.5 ml and 0.4 ml of methylene chloride extract showed 100 percent mortality with in 16 hours and 21 hours respectively. The percentage of mortality reduced to 84 with 0.3 ml of extract.

Comparison of the larvicidal effect of Aplidium indicum and Phallusia nigra: Methylene chloride extract of *Aplidium indicum* (0.6ml) and *Phallusia nigra* (0.5ml) appeared the most lethal showing 100 percent mortality to *Anopheles stephensi* within 20 hours and 21 hours respectively. *Culex quiquefasciatus* showed 100 percent mortality with in 22 hours on exposure to 0.6ml Methylene chloride extract of *Aplidium indicum.* Methylene chloride extract of *Phallusia nigra* indicated greater toxicity showing 100 percent mortality with in 16 hours with 0.5ml. Methylene chloride extract of *Aplidium indicum* was equally toxic to both the species of larvae where as methanol and methanol: water extracts were more toxic to larvae of *Culex quinquefasciatus* than to *Anopheles stephensi*. Methylene chloride and methanol extract of *Phallusia nigra* were more toxic to the larvae of *Culex quinquefasciatus* than to *Anopheles stephensi*. Methanol: water extracts were equally toxic to both the species of larvae.

DISCUSSION

A preliminary screening of the two ascidians, *Aplidium indicum* and *Phallusia nigra* against the larvae of *Anopheles stephensi* and *Culex quiquefasciatus* indicate their potency to control the larvae of mosquito. The present study showed that the mean mortality rate was dependent on the quantity of extract used. This can be attributed to the increase in the concentration of the toxic substance with the increase in the quantity of extract. Similar results have been reported with aqueous extract of *Azadirachta indica* to *Anopheles* larvae [7]. Among the three solvents used for extraction, methylene chloride extract was most lethal followed by methanol and methanol: water with both the species of ascidians to the larvae of mosquito. This may be due to the fact that methylene chloride extract contains the most active ingredient causing mortality. A comparative study of the anti-microbial activity of the methylene chloride and methanol extract of these two ascidians indicated that human pathogens are more sensitive to methylene chloride extract than to methanol extract [11].

A comparison of the larvicidal activity of the extracts showed that it was more toxic to the larvae of *Culex quienquefasciatus* than to *Anopheles stephensi.* Larvidcidal properties of the extracts may be due to feeding deterrent activity of the larvae [8]. Greater toxicity exhibited by larvae of *Culex quinquefasiciatus* may indicate mechanism other than feeding deterrent activity. Synergistic action of lamellarian alkaloids of *Didemnum obscurum* with *Beauveria bassiana*, potent larvicidal, insecticidal activity of sponge *Dendrilla nigra*, *Psammaplysilla purpurea*, *Haliclona cribricutis*, *Haliclona pigmentifera* and *Petrosia testudinaria* has also been reported [12,13,14]. The saponins present in the extracts of *Nematopalaemon tenuipes* and *Holothuria scabra* has been shown to have larvicidal activity against *Culex pipens fatigans* [15]. A prelimenary phytochemical screening has revealed the presence of compounds like alkaloids, terpenoids, saponins and a further GC- MS studies with the methanolic and ethanolic extract of *Phallusia nigra* has shown the presence of n-Hexadecanoic acid, (Z,Z,Z)-phenylmethyl ester of 6,9,12-Octadecatrienoic acid and (Z)- phenylmethyl ester of 9-Octadecenoic acid exhibiting pesticidal and insectifuge properties [16, 17].

CONCLUSION

In the present study it was found that both the species of ascidians posses natural products with larvicidal properties which could be developed and used as an effective anti larval mosquito control agent in controlling mosquito menance and protecting us from the threats of mosquito borne diseases.

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