Antagonistic activity of *Spirogyra micropunctata* against some multidrug resistant human pathogenic bacteria

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Abstract

The aim of present study is to investigate the antagonistic activity of *Spirogyra micropunctata* against multidrug resistant human pathogens. The test organisms include 3 Gram negative bacteria such as *Echerichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and 2 Gram positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. The algal cell mass was extracted in 90% Methanol and 90% Ethanol and further concentrations of 0.5, 1, 3, 5, 10, 20 mg/ml were made for each extract. Antagonistic effect was tested by using agar well-diffusion method. Methanolic crude extract showed strong antibacterial activity against all tested bacteria, while ethanolic crude extract showed moderate activity. These findings suggest the possibility of using the *Spirogyra micropunctata* as a novel source of natural antimicrobial agents in pharmaceutical industries.

Keywords: *Spirogyra micropunctata*, antimicrobial, extract, natural antimicrobial agents.

الفعالية المضادة لل* Spirogyra micropunctata* ضد بعض انواع البكتيريا الممرضه للإنسان والسائبة المقاومة للأدوية

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الخلاصة

الهدف من الدراسة الحاليه هو بحث نشاط طحلب *Spirogyra micropunctata* المضاد لبعض انواع البكتيريا الممرضه للإنسان والسائبة المقاومة للأدوية. الكائنات الحية تحت الاختبار تتضمن ثلاثة انواع من *Salmonella typhi*, *Klebsiella pneumoniae*, *Echerichia coli*, *Staphylococcus aureus*, و نوعين من البكتيريا الموجبة لملون غرام مثل *Staphylococcus epidermidis*. تم استخراج الكتلة الحية من الطحلب بعملية الاستخلاص ب 90% ميثانول و 90% أيثانول مع عمل تركيز مختلفة 0.5، 1، 3، 5، 10، 20 ملغ/مل لكل نوع مستخلص. تم اختبار تأثير الاضداد باستخدام طحلب *Spirogyra micropunctata* ونوعين من البكتيريا الموجبة لملون غرام مثل *Staphylococcus aureus*, *Staphylococcus epidermidis* باستخدام طاحب Agar well diffusion method. اظهرت الدراسه تأثير مضاد مناطق معينة وENSION في جميع انواع البكتيريا الممرضه للإنسان باستخدام طحلب *Spirogyra micropunctata* كمصدر للكائنات الممرضه في الصناعات الدوائية.

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Introduction

Nowadays the use of antibiotics increased significantly due to heavy infections of pathogenic bacteria and fungi which become resistant to drugs as a result of indiscriminate use of antibiotics. Resistance of pathogen to antibiotics has necessitated the development of new alter cation [1, 2]. There is an increasing demand for biodiversity in the screening programmes for selecting therapeutic drugs from natural products, the marine organisms especially seaweeds are of with interest [3]. Algae are primitive, autotrophic, chlorophyll- containing and non flowering organisms which have no true roots, stems, or leaves and capable to convert solar energy to chemical energy via photosynthesis [4]. Macroalgae are commonly refer to as seaweeds, are multi cellular plants growing in salts or fresh water (their size reach up to 60 m in length) [5] have been used as food stuff in Asia and coastal areas for centuries as it contains carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals essential for the human nutrition [6]. The huge algal diversity makes these organisms source of enrichment due to the presence of different compounds growing at various levels of conditions producing a variety of remarkable compounds collectively referred to as secondary metabolites [7]. These compounds possess interesting biological activities as antibacterial, antifungal, antiviral, antioxidant, antifeedant, antihelmintic and cytoxic agents [8]. In this investigation, antibacterial effects of macroalgae (Spirogyra micropunctata) belonging to chlorophyta, was studied against multidrug resistant bacterial pathogens (Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epiderimidis, Staphylococcus aureus).

Morphological Structure of Spirogyra micropunctata:

Spirogyra is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in fresh water areas. Spirogyra is un branched and the cell wall has two layers: the outer wall is composed of pectin that dissolves in water to make the filament slimy to touch while the inner wall is of cellulose. The chloroplast is serrated or scalloped, and spirally arranged, resulting the prominent and characteristic green spiral on each filament. Each chloroplast contains several pyrenoids, centers for the production of starches, appearing as small round bodies. It’s very common in relatively clean eutrophic water, developing slimy filamentous green mass. In spring spirogyra grows under water, but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubble between the tangled filaments. The filamentous mass come to the surface and become visible as slimy green mats [4].

Materials and methods:

Samples were collected from the depth of 30-40 cm from the slow running freshwater of the canal around University of Baghdad, Baghdad/ Iraq, and stored in plastic bags which transported to the laboratory and identified with the help of classical algal classification references [9, 10]. The collected algal samples were initially cleaned from epiphytes, necrotic parts. The samples were washed with fresh water to remove sand, salts and any adhering substances. The samples were rinsed with sterile water to remove any associated debris and air-dried. The dried samples were put in a mill in order to obtain powder which then stored in the refrigerator until the next step.

Preparation of alcoholic extract:

The alcoholic extract was prepared by soxhlet extraction according to Prakash [11]. In this process the dried powder of algae extracted by using 90% methanol and ethanol separately. The dried extract were collected and weighed to make different concentrations, then preserved in sterile test tubes at 4° C in refrigerator until required.

Bacterial strains:

Bacterial isolates used in this study were obtained from the Biology Department, College of Science University of Baghdad; they were Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epiderimidis and Staphylococcus aureus.

Antibacterial Assay:

Antibacterial test of algal extract were performed in vitro by using agar well diffusion method [12]. Nutrient agar was poured into numbers of petridishes, the entire agar surface of each plate was inoculated with bacterial cell’s. A number of wells were prepared in these plates, different concentrations of algal extract was introduced into those wells, and a control well was introduced with the organic solvent used in extraction. Plates were incubated at 37°C for 24-48hrs. The antibacterial activity was recorded by measuring the clear zone diameters of inhibition.
Results and discussion:
Evaluation of Antibacterial Activity:
The result obtained from the present study concerning the bioactivity of fresh water algae against different species of bacteria by agar well diffusion method were recorded in table-1 and -2.

Table 1-Antibacterial activity of methanolic crude extract of macro algae (inhibition zone was measured in millimeter).

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>20mg/ml</th>
<th>10mg/ml</th>
<th>concentrations</th>
<th>1mg/ml</th>
<th>0.5mg/ml</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>15.2</td>
<td>13.4</td>
<td>11.4, 9.3</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>18.8</td>
<td>15.0</td>
<td>11.3, 9.4</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>S. typhi</td>
<td>20.0</td>
<td>17.3</td>
<td>14.7, 13.0</td>
<td>11.0</td>
<td>9.6</td>
<td>_</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>15.2</td>
<td>11.3</td>
<td>9.0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>S. aureus</td>
<td>14.7</td>
<td>12.4</td>
<td>9.3</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

Table 2-Antibacterial activity of ethanolic crude extract of macroalgae (inhibition zone was measured in millimeter).

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>20mg/ml</th>
<th>10mg/ml</th>
<th>concentrations</th>
<th>1mg/ml</th>
<th>0.5mg/ml</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>13.3</td>
<td>11.6</td>
<td>10.0, 9.0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>13.3</td>
<td>11.7</td>
<td>10.0</td>
<td>9.3</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>S. typhi</td>
<td>12.3</td>
<td>11.0</td>
<td>9.3</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>13.1</td>
<td>10.8</td>
<td>9.1</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.7</td>
<td>11.6</td>
<td>9.5</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

The degree of activity was varied with references to concentration of algal extract. The methanolic crude extract showed strong antibacterial activity against all 5 tested bacterial organisms, and showed maximum inhibition against S. typhi with inhibition zone of 9.6, 11.0, 13.0, 14.7, 17.3, 20.0 mm in the concentrations 0.5, 1, 3, 5, 10, 20 mg/ml respectively, however the inhibition against both K. pneumoniae and E. coli with inhibition zone of 9.4, 11.3, 15.0, 18.8 mm and 9.3, 11.4, 13.4, 15.2 in the concentrations 3, 5, 10, 20 mg/ml respectively. S. epidermidis and S. aureus showed lowest inhibition zone of 9.0, 11.3, 15.2 mm and 9.3, 12.4, 14.7 mm in the concentration of 5, 10, 20 mg/ml respectively. Ethanolic crude extract showed moderate antibacterial activity against all 5 tested bacterial organisms. The maximum inhibition against K. pneumoniae and E. coli with inhibition zone of 9.3, 10.0, 11.8, 13.3 mm and 9.0, 10.0, 11.6, 13.3 mm in the concentration of 3, 5, 10, 20 mg/ml respectively, while the minimum inhibition against S. aureus, S. epidermidis and S. typhi with inhibition zone of 9.5, 11.6, 13.7 mm, 9.1, 10.8, 13.1 mm and 9.3, 11.0, 12.3 mm in the concentration of 5, 10, 20 mg/ml respectively. Therefore, methanol extract of S. micropunctata revealed very promising results of antibacterial activity.

Seaweeds are a great potential production of secondary metabolites, which are not found in terrestrial environment. Thus, marine algae are among the richest sources of known novel bioactive compounds [13, 14]. Antibacterial activity of methanolic and ethanolic crude extract with different concentrations (0.5, 1, 3, 5, 10, 20 mg/ml) has a great potential for the discovery of lead compounds that they could be used against infectious diseases and parasites [15]. Recent investigations on the antimicrobial efficiency was conducted [16] and reported that maximum antibacterial activity was observed in methanol followed by ethanol and aqueous water respectively and the choice of solvent for the extraction purpose was a helpful sign in determining antimicrobial activities. [17 - 20] investigated the methanolic extract of a blue green alga and two green algae which have shown good antimicrobial activity. [21] Has shown that in some stages of algal development of Spirogyra sp. in their cells contain bacteriostatic substances. The ability of marine algae to produce secondary metabolites of potential interest has been extensively documented [22]. According to earlier reports anti-bacterial activity depends on algal species, the efficiency of the extraction method, and the
resistance of the tested bacteria, Environmental factors also play a key role which can be associated with intra specific variability in the production of secondary metabolites and occasionally related to seasonal variations. Secondly, there may also be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods that would result in different susceptibilities of the target strains [23]. Most of the identified components with antimicrobial activity extracted from plant groups are aromatic or saturated, unsaturated fatty acids, organic compounds and phenolic compounds, and they are more soluble in methanol [24]. Similarly, in this study the methanol extracts exhibited higher activity followed by ethanol. The antibacterial activities of the algae tested could be attributed to the type and amount of free fatty acids which have a role in the overall defense against the studied pathogenic Gram-positive and Gram-negative bacteria [25]. The fats and fatty acids from marine algae may play an important role in the formation of many other bioactive secondary metabolites since some fatty acids have been shown to possess antibacterial activities, chemical nature of active extracts of algae is not so far totally explored [26, 27].

Our preliminary results suggest that antimicrobial activity observed against Gram-positive as well as Gram-negative bacteria could be due to more than one active principle. This hypothesis is to be further investigated and aimed for isolation and purification of active metabolites responsible for antimicrobial activity.

References:


