Evaluation of Some Biological Activities of *Chara hispida* L.

Tahir Atıcı¹  İlkay Orhan²  Puntip Wisepongpand³  Bilge Şener²

¹Dep. of Biology, Faculty of Education, Gazi University, 06500, Ankara, Türkiye.
²Dep. of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330, Ankara, Türkiye.
³Department of Marine Sciences, Faculty of Fisheries, Kasetsart University, 10900, Thailand


Deniz ve tahı su algılarından elde edilen birçok madde biyomedikal ve kozmetik alanda kullanıma girmiş veya girişikte işlemlerdir. Ülkemizin deniz ve tahı su kaynaklarını yönünden oldukça zengin olması rağmen, bu türler üzerinde çok az çalışma bulunmaktadır.

Bu araştırmada, daha önce üzerinde hiç biyolojik aktivite çalışması yapılmamış olan ve Salda Gölü’nden toplanan *Chara hispida* L.’dan hazırlanan ekstrelerin antibakteriyel, antialgal aktiviteleri ile ilişkisindeki ve tuzlu su karidesine karşı toksisiteleri incelenerek elde edilen sonuçlar değerlendirilmektedir.

Anahtar Kelimeler: *Chara hispida*, Antialgal aktivite, Antibakteriyel aktivite, Tuzlu su karidesi, Ilişkisitesi

Abstract: *Chara hispida* L. (Characeae), a species of Chlorophyta division, is a multicellular alga growing in fresh-waters and swamps. The studies on *Chara* species which are quite rich in calcium carbonate showed that these species contain gibberelic acid, peptide, protamine, sterol and fatty acids.

A lot of compounds obtained from marine and fresh-water algae have been used in biomedical area and cosmetic products. Although Turkey is quite rich in regard with marine and fresh-water resources, there are a few studies on aquatic species.

In this research, the results obtained by examining antibacterial, and antialgal activities along with ichthyotoxicity and toxicity against brine shrimp of the extracts prepared from *Chara hispida* L. on which no biological activity study has been carried out previously, collected from Salda Lake, are evaluated.

Key Words: *Chara hispida*, antialgal activity, antibacterial activity, Brine Shrimp, ichthyotoxicity
Introduction

As oceans cover 70% of the earth, they constitute a great part of ecosystem which hosts numerous species of organisms. A lot of compounds obtained from marine and fresh-water algae have been used in biomedical area and cosmetic products. Although Turkey is quite rich in regard with marine and fresh-water resources, there are a few studies on aquatic species. Chara hispida L. (Characeae), a species of Chlorophyta division (Van den Boek C. et al. 1995), is a multicellular alga growing in fresh-waters and swamps. The studies on Chara species which are quite rich in calcium carbonate showed that these species contain gibberellic acid, peptide, protamine, sterol and fatty acids. (Kazmierczak et al., 1999, Kazmierczak, 1999, Maszewski, 1999, Khaliq-Uz-Zaman et al., 1998, Reynolds, and Wolfe, 1984, Gosek and Kwiatkowska, 1991).

The biological activities screened in this study are important and useful in water ecology studies. For instance; the aquatic plant extracts or pure compounds isolated from these plants with antimicrobial activity have a possible role as defense against both microbial epibionts and larval settlement which is induced more favorably in the presence of bacterial film. Microorganisms and microalgae, the most common epibionts, might affect photosynthesis since clean leaves have a higher photosynthesis rates compared to heavily epiphytized ones. Antimicrobial agents produced by aquatic plants can act as fouling retardants. On the other hand, algalgal test helps establishing the interactions among marine organisms through the excretion of growth-inhibiting substances which have been suggested to play an important role in ecology.

In this study, the results obtained by examining antibacterial and antialgal activities along with ichthyotoxicity and toxicity against brine shrimp of the extracts prepared from Chara hispida L. of Salda Lake, on which no biological activity study has been carried out previously, are described.

Materials And Method

Collection and identification of the algal species Fresh samples of C. hispida L. were collected from Salda Lake, Burdur, Turkey in July, 2001. The material were stored freshly in formaldehyde solution at the Department of Biology, Faculty of Education, Gazi University, Ankara, Turkey.

Preparation of the crude extracts of C. hispida L.

The plant material was air-dried, powdered and weighed accurately. The materials were macerated with petroleum ether, chloroform, ethyl acetate and methanol, subsequently, for two days at room temperature. The extracts were filtered and concentrated to dryness under vacuum.

Test organisms

Gram-positive bacteria Staphylococcus aureus (DMST 4746) and Bacillus subtilis (DMST 5871), and gram-negative bacteria Vibrio parahaemolyticus (DMST 5665) and Escherichia coli (DMST 4212) used in antibacterial activity test were obtained from the culture collection of Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand. The microalgae Nitzschia sp. and Chlorella sp. were provided by
Faculty of Fisheries of Kasetsart University. Eggs of brine shrimp *Artemia salina* were purchased from the INVE company (Belgium) and hatched in the laboratory. Guppy fish *Lebistes reticulatus* used in the piscicidal activity test and prawn *Macrobranchium lancesteri* were scooped from the fish pond of Faculty of Fisheries of Kasetsart University, Thailand.

**Biological activity assays**

Antibacterial and antialgal activity by disk diffusion method, (Chan, 1980, Harrison and Chan, 1980, Tringali, 1991) ichthyotoxicity by using guppy fish *Lebistes reticulatus* and general toxicity tests against brine shrimp *Artemia salina* and the Riceland prawn *Macrobranchium lancesteri* de Man were carried out on the crude extracts of *C. hispida* according to the methods given in references.

**Results and Discussion**

According to the results we have obtained, petroleum-ether, chloroform and methanolic extracts of *C. hispida* showed moderate activity against *A. salina* at 100 mg/L concentration. In this test, the most active extract was the ethyl acetate extract, killing 5 larvae of *A. salina*. None of the extracts had antialgal activity. In the antibacterial test, chloroform and ethyl acetate extracts of *C. hispida* displayed activity only against *B. subtilis* at 500 μg/disk, causing 17 mm (in diameter) inhibition zone. Ethyl acetate extract of *C. hispida* possessed strong ichthyotoxicity against guppy fish (*Lebistes reticulatus*) at 100 and 500 μg/L concentrations, killing one fish for each concentration within 4 hours. The same extract was also toxic to Riceland prawn *Macrobranchium lancesteri* at 500 μg/L (Table 1).

As mentioned previously, nature has provided aquatic organisms with a number of noxious substances for both offensive and defensive purposes since they are sessile organisms and lack obvious mechanical devise. (Tringali, 1991, Meyer et at, 1982, Sam, 1993, Lincoln et al.,1996, Wongrat, 1996) In addition, the study of marine toxins has the utilitarian purpose of discovering compounds which are of potential therapeutic value, or can be used as pharmacological tools in biological and medical sciences. Among the extracts with various polarity of the fresh-water alga *C. hispida*, ethyl acetate extract is the most active one and deserves further phytochemical investigation in order to find out its active components. To best of our knowledge, this is the first study on biological activities of *C. hispida*. 

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Table 1. Biological activity results of the fresh-water alga *Chara hispida*.

<table>
<thead>
<tr>
<th>The extracts</th>
<th>Toxicity against <em>Artemia salina</em></th>
<th>Toxicity against <em>Macrobrachium lancersteri</em></th>
<th>Ichthyotoxicity</th>
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<tr>
<td></td>
<td>10 µg/L</td>
<td>100 µg/L</td>
<td>500 µg/L</td>
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<tr>
<td>CH-PE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>CH-CHCl&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>CH-EtOAc&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>CH-MeOH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>2</td>
<td>-</td>
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</table>

<sup>a</sup> Petroleum-ether extract of *C. hispida*, <sup>b</sup> Chloroform extract of *C. hispida*,<br> <sup>c</sup> Ethyl acetate extract of *C. hispida*, <sup>d</sup> Methanolic extract of *C. hispida*

References


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