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Short Communication

Antibacterial Activity of Two Brown Algae (*Cystoseria compressa* and *Padina pavonica*) Against Methicillin-Resistant *Staphylococcus aureus*

ABSTRACT

Aqueous and ethanolic extracts obtained from two brown algae (*Cystoseria compressa* (Esper) Gerloff et. Nizamuddin) and *Padina pavonica* (Linnaeus) Thivy) have been investigated for their ability to inhibit 35 hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Both aqueous and ethanolic extracts of the plant were effective on MRSA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic extract with the greatest antibacterial activity were those of *Cystoceria compressa* MIC 3.2-6.3 mg/mL and MBC 6.3-25 mg/mL, respectively.

Keywords*: Antibacterial activity, methicillin-resistant Staphylococcus aureus, brown algae*

1. INTRODUCTION

Staphylococcus aureus is one of the major causes of both community and hospital-acquired infections (1, 2). It produces numerous toxins, including super-antigens that cause unique diseases such as toxic shock syndrome and Staphylococcal scarlet fever (3). The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most serious issues in public health in developed countries because it does not only a high prevalence (<1-80%), but it has also become resistant to almost all the currently available antibiotics except teiocoplanin and vancomycin (4). The rapid development of resistance to vancomycin, the last resort antibiotics against MRSA, recently has been reported in several countries (5, 6).

Seaweeds provide a rich source of structurally diverse secondary metabolites. There are numerous of compounds derived from seaweeds with a broad range of biological activities, such as antibiotics, antivirals, antitumorals, and anti-inflammatories, as well as neurotoxins (7, 8). In western countries, seaweeds are mainly use as source of alginate, carrageenan and in addition to ingredients in the content of many beauty products. The greatest use of seaweeds in the worldwide is for food, most probably by reason of rich in non-digestible fibers, mineral salts, vitamins and protein, but low in fat content (9).

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To date, research on antimicrobial activity of seaweeds in Turkey is scarce. The aim of this study was to evaluate the antibacterial activity of two brown algae (Heterokontophyta) species; *Cystoseria compressa* (Esper) Gerloff et. Nizamuddin and *Padina pavonica* (Linnaeus) Thivy as wild-growing in Turkey against hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).

2. MATERIALS AND METHODS

2.1. Plant Materials

Seaweeds samples were collected at a depth of 1-2 m from the coast of Canakkale, Turkey in April, 2012 and were identified by Prof. Dr. Veysel Aysel from Dokuz Eylul University, Faculty of Science & Arts, Department of Biology, İzmir, Turkey. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris as described by Gonzales del Val et al. (10).

2.2. Microorganisms

Thirty-five clinical isolates of MRSA (BD01-BD35) were kindly provided by Research Hospital of Medical Faculty of Canakkale Onsekiz Mart University, Canakkale, Turkey and from Trakya University, Edirne, Turkey.

2.3. Preparation of extract

25 g of air-dried seaweed samples were extracted in 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24h by using Soxhlet equipment (11). The extract was filtered with Whatman filter, paper no.1, and the filtrate was evaporated under vacuum in a rotary evaporator at 55°C. The extract yield obtained was 7.2%. The dry extract, which was sticky and black, was stored in labeled sterile screw-capped bottles at -20 °C pending use. Prior to testing 2 g was dissolved in 0.4 L of dimethyl sulfoxide (DMSO) (5 mg/mL).

2.4. Screening for antimicrobial activity

The minimum inhibitory concentration (MIC) was determined by microdilution methods (12). The reconstituted extract was serially diluted two-fold in Mueller Hinton Broth (Oxoid,

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Hampshire, UK) medium. Duplicate tubes of each dilution (ranging from 50.0-0.1 mg/mL) were inoculated with $5x10^5$ cfu/mL of the best bacterial strain, and cultures incubated at 37°C for 48 h, minimum inhibitory concentration (MIC) was taken as the highest dilution (least concentration) of extract showing no detectable growth. Minimum bactericidal concentration (MBC) was determined by subculturing the best test dilution on the fresh extract-free solid medium and that yielded no single bacterial colony on a solid medium was taken as MBC.

3. RESULT AND DISCUSSION

Significant antibacterial activities, expressed as MICs and MBCs, of crude extracts obtained from *Cystoseria compressa* and *Padina pavonica* against the 35 hospital of MRSA are listed in Table 1.

Ethanolic extracts of *Cystoseria compressa* were the most active against the MRSA isolates with MICs and MBCs of 3.2–6.3 and 6.3–25 mg/mL, respectively. Notably, antibacterial assay indicated that the ethanol extracts of the algae used in this study were more efficient than those of the aqueous extracts. It is important to bear in mind that the concentration of extract used in this test may be correlated with high activity of its chemical components.

In previous study, it is determined that ethanol extract of *Padina pavonica* show antibacterial activity only against *Bacillus subtilis* (10). In another study, acetone, methanol and diethyl ether extracts of *P. pavonica* had no antibacterial or antifungal activities, but the ethanol extract of *P. pavonica* showed weak activity against *Candida* sp., *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* (13). In contrast, the methanol extract of *P. pavonica* inhibited both bacteria and the yeast cultures in another study (14). The methanol extracts shown average activity against *Staphylococcus aureus* ATCC 6538P (inhibiton zone is 12.8 mm). According to the findings obtained from present study, the ethanol extracts of the alga shown antibacterial activity against the MRSA isolates, with MICs and MBCs of 6.3–12.5 and 12.5–50 mg/mL, respectively. The differences between present results and the others may be due to several factors, for example the intra-specific variability in the production of secondary metabolites (15, 16). Also, as reported that efficacy of macro algae extracts against microorganisms is mostly influenced by factors such as location and seasonality (15).

The antimicrobial effect of several types of extracts (methanol, ethanol, diethyl ether, hexane, chloroform and water) of *Cystoseria tamariscifolia* (Hudson) Papenfuss was carried out on yeasts and only ethanol extracts showed antimicrobial activity against yeasts (17). In another

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study, extracts (methanol, dichloromethane, hexane and chloroform) obtained from *Cystoseria barbata* (Good et Woodw.) J.Agardh did not remarkably inhibit test microorganisms (18). Taskin et al. (19) reported that the methanolic extracts of *Cysteseria barbata* have shown broader activity spectrum against all the test microorganisms. The extracts showed moderate activity against *S. aureus* ATCC 6538P with inhibition zone at 12.66±0.57 mm. In our previous study, the methanolic extracts of *C. compressa* have high antibacterial activity against *S. aureus* ATCC 6538P with inhibition zone at 17.8 mm (14). According to the results obtained from in this study, ethanolic extracts *C. compressa* have high antibacterial activity against the MRSA isolates, with MICs and MBCs of 3.2–6.3 and 6.3–25 mg/mL, respectively. The differences between this study and the others may be due to species variation. Also, there may be differences in the extraction protocols to recover the active metabolites and differences in the assay methods.

Among the algal substances, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted (20). Terpenoids, polyphenols and C_{11} metabolites are broadly distributed among brown seaweeds (21). Several authors had found antibacterial activities of algae due to fatty acids. The mentioned substances may be responsible for the antimicrobial activity in seaweeds used in this study. So, these brown algae extracts should be analyzed further, as it might provide a new compound effective against pathogens. According to the latest report from the National Nosocomial Infection Surveillance System (NNISS), approximately 60% of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, representing an 11% increase in resistance compared to the preceding 5-year period (22).

5. CONCLUSIONS

The results clearly show that seaweeds are interesting source of biologically active compounds that may be useful for therapy against the MRSA infections. The most active extracts can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

Table 1. Antibacterial activity of the extracts of the algae on Methicillin-Resistant *Staphylococcus aureus*

	Aqueous extract	Ethanolic extract

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Plant tested	MIC	MBC	MIC	MBC
Cystoseria compressa	6.3–12.5	12.5–25	3.2–6.3	6.3–25
Padina pavonica	12.5–25	25–50	6.3–12.5	12.5–50

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