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Mosquito larvicidal and pupicidal activity of South peninsular coast sponge *Dendrilla nigra* extracts

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Abstract
Mosquito downside has become acute in recent years and lots of programmes are launched to manage these vectors. The utilization of biologically active plant materials with anti-larval properties has attracted hefty interest of scientists everywhere the globe. during this background, this study was undertaken to analyze the larvicidal and pupicidal potential of marine sponge Dendrilla Negro extracts against the dipteron species *Aedes aegypti*, genus *Anopheles stephensi* and *Culex quinquefasciatus*. By victimization 3 completely different solvent extracts (aqueous, acetone and methanol), LC50 and LC90 values were observed and therefore the pupae mortality was also analyzed. The results showed that the alcohol extract of sponge exhibited high degree of obstruction the event by induction of nice mortality of larvae and pupae. These findings might facilitate in developing a prospective various supply to manage the mosquitoes

Keywords: larvicidal activity, pupicidal activity, *dendrilla nigra*, *anopheles stephensi*, *aedes aegypti*, *culex quinquefasciatus*

Introduction
Mosquitoes serve as a vector for several diseases, causing serious health problems to human; they transmit diseases *viz*., yellow fever, human lymphatic filariasis and malaria (Abdel-Hameed, *et al*., 1994) [2]. Mosquito problem has become acute in recent years and many programmes have been launched to control these vectors. Synthetic insecticides are well recognized for their speedy action but a major drawback in their application is that they are non-selective and could be harmful to other beneficial organisms, animals and human beings (Abdel-Hameed, 1994) [2]. Besides their adverse environmental effects, pests and mosquito vector have become physiologically resistant to many of the synthetic pesticides (Rao*etal*., 1995).
All these restrictions on the usage of synthetic pesticides have stimulated investigations for environmentally safe, degradable and target specific insecticides against mosquitoes. Ultimately phytochemicals with anti larval properties, derived from various botanical sources are focused. Quite a lot of work has been carried out in higher plants on their biologically active material with anti larval properties (Saxena and Sumithra, 1985) [36]. Few attentions were focused on the larvicidal properties of the marine sponges (Semakov and Sirenko, 1985) [37]. Sponges are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from sponge.
The extract of sponges and essential oil of certain plants have been investigated, and its howed toxic effect against some public health pests (Hadjiakhoondi *etal*., 2006; Vatandoost *et al*., 2004) [17, 45]. Recently, the inhibitory substances biosynthesized by the sponges were reported (Husain *et al.,* 2020) [34]. Recent findings evidenced that sponges contained antibacterial (Priya *et al*., 2018a, b; Priya *et al*., 2020a, b) [31], antiviral (Serkedjieva, 2004 and Garg *et al*., 1992) [39], antifungal (Priya *et al*., 2020b), cytotoxic (Tang *et al*., 2002) [42] larvicidal and pupicidal potentials (Thangam and Kathiresan, 1991) [43]. Large numbers of plant samples have been screened for their insecticidal and /or repellent activities and a few of them have been found to be promising and their products are commercially available. Thangam and Kathiresan have in vestigated for the first time sponges, seagrasses and mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Some of them were effective in killing the larvae or repelling adult female mosquito potentials (Thangam and Kathiresan, 1991) [43]. In this background, the present study was initiated to explore the larvicidal and pupicidal potential in different solvent extracts of *Dendrilla nigra*.

Materials and Methods
Collection of Sponges
Fresh samples of *D. nigra* (Blackish green) specimens of the marine sponge were collected from the peninsular coast of India, especially Arokiapuram coast which is located about 6 km from Kanyakumari (Lat8° 4'N; Long 77° 50'E) to Vattakottai road (Lat 8°3'N; Long 77° 05'E) south east coastal region of TamilNadu, India.. An eco-friendly bulk collection of the sponges by bycatch was carried out during November and December, and April and August and taken-up for isolation and bioactivity screening of the secondary metabolites.

Preparation of sponge extracts
10 g of sponge was cut into small pieces, homogenized and extracted with different solvents: methanol (3×150 ml), acetone (3×150 ml) and Distilled water (3×150 ml). Each extraction was developed by mechanical shaking at room temperature. The extracts were filtered with Whatman filtere paper No. 1 and concentrated with rotary evaporator (McClintock and Gauthier, 1992; Sionov *et al*., 2005; Sepi *et al*., 2010) [25, 41, 38]. The extracts were collected in plastic

vials and stored in the refrigerator for further studies (Aseer *et al.*, 2009) [7]. One gram of the residue was dissolved in 100 ml of respective solvents to make a 1% stock solution. Six different concentration of the extracts (100,200, 300, 400and 500mg/L) were prepared from the stock solution for subsequent testing.

Mosquito larvae and pupae

Larvae of the three mosquito species *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were reared in enamel trays containing dechlorinated water. The larvae were fed with finely powdered mixture having 3:1 ratio of dog biscuits and dry yeast. The rearing water was changed daily. The pupae taken up for the study were assembled into two different category for three types of mosquito species (*Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*) as given below.

Table 1

1.	Larvae that emerged as pupae after larvicidal activity	L.P.
2.	Field pupae that were collected from the Stagnate water in the Field	F.P.

Larvicidal bioassay

Preliminary screening of mosquito larvicidal activity was carried out against the above mentioned three different mosquito larvae using the standard protocol of World Health Organization 1981 with minor modifications. In the present bioassay, 25 larvae were taken into six glass beakers (1500mLcapacity containing1000mLtap water) using a Pasteur pipette. Five different concentrations of the sponge extracts *viz*., 100, 200, 300, 400 and 500 mg/L taken up for the study. A control was also run simultaneously which comprised of water only. The experiment was carried out in glass beakers for 24 h at room temperature and after 24 hours the mortality rate was recorded and assessed. The study was undertaken intriplicates for further statistical analysis.

Pupicidal bioassay

Twenty pupae were taken into six glass beakers (1500 mL capacity containing 1000 mL tapwater) using a Pasteur pipette. Five different concentrations of the sponge extracts *viz*., 100, 200, 300, 400 and 500 mg/L taken up for the study. A control was also run simultaneously which comprised of water only. The experiment was carried out in glass beakers for 24 h at room temperature and after 24 hours the mortality rate was recorded and assessed. The pupae mortality in each concentration and control was recorded after 24 hours of exposure from the average of three replicates. The mortality percentage was calculated using the Abbotts (1925).

$$\text{Percentage mortality of pupae} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

Statistical analysis

The LC_{50}, LC_{90}, 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values were calculated by subjecting the average larvae mortality values to Sta tplus 2009 software on the guidelines of OECD recommended 'Probit' analysis (Finney, 1971) [13]. Using Abbotts (1925) formula, with the natural mortality observed in the negative controls, percentage mortalities were corrected.

Abbotts formula

P=PI–C/1–C

Where, PI and C denote the observed mortality rate and the natural mortality.

Results

Larvicidalactivity

The results of bio larvicidal activity of three different extracts (aqueous, acetone and methanol) of *D. nigra* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* were tabulated.

The values of larvicidal activity against *Aedes aegypti* were recorded and LC50 were found to be 18.74 mg/L of aqueous extract followed by 6427 mg/L of ethanol extract and 100.07mg/L of acetone extract of *D. nigra* against *Aedes aegypti*. The LC90 values of *D. nigra* of aqueous and acetone extracts were164.59 and 269.76 mg/L respectively. The order of hierarchy of bio larvicidal activity of the three different extracts of *D. nigra* against *Aedes aegypti* was found to be aqueous > methanol > aectone at the LC 50 level (Table-1).

Table 2: Effect of aqueous, acetone and methanol extracts of the *D. nigra* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* larvae

Mosquito species	Extract	LC50 (mg/L)	95% Confidence Limit		LC90 (mg/L)	95% Confidence Limit	
			LCL	UCL		LCL	UCL
A. aegypti	Aqueous	18.74	134.35	106.54	164.59	67.18	228.95
	Acetone	100.07	20.74	173.42	269.76	201.31	328.41
	Methanol	64.27	53.83	138.7	508	426.67	645.48
A.stephensi	Aqueous	66.62	50.67	139.41	472.03	400.45	586.34
	Acetone	76.35	25.07	143.85	184.4	110.89	237.87
	Methanol	88.18	33.67	163.3	212.78	131.06	272.04
C. quinquefasciatus	Aqueous	82.74	20.05	149.39	393.27	333.56	477.33
	Acetone	62.12	7.12	104.27	167.95	128.65	200.4
	Methanol	74.45	8.78	124.21	137.09	81.71	180.1

LC50-median lethal concentration; LC90-90% lethal concentration; LCL–Lower confidence limit; UCL – Upper confidence limit The LC50 values for aqueous extracts of *D. nigra* against *A. stephensi* were 66.618 mg/L. followed byacetone extract with 76.35 mg/L. The LC90 values for effective larvicidal activity were found to be acetone and it was 184.4mg/L. Successive effects was observed on methanol extract and it was 212.78 mg/L (Table-1).In the case of *C. quinquefasciatus*, acetone extracts was found to be high and the LC50 value was 2.12mg/L followed by

74.45 mg/L and 82.74mg/L or methanol and aqueous extracts respectively (Table-1). The above results inferred that out of the three Interestingly it is noticed that aqueous extract of *D. nigra* showed 90% pupicidal activity to the L.P. at 50 mg/L against *A. aegypti*.

Table 3: Mortality percentage of aqueous, acetone and methanol extracts of the sponge *D. nigra* against *A. aegypti* pupae

Extract	Concentration (mg/L)									
	10		20		30		40		50	
	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.
Aqueous	55	75	60	85	75	90	85	100	90	100
Methanol	10	50	10	60	20	70	25	80	35	85
Acetone	20	60	20	85	30	100	35	100	35	100

L.P.-Larvae that emerged as Pupae after larvicidal activity; F.P.-Field Pupae

Lethal effect of different extracts of *D. nigra* against *A. stephensi* showed one hundred percent mortality in aqueous extract at 50 mg/L concentration to the mortality percentage of *C. quinquefasciatus* showed hundred per cent pupicidal activity to the field pupae at 50mg/L concentration in acetone extracts while aqueous and mosquito larvae tested, exposure of *A. aegypti* to aqueous extracts of *D. nigra* showed the maximum larvicidal efficacy.

Pupicidal activity

The acetone extract of *D. nigra* showed hundred percent pupicidal effect at 30 mg/L concentration to the field pupae (F.P.) and aqueous extract showed 90% mortality followed by methanol extract that 70% pupicidal activity to the field pupae.larval pupae, whereas ethanol and acetone extracts showed a less significant effect. It is evident that 65and 35% mortality were recorded to the L.P where as 60% and 40% of lethal effects were observed to the F.P. in methanol and acetone extracts of *D. nigra* against *A. stephensi* mosquito pupae (Table 3). Ethanol extract showed insignificant effect to both field pupae and L.P. (Table.4).

Table 4: Mortality percentage of aqueous acetone and methanol extracts of the sponge *D. nigra* against *A. stephensi* pupae

Extract	Concentration (mg/L)									
	10		20		30		40		50	
	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.
Aqueous	20	20	35	40	60	45	80	50	100	55
Methanol	15	20	20	20	40	25	55	35	65	60
Acetone	5	10	15	10	20	25	25	30	35	40

L.P.-Larvae that emerged as Pupae after larvicidal activity; F.P.-Field Pupae

Table 5: Mortality percentage of aqueous acetone and ethanol extracts of the sponge *D. nigra* against *C. quinquefasciatus* pupae

Extract	Concentration (mg/L)									
	10		20		30		40		50	
	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.	L.P.	F.P.
Aqueous	10	15	15	35	15	40	20	45	25	60
Methanol	10	20	15	35	20	40	25	50	35	70
Acetone	30	25	40	50	50	70	55	85	65	100

L.P.-Larvae that emerged as Pupae after larvicidal activity: F.P.- Field Pupae

The values of pupicidal activity against *A. aegypti, A. stephensi* and *C. quinquefasciatus* were recorded and effective LC50 were found to be 5.64 mg/L of aqueous extract followed by 9.06 mg/L of acetone extract and 13.83 mg/L of methanol extract of *D. nigra* against *A. aegypti* pupa that collected from field (F.P) (Table - 5).

Table 6: The LC50 of aqueous, acetone and methanol extracts of the *D. nigra* against *A.aegypti*, *C. quinquefasciatus* and *A. stephensi* pupa that collected from field (F.P)

Extracts	LC50(mg/L)		
	A.aegypti	***A.stephensi***	***C.quinquefasciatus***
Aqueous	5.64	38.06	40.05
Acetone	9.06	80.8	18.12
Methanol	13.83	58.5	123.3

Whereas effective LC50 values of pupae that emerged after larvicidal activity (L.P) was found to be 10.16 mg/L of aqueous extract against *A. aegypti* followed by 36.82 mg/L and 38.06 mg/L of ethanol and aqueous extract respectively against *A. stephensi* (Table - 6).

Table 6: The LC50 of aqueous, acetone and ethanol extracts of the *D. nigra* against *A. aegypti*, *C. quinquefasciatus* and *A. stephensi* pupa that emerged after larvicidal activity (L.P)

Discussion

Though synthetic insecticides are effective they create many problems like development of insecticide resistance (Lin *et al*., 2005). Therefore, usage of indigenous plant based products, could provide standardized measure for protection to the human population against various disease caused by mosquito. Many approaches that have been developed to control the mosquito menace. One such approach to prevent mosquito- borne disease is to kill at its larval stage. Many studies made use of plant extracts for mosquito control approach.

Kamaraj *et al*., (2011) [21] reported that plants derived extracts using different solvents crude extracts have potential larvicidal activity. To evaluate the potential larvicidal activity of the plant preliminary screening is a good measure (Ali *et al*. 2012) [4]. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Kamaraj *et al*., 2009) [22].

Howard *et al*., 2007 [19] revealed that larval control can be the effective appropriate way in controlling the mosquitoes in breeding habitats, which are man-made. Fahd *et al*., 2013 reported that in early findings the effect of ethanol extract of *Annona squamosa* leaf was effective in larvicidal activity against *C. quinquefasciatus*. The results of the present study also confirm similar results with acetone extracts of *D. nigra*. Chloroform extract of *Millettia dura*, show higher larvicidal activity against the second instar larvae of *Aedes aegypti* was reported by Yenesew *et al*., (2003) [48]. Rahuman *et al*., (2008) [3] stated that the extracts of *Jatropha curcas* and *Euphorbia tirucalli* were highly effective against the larvae of *A aegypti*, and the LC50 values were 35.39, 256.77, 384.19, 703.76, and 13.14 ppm against *A aegypti*. Chowdhury *et al*., (2008) [11], stated that *Solanum villosum* offers promised as a potential bio control agent against *Aedes aegypti* particularly in its markedly larvicidal effect.

In the present study, larvicidal efficacy of three extracts of *D. nigra* was assessed. Aqueous extract of *D. nigra* was found to be more effective in larvicidal activity with a minimum LC50 value of 18.47 mg/L against *A. aegypti*. A similar effect was also observed by Anandhan and Sorna

kumari, (2011) [6] using *Gracilaria crassa* and *Hypnea valentia* in methanolic extract against *Aedes sp.* The LC50 values 66.618 mg/L for aqueous extracts of *D. nigra* was found to be promising against *A. stephensi* and mortality rate of *C. quinquefasciatus* was found to be high on the acetone extracts of *D. nigra*, and LC50 value was 62.12 mg/L. The results of Mullai and Jebanesan (2007) [26] on larvicidal effect of four different extracts of *Cucumis pubescens* leaf against *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* agrees with the relevant results of the present study. Sponges contains rich source of structurally diverse secondary metabolites. It is presumed that, the secondary metabolites offer a defence against mosquito larvae plays an effective role in larvicidal activity in the present investigation (Priya *et al*., 2018a, b; Priya *et al*., 2020a, b) [31]. Imaga *et al*., (2010) [20] indicated the presence of alkaloid, flavonoid, saponins and glycosides in the extract of *Carica papaya* leaves and these compounds have been found to possess high larvicidal activities against different species of mosquitoes (Shallan *et al*., 2005; Chapagain *et al*., 2008; Quevedo *et al*., 2012; Prakash *et al*., 2019) [10, 33, 29]. A similar study reported the evaluation of the use of *Parthenium hysterphourus* against mosquito *A. aegypti* (Muthukrishnan and Pushpalatha, 2001) [27] and combined effect of other phenolic acids *viz*., caffeic acid, vanillic acid, ansic acid, p-ansic acid, chlorogenic acid and parahydroxy benzoic acid may possess larvicidal and pupicidal property on *A. aegypti* and *C. quinquefasciatus*. Okumu, *et al*., (2007) [7] reported that the percentage emergence in most cases was less than the percentage pupation, which suggests some pupal mortality. The emergence inhibition (EI) values depicted with the neem oil formulation treatments were much lower than the respective lethal concentration (LC) values, an indication that the growth disruption activity of the neem product extended to pupal stages. In the present study, acetone extract of *D. nigra* showed one hundred per cent pupicidal effect at 30 mg/L concentration to the field pupae (F.P.) against *A. aegypti*; at 50 mg/L concentration of aqueous extract to the larval pupae against *A. stephensi* and hundred percent pupicidal activity to the field pupae at 50 mg/L concentration in acetone extracts of *D. nigra* against *C. quinquefasciatus* were observed. Kumar *et al*., (2012) [23] reported that *Sargassum wightii* crude extract treatment resulted in higher larval and pupal mortality which might be due to the multiple actions of bioactive compounds present in the seaweed. A similar effect was also observed in the present study. The present study revealed that treatment of sponge extracts on *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* contains a toxic chemical leading to remarkable mortality against subsequent developmental stages of mosquito life cycle. Observations showed that seaweed contained chemical that brought out such mortality to the larvae and pupae. Moreover it acts as a regulator of growth in immature larvae to the adult emergence.

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