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Antimicrobial activity of methanolic extracts of selected marine macroalgae against some pathogenic microorganisms

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ABSTRACT

Objective: To evaluate the antimicrobial activity of methanolic extracts of six marine macroalgae belonging to green algae (Chlorophyceae), brown algae (Phaeophyceae) and the red algae (Rhodophyceae) collected from the intertidal area of the Sudanese Red Sea coast near Port Sudan.

Methods: Methanol was used for extracting the active principles of the algae and the disc diffusion method was performed to examine the activity and the minimum inhibitory concentration of the samples against four pathogenic bacteria and two fungi.

Results: All tested algal extracts exhibited considerable bioactivity and inhibited the growth of all pathogenic microorganisms under investigation. The green alga *Caulerpa racemosa* produced the maximum inhibition zone (21 mm) against *Candida albicans* while the red alga *Laurencia papillosa* showed low antimicrobial activity with the minimum inhibition zone of 10 mm against *Pseudomonas aeruginosa*. The tested algal extracts did not show any special antimicrobial influence on the selected microorganisms when they were considered as Gram-positive and Gram-negative bacteria and fungi but the most efficient methanolic extracts in inhibiting microbial growth were those of green macroalgae followed by the brown and the red macroalgae respectively.

Conclusions: The study demonstrated that the tested marine macroalgae from Sudanese Red Sea coast may represent a potential and alternative source for secondary metabolites with antimicrobial activity.

1. Introduction

A growing concern is evident about the utilization of antimicrobial drugs in human medicine, agriculture, and aquaculture[1]. This is because many pathogens have developed potent resistance to the current antimicrobial drugs. In addition, the occurrences of frequent epidemic outbreaks caused by some pathogens like the recent outbreak of Zika virus have aggravated the need for novel antimicrobial drugs to combat the socioeconomic impacts of these pathogens. Therefore, new antimicrobial as well as other life saving drugs are sought for from both usual sources such as plant and unusual sources such as marine organisms. Of these organisms, marine algae or seaweeds have been reported to represent a significant resource of secondary metabolite with biological activities[2]. The biological activity of the seaweeds was attributed to the stressing environmental conditions prevailing in intertidal habitat[3]. These conditions stimulate seaweeds to produce protective secondary metabolites or compounds to combat the presence of the harmful free radical and reactive oxygen species commonly generated under such conditions. Accordingly, the biological activity of seaweed secondary metabolites obtained with different organic solvents has been investigated to search for novel drugs templates. These metabolites were proven to have antibacterial, antifungal, antiviral, antitumour, anti-inflammatory and antioxidant activities[4].

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In this study, an effort was carried out to evaluate the antimicrobial activity of methanolic extracts of six marine macroalgae belonging to Chlorophyceae [*Enteromorpha compressa* (*E. compressa*) and *Caulerpa racemosa* (*C. racemosa*)], Phaeophyceae [*Cystoseira myrica* (*C. myrica*) and *Sargassum* sp.] and Rhodophyceae [*Gracilaria* sp. and *Laurencia papillosa* (*L. papillosa*)] collected from the intertidal area of the Sudanese Red Sea coast near Port Sudan, against four bacterial pathogens and two fungi.

2. Materials and methods

2.1. Collection of macroalgae

Marine macroalgae were collected by hand picking from the shallow intertidal waters of the Sudanese Red Sea coast near Port Sudan harbor during June 2013 to June 2014. In the field, the samples were washed thoroughly with seawater then transported to the laboratory as soon as possible and kept away from direct sunlight during transportation. In the laboratory, epiphytic and extraneous matters were removed by washing samples with fresh water. Then the samples were shade dried, cut into small pieces and powdered in a mixer grinder.

2.2. Preparation of algal extracts

Fifty grams of the finely ground samples were weighed and mixed with 500 mL of 80% methanol (1:10, w/v). The mixtures were kept for two weeks at room temperature and mixed at regular intervals. After two weeks, the mixtures were filtered with Whatman filter paper No. 1. The filtrate (crude extract) was freed from solvent by evaporation at room temperature.

2.3. Microbial strains

The crude extracts obtained were finally tested for their inhibitory effects on four species of pathogenic bacteria, namely, *Escherichia coli* (ATCC 25922) (*E. coli*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Staphylococcus aureus* (ATCC 25923) (*S. aureus*) and *Bacillus subtilis* (NCTC 8236) (*B. subtilis*). The first two strains belong to the Gram-negative group and the two latter strains belong to the Gram-positive group. Additionally, two fungi, namely, *Candida albicans* (ATCC 7596) (*C. albicans*) and *Aspergillus niger* (ATCC 9763) (*A. niger*) obtained from the laboratory of Medicinal and Aromatic Plants Research Institute, the National Center for Research, Khartoum were also tested.

2.4. In vitro screening for antimicrobial activity of methanolic extracts

2.4.1. Antibacterial and antifungal assays

The screening of the antibacterial and antifungal activity of methanolic extracts was tested by the disc diffusion technique in nutrient agar-plated Petri dishes inoculated with 18-hour culture of the test pathogens using a cotton swab[5]. Two hundred milligrams of each algal extract were dissolved in 2 mL methanol to give 10% concentration. Twenty microlitres of this mixture were applied to sterile filter paper discs (6 mm) placed on agar test plates. The plates were incubated overnight at 37 ˚C and the diameter of inhibition zones of the bacteria and fungi around the discs was measured. The bacterial assay results were compared with those obtained when bacterial pathogens were exposed to commercial discs containing the antibiotic drug ciprofloxacin and the fungal assay results were compared with those obtained when the fungal pathogens were exposed to commercial discs containing the antifungal drug itraconazole. Each test was performed in duplicate and the two values obtained were averaged.

The activity was classified according to the diameter of the inhibition zone (mm) around the discs as follows: weak inhibition: $\leqslant$ 10 mm, 10 mm < moderate inhibition $\leqslant$ 15 mm, the highest inhibition: > 15 mm, – : no activity[6].

2.4.2. Determination of minimum inhibitory concentration (MIC)

The MIC test was done to determine the lowest concentration of methanol extract of each alga that inhibited the growth of the bacteria and fungi. The disc diffusion technique in agar-plated Petri dishes was performed with four serials of dilutions 5.000% (50.00 mg/mL), 2.500% (25.00 mg/mL), 1.250% (12.50 mg/mL) and 0.625% (6.25 mg/mL) and the inhibition zones around the discs were measured. For each dilution, only one disc was used and one result was obtained in each test.

The MIC value was defined as the lowest concentration that yielded no inhibition of bacterial and fungal growth[7].

3. Results

3.1. The antibacterial and antifungal assays

All tested algal species extracts showed considerable activity against all tested pathogens with zones of inhibition ranging between 10.0 and 20.0 mm for the bacterial pathogens and between 12.5 and 21.0 mm for the fungal pathogens (Table 1).

Table 1
Diameters of inhibition zones of methanolic extracts of the selected Red Sea marine macroalgae against the pathogenic microorganisms at 10% concentration (mm).

Algae species	Gram-negative		Gram-positive		Fungi	
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
E. compressa	19.0	16.0	15.0	17.5	21.0	17.5
C. racemosa	20.0	16.0	15.5	17.0	19.0	15.5
C. myrica	17.0	16.0	17.5	16.0	16.5	19.0
Sargassum sp.	18.0	18.0	16.0	15.5	16.5	14.5
Gracilaria sp.	15.0	16.0	13.0	13.5	15.5	14.5
L. papillosa	17.0	10.0	12.5	14.0	12.5	15.5

Of the algal species, *E. compressa* showed the highest activity with a maximum zone of inhibition of 21.0 mm against *C. albicans* while *L. papillosa* showed the lowest activity with a minimum inhibition zone of 10.0 mm against *P. aeruginosa*.

The antibiotic drug ciprofloxacin has inhibited the growth of all bacterial samples studied with zones of inhibition ranging between 21 mm against *P. aeruginosa* and *S. aureus* and 25 mm against *E. coli* and *B. subtilis* at 10% concentration (Table 2). The antifungal drug itraconazole inhibited the growth of both fungal species with a minimum inhibition zone of 20 mm against *A. niger* and a maximum inhibition zone of 25 mm against *C. albicans* at 10% concentration (Table 3).

Table 2
Diameters of inhibition zones of the antibiotic drug ciprofloxacin against the bacterial pathogens (mm).

Ciprofloxacin concentration	Inhibition zones			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
40%	30	29	26	30
20%	28	25	23	23
10%	25	21	21	25
5%	21	18	17	20

Table 3
Diameters of inhibition zones of the antifungal drug itraconazole against the fungal pathogens (mm).

Itraconazole concentration	Inhibition zones	
	C. albicans	*A. niger*
40%	27	24
20%	26	21
10%	25	20
5%	24	18

3.2. The MIC

From the results, all the four concentrations tested have inhibited the growth of the tested organisms. The minimum inhibitory zone obtained was 10 mm (Tables 4–9). This meant that MIC of each algal extract was lower than 6.25 mg/mL.

Table 4
Diameters of inhibition zones of four dilutions of methanol extract of *E. compressa* against the tested pathogens (mm).

E. compressa concentration	Inhibition zones					
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
5.000%	22	15	15	15	15	17
2.500%	15	14	14	13	11	15
1.250%	15	12	15	13	12	15
0.625%	12	13	12	11	13	11

Table 5
Diameters of inhibition zones of four dilutions of methanol extract of *C. racemosa* against the tested pathogens (mm).

C. racemosa concentration	Inhibition zones					
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
5.000%	16	12	15	14	15	14
2.500%	17	15	15	15	14	15
1.250%	14	11	14	14	13	14
0.625%	13	13	13	15	14	14

Table 6
Diameters of inhibition zones of four dilutions of methanol extract of *C. myrica* against the tested pathogens (mm).

C. myrica concentration	Inhibition zones					
	E. coli	*P. aeruginosa*	*S. aureus*	*B .subtilis*	*C. albicans*	*A. niger*
5.000%	17	15	15	17	13	17
2.500%	13	11	15	14	17	14
1.250%	15	12	14	14	10	13
0.625%	14	10	13	15	13	12

Table 7
Diameters of inhibition zones of four dilutions of methanol extract of *Sargassum* sp. against the tested pathogens (mm).

Sargassum sp. concentration	Inhibition zones					
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C.albicans*	*A. niger*
5.000%	17	15	16	13	13	16
2.500%	17	14	16	15	14	14
1.250%	15	13	14	15	14	11
0.625%	14	14	15	13	13	15

Table 8
Diameters of inhibition zones of four dilutions of methanol extract of *Gracilaria* sp. against the tested pathogens (mm).

Gracilaria sp. concentration	Inhibition zones					
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
5.000%	16	13	13	14	13	14
2.500%	16	14	14	14	14	14
1.250%	13	14	12	13	14	14
0.625%	13	13	12	13	11	12

Table 9
Diameters of inhibition zones of four dilutions of methanol extract of *L. papillosa* against the tested pathogens (mm).

L. papillosa concentration	Inhibition zones					
	E .coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
5.000%	15	14	15	15	15	17
2.500%	15	14	15	15	15	15
1.250%	14	13	13	14	14	14
0.625%	14	14	14	13	13	12

4. Discussion

The antibacterial and antifungal activities of methanol extracts of six marine macroalgae collected from the shallow waters of the Sudanese Red Sea near Port Sudan harbour were screened against the widely distributed *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *C. albicans* and *A. niger* pathogens, which cause serious problems for human health.

This study has demonstrated that the crude methanolic extracts of the present marine macroalgae have exhibited significant inhibition effect against these pathogens. Methanolic crude extract of seaweeds has been reported to have wide spectrum of biological activities. For example, it has been reported to have antibacterial, antifungal, and antitumor[8-10] and this supports the findings of this study. This means that the species investigated in these studies could possibly be used as a source of antimicrobial\antitumor drug templates.

In this investigation, no considerable difference was observed in the bioactivity of the macroalgal methanolic extracts against Gram-positive and Gram-negative bacteria and the fungi, however, a noticeable variation in the bioactivity of the methanolic extracts of the different macroalgal groups was evident. The most efficient

extracts were those of the chlorophycean macroalgae followed by the phaeophycean and then the rhodophycean.

This could be explained on two levels. On one level, this is the pattern of the natural distribution of these groups of macroalgae in the intertidal area along the Sudanese Red Sea. Commonly, starting from the water mark seawards, the chlorophycean macroalgae were found first in the shallow water depths, followed by the phaeophycean macroalgae, and then in more deeper water depths the rhodophycean were placed. This may reflect the exposure degree of these groups to the stressing abiotic and biotic conditions that may stimulate the production of bioactive metabolites in macroalgae.

On the second level, this could be attributed to the difference of exposure pattern of this macroalgal groups to the microorganisms from land-based sources such as sewage disposal which may again stimulate the production of the bioactive constituents. The density of indicator microorganisms of faecal origin was found to be greater in near shore water compared to off shore water[11]. This may indicate that density of pathogenic microorganisms increases near the shore where the chlorophycean macroalgae are found, and may stimulate this group to produce potent antimicrobial substance. Studies have established that macroalgae contain a wide range of bioactive compounds with significant pharmaceutical efficiency that could be used as a new drug template[12]. These pharmaceuticals were endogenously produced by seaweed as a defense mechanism against extreme environmental conditions[13]. For example, the antibacterial effect of the alkaloid extract of some green, brown, and red seaweed was reported. It was found that the inhibition zones of some green, brown and red macroalgae extracts against Gram-positive and Gram-negative bacteria were 12 to 29 mm, 13 to 35 mm, and 15 to 34 mm, respectively[14]. The values obtained in this study concur well with the above mentioned ranges.

The lowest MIC value reported in this study was in conformity with Alghazeer *et al.*[14] who reported similar values for the green algae *Codium tomentosum* and *Dictyopetris membranacea*. Higer MIC value of 100 mg/mL was also reported by the same author.

The macroalgae extracts exhibited higher and moderate inhibition activity on the tested microbial organisms. This study revealed that the Sudanese seaweeds from the Red Sea coast possess significant bioactive capacities, and thus deserve to be the subject of marine biotechnology programmes to examine their natural products properties and that they should be investigated for natural antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

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