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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF ORGANIC FRACTIONS OF SIX MARINE ALGAE FROM TUNISIAN MEDITERRANEAN COASTS

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ABSTRACT

Twenty four organic extracts obtained from six marine algae: *Cystoseira compressa, Cystoseira crinita, Cystoseira sedoides, Gelidium latifolium, Dictyopteris membranaceae* and *Halurus equisetifolius* collected from Tunisian Mediterranean coasts, were evaluated for their antibacterial and antifungal activities against eight human pathogenic bacteria and five human pathogenic yeast using the agar disk diffusion assay. The results showed that all marine algae extracts exhibited a moderate activity against four bacteria strains, *Staphylococus aureus, Echerichia coli, Staphylococcus epidermis* and *Micrococcus luteus.*. No activity was observed against *Staphylococcus thyphymerium, Pseudomonas aeruginosa, Enterococcus feacium* and *Listeria monocytogenes..* However, the chloroformic and the ethyl acetate extract obtained from *Cystoseira crinita* and *Cystoseira sedoides* showed a higher antifungal activity against four *Candida* strains. These finding suggest that the chloroformic and ethyl acetate extracts of the brown algae could be contained a new antifungal compound(s). The purification and the determination of chemical structure of compound(s) of these active extracts are under investigation.

Keywords: Antibacterial activity; Antifungal activity; Marine algae; *Cystoseira sp*.

INTRODUCTION

The marine environment is an exceptional reservoir of bioactive natural compounds, which exhibit structural/ chemical features not found in terrestrial natural products [1]. Several marine organisms produce bioactive metabolites in response to ecological pressures, including competition for space, the fouling of the surface [2]. In addition, they develop a chemical strategy for defence to ensure their survival, and to synthesize extremely active molecules, since having to act as aqueous medium much diluted [3].

Researchers have isolated approximately 7000 marine natural products, 25% of which are from algae, 33% from sponges, and 24% representatives of other invertebrates, molluscs, echinoderms [4]. This diversity has been the source of unique chemical compounds with the potential for industrial development as pharmaceuticals, cosmetics, nutritional supplements, and molecular probes. In recent years, a significant number of novel metabolites with pharmacological properties have been discovered from the marine organisms [5].

In the past few decades, macroalgae have been widely recognised as producers of a broad range of bioactive metabolites [6, 7]. Numerous reports have described active compounds derived from macroalgae which has a broad range of biological activities, such as antibiotics antifouling, anti-inflammatory, cytotoxic and antimitotic activity [8,9,10,11,12,13]. Harder [14] was the first who reported the antimicrobial substances secreted by algae. It was not until the 1970s that large-scale screening of antimicrobial activity was carried out [15,16,17]. However, the results obtained suggest that the production of antimicrobial substances by the same species varies [18]. This intraspecific variability may be due to ecology, the stage of active growth or sexual maturity [19]. To date, research on biologically active substances of Mediterranean seaweeds has been scarce [20,21].

The purpose of this work was to evaluate the antibacterial and antifungal activity of six Mediterranean marine macroalgae collected from the coastline of Tunisia, with various extraction solvents (Petroleum ether, chloroform, ethyl acetate and methanol) against eight human pathogenic bacteria and five human pathogenic yeast in order to discover new antibacterial or / and antifungal metabolites.

MATERIALS AND METHODS

Sample collection

Fresh six seaweeds: *Cystoseira compressa Cystoseira crinita*, *Cystoseira sedoides, Gelidium latifollium*, *Dictyopteris membranaceae* and *Halurus equisetifolius* were harvested at various sites along the Mediterranean Tunisian coastline (Tabarka, Bizerte et Monastir), during June 2006, at depth of 1- 3 m. The seaweeds were identified at the National Institute of Oceanology of Tunisia (INSTM). After collection, the samples were rinsed with fresh seawater and distilled water to remove associated debris and epiphytes. The cleaned material was then air dried to dryness in the shade at 30°C. The dried samples were finely powdered and stored at -20°C until use.

Preparation of extracts

For extraction of bioactive in shade dried seaweeds, 600 g of finely powdered algal material were packed in small bags (5x 10 cm) of Whatman filter paper # 1 and all bags were sealed and soaked three times in an organic solvent bath for steeping during 24h. The extraction was carried out, separately, with different organic solvents in the order of increase polarity: Petroleum ether, chloroform, ethyl acetate and methanol. The organic extracts were concentrated to solvent free by evaporation in a rotary vacuum evaporator (Buchi, B-480) at 45°C. The residues obtained were finally dried in a vacuum dessicator and dissolved in the respective solvent.

Screening for antibacterial activity of algae extracts

The antibacterial activity of algae extracts was performed using the agar-disk diffusion method [22], using bacterial cell suspension whose concentration was equilibrated to a 0.5 Mc Farland standard [23, 24]. Eight human pathogens bacteria were used: *Staphylococcus epidermidis* CIP 106510, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB 8166, *Enterococcus feacium* ATCC 29212, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella thyphymerium* LT2 and *Listeria monocytogenes* ATCC 19115. The bacterial cultures were first grown on Muller Hinton infusion agar (MH) plates at 37 °C for 24h prior to use.

Several colonies of similar morphology of the respective bacteria were transferred into API suspension medium (Biomérieux). The inocula of the respective bacteria were streaked into the MH agar plates and were then dried.

A sterile filter disk 5 mm in diameter (Whatman paper # 3) was placed on the infusion agar seeded with bacteria and 10 µl of the respective extracts was dropped into each paper disk (10 mg/ disk). The same procedure was done with the solvent only as a negative control. Standard disks of the antibiotic gentamycin (10 UI) served as positive controls according to the CASFM 2005 guidelines. The treated Petri dishes were incubated at 37°C during 24h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones surrounding the disks. The experiment was performed in triplicate

Screening for antifungal activity of algae extracts

For screening the antifungal activity of algae extracts, the agar-disk diffusion method was used as previously described [25]. Five strains isolated from patients suffering from candidosis: *Candida albicans, Candida glabrata, Candida krusei, Candida dubliniensis and Candida kefyr* were used. All Candida strains were first grown on Sabouraud chloramphenicol agar plate at 30°C for 18-24h. Several colonies were transferred into API suspension medium and adjusted to 2 McFarland turbidity standards with a densimat (biomérieux, France). The inocula of the respective yeast was streaked into Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterile filter disk, diameter 5 mm (Whatman paper # 3) was placed in the plate. An amount of ten microliters of the extract were dropped on each paper disc (10 mg/ disk). The treated Petri dishes were incubated at 30°C for 18- 24h. The antifungal activity was evaluated by measuring the diameter of the inhibition zones around the disks. The susceptibility of the standard drug was determined using a disk paper containing 20 µg of Amphoterecin B. Each experiment was carried out in triplicate and the mean diameter of the inhibition zones was recorded.

RESULTS

The paper describes the antimicrobial activity of six different species of marine algae collected from the Mediterranean Tunisian coastline. The antibacterial activities against eight pathogenic bacteria were summarized in Table 1. The antifungal activities against five yeast strains were represented in Table 2. Antimicrobial activities of different organic extracts of marine algae, against tested microorganisms in the present study and their potency were qualitatively and quantitatively assessed by the presence or the absence of inhibition zone diameter.

Antibacterial activity

The antibacterial screening of twenty four organic extracts (petroleum ether, chloroform, ethyl acetate and methanol), obtained from six seaweeds species, and against eight pathogenic bacteria was studied in comparison to the reference drug Gentamycin (10 UI).

Table 1: Antibacterial activity of organic fractions of six marine algae

Marine algae		**Microorganisms**							
Species	**Organic extracts (10 mg/disc)**	***S. aureus* ATCC25923**	***S. epidermidis* CIP106510**	***E.feacium* ATCC 9212**	***M. luteus* NCIMB 8166**	***E. coli* ATCC 35218**	***S. thypheme rium* LT2**	***P. aerugino sa* ATCC 27853**	***L. monocy togenes* ATCC19 115**
Cystoseira sedoides	Petroleum Ether	7.5 ± 0.7	9.5 ± 0.7	-	6	12 ± 1	-	-	-
	Chloroform	7 ± 1.4	9 ± 1.1	-	10 ± 1.4	6 ± 1.5	-	-	-
	Ethyl acetate	6	9 ± 1.4	-	6.5 ± 0.7	-	-	-	-
	Methanol	7	6	-	6.5 ± 0.7	-	-	-	-
Cystoseira crinita	Petroleum Ether	8 ± 0.5	9 ± 0.7	-	7 ± 0.5	13 ± 1.1	-	-	-
	Chloroform	9 ± 0.5	9	-	10 ± 0.5	7 ± 0.5	-	-	-
	Ethyl acetate	6.5 ± 0.7	6.6 ± 0.5	-	6.5 ± 0.7		-	-	-
	Methanol	6	7	-	6	6	-	-	-
Cystoseira compressa	Petroleum Ether	11 ± 0.5	6.3 ± 0.5	-	-	-	-	-	-
	Chloroform	9 ± 0.5	14	-	-	-	-	-	-
	Ethyl acetate	7 ± 1.4	7	-	6	-	-	-	-
	Methanol	6.5 ± 0.7		-	-	7	-	-	-
Dictyopteris membranaceae	Petroleum Ether	6	-	-	7 ± 0.5	-	-	-	-
	Chloroform	10 ± 1	6.5 ± 0.5	-	7.5 ± 0.7	-	-	-	-
	Ethyl acetate	11± 1.4	6.5 ± 0.7	-	11 ± 0.5	-	-	-	-
	Methanol	6	6	-	6	-	-	-	-
Gelidium latifollium	Petroleum Ether	6	-	-	7	-	-	-	-
	Chloroform	10 ± 0.5	9		7.5 ± 0.5	7 ± 0.5	-	-	-
	Ethyl acetate	7 ± 1.4	10.5 ± 0.7	-	8.5 ± 0.7	-	-	-	-
	Methanol	7	8.5 ± 0.5	-		-	-	-	-
Halurus equisetifolius	Petroleum Ether	6	7 ± 1	-	7.5 ± 0.7	-	-	-	-
	Chloroform	10 ± 0.5	13 ± 0.7	-	10.5 ± 1.1	-	-	-	-
	Ethyl acetate	6	9 ± 1.4	-	6.5 ± 0.7	-	-	-	-
	Methanol	6	9.5 ± 1	-	-	6	-	-	-
Gentamycine (Reference drug , 10 UI / disc)		19 ± 1.1	22 ± 1.4	16 ± 1	21.5 ± 0.7	20.6 ± 0.5	20.6 ± 1.5	16 ± 0.5	ND

Results are presented by the mean diameter of the inhibition zones (D ± SD, mm)

As shown, in Table 1, marine algae extracts exhibited a moderate to weak activity against four bacteria strains (*S. aureus*; *S. epidermis, E. coli* and *Micrococcus luteus*). The diameter of the inhibition zones against these strains was ranged from 6 to 12 mm. whereas, the chloroformic extract of *Cystoseira compressa* and *Halurus equisetifolius* presented a significant antibacterial activity against *S. epidermis* with a diameter of inhibition zone ranged from 13 mm to 14 mm. And the chloroformic extract of *Dictyopteris membranaceae and Gelidium latifolium* showed a diameter of inhibition zone of 10 mm against *S. aureus.*

We noted also that only petroleum ether extracts of *C. sedoides* and *C. crinita* exhibited a moderate antibacterial activity against *E. coli,* the diameter of the inhibition zones were 12 and 13 mm, respectively. No activity was observed against *S. thyphymerium*, *Pseudomonas aeruginosa, Listeria monocytogenes,* and *Enterococcus feacium* of all the extracts of the six marine algae. The methanol extracts of these species did not show any antibacterial activity against the eight bacteria strains.

Antifungal activity

As shown in Table 2, the antifungal activity was observed particularly with the chloroformic and the ethyl acetate extracts of two brown algae *C. crinita* and *C. sedoides* against five strains of *Candida.* The highest antifungal activity was produced, at the concentration of 10 mg/ disk, against two *Candida* strains: *C. krusei and C. kefyr*. The diameter of the inhibition zones of *C. crinita* and *C. sedoides* extracts, against C*. Krusei*, were 23 mm and 33.3 mm respectively for the chloroformic extract; and 17.6 mm and 24 mm , for the ethyl acetate. The diameter of the inhibition zones of *C. crinita* and *C. sedoides* against *C. Kefyr*, were 44.3 mm and 42.6 mm, respectively for the chloroformic extract (10mg/ disk), and 20.3 mm and 28.3 mm, respectively for the ethyl acetate extract.

The diameters of the inhibition zone produced by the brown algae were greater than produced by the reference drug, amphotericin B (10 µg/ disk). In addition, the chloroformic and the ethyl acetate extracts of *D. membranaceae showed* a moderate antifungal activity against *C. kefyr and* the diameter of the inhibition zones *were* in the range of 11 mm.

Also, we noted that, the petroleum ether extract of *C. sedoides* showed a diameter of the inhibition zone of 14 mm against *C. krusei and* the diameter of the inhibition zone of the petroleum ether extract of *C. crinita* against *C. kefyr and C.* albicans were 15 mm and 17 mm, respectively. Whereas The petroleum ether extract of *Halurus equisetifolius* and *Gelidium latifolium* presented antifungal activity with a diameter of inhibition zone in the range of 11 mm against *Candida glabrata*.

Table 2: Antifungal activity of organic fractions of six marine algae

Marine algae		**Microorganisms**				
Species	**Organic extracts(10 mg/ disc)**	***Candida albicans***	***Candida glabrata***	***Candida krusei***	***Candida dubliniensis***	***Candida kefyr***
Cystoseira sedoides	Petroleum Ether	10.3 ± 1.1	8 ± 0.5	14.3 ± 1.5	-	-
	Chloroform	25.6 ± 0.5	20 ± 0.7	33.3 ± 0.5	33 ± 1	42.6 ± 1.5
	Ethyl acetate	13.6 ± 0.7	10.5 ± 0.5	24 ± 1	15 ± 1.4	29.3 ± 0.7
	Methanol	-	-	-	-	-
Cystoseira crinita	Petroleum Ether	17 ± 0.5	6 ± 0.5	6 ± 0.7	13 ± 0.5	15 ± 1.4
	Chloroform	19.6 ± 1	10 ± 0.7	23 ± 1.4	20.5 ± 2.1	44.3 ± 0.5
	Ethyl acetate	10.6 ± 0.5	9 ± 0.5	17.6 ± 1.5	11.5 ± 0.7	20.3 ± 1.5
	Methanol	-	-	-	-	-
Cystoseira compressa	Petroleum Ether	-	-	-	-	6
	Chloroform	-	-	-	-	6
	Ethyl acetate	-	-	-	-	-
	Methanol	-	-	-	-	-
Dictyopteris membranaceæ	Petroleum Ether	-	-	-	-	-
	Chloroform	9 ± 1	-	-	10 ± 0.5	11± 0.7
	Ethyl acetate	11.5 ± 0.7	-	7.5 ± 0.7	-	11.5 ± 1
	Methanol	7.5 ± 0.5	-	-	-	-
Gelidium latifolium	Petroleum Ether	-	11± 0.5	-	-	10.5 ± 0.7
	Chloroform	-	10 ± 0.7	-	7 ± 0.5	10.6 ± 0.5
	Ethyl acetate	9 ± 1	7 ± 0.5	-	-	9. 5 ± 0.7
	Methanol	-	6 ± 0.5	11.6 ± 0.5	-	-
Halurus equisetifolius	Petroleum Ether	-	11 ± 1	6	6	6. 5 ± 0.7
	Chloroform	-	9 ± 0.7	-	-	-
	Ethyl acetate	-	10± 0.5	-	-	-
	Methanol	-	-	-	-	-
Amphotericin B (Reference drug, 20 µg/ disk)		13 ± 0.5	13 ± 0.7	16 ± 1	17 ± 1.4	7± 0.5

Results are presented by the mean diameter of the inhibition zones (D ± SD, mm).

DISCUSSION

In this present investigation, our results showed that petroleum ether and chloroformic extracts of tested marine algae exhibited a moderate to weak antibacterial activity whereas, the methanolic extracts of these algae didn't show any antibacterial activity. The type of extraction solvent had a big influence on the antimicrobial properties of obtained extracts, suggesting that antimicrobial activity depends on both algal species and the efficiency of the extraction method. Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than petroleum ether and ethyl acetate [26,27]. Whereas others authors reported that chloroform is better than methanol and benzene [28].

A good number of authors [29] have documented the antimicrobial potency of organic extracts and of some compounds isolated from marine algae of the genus of *Cystoseira*: such as terpenes [30,31]. From our results it seems that the antibacterial actions of the organic extracts were more pronounced with brown algae than the

red ones. This is agree with Rizvi and Shameel [26], who reported that some species of marine brown algae collected from different coastal areas of Karachi (Pakistan) showed greater antibacterial activity than the green and red ones. Bennamara et al. [32] isolated a meroditerpenoid metabolite from the brown alga *Cystoseira tamariscifolia* and characterized as Methoxybifurcarenone, in which they found antimicrobial activity.

For the antifungal activity, our results showed that the chloroformic extracts obtained from *C. crinita* and *C. sedoides* have a strong antifungal activity against Candida strains, which were slightly greater than produced by the ethyl acetate extracts. The highest activities were observed with species of the genus of *Cystoseira.* With regards to the components responsible for the antifungal activity, both chloroform and ethyl acetate extracts from *C. crinita* and *C. sedoides* showed remarkable activity strongly suggesting that several compounds of distinct nature were actives as antifungal agents. This is not surprising because the seaweeds belonging to the genus of *Cystoseira* possess a wide variety of compounds with different biological activities [32,13]. Ballesteros et al. [20] reported that the most dominant plants in the Mediterranean phytobentic communities such as the *Seagrasses*, *Cystoseira sp*, *Halopteris sp*. *Codium sp*, and *Mesophyllum lichenoides* strongly inhibited the growth of fungi which is in accordance with our results.

In the other hand, these results are of interest as we are dealing with an extract and not a pure product. It is important to consider that these extracts were unpurified and may contain both polar and apolar compounds, therefore the antimicrobial activity may be due to different compounds and related to the presence of bioactive metabolites.

In addition, this extensive and high activity found in our samples had never been observed in other Mediterranean surveys [33,34]. Seasonal and geographical changes in activity must also be taken into account to explain differences between our results and those found by others authors. Nevertheless, no reasons seem to explain this high level of antifungal activity, coupled with a low level of antibacterial activity exhibited by our samples, since the antimicrobial activity in other species of the genus of *Cystoseira* has been found with the steroid and terpenes [13]. It is possible that these compounds could be responsible for the antifungal properties reported here. In a recent study, Zineb et al. [36] reported that ethanolic extracts from marine alga, *Cystoseira tamariscifolia*, at the concentration of 10%, totally inhibited the growth of *Aspergillus flavus*. Also, Haliki et al. [37] have reported the antifungal activities of some marine algae (C*ystoseira barbata*, *Cystoseira compressa*, *Dictyopteris membranaceae*) against yeast and fungi.

In summary, to the best of our knowledge, the antibacterial and the antifungal activities of the different marine algae is being reported for the first time especially for the following species: *C. crinita, C. sedoides* and *H. equisetifolius.*

CONCLUSION

Seaweeds collected from Tunisian Mediterranean coasts has been shown to possess a specific antimicrobial activity. The most interesting species were: *C. crinita* and *C. sedoides*. These observations showed their importance as a potential source for biological active compounds such as antibacterial and antifungal substances. Further study need to be completed for the isolation and the identification of active molecules.

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