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 (CHCl₃), 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 LC₅₀ and LC₉₀ 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 Kerman¹. 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 country²–⁷.

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 pests⁸–¹⁰. Several species of marine algae from coastlines of Iranian islands and Hormozgan province have been reported¹¹. Marine algae produce different secondary metabolites with a wide range of biological activities¹². Many studies have been achieved on the screening of biological effects of marine organisms and many active compounds were isolated and characterized¹³. 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* species¹⁵–¹⁶. 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 Asaluyeh-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-H₂O (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, CHCl₃, EtOAc and Methanol. Removal of the solvents resulted in the production of n-Hexane, CHCl₃, EtOAc and MeOH-H₂O 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. For each concentration, at least 4 replicates of 25 individuals were used.

**Statistical analysis**

The mortality data were subjected to probit analysis using Finney studies. From the regression line between logarithmic dose and probit mortality all the parameters including LC₅₀ and 95% confidence interval, LC₉₀ and 95% confidence interval were determined. 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. LC₅₀ and LC₉₀ 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.
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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 IC₅₀ 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 mortality²⁴. Some researchers have shown ethanol extract of aerial parts of *Tagetes minuta* had larvicidal effects with LC₅₀ value about 2.5 mg/l²⁵. Also for *Conyza albida*, LC₅₀ value of 2 mg/l and for *Artmisisa afric*, LC₅₀ of 5 mg/l has been determined²⁶. In another report for *Maytenus senegalensis*, LC₅₀ value was about 3.9 mg/l and for *Harrisonia abyssinica* LC₅₀ 4.7 mg/l have been reported²⁷.

**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.

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**REFERENCES**


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