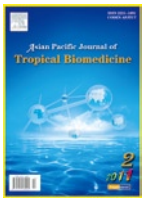
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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



Document heading

Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*

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

[1]*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Division of Marine Microbiology and Infectious Medicine, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India*
[2]*Department of Chemistry Scott Christian College (Autonomous) Nagercoil, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 10 September 2011
Accepted 30 September 2011
Available online 15 October 2011

Keywords:
Aedes aegypti
Dengue fever
Larvicidal activity
Mosquito control
Seaweeds
Enteromorpha intestinalis
Dictyota dichotoma
Acanthopora spicifera

ABSTRACT

Objective: To identify the larvicidal activity of the seaweed extracts. **Methods:** Seaweed extracts of *Enteromorpha intestinalis* (*E. intestinalis*), *Dictyota dichotoma* (*D. dichotoma*) and *Acanthopora spicifera* (*A. spicifera*) were dissolved in dimethylsulfoxide (DMSO) to prepare a graded series of concentration. Batches of 25 early 4th instars larvae of *Aedes aegypti* were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (0.01–0.1 mg). Each experiment was conducted in three replicates. A control group consisted of 1 mL of DMSO and 99 mL of distilled water only. After 24 h, the percentage of mortality was identified with the formula: % of mortality=[(% of test mortality − % of control mortality) / (100 − % of control mortality)]×100. **Results:** The extract of *D. dichotoma* showed minimum level of LC_{50} value (0.068 3±0.008 4 μg/mL) and LC_{90} value was 0.140 1. The regression equations of *D. dichotoma* and *E. intestinalis* for 4th instar larvae were Y=0.333 + 0.684x (R^2=0.946) and Y=0.600 + 0.781x (R^2=0.812), respectively. The results of the preliminary phytochemical constituents showed the presence of saponin, steroids, terpenoid, phenols, protein and sugars. **Conclusions:** It can be concluded from the present study that, the ethanolic extracts of seaweed of *D. dichotoma* possess active compounds for development of larvicidal ativity.

1. Introduction

Mosquito borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases[1]. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, *etc.*, causing millions of deaths every year. *Aedes aegypti* (*Ae. aegypti*), a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. *Ae. ageypti* is also the vector of dengue hemorragic fever, which is endemic to South East Asia, the Pacific islands area, Africa and the America[2]. Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and also 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 such as closets. The only successful way of reducing mosquito densities to a level where dengue 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]. Natural products of plant origin with insecticidal properties have been tried in the recent past for the control of variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals[5] and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-

*Corresponding author: Ravikumar S, School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Division of Marine Microbiology and Infectious Medicine, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India.
E-mail: ravibiotech201321@gmail.com
Foundation Project: This work was financially supported by Ministry of Environment and Forest, NewDelhi, India [grant No. D.O.NO.3/2/2004 CS(M)-11.12.2006].

target organisms and due to their innate biodegradability. Several seaweeds have shown a wide range of bioactive properties[6-14]. In this connection, the present study was made an attempt to find out the larvicidal efficacy of ethanolic extracts of seaweeds *i.e. Enteromorpha intestinalis* (*E. intestinalis*), *Dictyota dichotoma* (*D. dichotoma*) and *Acanthopora spicifera* (*A. spicifera*) against dengue fever vector *Ae. aegypti*.

2. Materials and methods

2.1. Plant materials

Fresh samples of *E. intestinalis*, *D. dichotoma* and *A. spicifera* were collected from Mandapam (Latitude 9° 16′ N and Longitude 79° 07′ E) of South East coast of India and were botanically authenticated by Dr. K Eswaran, Scientist, Central Salt and Marine Chemical Research Institute (CSMCRI), Mandapam Camp, Ramanathapuram District, Tamil Nadu, India. A sample voucher specimen was deposited in the herbarium facility and maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India sponsored by Indian Council of Medical Research, New Delhi. 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 seaweeds 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 ℃) and then freeze-dried (-80 ℃) to obtain solid residue. The percentage of extraction was calculated by using the following formula: % of extraction=weight of the extract / weight of the plant material×100. The extracts of seaweeds were further tested for the presence of phytochemical constituents by following the method of Sofowora and Kepam[15,16].

2.3. Mosquito larval culture

To satisfy the enormous number of mosquitoes need 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 (VCRC), Puducherry, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30-40 min, and time was enough to allow for eggs to hatch into larvae. They were reared indoors at (28±2) ℃ temperature and 14:10 h light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and yeast powder in 3:1 ratio. Five days after emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with a 10% 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 larvicidal effect of ethanolic extracts of 3 seaweeds species *viz.*, *E. intestinalis*, *D. dichotoma* and *A. spicifera* against *Ae. aegypti* was conducted in accordance with the WHO standard method[17]. Each seaweed extract was dissolved in dimethylsulfoxide (DMSO) to prepare a graded series of concentration. Batches of 25 early 4th instar larvae of *Ae. aegypti* were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (0.01-0.1 mg). Each experiment was conducted in three replicates. A control group consisted of 1 mL of DMSO and 99 mL of distilled water only. After treatment, symptoms in treated larvae were observed and recorded immediately at different time intervals and no food was offered to the larvae at this time. 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 World Health Organization'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 percentage of mortality was calculated by using Abbott's formula: % of mortality=[(% of test mortality − % of control mortality) / (100 − % of control mortality)]×100.

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), regression equation, *Chi*-square and analysis variation values were calculated using the Stat plus 2009 software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The percentage yields of extracts ranged from 1.51% to 5.01% (Table 1). It revealed that, *D. dichotoma* (5.01%) showed maximum yield followed by *E. intestinalis* (2.06%). The LC_{50} and LC_{90} values of the seaweeds extracts against *Ae. aegypti* were listed in Table 2. The extract of *D. dichotoma* showed minimum level of LC_{50} value (0.0683±0.0084 μg/mL) and LC_{90} value was 0.1401 μg/mL followed by extract of *E. intestinalis* with LC_{50} (0.0744±0.0086 μg/mL) and LC_{90} (0.1399 μg/mL) and *A. spicifera* did not showed any mortality. The regression equations of *D. dichotoma* and *E. intestinalis*

Table 1
Percentage of ethanolic extracts from seaweed species.

Botanical name	Family	Weight of plant part (g)	Yield of extract	
			g	%
E. intestinalis	Ulvaceae	20	0.41	2.06
D. dichotoma	Rhodomelaceae	31	6.71	5.01
A. spicifera	Rhodomelaceae	62	0.94	1.51

Table 2
Larvicidal activity of ethanolic extracts of seaweed plants against *Ae. aegypti*.

Name of the species	Parts	LC_{50} (LCL–UCL)	LC_{90}	Regression Equation	R^2	χ^2	*P*–value
E. intestinalis	Whole plant	0.0744±0.0086 (0.0571–0.0916)	0.1399	Y=0.600 + 0.781x	0.812	4.3513	0.8241
D. dichotoma	Whole plant	0.0683±0.0084 (0.0516–0.0849)	0.1401	Y=0.333 + 0.684x	0.946*	0.5688*	0.0480*
A. spicifera	Whole plant	No mortality					

* Significant at $P<0.05$ level; LCL: Lower confidence level; UCL: Upper confidence level; χ^2: *Chi*–square.

Table 3
Phytochemical constituents in seaweed species

Seaweeds plants	Alkaloids	Carboxylic acid	Coumarins	Flavanoids	Quinones	Phenols	Saponins	Xanthoproteins	Protein	Resins	Steroids	Tannins	Sugars
E. intestinalis	–	–	–	–	–	–	+	–	+	–	–	–	+
D. dichotoma	–	–	–	–	–	–	++	–	++	–	–	–	++
A. spicifera	–	–	–	–	–	–	–	–	–	–	–	–	–

–: Absent; +: Medium; ++: High.

for 4th instar larvae were Y=0.333 + 0.684x (R^2=0.946) and Y=0.600 + 0.781x (R^2 =0.812), respectively. The *Chi*–square value was significant at $P<0.05$ level (Table 2). The preliminary phytochemical study revealed that, the extracts from seaweeds have variety of phytochemical constituents, namely, saponin, steroids, terpenoid, phenols, protein and sugars (Table 3).

4. Discussion

The marine environment is a incomparable reservoir of bioactive natural products, many of which exhibit structural features that have not been found in terrestrial natural products[18]. Each year, an increasing number of novel marine metabolites are reported in the literature, indicating that the marine environment is likely to continue to be a prolific sources of more natural products for many years to come. Marine algae have been found to be vital source of useful bioactive substances since two decades ago. Several studies have demonstrated that, seaweeds are an excellent source of components with biological activity such as antibacterial[10,12,19,20], antifungal[11,21,22], antiviral[8,23], anti–inflammatory[24,25], cytotoxic[20,26], nematicidal[26,27], antifeedant[26], larvicidal[26], phytotoxic[20] and anticoagulant activities[28]. The studies on larvicidal activities with seaweed extracts are too restricted[29–32]; hence, the present study was investigated with several seaweed extracts. Among the seaweeds, *D. dichotoma* showed minimum LC_{50} values when compared with other seaweeds species due to the presence of polysaccharides[33–36]. Osman *et al*[37] demonstrated that, the post coital contraceptive activity from a crude extract in marine red algae *Gelidiella acerosa* is due to the presence of various phytochemical components such as alkaloids, flavonoids, phenols, amino acid, steroids, tannins and carbohydrates. Chapagain *et al*[38] reported that, saponins can also serve as natural larvicidal compounds. Cetin *et al*[39] reported that, maximum larvicidal activity was observed in the case of *D. dichotoma* which caused 50 percent mortality of the larvae of *Anopheles stephensi* and *Culex quinqefasciatus* at the concentartions of 22 and 34 ppm and caused total mortality at 80 and 100 ppm. Ravikumar *et al*[13] reported that, the seaweed *Chaetomorpha antennina* showed excellent *in vitro* and *in vivo* antiplasmodial activity. The larvicidal activity of terrestrial plant extracts has been reported by many workers. Among them, *Pedalium murax*[40] and *Cleome viscose*[41] have been found promising against *Culex quinquefasciatus* with the minimum LC_{50} of 23 and 10.7 ppm, respectively. *Adhatoda* species[40] and *Eucalyptus tereticornis*[42] have been found most effective against *Anopheles stephensi* with the minimum LC_{50} of 21.7 and 63 ppm, respectively. Our finding reveals that, the seaweed extract of *D. dichotoma* is highly effective in causing mortality of mosquito larvae with the minimum lethal concentration. It is concluded from the present study that, the seaweeds which were collected from South East coast of India showed enormous potential for mosquito larvicidal activities and would make it economical for field use in mosquito control programme.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the Authorities of the Alagappa University for providing the required facilities and also to the Ministry of Environment and Forest, Delhi for providing financial assistance to carry out Ph.D degree for M Syed Ali, Junior Research Fellow.

References

[1] de Omena MC, Navarro DM, de Paula JE, Luna JS, Ferreira de Lima MR, Sant'Ana AE. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. *Bioresour Technol* 2007; **98**: 2549–2556.

[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**: 959–962.

[3] 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*. *Bioresour Technol* 2008; **99**: 763–768.

[4] Pontes WJT, de Oliveira JCS, da Câmara CAG, Gondim–Júnior MGC, de Oliveira JV, Schwartz MOE. Acaricidal activity of the essential oils of leaves and fruits of *Xylopia sericea* St. Hill. on the two spotted spider mite (*Tetranychus urticae* Koch.). *Quim Nova*

2007; **30**: 838-841.
[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] Barbosa JP, Fleury BG, da Gama BAP, Teixeira VL, Pereira RC. Natural products as antifoulants in the Brazilian brown alga *Dictyota pfaffii* (Phaeophyta, Dictyotales). *Biochem Syst Ecol* 2007; **35**: 549-555.
[7] Sabina H, Aliya R. Seaweed as a new source of flavone, scutellarein 4′-methyl ether. *Pak J Bot* 2009; **41**(4): 1927-1930.
[8] Wang H, Vincent E, Put O, Ang JR. Antiviral activities of extracts from Hong Kong seaweeds. *J Zhejiang Univ Sci B* 2008: **9**(12): 969-976.
[9] Bazes A, Silkina A, Douzenel P, Fay F, Kervarec N, Morin D, et al. Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. *J Appl Phycol* 2009; **21**: 395-403.
[10] Ravikumar S, Anburajan L, Ramanathan G, Kaliaperumal N. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens. *Seaweed Res Util* 2002; **24**(1): 95-99.
[11] Ravikumar S, Ramanathan G, Subhakaran M, Inbaneson SJ. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int J Med Sci* 2009; **1**(5): 184-191.
[12] Ravikumar S, Nazar S, Shiefa NA, Abideen S. Antibacterial activity of traditional therapeutic coastal medicinal plants against some pathogen. *J Environ Biol* 2005; **26**: 383-386.
[13] Ravikumar S, Ramanathan G, Inbaneson SJ, Ramu A. Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. *Parasitol Res* 2011; **108**: 107-113.
[14] Ravikumar S, Inbaneson SJ, 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**: 873-878.
[15] Sofowora A. *Medicinal plants and traditional medicine in Africa*. New York: Wiley; 1982, p. 251.
[16] Kepam W. *Qualitative organic analysis (spectrochemical techniques)*. 2nd ed. London: McGraw Hill; 1986, p. 40-58.
[17] WHO. *Report of the WHO informal consultation on the evaluation on the testing of insecticides*. Geneva: WHO; 2009, p. 69.
[18] Samee H, Li ZX, Lin H, Khalid J, Guo YC. Anti-allergic effects of ethanol extracts from brown seaweeds. *J Zhejiang Univ Sci B* 2009; **10**(2): 147-153.
[19] Kumar SS, John JAC, Ravikumar S. Antimicrobial activity of acetone extracts of seaweeds against human pathogens. *Seaweed Res Util* 2002; **24**: 111-115.
[20] Jimenez JK, Connell SO, Lyons H, Bradley B, Hall M. Antioxidant, antimicrobial and tyrosinase inhibition activities of acetone extract of *Ascophyllum nodosum*. *Chem Pap* 2010; **64**(4): 434-442.
[21] Solomon RDJ, Santhi VS. Purification of bioactive natural product against human microbial pathogens from marine seaweed *Dictyoto acutiloba*. *World J Microbiol Biotechnol* 2008; **24**: 1747-1752.
[22] Patra JK, Rath SK, Jena K, Rathod VK, Thatoi H. Evaluation of antioxidant and antimicrobial activity of seaweeds (*Sargassum* sp.) extract: a study on inhibition of glutathione-s-transferase activity. *Turk J Biol* 2008; **32**: 119-125.
[23] Vallim MA, Barbosa JE, Cavalcanti DN, De Paula JC, Da Silva VAG, Teixeira VL, et al. *In vitro* antiviral activity of diterpenes isolated from the Brazilian brown alga *Canistrocarpus cervicornis*. *J Med Plant Res* 2010; **4**(22): 2379-2382.
[24] Tan LT, Williamson RT, Gerwick WH, Watts KS, McGough K, Jacobs R. Cis, cis- and trans, trans-ceratospongamide, new bioactive cyclic heptapeptides from the Indonesian red alga *Ceratodictyon spongiosum* and symbiotic sponge *Sigmadocia symbiotica*. *J Org Chem* 2000; **28**: 419-425.
[25] Margret RJ, Kumaresan S, Ravikumar S. A preliminary study on the anti-inflammatory activity of methanol extract of *Ulva lactuca* in rat. *J Environ Biol* 2009; **30**(5 Suppl): 899-902.
[26] Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi R, et al. Biopotentials of seaweeds collected from southwest coast of India. *J Mar Sci Technol* 2009; **17**(1): 67-73.
[27] Rizvi MA, Shameel M. *In vitro* nematicidal activities of seaweed extracts from Karachi coast. *Pak J Bot* 2006; **38**(4): 1245-1248.
[28] Ganesh EA, Das S, Arun G, Balamurugan S, Raj RR. Heparin like compound from green alga *Chaetomorpha antennina* as potential anticoagulant agent. *Asian J Med Sci* 2009; **3**: 114-116.
[29] Subhash C Arya, Nirmala Agarwal, Satish C Parikh. Detection of dengue NS1 antigen, alongside IgM plus IgG and concurrent platelet enumeration during an outbreak. *Asian Pac J Trop Med* 2011; **4**(8): 672.
[30] Jhamb R, Kashyap B, Ranga GS, Kumar A. Dengue fever presenting as acute liver failure- a case report. *Asian Pac J Trop Med* 2011; **4**(4): 323-324.
[31] Abdel-Hady El-Gilany, Eldeib A, Hammad S. Clinico-epidemiological features of dengue fever in Saudi Arabia. *Asian Pac J Trop Med* 2011; **4**(4): 220-223.
[32] Aziz AT, Dieng H, Hassan AA, Satho T, Miake F, Salmah MRC, *et al.* Insecticide susceptibility of the dengue vector *Aedes aegypti* (Diptera: culicidae) in Makkah City, Saudi Arabia. *Asian Pac J Trop Dis* 2011; **1**(2): 94-99.
[33] Andrews KT, Klatt N, Adams Y, Mischnick P, Schwartz-Albiez R. Inhibition of chondroitin-4-sulfate-specific adhesion of *Plasmodium falciparum* infected erythrocytes by sulfated polysaccharides. *Infect Immun* 2005; **73**: 4288-4294.
[34] Clark DL, Su S, Davidson EA. Saccharide anions as inhibitors of the malaria parasite. *Glycoconj J* 1997; **14**: 473-479.
[35] Xiao L, Yang C, Patterson PS, Udhayakumar V, Lal AA. Sulfated polyanions inhibit invasion of erythrocytes by plasmodial merozoites and cytoadherence of endothelial cells to parasitized erythrocytes. *Infect Immun* 1996; **64**: 1373-1378.
[36] Kisilevsky R, Crandall I, Szarek WA, Bhat S, Tan C, Boudreau L, et al. Short-chain aliphatic polysulfonates inhibit the entry of *Plasmodium* into red blood cells. *Antimicrob Agents Chemother* 2002; **46**(8): 2619-2626.
[37] Osman MEH, Abushady AM, Elshobary ME. *In vitro* screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria Egypt. *Afr J Biotechnol* 2010; **9**(12): 7203-7208.
[38] Chapagain BP, Saharan V, Wiesman Z. Larvicidal activity of saponins from *Balanites aegyptiaca* callus against *Aedes aegypti* mosquito. *Bioresour Technol* 2008; **99**(5): 1165-1168.
[39] Cetin H, Gokoglu M, Emre O. Larvicidal activity of the extract of seaweed, *Caulerpa scalpelliformis*, against *Culex pipiens*. *J Am Mosq Control Assoc* 2010; **26**(4): 433-435.
[40] Kalyanasundaram M, Das PK. Larvicidal and synergestic activity of plant extract for mosquito control. *Indian J Med Res* 1985; **82**: 19-21.
[41] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vector Borne Dis* 2007; **44**: 145-148.
[42] Nathan SS. The use of *Eucalyptus tereticorniss* Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour Technol* 2007; **98**(9): 1856-1860.