Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):225-229

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial activities of the Tunisian marine brown algae *Cystoseira schiffneri*

Abdelkader Ben Salem[a,b], Adel Mahfoudhi[a,c], Meriem Garreb[d], Saousen Hammami[a], Mohamed Gorcii[d], Maha Mastouri[d] and Zine Mighri[a*]

[a]*Research Unit Applied Chemistry and Environment (UR13ES63), Faculty of Sciences of Monastir, University of Monastir, Tunisia*
[b]*Faculty of Sciences of Sfax, University of Sfax, Tunisia*
[c]*Faculty of Sciences of Bizerte, University of Carthage, Tunisia*
[d]*Laboratory of Microbiology, CHU Fattouma Bourguiba Monastir, Tunisia*

ABSTRACT

Marine organisms exhibit a rich chemical content that possesses unique structural features as compared to terrestrial metabolites. Among marine resources, marine algae are a rich source of chemically diverse compounds with the possibility of their potential use as a novel class of antimicrobial agents. The antimicrobial activities of Cystoseira schiffneri extracts were tested toward Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa. The ethyl acetate extracts for both fresh and dried Cystoseira schiffneri exhibited the highest activities with MICs values ranged from 0.078 to 0.729 mg/mL. The antifungal activities were evaluated against Candida albicans, Candida krusei, Candida parapsilosis and Candida glabrata. Ethyl acetate and dichloromethane extracts from fresh Cystoseira schiffneri recorded inhibitory activities higher than the positive control "fluconazole".

Key words: *Cystoseira schiffneri*; Extracts;; Antibacterial activities; Antifungal activities.

INTRODUCTION

Increasing resistances of clinically important bacteria and fungi to existing antibiotics are a major problem throughout the world. One of the ways of preventing antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents. Thus, the search for novel natural sources from marine ecosystems could lead to the isolation of new antibiotics [1].

Since the 1970s, more than 21,855 structurally diverse bioactive natural products have been discovered from marine microbes, algae and invertebrates [2]. Even though, the ocean covers more than 70% of the earth's surface, less than 10% of the total ocean area are explored [3].

Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols[4].

The algal family Cystoseiraceae (order Fucales, class Phaeophyceae) includes the genera *Acrocarpia*, *Acystis*, *Bifurcaria*, *Bifurcariopsis*, *Carpoglossum*, *Caulocystis*, *Coccophora*, *Cystophora*, *Cystoseria*, *Halidrys*, *Hormophysa*, *Landsburgia*, *Myriodesma*, *Scaberia* and *Stolonophora*. Among these *Cystoseira* and *Cystophora*, the richest in species, are the most representative of the family. *Cystoseira* is a genus of worldwide distribution with

about 80% of the species occurring along the Mediterranean and adjoining Atlantic coasts. Members of the genus *Cystoseira* generally synthesise tetraprenyltoluquinols[5].

In the present study, we report the antimicrobial activities of fresh and dried *Cystoseira schiffneri* extracts evaluated against the following pathogenic bacteria "*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*, and fungi"*Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*".

EXPERIMENTAL SECTION

2.1. Algae

Algal materials were collected, in May 2011, from the littoral zone of Chebba region, Governorate of Mahdia, Tunisia (Lat35° 14'14" N, Long 11° 6'54'' E) between 0.5–1.0 m depth.

The collected algal samples were initially washed thoroughly with seawater to remove the macroscopic epiphytes, sand particles and other extraneous matter and then rinsed with distilled water to remove salts.

This material was then air-dried under shade at room temperature for one month and then ground to a fine powder which was used for subsequent analysis.

The algal species were identified by Dr. Lotfi Mabrouk and Pr. Asma Hamza (National Institute of Marine Sciences and Technology (INSTM), Tunisia) and saved in the Laboratory of Applied Chemistry and Environment, Department of Chemistry, Faculty of Science of Monastir, University of Monastir, Tunisia.

2.2. Microorganisms

The tested pathogenic bacteria were *Staphylococcus aureus* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The pathogenic fungi were *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019).

2.3. Preparation of *Cystoseira schiffneri* extracts

2.3.1. Preparation of extracts from fresh *Cystoseira schiffneri*

200 g of fresh *Cystoseira schiffneri* were soaked three times in 200 mL of methanol for 72 h. Later the obtained samples were homogenized, filtered, and concentrated to dryness under vacuum. The residue was submitted to successive macerations with 200 mL each of ethyl acetate, butanol and water under the same methanolic extraction conditions.

2.3.2. Preparation of extracts from dried *Cystoseira schiffneri*

Extraction of *Cystoseira schiffneri* dried powder was done using dichloromethane, ethyl acetate, butanol and water. Aliquots of 780 g of the powdered algal samples were soaked three times in 500 mL of dichloromethane for 72 h. Later the soaked sample was homogenized, filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was submitted to successive macerations with 500 mL each of ethyl acetate, butanol and water under the same dichloromethane extraction conditions.

2.4. Antimicrobial assay

2.4.1. Antibacterial activity of *Cystoseira schiffneri* extracts

2.4.1.1. Determination of the minimum inhibitory concentration (MIC)

To determine the MICs of the studied extracts, we used the microdilution technique using 96-well ELISA plates round bottom [6, 7]. The initial extract concentration, prepared by dissolving in 10% DMSO, was 10 mg/mL. The antibacterial test for each sample is repeated three times.

The wells of column one and two were used as negative control: the wells of column one, 150 µL of the bacterial inoculum and those in column two is added, is placed 100 µL of 10% DMSO and 50 µL of the bacterial inoculum.
The last column of the wells was used as positive controls where each well contains 100 µL of 10% DMSO and 50 µL of broth. Is deposited in the wells of the third to the ninth column 100 µL of 10 % DMSO and is brought with the data for column three, 200 µL of the sample to be tested (10 mg / mL), then serially diluted in half each time realized by taking 100 µL of the column three, the diluent in the column four and so on until in column nine. Series of dilutions being made is placed in the treated wells 50 µL of the bacterial suspension tested.

Then all plates incubated in an incubator at 37°C for ten-eight hours then proceeds to their observation with the naked eye: the lowest concentration that gave an inhibition of bacterial growth in the minimum inhibitory concentration (MIC) of the sample with respect to the test strain.

2.4.1.2. Determination of the minimum bactericidal concentration (MBC)
MBC corresponds to the lowest concentration, which can kill 99.9% or more of the initial inoculums during twenty-four hours of incubation at 37°C [8, 9]. The MBC is determined by streaking, of 10 µL of the contents of each well whose concentration is equal or superior to the MIC, on nutrient agar.

2.4.2. Antifungal activity of *Cystoseira schiffneri* extracts
Growth inhibition of *Candida* specie was assessed using the disc diffusion method on solid medium (Sabouraud Chloamphenicol). Before each experiment, all reference species were grown on Sabouraud Dextrose Agar *(SDA; Merck, Germany)* for 24 h at 37◦C. Yeast cells were suspended in 5 ml of sterile saline solution (0.9%). The resulting yeast suspension was mixed and adjusted to 1 McFarland turbidity standard using a densitometer (*Densimat®, BioMérieux)*.

The dried surfaces of Sabouraud chloramphenicol agar in square Petri dishes were inoculated by 2-3 ml of each fungal suspension. Petri dishes were rotated approximately 60° to ensure a uniform distribution of inoculum. Next, the Petri dishes were incubated at 37°C for 15 min.

Sterile Whatman paper disks (diameter 6 mm, Whatman paper N° 1) were impregnated with 10µL of the tested samples (100 mg/mL). Discs impregnated with 10Ml of methanol (used solvent) were included as negative controls and discs impregnated with fluconazole (40 µg/disc) were included as positive controls.

All discs were placed aseptically on the inoculated Petri dishes that are subsequently incubated at 37°C for 24h.

After incubating Petri dishes at 37°C for 24 hours, the diameters of the inhibition zones (ϕ-IZ) surrounding the discs were measured [10].

RESULTS AND DISCUSSION

3.1 Preparation of extracts
Different methods were tried to prepare extracts from the brown algae *Cystoseira schiffneri*. The yields of fresh and dried algal material obtained by different extraction methods were compared. In general, the largest amount of dry material in all samples was obtained by the dried *Cystoseira schiffneri*. The yields of butanolic extracts were highest in all the organic solvent extracts (Table 1).

Table 1. Yields of *Cystoseira schiffneri* extracts

Cystoseiraschiffneri	Solvents	Yields (%)
Fresh algae	Dichloromethane (CF1)	0.07
	Ethyl acetate (CF2)	0.04
	Butanol(CF3)	0.39
Dried algae	Dichloromethane (C1)	0.19
	Ethyl acetate (C2)	0.16
	Butanol(C3)	0.30

3.2. Antibacterial activity of *Cystoseira schiffneri* extracts
Different solvent polarities, including dichloromethane, ethyl acetate, butanol and water, used for extracting the bioactive compounds from *Cystoseira schiffneri*, were screened for their antibacterial activities. The results of the antibacterial screening are presented in Table 2.

In order of solvent type, the highest activities were recorded for the ethyl acetate extracts for both fresh and dried *Cystoseira schiffneri*. MICs values were ranged from 0.078 to 0.156 mg/mL for fresh *Cystoseira schiffneri* extracts and from 0.312 to 0.729 mg/mL for dried *Cystoseira schiffneri* ones. Whereas, fresh *Cystoseira schiffneri* water extracts presented the lowest antibacterial activities against all the tested bacteria. The MICs values were between 0.625 and 3.281 mg/mL.

Table2. Antibacterial effects of *Cystoseira schiffneri* extracts (10 mg/mL) toward four pathogenic bacteria using the micro dilution method

	Extracts from fresh *Cystoseira schiffneri*			
	CF1	**CF2**	**CF3**	**CF4**
	MIC (mg/mL)			
Gram-positive bacteria				
S. aureus(ATCC25923)	0.625	0.078	1.25	2.5
E. feacalis(ATCC29212)	0.312	0.078	0.156	3.281
Gram-negative bacteria				
E. coli (ATCC25922)	0.312	0.078	0.520	2.5
P. aeruginosa(ATCC27853)	0.625	0.156 **	0.312	0.625
	Extracts from dried *Cystoseira schiffneri*			
	C1	**C2**	**C3**	**C4**
	MIC (mg/mL)			
Gram-positive bacteria				
S. aureus (ATCC25923)	1.25	0.312	1.25	5
E. feacalis(ATCC29212)	1.25	0.312	1.25	5
Gram-negativebacteria				
E. coli (ATCC25922)	2.5	0.729	2.19	0.625
P. aeruginosa(ATCC27853)	2.5	0.312	0.625	0.312

*CF1, C1: Dichloromethane; CF2, C2: Ethyl acetate; CF3, C3: Butanol; CF4, C4: water; ** MBC = 5 mg/mL*

From the results reported in the present study, it appears that, organic solvents always have higher efficiency in extracting antibacterial compounds compared to the water as extractant. These results are in accordance with those reported earlier by Faulkner (2002) [11] and Saleh Abdu-llah Al-Saif et al. (2014)[12].

4.2. Antifungal activities of *Cystoseira schiffneri* extracts

The results of the antifungal activities of *Cystoseira schiffneri* extracts toward the pathogenic *Candida* species are summarized in Table 3.

Table 3 revealed that the algal extracts prepared with dichloromethane, ethyl acetate and butanol inhibit the growth of the pathogenic Candida. However, water extracts of fresh and dried *Cystoseira schiffneri* did not record antifungal activity against all the tested pathogenic fungi.

Ethyl acetate and dichloromethane extracts from fresh *Cystoseira schiffneri* recorded inhibitory activities higher than fluconazole respectively toward *C. albicans* (ϕIZ = 15 mm) and *C. krusei* (ϕIZ = 20 mm).

Ethyl acetate extract from dried *Cystoseira schiffneri* possesses antifungal activity higher than fluconazole toward *C. glabrata* (ϕIZ = 20 mm).

Table3. Antifungal activities of *Cystoseira schiffneri* extracts (100 mg/ mL) towards four *Candida* species using the method of diffusion in a solid medium

	Extracts from fresh*Cystoseiraschiffneri*				Fluconazole
	CF1	**CF2**	**CF3**	**CF4**	
	ϕIZ (mm)				
C. albicans (ATCC90028)	10	15	10	0	10
C. parapsilosis (ATCC22019)	20	15	10	0	25
C. glabrata (ATCC2001)	7	0	7	0	15
C. krusei (ATCC6258)	20	10	10	0	10
	Extracts from dried*Cystoseiraschiffneri*				**Fluconazole**
	C1	**C2**	**C3**	**C4**	
	ϕIZ (mm)				
C. albicans (ATCC90028)	8	10	15	0	10
C. parapsilosis (ATCC22019)	20	15	10	0	25
C. glabrata (ATCC2001)	0	20	8	0	15
C. krusei (ATCC6258)	10	8	0	0	10

*CF1, C1: Dichloromethane; CF2, C2: Ethyl acetate; CF3, C3: Butanol; CF4, C4: water; ** MBC = 5 mg/mL*

The results reported in this study are in accordance with several published reports, which have demonstrated the antifungal potential of marine algae [13-15].

CONCLUSION

Our antimicrobial studies give a few indications of the presence of promising antibacterial and antifungal compounds in the algae *Cystoseira schiffneri*. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activities of these extracts against human bacteria and fungi pathogens.

REFERENCES

[1] E Sung-Hwan ; K Young-Mog ; K Se-Kwon, *Food ChemToxicol*, **2012**, 50, 3251-3255.
[2] JW Blunt ; BR Copp ; MHG Munro; PT Northcote ; MR Prinsep, *Nat. Prod. Rep.*, **2012**, 29, 144-222.
[3] RE Schultes, The kingdom of plants. In: Thomson, W.A.R. (Ed.), Medicines From The Earth. Mc Graw-Hill Book Co., New York, **1978**, p. 208.
[4] M Cabrita ; C Vale ; A Rauter, *Mar. Drugs*,**2010**, 8, 2301-2317.
[5] V Amico, *Phytochemistry*, **1995**, 39, 1257-1279.
[6] CLSI, Reference Method for broth dilution antifungal susceptibility testing of yeasts, 3rd ed. Approved Standard M27-A3. Wayne, PA: Clinical and Laboratory Standard Institute. **2008**.
[7] SM Mousavi; D Raftos, *Middle-East J. Sci. Res*., **2012**, 11, 156-161.
[8] CJ Fernandes ; MVN O'sullivan ;Y Cai;F Kong ; X Zeng ; GL Gilbert ; G Kotsiou, *Clin. Microbiol.*,**2007**, 45, 4018-4020.
[9] N Canillac ; A Mourey, *Food Control*., **2001**, 18, 261-268.
[10] KP Lutta; C Bii; AT Akenga; WC Wanjala. *Rec. Nat. Prod*., **2008**, 2, 116-127.
[11] DJ Faulkner, *Nat. Prod. Rep*., **2002**, 19, 1-48.
[12] S Saleh Abdu-llah Al-Saif; N Abdel-Raouf; H A El-Wazanani; I A Aref, *Saudi J BiolSci*, **2014**, 21, 57-64.
[13] DC Carter; RE Moore; JS Mynderse; WP Niemczura; JS Todd, *J. Org. Chem*, **1984**, 49,236-241.
[14] R De Felício ; S De Albuquerque ; MC Marx Young ; N SumieYokoya ; Debonsi HM, *J Pharm Biomed Anal*, **2010**, 52, 763-769.
[15] P Vijayabaskar; N Vaseela; G Thirumaran, *Chin J Nat Med.*, **2012**, 10, 421-428