British Microbiology Research Journal
4(8): 918-923, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

Gorkem Dulger[1*] and Basaran Dulger[2]

[1]Department of Medical Biology, Faculty of Medicine, Duzce University, 81620 Konuralp/Duzce, Turkey.
[2]Department of Biology, Faculty of Science and Arts, Duzce University, 81620 Konuralp/Duzce, Turkey.

Authors' contributions

This work was carried out in collaboration between both authors. Author GD designed the study, did the field work samples collection, laboratory work. Authors BD participated in the literature search, was also involved in some laboratory work. Both authors read and approved the final manuscript.

Short Communication

Received 28th March 2014
Accepted 19th April 2014
Published 6th May 2014

ABSTRACT

Aqueous and ethanolic extracts obtained from two brown algae (*Cystoseira 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 *Cystoseira compressa* MIC 3.2-6.3mg/mL and MBC 6.3-25mg/mL, respectively.

Keywords: Antibacterial activity; methicillin-resistant Staphylococcus aureus; brown algae.

**Corresponding author: Email: gorkemdulger@yandex.com;*

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 have 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 used 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].

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; *Cystoseira 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 1-2m 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 Extracts

25 g of air-dried seaweed samples were extracted in 150mL of 95% ethanol (Merck, Darmstadt, Germany) and 150mL of distilled water for 24h by using Soxhlet equipment, separately [11]. The extracts were filtered with Whatman filter, paper no.1, and the filtrates were evaporated under vacuum in a rotary evaporator at 55°C. The yields obtained were 7.2% for the ethanol extract and 6.8% for the aqueous extract**.** The dry extracts, which were sticky and black, were stored in labeled sterile screw-capped bottles at -20°C pending use.

Prior to testing, 2g from each extract was dissolved in 0.4L of dimethyl sulfoxide (DMSO) (5mg/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, Hampshire, UK) medium. Duplicate tubes of each dilution (ranging from 50.0-0.1mg/mL) were inoculated with $5x10^5$cfu/mL of the best bacterial strain, and cultures incubated at 37°C for 48h, 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. RESULTS AND DISCUSSION

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

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

Plant tested	Aqueous extract		Ethanolic extract	
	MIC	MBC	MIC	MBC
Cystoseira 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

Ethanolic extracts of *Cystoseira compressa* were the most active inhibitors against the MRSA isolates with MICs and MBCs of 3.2–6.3 and 6.3–25mg/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 12.8mm). 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 12.5–50mg/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 *Cystoseira tamariscifolia* (Hudson) Papenfuss was carried out on yeasts and only ethanol extracts showed antimicrobial activity against yeasts [17]. In another study, extracts (methanol, dichloromethane, hexane and chloroform) obtained from *Cystoseira 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±0.57mm. In our previous study, the methanolic extracts of *C. compressa* have high antibacterial activity against *S. aureus* ATCC 6538P with inhibition zone at 17.8mm [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 6.3–25mg/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.

ACKNOWLEDGEMENTS

The authors are thankful to Prof.Dr. Veysel Aysel for the identification of seaweeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: Genetic basis. Microbiol Rev. 1987;51(1):88-134.
2. Petinaki E, Miriagou V, Tzouvelekis LS, Pournaras S, Hatzi F, Kontos F, Maniati M, Maniatis AN. Methicillin-resistant *Staphylococcus aureus* in the hospitals of central Greece. Int J Antimicrob Agents. 2001;18:61-65.

3. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev. 2000;13(1):16-34.
4. Hwang JM, Yeom SH, Jung KY. Synthesis of oxazolidinone phosphonates as antibacterial agents. J Ind Eng Chem. 2007;13(3):474-479.
5. Isnansetyo A, Cui L, Hiramatsu K, Kamei Y. Antibacterial activity of 2,4-diacetylphloroglucinol produced by *Pseudomonas* sp. AMSN isolated from a marine alga, against vancomycin-resistant *Staphylococcus aureus.* Int J Antimicrob Agents. 2003;22:545-547.
6. Lee HW, Yoon JH, Sohn JH, Lee KH, Yeh BI, Park DW, Kim HW, Choi JW. Detection of MecA gene in clinical isolates of *Staphylococcus aureus* by multiplex-PCR, and antimicrobial susceptibility of MRSA. J Microbiol Biotechnol. 2003;13: 354-359.
7. Scheuer PJ. Some marine ecological phenomena: chemical basis and biomedical potential. Science 1990;248:173-177.
8. Attaway DH, Zarborsky OR. Marine biotechnology, pharmaceutical and bioactive Natural Products. Plenum Press, New York. 1993;459.
9. Bongiorni L, Pietra F. Marine natural products for industrial application. Chem Ind. 1996;2:54-58.
10. Gonzales del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, Vicente F, Portillo E, Jimenez del Rio M, Reina GG, Pelaez F. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int Microbiol. 2001;4(1):35-40.
11. Khan NH, nur- E Kamal MSA, Rahman M. Antimicrobial activity of *Euphorbia thymifolia* Linn. Indian J Res. 1988;87:395-397.
12. CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Eleventh Edition. CLSI Document M0 – A11, Wayne: Pennsylvania, USA; 2012.
13. Tuney I, Cadirci BH, Unal D, Sukatar A. Antimicrobial activities in extracts of marine algae from the coast of Urla (Izmir). Tr J Biol. 2006;30:171-175.
14. Dulger B, Hacioglu N, Erdugan H, Aysel V. Antimicrobial activity of some brown algae from Turkey. Asian J Chem. 2009; 21(5):4113-4117.
15. Febles CI, Arias A, Gil-Rodriguez MC, Hardisson A, Lopez AS. In vitro study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuario del Estudios Canarios. 1995;34:181-192.
16. Tariq VN. Antifungal activity in crude extracts of marine red algae. Mycol Res. 1991;95:1433-1440.
17. Zinedine, A. Elakhdari S. Faid M. Benlemlih M. Antifungal and anti-aflatoxinogenic activity of the brown algae *Cystoseira tamarisscifolia*. Journal de Mycologie Medicale. 2004;14(4):201-205.
18. Ozdemir G, Horzum Z, Sukatar A, Karabay-Yavasoglu NU. Antimicrobial activities of volatile components and various extracts of *Dictyopteris membranaceae* and *Cystoseira barbata* from the Coast of Izmir, Turkey. Pharm Biol. 2006;44:183-188.
19. Taskin E, Ozturk M, Taskin E, Kurt O. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr J Biotechnol. 2007;6:2746-2751.
20. Moreau J, Pesando D, Caram B. Antifungal and Antibacterial screening of Dictyotales from the French Mediterranean coast. Hydrobiologia. 1984;116(117):521-524.
21. Schnitzler I, Pohnert G, Hay M, Boland W. Chemical defense of brown algae (*Dictopteris* spp.) against the herbivorous amphipod *Ampithoe longimana*. Oecologia 2001;126:515-521.

22. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. American Journal of Infection Control. 2004;32:470-485.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=489&id=8&aid=4480