Mosquito larvicidal efficacy of phenolic acids of seaweed
*Chaetomorpha antennina* (Bory) Kuetz. against *Aedes aegypti*

Subramanium VIMALADEVI¹, Ayyavu MAHESH²*, Balaji N. DHAYANITHI³ & Nattarayan KARTHIKEYAN⁴

¹Department of Medical Biotechnology, Chettinad University, Chennai 603 103, Tamil Nadu, India
²Department of Plant Genetics, Institute of Plant Sciences, ARO, The Volcani Center, Bet-Dagan 50250, Israel; e-mail: a.mahesh05@gmail.com
³Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, Tamil Nadu, India
⁴Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

Abstract: Mosquito larvicidal and repellent activities of phenolic acids of *Chaetomorpha antennina* (Bory) Kuetz. against the third instar larvae of *Aedes aegypti* were investigated. The larval mortality was observed after 24 h exposure. Results of mosquito larvicidal tests revealed that insoluble bound phenolic acids and soluble conjugated phenolic acid fractions of *C. antennina* had an excellent inhibitory effect against *A. aegypti* and its LC₅₀ values were 23.4 and 44.6 µg ml⁻¹, respectively. The repellency assay of insoluble bound phenolic acids and soluble conjugated phenolic acid fractions of *C. antennina*, at 10 µg cm⁻² concentration gave 100% protection up to 120 min. The results indicate that phenolic acids of *C. antennina* have a wide spectrum of larvicidal and repellent activities against *Aedes aegypti*.

Key words: Cladophoraceae; repellency; larvicidal activity; phenolic acids; *Aedes aegypti*

Introduction

Mosquitoes are the most important group of insects in terms of public health importance as they transmit a number of causative agents of diseases, such as malaria, filariasis, dengue fever, etc. causing millions of deaths every year (Hales et al. 2002). Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (Peng et al. 2004). Mosquito borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Fradin & Day 2002). The yellow fever mosquito, *Aedes aegypti* (L., 1762), is responsible for dengue fever in India, where the number of dengue fever cases has increased significantly in recent years. Some 2.5 billion people two-fifths of the world’s population are now at risk from dengue. The WHO has estimated that there may be 50 million dengue infections worldwide every year. The disease is now endemic in more than 100 countries in Africa, America, Eastern Mediterranean, South-east Asia and the Western Pacific, the last two being the most seriously affected (WHO 2008). In the absence of effective vaccine and drugs, dengue prevention and control programs have depended on vector control. Management of this disease vector using synthetic organic chemical insecticides has failed because *Aedes* mosquitoes have developed insecticide resistance (Scott et al. 1993). For the control of mosquito vectors, dichlorodiphenyl trichloroethane, organophosphates and pyrethroids are being used as adulticides/larvicides for the latest several decades in the National Vector Borne Disease Control programme in India, depending on the requirements of the given disease control programme (Raghavendra & Subbarao 2002). Although effective repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organisms (Yang et al. 2002).

In situations where mosquitoes have developed resistance to all conventional larvicides, consideration may be given to using larvicidal oils, bacterial larvicides such as *Bacillus* spp., or more expensive insect growth regulators as alternatives. However, the implementation of these agents in the field has failed to show satisfactory results in many parts of the world (Liu et al. 1996; Chevillon et al. 2001). These partial failures have prompted renewed interest in the search and development of better vector control strategies that destroy vectors over a wide range, but cause no harm...
to non-target organisms and the environment. The use of natural products for vector control might be one of the potentially alternative approaches. Although certain plant-based products provide lower and short-lived efficacy compared with synthetic chemicals, safety considerations of life and the environment are invigorating further investigations of plants potential.

The biodiversity of the marine ecosystem provides an important source of chemical compounds which, due to antiviral, antibacterial, antifungal and anticancer activities, have many therapeutic applications (Solomon & Santhi 2008). Many of the marine macro algae produce a variety of secondary metabolites, such as polyphenols, terpenes, steroids, lectins, inhibitors of proteinases, fatty acids, enzymes, and polysaccharides (Smit 2004; Barabanova et al. 2005; Ermak et al. 2006) which act as potential bioactive compounds of interest for pharmaceutical applications.

To the best of our knowledge, there is no documented literature on larvicidal activities of phenolic acids of Chaetomorpha antennina. In the present study, an attempt has been made to evaluate the mosquito larvicidal and repellent efficacy of phenolic acids of C. antennina against A. aegypti.

Material and methods

Plant material

Chaetomorpha antennina (Bory) Kuetz. (Cladophoraceae) was collected from the coastal waters near Rameshwaram, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. Soosairaj, Department of Plant Biology and Plant Biotechnology, St. Joseph’s College, Tiruchirappalli, India. The authenticated samples were used for the further study.

Extraction of phenolic compounds

The collected sea weeds were washed with distilled water and then the sea weed samples were shade dried at room temperature. The dried sea weeds were powdered in an electrical blender and 15 g samples were defatted with hexane. The phenolic compounds were extracted according to Zhou (2007) with slight modification. Phenolic compound were prepared and extracted as three phases (soluble conjugated phenolic acids, free phenolic acids and insoluble bound phenolic acids). The extracts were concentrated by a rotary vacuum evaporator until the solvent completely evaporated. The concentrated extracts were used for further studies.

Estimation of phenolic acids

The concentration of phenolic acids in the extracts was determined according to the method described by Jayaprakash et al. (2007) with slight modification. The results were expressed as gallic acid equivalents. The C. antennina extract and its fractions (2 mg) were dissolved in 1 ml methanol. The MeOH extract and its various soluble fractions (100 µg) and different concentrations (10–100 µg) of gallic acid in 0.1 ml were mixed with 0.5 ml of a ten-fold diluted Folin-Ciocalteu reagent and 0.4 ml of 7.5% sodium carbonate solution. After incubation at ambient temperature for 30 min, the absorbance was measured at 750 nm using a multiplate spectrophotometer. The estimation of phenolic acids content in the MeOH extract and fractions was calculated using a standard graph (gallic acid).

Mosquito larvicidal assay

Egg masses of Aedes aegypti were collected from the drainage of a local residential area of Chennai, Tamil Nadu, India. The collected egg masses were incubated in laboratory (28 ± 3°C, 75–85% RH). The hatched larvae were fed with Brewer’s yeast/dog biscuit (1:3). Laboratory reared third instars larvae were used for further investigation.

The larval mortality bioassays were carried out according to the test method of larval susceptibility as suggested by the World Health Organization (WHO 1981). Twenty larvae were placed in a glass beaker with 100 ml of aqueous suspension of phenolic acid at various concentrations (from 10–100 µg L⁻¹) depending on the kind of compound. Five replicates were made per concentration, and a control treatment with tap water was included in each bioassay. The larval mortality was calculated after a 24 h exposure period. In records of the effect of each phenolic acid concentration, moribund and dead larvae were considered as affected. LC₅₀ and LC₉₀ value indicated 50% and 90% mortality, respectively; 95% confidence limit of upper and lower confidence levels were calculated from a series of “exposure” concentration by comparing the percentage of mortality in the treated group to control by probit analysis (SPSS, version 13).

Mosquito repellent assay

The repellency of three phenolic acids of C. antennina was evaluated using the human-bait technique (Schreck & McGovern 1989; WHO 1996). Evaluation was carried out in a net cage (45 × 30 × 25 cm²) containing 100 blood-starved female A. aegypti of an age of 3–4 days at 28 ± 2°C and relative humidity of 75–85%. Arms of volunteers (only 25 cm² of the dorsal side of the skin on each arm) were exposed to starving mosquitoes and the remaining parts were covered by rubber gloves. Soluble conjugated phenolic acids, free phenolic acids and insoluble bound phenolic acids (10 µg cm⁻²) were applied separately on the exposed area of the fore arm. Ethanol served as a control. The volunteers conducted their test of each concentration by inserting the treated and control arm into the same cage for one minute in 5 min intervals. Numbers of mosquitoes that landed on the arms were recorded and then shaken off before imbibing any blood, making out a 5-min protection. The percentage of repellency was calculated by the following formula:

\[
\text{% Repellency} = \left( \frac{C - T}{C} \right) \times 100
\]

where \( C \) is the number of mosquitoes in the control group and \( T \) is the number of mosquitoes in the treated group. Each test was repeated five times in separate cages, and in each replicate.

Results and discussion

The present preliminary investigations provided a list of bioactive phenolic acids of C. antennina, which possess larvicidal and bactericidal activities. These bioactive compounds could be used for obtaining new leads to isolate bioactive molecules of marine origin. The content of insoluble bound phenolic acids, soluble conjugated phenolic acid and free phenolic acids in lyophilized sea weed C. antennina was investigated. Accumulation of insoluble bound phenolic acids of C. antennina (15.14 ± 0.04 mg g⁻¹) was significantly higher than the accumulation of soluble conjugated phenolic acid and free phenolic acids (10.1 ± 0.01 mg g⁻¹ and 7.9 ± 0.03 mg g⁻¹).
respectively). Phenolic compounds are commonly found in the edible brown, green and red seaweeds (Jimenez-Escrig et al. 2001; Kuda et al. 2005; Ganesan et al. 2008), and the bioactive property has been correlated to their phenolic content (Tilquin et al. 2002; Roh et al. 2008; Pandimadevi et al. 2008). For example, high concentrations of polyphenols such as catechin, epicatechin, epigallocatechin gallate and gallic acid are reported in the seaweed *Halimeda* spp. (Yoshie et al. 2002). Halogen-containing terpenoids, acetylens and phenols have been identified in several seaweed species as biologically active compounds (Vairappan et al. 2001).

**Larvicidal activity**

The use of bioactive compounds of seaweeds is an alternative pest control method, which can help to minimize the usage of toxic pesticides and their deleterious effects on non-target insect species in the environment. Larvicidal activity of different phenolic acids screened against third instar larvae of *A. aegypti* are depicted in Table 1. An exposure of larval diet containing soluble conjugated phenolic acid, free phenolic acid and insoluble bound phenolic acid extracted from the sea weed *C. antennina* increased the mortality of mosquito larvae in a concentration-dependent manner. Among the three tested phenolic acids of *C. antennina*, insoluble bound phenolic acids were found to exhibit relatively high larvicidal activity against *A. aegypti*. The LC50 and LC90 values of insoluble bound phenolic acid were 23.4 and 74.8 µg L⁻¹, respectively. This was closely followed by soluble conjugated phenolic acid with LC50 and LC90 of 44.6 and 85.2 µg L⁻¹, respectively. The free phenolic acid also showed larvicidal activity with LC50 and LC90 of 60.8 and 116.6 µg L⁻¹, respectively. In general, *A. aegypti* is used in insecticide-screening trials because it is usually less susceptible and easy to colonize in the laboratory (Shaalan et al. 2005). During the last decade, various studies on natural marine products against mosquito vectors indicated them as possible alternatives to synthetic insecticides (Venkateswara Rao et al. 2008; Chen et al. 2009). Previous literature also indicated that the dichloromethane: methanolic crude extract of *C. antennina*, showed larvicidal activity against *Culex quinquefasciatus* Say, 1823 second instar larvae with a LC50 value of 100 µg ml⁻¹, whereas *C. antennina* had less lethal effect against *C. quinquefasciatus* third instar larvae with a LC50 value 400 µg ml⁻¹ (Manilal et al. 2009). *In vitro* and *in vivo* antiplasmodial activity of polyherbal extracts of *C. antennina* have been reported against *Plasmodium falciparum* (Ravikumar et al. 2011), however, *C. antennina* exhibited higher antiplasmodial activity than *Aegiceras corniculatum*.

The larvicidal activity of insoluble phenolic compounds of alder leaf litter against *A. aegypti* has been reported (Tilquin et al. 2002). The finding of acute larvicidal effects of polyphenols against certain larval Culicidae, Chironomidae and Simuliidae has already suggested the prospect of using these polyphenols in dipteran pest control (Rey et al. 2001; Pautou et al. 2000). Polyphenols would be regarded as relevant only to target mosquito taxa associated with a poor non-target arthropod fauna. This is the case for anthropophilic *Aedes* and *Culex* taxa in urban and suburban areas, since alternative strategies for controlling these pests are being actively investigated (Rey et al. 2001). Previous reports on extracts of *Psammaphyllis purpurea* and *Haliclona cricributa* showed LC50 values of < 50 ppm against *A. aegypti* (Venkateswara Rao et al. 2008), whereas fucoidan derived from *Undaria pinnatifida* seaweed showed LC50 values of 9.17 µg ml⁻¹ against *P. falciparum* (Chen et al. 2009). Watanabe et al. (1989, 1990) reported insecticidal activity of the polyhalogenated monoterpenes, aplysianterenoid A and telfairine isolated from *Placium telfairiae*, against *Culex pipiens pallens* and *Anopheles gambiae*.

**Repellent activity**

The results from the repellency activity of phenolic acids of *C. antennina* against blood-starved adult female of *A. aegypti* are given in Table 2. The protection period of insoluble bound phenolic acid against *A. aegypti* was 120 min and that of soluble conjugated phenolic acid 90 min. The free phenolic acid compounds

---

**Table 1. Mosquito larvicidal activity of phenolic acids of Chaetomorpha antennina (Bory) Kuent. against Aedes aegypti.**

<table>
<thead>
<tr>
<th>Conc. (µg L⁻¹)</th>
<th>Soluble conjugated phenolic acids</th>
<th>95% confidence interval</th>
<th>Free phenolic acids</th>
<th>95% confidence interval</th>
<th>Insoluble bound phenolic acids</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% mortality LC50 LC90 ± SE</td>
<td>% mortality LC50 LC90 ± SE</td>
<td>% mortality LC50 LC90 ± SE</td>
<td>% mortality LC50 LC90 ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>24.66±3.71</td>
<td>18.33±3.75</td>
<td>33.66±3.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>38.00±6.11</td>
<td>28.66±1.76</td>
<td>47.33±1.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>41.00±5.50</td>
<td>34.33±4.33</td>
<td>55.00±2.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>43.66±2.18</td>
<td>39.33±4.37</td>
<td>61.33±4.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>55.66±2.60</td>
<td>42.33±2.72</td>
<td>71.66±2.02</td>
<td>23.4 74.8 46.04 63.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>70.33±3.28</td>
<td>49.00±1.73</td>
<td>78.33±3.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>75.00±4.72</td>
<td>58.66±1.45</td>
<td>85.00±4.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>86.00±2.08</td>
<td>72.33±2.96</td>
<td>96.00±2.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>95.00±2.09</td>
<td>76.00±4.04</td>
<td>98.66±1.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>97.66±1.85</td>
<td>85.33±1.85</td>
<td>99.33±0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Explanations: Each value represents the mean ± SE of six replications.
provided only a 30 min protection. In contrast, treatment of an arm with ethanol provided less than 30 min protection against the bite of A. aegypti mosquitoes. The percentage of repellency for A. aegypti ranged between 100% for insoluble bound phenolic acid and 91.2 ± 1.17% for soluble conjugated phenolic acid after 2 h. The free phenolic acid exhibited only 66.6 ± 0.71% repellency after 2 h. A diluted ethanolic solution of phenolic acids applied to skin of the forearm was shown to be very effective in protection against A. aegypti. The repellent properties of natural products to mosquitoes and other pest insects were well known before the advent of synthetic repellents (Curits et al. 1989). However, the interest in plant-based repellents was reduced because of the advent of synthetic repellents. Only in recent years, interest in plant-based products has been revived because of the rising cost and other noxious effects of synthetic repellents. In the present study, no adverse effects of phenolic compounds of C. antennina on the forearm of the human volunteers were observed during the 2 h study.

In conclusion, C. antennina offers a promising and potential biocontrol agent against A. aegypti. Furthermore, safety considerations and the emergence of mosquito resistance to conventional insecticides make larvicide of natural origin preferable to synthetic compounds. Natural insecticides, especially those derived from plants that are more selective and easily degradable, are more promising in this aspect. The isolated bioactive phytochemical from the seaweed could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, the exact mechanism and the compound responsible for the larvicidal activities are currently unclear. Therefore, further studies on the identification of the active compounds involved and their mode of action in field trials are needed to recommend C. antennina as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

References


Received November 2, 2010
Accepted September 28, 2011