J Vector Borne Dis 48, December 2011, pp. 241–244

Larvicidal activity of marine algae, *Sargassum swartzii* and *Chondria dasyphylla,* against malaria vector *Anopheles stephensi*

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ABSTRACT

Objectives: The objective of this study was to evaluate larvicidal activity of native marine algae against main malaria vector *Anopheles stephensi*.

Study design: The total 70% methanol (MeOH) extract and partition fractions of chloroform ($CHCl_3$), ethylacetate (EtOAc), and MeOH from two algae, *Sargassum swartzii* and *Chondria dasyphylla* were investigated for larvicidal activities against late III and early IV instar larvae of malaria vector *An. stephensi.*

Results: Among all the fractions tested against larvae, EtOAc fraction of *S. swartzii* and *C. dasyphylla*, showed mortality rate of 96 and 95%, respectively. Probit analysis of logarithmic concentration from regression line exhibited the LC_{50} and LC_{90} values of 11.75 and 53.47 ppm respectively for *S. swartzii* and 10.62 and 56.39 ppm respectively for *C. dasyphylla*.

Conclusion: This is the first report of larvicidal activities of two native algae against *An. stephensi*. We propose that the larvicidal activity of EtOAc fraction is related to the presence of semi-polar compounds. Further isolation and purification could lead to identify more potent compounds.

Key words *Anopheles stephensi; Chondria dasyphylla;* Iran; larvicidal activity; *Sargassum swartzii*

INTRODUCTION

Malaria is still a major endemic disease in foci located in south and southeast of Iran. The annual malaria cases have been reported from 66,075 to 6,211 during 1995–2009, indicating the sharp decline of disease. It is unstable with two seasonal peaks mainly in spring and autumn. These areas include the provinces of Sistan and Baluchistan, Hormozgan and Kerman[1]. In this part of the country, six anopheline mosquitoes including *An. culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. superpictus*, and *An. pulcherrimus* (Diptera: Culicidae) are known to be the malaria vectors and *An. sacharovi* and *An. maculipennis* are considered as malaria vectors in northern part of the country[2–7].

Chemical control methods have been applied against either the immature or the adult of malaria vectors. Applying chemical parricides is the most important part of such program. Mosquito control, using chemical larvicides has been performed during the fight against malaria in Iran and still considered as an important part of vector control. Chemical larvicides are now considered as toxic material to fish and other non-target organisms as well as the environment. They are also responsible for increase of insecticide resistance in arthropods. The extract of whole leaf and essential oil of certain plants have been investigated, and showed toxic effect against some public health pests[8–10]. Several species of marine algae from coastlines of Iranian islands and Hormozgan province have been reported[11]. Marine algae produce different secondary metabolites with a wide range of biological activities[12]. Many studies have been achieved on the screening of biological effects of marine organisms and many active compounds were isolated and characterized[13]. Red algae from genus *Chondria* are known as a producer of cyclic polysulfides, terpenoids, amino acids and amines. Domoic acid derivatives with larvicidal and lowering blood pressure activity have been identified in *Chondria armata*[14]. Secondary metabolites with cytotoxic and antitumor activity have been extracted and identified in *Sargassum* species[15–16]. This study was aimed to determine the larvicidal activity of different extracts of *S. swartzii* and *C. dasyphylla*, collected from coastlines of the Persian Gulf, southern Iran, against main malaria vector *An. stephensi*.

MATERIAL & METHODS

Plant material

Brown algae, *Sargassum swartzii* C. Agardh (Sargassaceae), *Chondria dasyphylla* (Woodward) C.

Agardh *(Rodomelacea)*, were collected from Asaluye-Niband marine protected area of the Persian Gulf in February 2008. The algae were identified by Dr J. Sohrabipour at the Agriculture and Natural Resource Research Center of Hormozgan (herbarium numbers are 20,424, 20,426 respectively) and the voucher specimens were deposited at this center.

Extraction of marine algae

The algae were air-dried in the shade at room temperature and were smashed to make a powder with a mortar and pestle. Each sample of 200 g was extracted with MeOH-H_2O (70:30) (5×200 ml) at room temperature. The combined extracts were evaporated under vacuum. The residues were subjected to Silica gel (230) mesh and diluted successively with n-Hexane, $CHCl_3$, EtOAc and Methanol. Removal of the solvents resulted in the production of n-Hexane, $CHCl_3$, EtOAc and MeOH-H_2O fractions.

Biological study

Different extracts of *S. swartzii* and *C. dasyphylla* were evaluated against late III and early IV instar larvae of *An. stephensi*. The mosquitoes were collected from malarious areas of Iran, and then were maintained at the insectary of School of Public Health & National Institute of Health Research, Tehran. The reared susceptible larvae to different insecticides were exposed to different concentrations of the *S. swartzii* and *C. dasyphylla* extracts which were prepared in methanol. The minimum concentration was 2.5 mg/l and the maximum was 40 mg/l. These concentrations gain the appropriate mortality to plot the regression line. Mortality was determined after 24 h exposure period. All the tests were conducted at 30 ± 1ºC and 60 ± 5% relative humidity, and 10 : 14, dark : light periods respectively in the laboratory conditions[17,18]. For each concentration, at least 4 replicates of 25 individuals were used[19].

Statistical analysis

The mortality data were subjected to probit analysis using Finney studies[20]. From the regression line between logarithmic dose and probit mortality all the parameters including LC_{50} and 95% confidence interval, LC_{90} and 95% confidence interval were determined[21]. The regression line was plotted using Microsoft Excel.

RESULTS

Mortality data of *An. stephensi* exposed to different extracts of two algae, *S. swartzii* and *C. dasyphylla* are shown in Table 1. The EtOAc fraction of both *S. swartzii* and *C. dasyphylla* were found to be more effective than the other fractions and total extract. Other fractions didn't show significant larvicidal effect against *An. stephensi.* For EtOAc fractions the chi-square values were significant at $p < 0.05$ level[22]. LC_{50} and LC_{90} values for *S. swartzii* were 11.7584 and 53.472 ppm respectively, and values for *C. dasyphylla* were 10.625 and 56.394 ppm, respectively (Table 2). The probit regression line is plotted in Fig. 1. From this probit regression line different parameters about efficacy of product against malaria vector can be calculated.

DISCUSSION

Secondary metabolites with broad range of activities have been found in marine algae. To evaluate the larvicidal effect of the algae from the Persian Gulf against *An.*

Table 1. Comparison of larvicidal effect of different extracts of *Sargassum swartzii* and *Chondria dasyphylla* on *An. stephensi* larvae

Samples	Concentration (ppm)	Total tested	Total dead	Mortality (%)
Control	70% (methanol)	50	0	0
Sargassum swartzii				
Total extract	40	50	4	8
Chloroform	40	50	0	0
Ethyl acetate	40	52	50	96.1
MeOH	40	49	3	6.1
Chondria dasyphylla				
Total extract	40	50	3	6
Chloroform	40	49	0	0
Ethyl acetate	40	51	47	92.1
MeOH	40	50	2	4

Note: The larvae were exposed to a 40 ppm concentration of different extracts of *Sargassum swartzii* and *Chondria dasyphylla* which were prepared in methanol. Mortality was determined after 24 h exposure period. For each extract, at least 2 replicates were used.

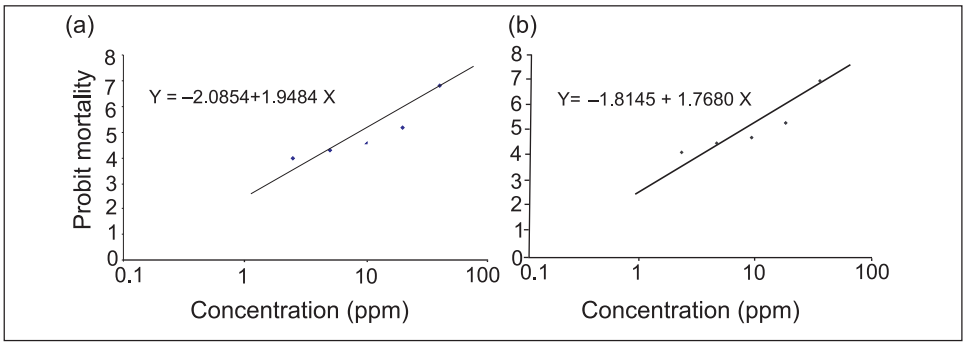

Fig. 1: Probit regression line of ethyl acetate fraction of (a) *Sargassum swartzii* and (b) *Chondria dasyphylla* against *An. stephensi* larvae

Table 2. Probit regression line parameters of extract of *S. swartzii* and *C. dasyphylla* against larvae of *An. stephensi*

Intercept (a)	Slope (b ± S.E.)	LC_{50} in ppm (95% C.L.)	LC_{90} in ppm (95% C.L.)	$ë^2$ (heterogeneity)	$ë^2$ table (df)	*p*-value
S. swartzii						
–2.0854	1.9484 ± 0.446	4.9945 (11.75–33.78)	41.2960 (53.47–75.35)	23.515	7.81 (3)	0.05
C. dasyphylla						
–1.8145	1.7680 ± 0.385	4.5996 (10.62–12.46)	42.2081 (56.39–83.64)	18.334	7.81 (3)	0.05

stephensi, the samples were extracted with methanol (70%) and fractions were obtained by using various polar and non-polar solvents.

In a previous study on antiplasmodial and antimicrobial activities of South African marine algal extracts, the dichloromethane fraction of *Sargassum heterophyllum* showed the most antiplasmodial effect with IC_{50} value of 2.8 µg/ml against chloroquine sensitive strain of *Plasmodium falciparum* (D10)[23].

Exposure of *An. stephensi* larvae to sub-lethal doses of neem extracts in the laboratory prolonged larval development, reduced pupal weight, high oviposition deterrence and high mortality[24]. Some researchers have shown ethanol extract of aerial parts of *Tagetes minuta* had larvicidal effects with LC_{50} value about 2.5 mg/l[25]. Also for *Conyza albida,* LC_{50} value of 2 mg/l and for *Artmisisa afra,* LC_{50} of 5 mg/l has been determined[26]. In another report for *Maytenus senegalensis*, LC_{50} value was about 3.9 mg/l and for *Harrisonia abyssinica* LC_{50} 4.7 mg/l have been reported[27].

CONCLUSION

In conclusion, larvicidal effects of EtoAc fractions of *S. swartzii* and *C. dasyphylla* could be related to semi-polar compounds existing in both algae. The extracts from these plants may be useful for improvement of new natural insecticides, however, further investigations are needed to identify and purify the effective components and their mechanisms of actions of these algae.

ACKNOWLEDGEMENT

This study is a part of Pharm. D. thesis funded and supported by the Tehran University of Medical Sciences (TUMS).

REFERENCES

1. Manouchehri AV, Zaim M, Emadi AM. A review of malaria in Iran, 1957–1990. *J Amer Mosquito Control Assoc* 1992; *8(*4): 381–5.
2. Abai MR, Mehravaran A, Vatandoost H, Oshaghi MA, Javadian E, Mashayekhi M, *et al*. Comparative performance of imagicides on *Anopheles stephensi,* main malaria vector in a malarious area, southern Iran. *J Vector Borne Dis* 2008; *45*(4): 307–12.
3. Oshaghi MA, Sedaghat MM, Vatandoost H. Molecular characterization of the *Anopheles maculipennis* complex in the Islamic Republic of Iran. *East Mediterr Health J* 2003; *9*(4): 59–66.
4. Sedaghat MM, Linton YM, Nicolescu G, Smith L, Koliopoulos G, Zounos AK, *et al*. Morphological and molecular characterization of *Anopheles* (*Anopheles*) *sacharovi* Favre, a primary vector of malaria in the Middle East. *Systematic Entomol* 2003; *28:* 241–56.
5. Sedaghat MM, Harbach RE. An annotated checklist of the *Anopheles* mosquitoes (Diptera: Culicidae) in Iran. *J Vector Ecol* 2005; *30:* 272–6.

6. Zahirnia AH, Vatandoost H, Nateghpour M, Javadian E. Insecticide resistance/susceptibility monitoring in *Anopheles pulcherrimus* (Diptera: Culicidae) in Ghasreghand district, Sistan and Baluchistan province. *Hakim* 1998; *1:* 97–106.
7. Zahirnia AH, Taherkhani H, Vatandoost H. Observation of malaria sporozoite in *Anopheles culicifacies* (Diptera: Culicidae) in Ghasreghand district, Sistan & Baluchistan province. *Hakim* 2001; *4:* 149–53.
8. Hadjiakhoondi A, Sadeghipour-Roodsari HR, Vatandoost H, Khanavi M, Abaee MR, Vosoughi M, *et al*. Fatty acid composition and toxicity of *Melia azedarach* L. fruits against malaria vector *Anopheles stephensi. Iranian J Pharm Sci* 2006; *2(*2): 97–102.
9. Hadjiakhoondi A, Vatandoost H, Jamshidi A, Bagherj Amiri E. Chemical constituents and efficacy of *Cymbopogon olivieri* (Boiss) bar essential oil against malaria vector, *Anopheles stephensi*. *Daru* 2003; *11*(3): 125–8.
10. Vatandoost H, Moinvaziri VM. Larvicidal activity of neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. *Eastern Med Health J* 2004; 10: 573–8.
11. Sohrabipour J, Rabii R. A list of marine algae of sea shores of the Persian Gulf and Oman Sea in the Hormozgan province. *Iran J Bot* 1999; *8*(1): 131–62.
12. Mayer AMS, Rodriguez AD, Berlinck RGS, Hamann MT. Marine pharmacology in 2003-04. *Comp Biochem Phys* 2007;*145*(c), 553–81.
13. Blunden G. Biologically active compounds from marine organisms. *Phytother Res* 2001; *15:* 89–94.
14. Mangala B, Solimabi W. Constituents of *Chondria armata*. *Phytochem* 2000; *54*(8): 979–81.
15. Numata A, Kanbara S, Takahashi C, Fujiki R, Yoneda M, Fujita E, *et al*. Cytotoxic activity of marine algae and a cytotoxic principle of the brown alga *Sargassum tortile*. *Chem Pharm Bull* 1991; *39*(8): 2129–31.
16. Tang HF, Yi YH, Yao XS, Xu QZ, Zhang SY, Lin HW. Bioactive steroids from the brown alga *Sargassum carpophyllum*. *J Asian Nat Prod Res* 2002; *4:* 95–105.
17. Senthil Nathan S, Kalaivani K, Murugan K, Chung PG. Effects of neem limonoids on malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Trop* 2005; *96:* 47–55.
18. Senthil Nathan S, Kalaivani K, Sehoon K. Effects of *Dysoxylum malabaricum* Bedd. (Meliaceae) extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Biores Technol* 2006b; *97:* 2077–83.
19. *Instructions for determining susceptibility or resistance of mosquito larvae to insecticides*. WHO/VBC-81, 1981; p. 807.
20. Finney DJ. *Probit analysis*. III edn. Cambridge: Cambridge University Press 1971; p. 42–6.
21. Cary NC, Saxena SC, Sumithra L. Laboratory evaluation of leaf extract of new plant to suppress the population of malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Curr Sci* 1985; *54:* 201–2.
22. Wandscheer CB, Duque JE, Da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, Fontana JD. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon* 2004; *44:* 829–35.
23. Lategan C, Kellerman T, Afolayan AF, Mann MG, Antunes EM, Smith PJ, *et al*. Antiplasmodial and antimicrobial activities of South African marine algal extracts. *Pharm Biol* 2009; *47*(5): 408–13.
24. Su T, Mulla MR. Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus* to neem products containing azadirachtin. *Entomol Exp Appl* 1999; *91:* 337–45.
25. Hadjiakhoondi A, Vatandoost H, Khanavi M, Abaee M, Karami M. Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L. on *Anopheles stephensi* larvae. *Iran J Pharm Sci* 2005; *2:* 81–4.
26. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, Bhagwandin N, Smith PJ, Folb PI. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *J Pharmacol* 2004; *92*(2–3): 177–91.
27. El Tahir A, Satti G, Khalid S. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam) Exell. *J Ethnopharmacol* 1999; *64*(3): 227–33.

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Received: 13 May 2011 *Accepted in revised form:* 13 December 2011