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Full Length Research Paper

Antibacterial effect of the brown alga *Cystoseira trinodis*

Saeed Tajbakhsh[1,2]*, Mahsa Ilkhani[3], Abdolhossein Rustaiyan[4], Kambiz Larijani[4], Kohzad Sartavi[5], Rahim Tahmasebi[6] and Golandam Asayesh[1]

[1]The Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Sciences, Bushehr, Iran.
[2]Department of Microbiology and Parasitology, Faculty of Medicine, The Persian Gulf Tropical and Infectious Diseases Research Center, Bushehr University of Medical Sciences, Bushehr, Iran.
[3]Department of Marine Chemistry, Faculty of Marine Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran.
[4]Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[5]Jahad Keshavarzi Research Center, Bushehr, Iran.
[6]Faculty of Health, Bushehr University of Medical Sciences, Bushehr, Iran.

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It is understood that bacteria can become resistant to the existing antibiotics. Thus, finding the new antibacterial substances is an important necessity. Since algae have been known to contain biologically active compounds, in the present investigation we attempted to study of antibacterial effect of the brown alga *Cystoseira trinodis* harvested from the Persian Gulf. This investigation was an *in vitro* study. The activity of the extract of *C. trinodis* was examined against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). The minimum inhibitory concentration (MIC) of the extract was determined for each test strain. Extract of *C. trinodis* showed antibacterial activity against all test organisms. The MIC of extract was 1.031 mg/ml for *S. aureus*, 0.687 mg/ml for *S. epidermidis*, 4.125 mg/ml for *E. coli*, and 6.6 mg/ml for *P. aeruginosa*. The extract was active against both gram-positive and gram-negative species which were tested in this study. *C. trinodis* could be a useful natural resource for preparation of antibacterial agents.

Key words: Antibacterial effect, *Cystoseira trinodis*, alga.

INTRODUCTION

There are biological and chemical diversities in the marine environment; therefore, marine algae contain numerous structurally novel and biologically active compounds (Sandsdalen et al., 2003; Tüney et al., 2006). They are used for the industrial production of some substances such as alginate, carrageenan, and agar-agar (Taskin et al., 2007; Rajasulochana et al., 2009). Some bioactive metabolites derived from algae are brominated, aromatics, nitrogen-heterocyclic, sterols, proteins, and sulfated polysaccharides (Kolanjinathan et al., 2009). Since algae have been used in traditional medicine for a long time, many researchers are interested to evaluate pharmacological properties of these organisms. Evaluation of antioxidant property of brown alga *Colpomenia sinuosa* (Lekameera et al., 2008), antiulcer effects of plastoquinones from the brown algae *Sargassum micracanthum* (Mori et al., 2006), antitumor activity of *Sargassum oligocystum* water extract (Zandi et al., 2010a) and *Gracilaria corticata* (Zandi et al., 2010b), antifungal activity of *Cystoseira mediterranea* (Taskin et al., 2010), antiprotozoal activity of compounds from the red alga *Asparagopsis* against *Leishmania* (Genovese et al., 2009), and antiviral activity of *Symphyocladia latiuscula* (Park et al., 2005) and *Cystoseira myrica* (Zandi et al., 2007) against herpes simplex virus type-1, are examples of numerous studies which have been performed on algae. Bacterial infections cause high rate mortality in humans worldwide. It is well known that bacteria can exhibit

*Corresponding author. E-mail: tajbakhshsaeed@yahoo.com or tajbakhsh@bpums.ac.ir. Tel: +98-917-774 6164. Fax: +98-771-2520657.

Abbreviations: MIC, Minimum inhibitory concentration; **ATCC,** American type culture collection; **CFU,** colony forming units; **GC/MS,** gas chromatography-mass spectrometery.

Table 1. Antibacterial activity and MIC of the extract of *C. trinodis*

Microorganisms	Antibacterial activity of extract	MIC (mg/ml)
S. aureus ATCC 25923	+	1.031
S. epidermidis ATCC 14990	+	0.687
E. coli ATCC 25922	+	4.125
P. aeruginosa ATCC 27853	+	6.6

+: Shows activity.

resistance to the available antibiotics. Thus, there is an increasing need for new antibacterial substances (Sandsdalen et al., 2003). Antibacterial properties of marine algae have been extensively investigated in many studies (Nagayama et al., 2002; Tüney et al., 2006; Taskin et al., 2007; Rajasulochana et al., 2009; Taskin et al., 2010). *C. trinodis* belongs to the class Phaeophyceae and we had access to this brown alga in the Bushehr coast of Persian Gulf (south west of Iran). The purpose of our work was *in vitro* investigation of antibacterial effect of *C. trinodis* from the Persian Gulf.

MATERIALS AND METHODS

Collection of alga and preparation of extract

The sample of *C. trinodis* was collected from the Bushehr coast of Persian Gulf (south west of Iran) in August 2009. The alga was rinsed with distilled water to remove epiphytes and sediments. For extraction, the algal sample was put into a mixture of diethyl ether, ethanol and normal hexane (1:1:1) for 48 h. After filtration, the extract was concentrated by a rotary evaporator.

Test organisms and maintenance procedure

Four ATCC bacterial strains in our study were *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922). These strains were cultured on brain heart agar (Merck, Darmstadt, Germany). The grown bacteria were then picked from the medium, and maintained in skim milk (Merck, Darmstadt, Germany) containing 10% glycerol (Merck, Darmstadt, Germany) at -20ºC until testing (Tajbakhsh et al., 2008).

Antibacterial activity assay

Fresh cultures of aforesaid microbial strains were prepared. Antibacterial activity of extract against *S. aureus* and *E. coli* was investigated by following procedure: in the test tube containing Mueller Hinton broth (Merck, Darmstadt, Germany), the extract with concentration of 8.25 mg/ml was tested on a bacterial concentration of 5×10^5 colony forming units (CFU)/ml (Forbes et al., 2007). Also, a tube of Mueller Hinton broth containing the identical bacterial concentration, but lacking the extract, was used as growth control, and another tube of the uninoculated Mueller Hinton broth containing the same concentration of the extract was utilized as negative growth control (Forbes et al., 2007). In addition, a tube of Mueller Hinton broth containing the same bacterial concentration and solvents, but lacking the extract, was used to control and rule out antibacterial activity of the solvents in the test tube. The test tube as well as control tubes were incubated at 37ºC. After 24 h incubation, antibacterial effect of the extract in the test tube was distinguished by lack of turbidity (similar the negative growth control) which show the inhibition of microbial growth (Talaro and Talaro, 2002; Forbes et al., 2007). Examination of antibacterial activity of the extract against *S. epidermidis* and *P. aeruginosa* was also carried out by the same procedure except that the concentrations of extract in the test tubes of *S. epidermidis* and *P. aeruginosa* were 5.5 and 6.6 mg/ml, respectively.

Minimum inhibitory concentration (MIC)

The technique for determination of MIC of the extract was broth dilution method (Talaro and Talaro, 2002; Forbes et al., 2007). The MIC of extract for *S. aureus* and *E. coli* was determined as follows: the extract was added as serial dilutions to a series of tubes containing Mueller Hinton broth so that the concentrations ranged from 8.25 to 0.032 mg/ml.

The bacterial concentration in all tubes was 5×10^5 CFU/ml. Control tubes were also prepared as aforementioned. MIC of the extract for *S. epidermidis* and *P. aeruginosa* was determined by the same method, but the concentrations of the extract ranged from 5.5 to 0.021 mg/ml for testing on *S. epidermidis* and from 6.6 to 0.026 mg/ml for testing on *P. aeruginosa*. All test and control tubes were incubated at 37ºC for 24 h. After incubation, the lowest concentration of extract that causes inhibition of bacterial growth was considered as MIC.

RESULTS

Table 1 shows the results of antibacterial effect of the extract from *C. trinodis* against *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). In the first step of study, strikingly, the extract showed antibacterial activity on all the four test bacteria so that the concentration of 8.25 mg/ml of extract was effective against *S. aureus* and *E. coli*; also the concentrations of 5.5 and 6.6 mg/ml of the extract showed activity against *S. epidermidis* and *P. aeruginosa*, respectively. In the next step of study, the MIC of extract for each test organism was determined (Table 1).

The MIC of extract was 1.031 mg/ml for *S. aureus*, 0.687 mg/ml for *S. epidermidis*, 4.125 mg/ml for *E. coli*, and 6.6 mg/ml for *P. aeruginosa*. Therefore, we found that the MIC for gram-positive bacteria was lower than the MIC for gram-negative bacteria.

DISCUSSION

Marine algae are among the major source of various compounds which may be useful for humans. Some algae have been used as food resource because they contain a significant amount of proteins, carbohydrates, unsaturated fatty acids and minerals (Fayaz et al., 2005). It should be also emphasized that an abundant bioactive substances are produced by algae. These substances could have medicinal properties including antibacterial effect (Sandsdalen et al., 2003). In the present study, we attempted to evaluate the antibacterial activity of *C. trinodis* from the Persian Gulf against *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. The mentioned bacteria have been used as test microorganisms in many studies in order to find antibacterial substances (Sandsdalen et al., 2003; Tüney et al., 2006; Govindarajan et al., 2008; Tajbakhsh et al., 2011). In our study, fresh algal sample was used for extraction. It has been reported that extracts obtained from fresh algae exhibit more antibacterial activity than extracts from air-dried algae because some substances may loss during drying process. For example, it has been shown that extracts of fresh *Gracilaria gracilis* and *Ectocarpus siliculosus* exhibit antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa* and *Enterococcus faecalis*, however, extracts from dried samples of these algae do not show antibacterial activity on mentioned tested bacteria (Tüney et al., 2006). Results of the present study revealed that the extract of *C. trinodis* was effective against all test organisms, but the MIC for gram-positive species was lower than the MIC for gram-negative species (Table 1). Thus, in this study, gram-positive bacteria were more susceptible to extract of *C. trinodis* than gram-negative bacteria. More susceptibility of gram-positive species to extract of *Sargassum oligocystum* has also been reported previously (Tajbakhsh et al., 2011). Tüney et al. (2006) used methanol, acetone, diethyl ether, and ethanol for extraction from several marine algae such as *C. mediterranea* collected from the coast of Urla (Izmir, Turkey). In their study, diethyl ether was the best solvent for extracting the antimicrobial materials from the algae. Similar to our results, extract of *C. mediterranea* exhibited antibacterial activity on all test bacteria including *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. In another study conducted by Taskin et al. (2010), methanol extract of *C. mediterranea* harvested from the eastern Mediterranean sea was shown to be effective against *E. coli* O157:H7.

Antibacterial activity of methanolic extract of *C. barbata* from the Aegean Sea (Turkey) was investigated in another study (Taskin et al., 2007). Like our results, *C. barbata* exhibited antibacterial effect against all test bacteria including *S. aureus* and *E. coli*. Chiheb el al. (2009) investigated the antibacterial activities of methanol extract from several species of *Cystoseira* which were collected from the coast of Morocco. Similar to our results, extract of *C. tamariscifolia* showed activity against *S. aureus*, *E. coli*, and other test bacteria. Also, extracts of *C. mediterranea*, *Cystoseira humilis*, and *Cystoseira usneoides* had antibacterial effect on *S. aureus* and *E. coli*. Whereas, in contrast to our work, extract of *Cystoseira crinita* was not effective against *E. coli*; and the extract from *Cystoseira compressa* did not show antibacterial effect on any of the test bacteria. It is obvious that there are both similarities and dissimilarities between the results of mentioned articles and our results.

The reasons for these dissimilarities could be the differences in various algal species or strains for production of substances, geographical zones and habitats, seasonal variations, life phase, as well as solvents and protocols used for extraction (Zheng et al., 2001; Sandsdalen et al., 2003; Tüney et al., 2006). The extract of *C. trinodis* from the Persian Gulf has been analysed by gas chromatography-mass spectrometery (GC/MS); among identified constituents, α-pinene (15.84%) was one of the major components (Ilkhani et al., article submitted for publication). Identification of chemical constituents of some plant materials such as essential oil of Thyme (*Thymus vulgaris*) (Imelouane et al., 2009) or essential oil isolated from fruits of *Prangos ferulacea* (Massumi et al., 2007) was also revealed that α-pinene is a major component. α-pinene has been shown to possess antibacterial effect (Dormans and Deans, 2000). Therefore, in our study, the antibacterial potential of the extract could be due to α-pinene.

Finally, we concluded that *C. trinodis* from the Persian Gulf could be a useful natural source for preparation of antibacterial compounds. In addition, using of this alga for further *in vivo* investigations is suggested.

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