Saudi Journal of Pathology and Microbiology (SJPM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Antimicrobial Activity of solvent Extracts of Selected Red Sea Macroalgae against Some Pathogenic Microorganisms

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Original Research Article

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Article History
Received: 13.03.2018
Accepted: 22.03.2018
Published: 30.03.2018

DOI:
10.21276/sjpm.2018.3.3.6

Abstract: This study was carried out to evaluate the antimicrobial activity of hexane, chloroform, ethyl acetate and water extracts of six marine macroalgae belonging to different families namely 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. Successive extraction techniques using hexane, chloroform, ethyl acetate and water were used for extracting the active principles of the algae and the disc-diffusion method was performed to examine the activity of the crude extracts against four pathogenic bacteria and one fungus. Most of the tested algal extracts exhibited considerable bioactivity and inhibited the growth of most of pathogenic microorganisms under investigation. The chloroform extract of the red alga *Gracillaria* sp produced a maximum inhibition zone of 22 mm against *Escherichia coli* whereas the chloroform extract of the red alga *Laurencia papillosa* showed a low antimicrobial activity with a minimum inhibition zone of 5 mm against *Bacillus subtilis*. Some extracts had no antimicrobial activity against the tested pathogens. The tested algal extracts did not show any different antimicrobial effects on the selected microorganisms when considered as Gram-positive or Gram-negative bacteria, however the fungal sample tested was less sensitive when compared with the bacterial samples. The results showed that chloroform and ethyl acetate were the appropriate solvents for extracting the bio-active agents. The study demonstrated clearly that the tested marine macroalgae from Sudanese Red Sea coast represent a potential and alternative source of secondary metabolites with antimicrobial activity.
Keywords: Algal extracts, Antimicrobial activity, Pathogens, Red Sea, Sudan.

INTRODUCTION

Bacterial pathogens cause diseases in humans and in aquaculture organisms, sometimes with mortality. For example *Staphylococcus aureus* causes food poisoning, located infections, blood poisoning and generalized infections in debilitated subjects. *Bacillus cereus* is responsible for causing food borne gastroenteritis. *Escherichia coli* and *Pseudomonas aeruginosa* cause diseases like gastroenteritis, urinary tract infections and upper respiratory tract complications, while *Salmonella* sp cause diarrhea and typhoid fever [1]. *Pseudomonas aeruginosa* is an important and prevalent pathogen among burnt patients and is capable of causing a life-threatening illness [2].

Prevention or treatment of disease outbreaks with drugs or chemicals may reduce the incidence. Recently, the improper use of antibiotics resulted the emergence of resistant pathogenic bacteria [3]. Moreover the cost of drugs is high and these drugs may cause adverse effects on the host, which include hypersensitivity and depletion of beneficial microorganisms in the gut [4].

Decreased efficiency and resistance of pathogens to antibiotics necessitated the development of new alternatives [5]. Accordingly, pharmaceutical industries while searching for new alternatives are giving importance to compounds derived from traditional sources like soil and plants and less traditional sources especially marine organisms [6]. Hence, interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during recent years [7].

Many compounds which are diverse, novel and bioactive have been isolated from marine organisms. Among the marine organisms, the macroalgae (seaweeds) occupy an important place as a source of biomedical compounds [8].

Algae are primitive non-flowering plants without true stems and leaves. They are abundant in intertidal, shallow, coastal estuaries and backwaters and flourish wherever the substratum is available. They grow on rocks, dead corals, stones, pebbles, solid substances and on other plants. Recently, marine pharmacology attacked global attention [9].

Marine algae are exploited mainly for the industrial production of phycocolloids such as agar, agarose, alginate and carrageenan, not for health aspects [10]. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. Edible seaweeds contain a significant amount of protein, carotenoids, dietary fibers, essential fatty acids, vitamins and minerals essential for the human nutrition [11].

The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvents, for example, acetone, methanol-toluene, ether and chloroform-methanol [12]. Use of organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity [13]. Many extractable compounds from seaweeds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms, and are therefore, responsible for the antimicrobial activity of some seaweed [14].

Screening of organic extracts from marine algae and other marine organisms is a common approach to identify their compounds of biomedical importance [15]. The potential biological resources of marine environments of Sudan represented by the Red Sea have not been adequately explored and harnessed for biotechnological applications and deriving biopharmaceuticals. In this context a study was undertaken to evaluate the antimicrobial activity of hexane, chloroform, ethyl acetate and water extracts of six marine macroalgae belonging to *Chlorophyceae* (*Enteromorpha compressa* and *Caulerpa racemosa*), *Phaeophyceae* (*Cystoseira myrica* and *Sargassum* sp) and *Rhodophyceae* (*Gracilaria* sp and *Laurencia papillosa*) collected from the intertidal area of the Sudanese Red Sea coast near Port Sudan, against four bacterial pathogens and one fungus in search of a promising source of pharmaceutical agents.

MATERIALS AND METHODS

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, 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. Samples were authenticated and herbarium specimens were deposited at the Red Sea University. The samples were then shade dried, cut into small pieces and powdered in a mixer grinder.

Preparation of algal extracts

Successive extraction techniques using solvents was used for extracting bio-active principles in algae. Fifty grams of each finely ground sample 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 through Whatman filter paper No. 1. The filtrates (crude extracts) were freed from solvent by evaporation at room temperature.

The methanol extracts were dissolved in water and fractioned to hexane extracts, chloroform extracts, ethyl acetate extracts and aqueous extracts by adding 100 ml of hexane, chloroform and ethyl acetate respectively to the water layer in the separation funnels. Finally the filtrates (crude extracts) were freed from solvents and water by evaporation at room and refrigerator temperature.

Microbial strains

The crude extracts were tested for their antimicrobial effects against two Gram-negative pathogenic bacteria namely; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and two Gram-positive bacteria namely; *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (NCTC 8236) and one fungus namely; *Candida albicans* (ATCC 7596).

In vitro screening for antimicrobial activity of algal extracts

Hexane, chloroform, ethyl acetate and water extracts were screened for their antimicrobial activity by the disc diffusion technique [16]. Nutrient agar-plates were inoculated from an 18 h cultures of the test pathogens using cotton swabs. Two hundred milligrams of each algal extract were dissolved in 2 ml of the extraction solvent to give 10% concentration. Twenty microlitres of this mixture were applied to sterile filter paper discs (6 mm) which had been placed on the agar test plates. The plates were incubated overnight at 37°C and diameter of the inhibition zones around the discs were measured. The bacterial assay results were compared with those obtained when the bacterial pathogens were tested using commercial discs containing the antibiotics Ciprofloxin and Gentamycin. The fungal assay results were compared with those obtained when the fungal pathogens were tested using commercial discs containing the antifungal Itraconazole.

Each test was performed in duplicate and the two values obtained were averaged. Antimicrobial activity was classified according to the diameter of the inhibition zone (in mm) around the discs as follows:

Weak inhibition: ≤ 10 mm, 10 mm < moderate inhibition ≤ 15 mm, high inhibition: > 15 mm, 0: no activity [17].

RESULTS

The antimicrobial assays of algal solvents extracts

The antimicrobial assays of hexane extracts

Most of hexane extracts of the tested algal species showed considerable activity (ranging between moderate and high inhibition) against most of the tested pathogens with zones of inhibition ranging between 9 and 16.5 mm for the bacterial samples. Only the hexane extract of *Gracillaria* sp showed weak activity against *C. albicans* with an inhibition zone of 8 mm. Some algal extracts showed no activity against the tested bacterial strains.

Table-1: Diameters of inhibition zones (mm) of hexane extracts of the selected Red Sea marine macroalgae against the tested microorganisms at 10% concentration.

Algae species	Inhibition zone (mm)				
	Gram-negative		Gram-positive		Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Enteromorpha compressa	12.5	10	12	0	0
Caulerpa racemosa	16.5	0	16	9	0
Cystoseira myrica	ND	ND	ND	ND	ND
Sargassum sp	0	0	12	16	0
Gracilaria sp	16.5	15.5	16	15	8
Laurencia papillosa	0	0	11	16	0

ND=Not done

The antimicrobial assays of chloroform extracts

Most of chloroform extracts of the tested algal species showed considerable activity (ranging between moderate and high inhibition) against most of the tested bacteria with zones of inhibition ranging between 5 and 22 mm but no activity against *C. albicans* was recorded. Some algal extracts showed no activity against bacterial samples

Table-2: Diameters of inhibition zones (mm) of chloroform extracts of the selected Red Sea marine macroalgae against the tested microorganisms at 10% concentration.

Algae species	Inhibition zone (mm)				
	Gram-negative		Gram-positive		Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Enteromorpha compressa	ND	ND	ND	ND	ND
Caulerpa racemosa	0	20	0	0	0
Cystoseira myrica	11.5	13.5	10.5	0	0
Sargassum sp	17	13.5	0	7	0
Gracilaria sp	22	17	13	9	0
Laurencia papillosa	15	12	17	5	0

ND=Not done

The antimicrobial assays of ethyl acetate extracts

Most of ethyl acetate extracts of the tested algal species showed considerable activity (ranging between moderate and high inhibition) against most of the tested microorganisms with zones of inhibition ranging between 10 and 18.5 mm for the bacterial samples and moderate activity against *C. albicans* with inhibition zones ranging between 10 and 13mm. Some algal extracts showed no activity against some tested microorganisms

Table-3: Diameters of inhibition zones (mm) of ethyl acetate extracts of the selected Red Sea marine macroalgae against the tested microorganisms at 10% concentration.

Algae species	Inhibition zone (mm)				
	Gram-negative		Gram-positive		Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Enteromorpha compressa	15	0	15	18.5	12.5
Caulerpa racemosa	0	15	16	15	13
Cystoseira myrica	0	0	14	0	0
Sargassum sp	0	0	12.5	0	0
Gracilaria sp	15	10	12.5	17	10
Laurencia papillosa	0	15	15	0	0

The antimicrobial assays of aqueous extracts

Most of aqueous extracts of the tested algal species showed considerable activity (ranging between moderate and high inhibition) against most of the tested bacteria with zones of inhibition ranging between 10 and 17 mm. No activity against *C. albicans* and some bacterial species was recorded.

Table-4: Diameters of inhibition zones (mm) of aqueous extracts of the selected Red Sea marine macroalgae against the tested microorganisms at 10% concentration.

Algae species	Inhibition zone (mm)				
	Gram-negative		Gram-positive		Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Enteromorpha compressa	10	10	12.5	14	0
Caulerpa racemosa	12	13	17	14.5	0
Cystoseira myrica	0	14.5	13.5	12	0
Sargassum sp	10	10	11	0	0
Gracilaria sp	13	13	14	0	0
Laurencia papillosa	0	10	12.5	0	0

The antibacterial activity of commercial antibiotics

Ciprofloxin antibiotic showed high activity against the tested bacteria and at all concentrations with diameters of inhibition zones ranging between 17 and 30 mm while Gentamycin showed high activity against the Gram-positive bacteria and less activity against *E. coli* and no activity against *P. aeruginosa* at 10% concentration.

The antifungal activity of Itraconazole:

Itraconazole showed high activity against *C. albicans* and at all concentrations with diameters of inhibition zones ranging between 18 and 27mm (Table-6).

Table-5: Diameters of inhibition zones of the antibiotics Ciprofloxin and Gentamycin against the tested bacteria in mm.

Antibiotic	concentration	Gram-negative		Gram-positive	
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Ciprofloxin	40%	30	29	26	30
	20%	28	25	23	23
	10%	25	21	21	25
	5%	21	18	17	20
Gentamycin	10%	15	0	20	24

Table-6: Diameters of inhibition zones of the antifungal Itraconazole against *C. albicans* in mm.

concentration	*C. albicans*
40%	27
20%	26
10%	25
5%	24

DISCUSSION

The antimicrobial activity of hexane, chloroform, ethyl acetate and water extracts of six marine macroalgae collected from the shallow waters of the Red Sea near Port Sudan was screened against the widely distributed *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *C. albicans* pathogens, which may cause health problems in man.

The study demonstrated that chloroform and ethyl acetate extracts contained more active compounds than those of hexane and water which is in agreement with Patra *et al*., [18] who found that chloroform and ethyl acetate extracts were active against most of the bacterial pathogens investigated. Also this agrees with a study carried out to evaluate the antimicrobial activity of methanol, chloroform, ethyl acetate, hexane and aqueous extracts of the marine brown alga *Spatoglossum asperum* which tested against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* by the disc diffusion method. The chloroform and methanolic extracts showed maximum activity against *Staphylococcus aureus*, the aqueous extract showed moderate activity against *Bacillus cereus* and ethyl acetate extract showed minimum activity against *Bacillus cereus*, whereas no activity was observed with the hexane extract. The study established that methanol extract of brown seaweed *S. asperum* was highly effective, against most of the bacterial strains tested [19].

However, our results are in disagreement with the findings of Abdalla *et al*., [20] who demonstrated

that methanol was a good solvent for extracting active principles from algae and all tested methanolic algal extracts exhibited considerable bioactivity and inhibited the growth of all pathogenic microorganisms under investigation. A study of six organic and aqueous extracts of 23 red marine algae collected along the Atlantic coast of Morocco for anti-inflammatory and antimicrobial activities, showed that positive antibacterial activity with inhibition zones more than 10 mm was obtained with methanolic and the methanol–dichloromethane (50:50) extracts and Gram-positive bacteria were more sensitive than the Gram-negative bacteria and *Staphylococcus aureus ssp aureus* was the most sensitive [21].

Al-Saif *et al.*, [22] assayed the antibacterial activity of extracts of *Ulva reticulata*, *Caulerpa occidentalis*, *Cladophora socialis*, *Dictyota ciliolata*, and *Gracilaria dendroides* isolated from Red sea coastal waters of Jeddah, Saudi Arabia, prepared using ethanol, chloroform, petroleum ether and water, found that chloroform extracts was most effective followed by ethanol, petroleum ether and aqueous extracts respectively. Results also indicated that the extracts of the red alga *G. dendroides* were more effective against the tested bacterial strains followed by those of the green alga *U. reticulata*, and the brown alga *D. ciliolate*. Our study found that chloroform extract of the red alga *Gracillaria* sp produced a maximum inhibition zone against *E. coli* and this may be due to the growth pattern of this alga and its exposure to environmental conditions that stimulated the production of more active secondary metabolites as a defense mechanism.

In this investigation no considerable differences were observed in the bioactivity of the macroalgal extracts against Gram-positive and Gram-negative bacteria, but *C. albicans* was found less sensitive to algal extracts when compared with bacteria samples.

Nevertheless, and regardless of the solvent used, all these investigations demonstrated that marine macroalgae produced secondary metabolites that had significant inhibitory effect against a broad spectrum of microorganisms and many organic solvents can be used for extracting these active compounds from algae.

Priyadharshini *et al.*, [23] reported that seaweeds were an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids that exhibited different biological activities. Depending upon their solubility and polarity, different solvents shows the different antimicrobial activity. So chemical compounds should be extracted from different seaweeds in order to optimize their antibacterial activity by selecting the best solvent system [24].

ACKNOWLEDGMENTS

Authors would like to acknowledge the technical assistance of laboratory staff of the Faculty of Marine Sciences and Fisheries, Red Sea University, Port Sudan, during sampling and extraction and laboratory staff of Medicinal and Aromatic Plants and Traditional Medicine Research Institute, The National Center for Research, Khartoum, during laboratory analysis.

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