The antibacterial activity evaluation of *Cystoseira barbata* biomass and some alginates upon bacteria from oropharyngeal cavity

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VICTORIA BADEA*, DOINA PAULA BALABAN*, GABRIELA RAPEANU**, CORNELIU AMARIEI*, CIPRIAN FLORIN BADEA*

* “Ovidius” University of Constanta, Faculty of Dentistry Medicine, 7 Ilarie Voronca Street, Constanta, Romania, badea_victoria@yahoo.com, 0722880254
**Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, Galati, Romania

Abstract

The study was done to assess the antibacterial activity of the dry biomass from the brown algae *Cystoseira barbata* when compared to sodium and magnesium alginates isolated from the same algae.

The bacterial species tested in the present study were isolated from oropharyngeal cavity. Antimicrobial activity was observed through the radial diffusion technique by placing samples in the quantity of 30 mg directly on the culture media inoculated with the following bacterial strains: *Staphylococcus* coagulase negative species and *Streptococcus* sp. group D (Gram positive bacteria), *Klebsiella* sp. and *Pseudomonas* sp. (Gram negative bacteria) isolated from the oropharyngeal human cavity microbiota.

An increased sensitivity of Gram positive bacteria than Gram negative bacteria was observed. In the same time, bacteria included in *Pseudomonas* sp. with potential resistance set were resistant to all tested samples.

The highest antibacterial effect upon both Gram-positive cocci as well as Gram-negative bacilli was observed for magnesium alginate sample S2 which had the highest content of magnesium (70313 mgKg\(^{-1}\)).

Keywords: *Cystoseira barbata*; alginates, antibacterial activity, oropharyngeal human cavity bacteria

Introduction

Nowadays seaweed, ocean ecosystems and the great worldwide represent an inexhaustible reservoir of raw materials used in pharmaceutical, food industries, medicine and cosmetics. By their content, seaweed provides mostly needs agar, collagen, vitamins and sterols used in the pharmaceutical industry.

The existence of active principles with bactericidal effect and/or bacteriostatic contained by seaweed have been intentioned and used in traditional medicine many years ago [1,2]. These observations were taken over by modern medicine and in different corners of the world [3,4,5] continued to study the effect of some antibacterial extracts from algae such as alginates, sterols, catechic tannin, phenolic compounds.

It was studied and demonstrated that marine algae have not only an important antibacterial activity [6,7], but also antiviral [8, 9,10], antifungal [11,12], antihypertensive, antitumoral [13,14], immunomodulatory [15] activities.
In the Black Sea cohabitates a wide range of algae, including *Cystoseira barbata*-brown algae (large size 1.5-2m) that develops in the form of associations perennial, fixed on the sea depth rocks.

In literature, studies reported the existence of specific antibacterial properties of some extracts from this algae [16,17] from different origins (Turkey, Italy) but not studies were done until now in Romania.

Antimicrobial activity of several extracts of the algae *Cystoseira barbata* has been investigated by using hexane, methanol, dichloromethane and chloroform extracts against four Gram-positive bacteria, four Gram-negative bacteria and *Candida albicans* ATCC 10239 yeast. Hexane extracts showed higher antimicrobial activity than methanol, dichloromethane and chloroform extracts. The volatile oils of these algae did not remarkably inhibit the growth of tested microorganisms. In these volatile oils authors identified hydrocarbon compounds such as docosane (7.61%) and tetraatriacontane (7.47%), among others.

The antifungal and anti-aflatoxinogenic activity of the brown algae *Cystoseira tamariscifolia* has been studied against pathogenic strains of yeasts and moulds. The antimicrobial effect of different algae extracts (using methanol, ethanol, diethyl ether, hexane, chloroform and water) was investigated. Results from inhibitory tests showed that only ethanolic extracts present antimicrobial activity against moulds and yeasts. Results showed that the reduction on aflatoxin B1 biosynthesis was, respectively, about 25.4%, 37.6%, 75.8%, and 96.3% by 10, 25, 50, and 100 ppm of the ethanolic extract.

Starting from these considerations, this study aims to evaluate the antibacterial action of dry biomass and of some alginates extracted from the brown algae *Cystoseira barbata* from Black Sea (Romania).

**Materials and methods**

1. **Materials**

   The brown algae, *Cystoseira barbata* was harvested in the Vama Veche area during the October 2008, according to [15, 18, 19] related to the content of alginic acid which exists in the algae in great concentration only in April and October months.

   Dry biomass from algae was obtained by washing algae with sterile distilled water, dried at 46°C and milled.

   Sodium alginate was acquired by using the classical method of extraction from brown algae *Cystoseira barbata* (S1) [18].

   Three samples of magnesium alginate with the different concentrations of Mg [70313 mgKg⁻¹ (S2), 65300 mg Kg⁻¹ (S3), 56250 mgKg⁻¹ (S4)], were prepared from *Cystoseira barbata* by precipitation with absolute ethyl alcohol in the presence of magnesium chloride. Magnesium was fixed on the alginate via the carboxyl and hydroxyl groupings [19].

2. **The isolation and identification of bacterial species**

   The bacteria used as indicator microorganisms were isolated from pharyngeal exudates, saliva and gingival fluid products collected from patients from the Social Center of Dentistry of the Faculty of Dentistry Medicine of Constanta, Romania.

   The pathological samples isolated from patients were inoculated on blood-agar medium and AABTL (agar, blue thymol bromine, lactose) and the identification was done according to NCCLS standards.

   The motivation of choosing these bacterial species (*Staphylococcus sp.*, *Streptococcus sp.*, *Klebsiella sp.* and *Pseudomonas sp.*) has been justified on the one hand by the possibility to
The antibacterial activity evaluation of *Cystoseira barbata* biomass and some alginates upon bacteria from oropharyngeal cavity demonstrate the different antibacterial activity, generated by different wall structure when the Gram-positive bacteria is compared with Gram-negative bacteria. In the same time, the capsula presence at *Klebsiella sp.* may confer a greater resistance to the action of chemical factors.

3. **The antimicrobial activity assessment**

The antibacterial activity was evaluated upon Gram-positive cocci: *Staphylococcus sp.* and the *Streptococcus sp.*, but also on Gram-negative bacilli, *Klebsiella sp.* and *Pseudomonas sp.* The antimicrobial activity was quantified by using the tested substances in the quantity of 30 mg powder applied directly on Müller-Hinton agar medium in Petri dishes where selected bacteria were inoculated. Next, the Petri plates were incubated at 37°C during 24 hours. The results were interpreted on the same principle as for the qualitative antibiogram data (antibiotic sensitivity report). This data is based on the existence of a direct relationship between the sensitivity level to the tested substances and to the diameter size (D) of the zone inhibition developed around the substances tested.

**Results and discussions**

The antibacterial effect of the five tested substances (dry biomass algae, sodium alginate and three samples of magnesium alginates) was evaluated through diameter of the inhibition zones estimation (Table 1).

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>Staphylococcus</em> sp.</th>
<th><em>Streptococcus</em> sp.</th>
<th><em>Pseudomonas</em> sp.</th>
<th><em>Klebsiella</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry biomass algae</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Na-alginate (S1)</td>
<td>15</td>
<td>19</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Mg-alginate (70313 mgKg⁻¹ Mg) - (S2)</td>
<td>25</td>
<td>23</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Mg-alginate (65300 mgKg⁻¹ Mg) - (S3)</td>
<td>10</td>
<td>15</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Mg-alginate (56250 mgKg⁻¹ Mg) - (S4)</td>
<td>8</td>
<td>14</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

The dry biomass algae evolve a moderate antibacterial effect on Gram positive bacteria *Staphylococcus* species with a diameter of inhibition zone by 5 mm around the dry biomass algae (Fig. 1).

**Figure 1.** The antibacterial effect of dry biomass algae upon *Staphylococcus sp.*
A weak antibacterial effect on Gram negative *Klebsiella sp.* with 1 mm diameter of inhibition zone around the dry biomass algae was observed (Fig. 2). No effect was noticed on *Pseudomonas sp.* (Fig. 3).

![Figure 2](image1.png)

**Figure 2.** The antibacterial effect of dry biomass algae upon *Klebsiella sp.*

Also, the sodium alginate sample (S1) and the 70313 mg Kg\(^{-1}\), 65300 mg Kg\(^{-1}\), 56250 mg Kg\(^{-1}\) magnesium alginate samples (S2, S3 and S4) were evaluated for antibacterial activity. A moderate antibacterial effect of the sodium alginate (S1) on both Gram positive bacteria as well as upon the Gram negative bacteria was observed (Fig 4, 5, 6 bottom right). The magnesium alginate (S2) developed an increased antimicrobial activity on both types of bacteria, but induce a greater inhibition zone upon Gram-positive bacteria (coagulase negative *Staphylococcus sp.*) with a diameter of inhibition zone of D = 25 mm (Fig.4 top right).

![Figure 3](image2.png)

**Figure 3.** The antibacterial effect of dry biomass algae upon *Pseudomonas sp.*

![Figure 4](image3.png)

**Figure 4.** The antibacterial effect of sodium alginate (S1) and magnesium alginites (S2, S3 and S4) upon *Staphylococcus sp.*
The antibacterial activity evaluation of *Cystoseira barbata* biomass and some alginates upon bacteria from oropharyngeal cavity

Upon Gram negative bacteria *Klebsiella sp.*, the magnesium alginate had a lower antibacterial effect with a diameter of inhibition zone of \( D = 20 \) mm (Fig 5 right up).

![Figure 5](image)

**Figure 5.** The antibacterial effect of sodium alginate (S1) and magnesium alginates (S2, S3 and S4) upon *Klebsiella sp.*

When antibacterial effect of sodium alginate (S1) and magnesium alginates (S2, S3 and S4) on *Pseudomonas sp.* including bacteria with a special resistance to a large number of antibiotics, antiseptics and disinfectants was evaluated, a non-inhibition zone was observed after 24 hours of incubation, although after first 6 hours of incubation a little size of zone inhibition was noticed.

The occurrence of bacteria resistant to samples experimented in the zone where initially the bacterial growth of *Pseudomonas sp.* were inhibited, was observed around sodium alginate, as well as on magnesium alginate samples tested (Fig. 6).

![Figure 6](image)

**Figure 6.** The antibacterial effect of sodium alginate (S1) and magnesium alginates (S2, S3 and S4) upon *Pseudomonas sp.*

The microscopic examination of the bacterial cells developed in the initial inhibition zone (colonies of mutant bacteria resistant) reveals an “aggregation” phenomenon of bacteria. In fact, this “aggregation” is able to explain the existence of attraction forces between the bacterial walls modified due to the suppressive action of the dry biomass algae, sodium and magnesium alginates on the bacteria during the multiplication phase.

**Conclusions**

The Gram-positive cocci were more sensitive to all products obtained from the *Cystoseira barbata* alga, when compared to Gram-negative bacteria.
The highest antibacterial effect upon both Gram-positive cocci as well as Gram-negative bacilli was observed for magnesium alginate sample S2 which had the highest content of magnesium (70313 mgKg⁻¹).

By using magnesium alginate with the highest content of magnesium, the inhibition zone upon Gram-positive cocci was higher (D = 25 mm) when compared with the Gram-negative bacilli (D = 20 mm);

Bacteria with high resistance potential *Pseudomonas sp.* were resistant to all tested samples (powder, sodium and magnesium alginates) isolated from the *Cystoseira barbata* algae (Black Sea, Romania).

Microscopical studies demonstrated the possibility of bacterial wall modification into a “trauma” sense by the magnesium alginate effect.

This study demonstrates the possibility to use the dry biomass algae, sodium alginate and magnesium alginates obtained from the *Cystoseira barbata* algae (Black Sea, Romania) for the antibacterial action at the level oropharyngeal cavity.

Reference

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