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ANTIBACTERIAL ACTIVITY OF METHANOL CRUDE EXTRACTS OF MARINE MACRO ALGAE FROM THE THIKKODI CALICUT WEST COAST OF INDIA

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ABSTRACT

The antibacterial activities of methanol extracts of *Caulerpa racemosa* (Forsk), *Caulerpa sertularioides* (Gmelin) Howe, *Ulva lactuca* Linn, *Padina gymnospora* (Kutzing) Sonder., *Dictyota dichotoma* (Hudson) Lamouroux. and *Gracilaria multipartita* (Clemente) Harvey against bacterial strains *viz., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Salmonella enterica* were studied by using disc diffusion method, determination of MIC and MBC. The methanol extracts of *P. gymnospora* and *D. dichotoma* showed the highest antibacterial activity against all the bacterial strains tested. The mean zones of inhibition produced by the extracts in agar disc diffusion assays were from 6.3 to 19.5 mm. The MIC was between 125 and 500 µg/ml and MBC was between 250 and 1000 µg/ml. The highest mean zone of inhibition (19.5 mm) was observed with methanol extract of *Padina gymnospora* against *S. aureus.* The lowest MIC (125 µg/ml) and MBC (250 µg/ml) values were observed in methanol extract of *P. gymnospora* and *D. dichotoma* against *S. aureus*. These finding suggest that methanol crude extract of *P. gymnospora* have potential antibacterial activity.

KEYWORDS: **Marine macroalgae, Antibacterial activity, MIC, MBC**

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INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide[1]. Bacterial infection causes high rate of mortality in human population and aquaculture organisms. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems [2]. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs[3] has been the reason for an extended search for newer drugs to treat opportunistic fungal infections[4]. Nowadays the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alteration [5]. Many antifungal drugs, including imidazoles, butenafine and terbinafine, have been used clinically for the topical treatment of dermathophytosis[6]. Triazoles, griseofulvin and terbinafine are used as oral antifungal drugs for systemic therapy of severe dermatophytosys[7], but the prolonged duration of treatment, drug toxicity and interactions, fungal resistance and high costs are encountered difficulties[8]. These factors render the development of new more efficient and safe antifungal drugs. Secondary metabolites produced by plants constitute a major source of bioactive substances. The scientific interest in these metabolites has increased today with the search of new therapeutic agents from plant source, due to the increasing development of the resistance pattern of microorganisms to most currently used antimicrobial drugs. According to World Health Report of infectious diseases, overcoming antibiotic resistance is one of the major issues of the WHO for the present millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management [9]. Algae appear to be an interesting source for ethno medicinal and phytochemical studies. The power of algal resources has been sought for thousands of years for their ability to prevent disease and prolong life. Algae contain minerals, an abundance of vitamins, variety of trace elements and have shown high potential in controlling antimicrobial, antitumor, anticoagulant and cytotoxic activity[10]. Seaweed extracts were also reported to exhibit antimicrobial activity[11,12]. Active compounds from seaweeds were found to be effective against human bacterial pathogens, fish bacterial pathogens[13], leaf spot disease of plants and marine pathogenic microorganisms [14]. Many chemically unique compounds of marine algae with biological activities have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals such as brominated phenols, sterols, terpenoids, polysaccharides, peptides, proteins, acrylic acid, terpenes, chlorophyllides, phenols and heterocyclic carbons etc.[15]. Most identified active antimicrobial compounds are water insoluble and thus organic solvent extracts have been found more potent[16]. Hence, the present study was made to evaluvate the antibacterial activity of methanol extracts of *U. lactuca, C. racemosa, C. sertularioides, P. gymnospora, D. dichotoma,* and *G. multipartita* against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis* and *S. enterica* against bacterial strains to develop new antibacterial agent with no side effect.

MATERIALS AND METHODS

Algal sample collection

Ulva lactuca, Caulerpa racemosa, Caulerpa sertularioides (Chlorophyceae) *Padina gymnospora, Dictyota dichotoma* (Phaeophyceae) and *Gracilaria verrucosa* (Rhodophyceae) were collected by hand picking from the submerged marine rocks at Thikkodi village (Lat. 8°30′N; Long. 78°8′E), the Calicut West Coast, Kerala, India. Seaweeds collections were made during the month of August, 2012. The algae were identified by Dr. R. Selvaraj, Former Professor, Department of Botany, Annamalai University and the museum specimens are deposited in the Department of Botany, Annamalai University, Annamalainagar Tamilnadu, India.

Preparation of extracts

The algae were handpicked during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal castings and adhering sand particles etc., morphologically distinct thallus of alga were placed separately in new polythene bags and were kept in a ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. Then the seaweeds were shade dried under room temperature and kept in a hot air oven for 50 ºC for half an hour. Oven dried that the material were ground by using electric blender. The powdered materials were stored in air tight container. Five hundred gram of seaweed materials was packed inside a Soxhlet apparatus and successive extraction was carried out using methanol for 72 hours. The solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4 ºC until further assay.

Microorganisms

The gram positive bacterial strain *viz., Staphylococcus aureus* (MTCC 2063) and gram negative bacterial strains such as *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443), *Proteus mirabilis* (MTCC 425) and *Salmonella entrica* (MTCC 3231) were used in this study. These standard bacterial strains were obtained from Microbial Type Culture Collection (MTCC) Chandigarh, India. The stock cultures were maintained on Nutrient Agar at 4 °C. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) and they were obtained from Himedia Ltd., Mumbai.

Disc diffusion method

The disc diffusion method of Bauer *et al.* [17] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of MHA. Then the plates were allowed to solidify and used in susceptibility test. The standard inoculum using bacterial suspensions

containing 10^8 colony forming units (CFU) per ml, were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. The methanol extract was dissolved in 10 per cent Dimethyl sulfoxide (DMSO) and under aseptic conditions. The sterile discs were impregnated with 20 µl of different concentrations. The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Methicillin (5 µg/disc) for *S. aureus* and Ciprofloxacin (10 µg/disc) for all the bacteria were used as positive control and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h for all bacterial strains. The zone of inhibitions were observed and measured in millimeters. The assay in this experiment was repeated three times.

Determination of the Minimum Inhibitory Concentration (MIC) for bacteria

To determine the MIC for the algae crude extracts, a modified reaszurin microtitre plate assay was carried out according to the method of Sarker *et al.* [18]. 50 µl of Sterile MHB were transferred in to each well of a sterile 96-well micro titer plate. The algae extract was dissolved in 10 per cent DMSO to obtain 1000 µg/ml stock solution. 50 µl of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth, 50 µl of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625 µg/ml of the extract in each well. To each well, 10 µL of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5 $\times 10^5$ CFU/mL. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10 % DMSO solution as a negative control. The plates were incubated at 37 °C for 24 h for all the bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of algae extracts that exhibited the growth of the organisms the values by visual reading.

Determination of the Minimum bactericidal concentration (MBC)

MBC was determined by plating loop full samples from each MIC assay well with growth inhibition in to freshly prepared MHA for bacteria strains. The plates were incubated at 37 °C for 24 h for all bacterial strains. The MBC was recorded as the lowest concentration of the extracts that did not permit any visible bacterial growth after the period of incubation.

Statistical Analysis

The experimental data are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Comparison of means for *in vitro* antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. *P* value < 0.05 were considered statistically significant.

RESULTS

Table 1
Antibacterial activity of methanol extracts of Caulerpa racemosa and Caulerpa sertularioides

S. No.	Name of the microorganisms	Mean zone of inhibition[a] (mm)[b]						Control (Me/ Cip)	*Caulerpa racemosa*		*Caulerpa sertularioides*	
		Concentration of the disc (µg/disc)			Concentration of the disc (µg/disc)				MIC µg /ml	MBC µg /ml	MIC µg/ml	MBC µg/ml
		Caulerpa racemosa			*Caulerpa sertularioides*							
		1000	500	250	1000	500	250					
1	*Staphylococcus aureus*	16.5 ± 1.0	12.8 ± 1.3	9.3 ± 1.3	13.3 ± 1.3	10.5 ± 1.0	7.3 ± 0.8	7.5 ± 2.4	250	500	500	1000
2	*Klebsiella pneumoniae*	13.0 ± 1.2	9.8 ± 1.0	7.0 ± 0.8	11.3 ± 1.3	9.0 ± 0.8	6.5 ± 0.6	24.3 ± 2.1	250	500	500	1000
3	*Pseudomonas aeruginosa*	12.5 ± 1.7	9.3 ± 1.5	6.5 ± 1.3	11.5 ± 1.3	9.3 ± 1.3	6.5 ± 1.0	30.5 ± 2.4	500	1000	500	1000
4	*Escherichia coli*	10.0 ± 1.4	8.5 ± 1.3	6.5 ± 1.0	12.5 ± 1.3	9.0 ± 1.2	7.3 ± 1.0	29.0 ± 1.8	500	1000	500	1000
5	*Proteus mirabilis*	12.0± 1.0	9.0 ± 0.8	7.0 ± 0.8	10.3 ± 1.0	8.5 ± 0.6	6.3 ± 0.5	27.0 ± 1.8	500	1000	500	1000
6.	*Salmonella entrica*	13.8 ± 1.0	9.0 ± 0.8	7.0 ± 0.8	11.3 ± 1.0	8.5 ± 0.6	6.3 ± 0.5	28.5 ± 1.7	500	1000	500	1000

a- Diameter of zone of inhibition (mm) including disc diameter of 6 mm; b- Mean of four assays; ± standard deviation; Me – Methicillin for Staphylococcus aureus (5 µg/disc); Cip – Ciprofloxacin for all the bacterial except S. aureus strains (10 µg/disc)

Table 2
Antibacterial activity of methanol extracts of Ulva lactuca and Padina gymnospora

S. No.	Name of the microorganisms	Mean zone of inhibition[a] (mm)[b]						Control (Me/ Cip)	*Ulva lactuca*		*Padina gymnospora*	
		Concentration of the disc (µg/disc)			Concentration of the disc (µg/disc)				MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
		Ulva lactuca			*Padina gymnospora*							
		1000	500	250	1000	500	250					
1	*Staphylococcus aureus*	13.7 ± 1.6	10.6 ± 1.4	7.1 ± 1.2	19.5 ± 1.3	14.0 ± 1.0	9.3 ± 1.3	9.5 ± 1.5	500	1000	125	250
2	*Klebsiella pneumoniae*	10.0 ± 1.2	8.5 ± 1.0	6.0 ± 0.3	14.1 ± 1.2	11.0 ± 0.6	8.5 ± 0.5	25.0 ± 1.8	250	500	500	1000
3	*Pseudomonas aeruginosa*	11.5 ± 1.5	9.3 ± 1.2	6.5 ± 1.8	14.1 ± 1.0	11.6 ± 1.5	9.3 ± 1.0	28.5 ± 1.4	500	1000	250	500
4	*Escherichia coli*	10.0 ± 1.2	8.1 ± 1.1	6.3 ± 1.0	14.2± 1.5	11.0 ± 1.0	9.3 ± 1.5	29.0 ± 1.8	500	1000	500	1000
5	*Proteus mirabilis*	11.8 ± 1.2	8.0 ± 0.3	7.0 ± 0.8	13.3 ± 1.4	11.5 ± 1.8	7.3 ± 1.7	27.0 ± 1.5	500	1000	250	500
6.	*Salmonella entrica*	12.4 ± 1.5	9.0 ± 1.8	7.0 ± 1.2	14.3 ± 1.3	12.5 ± 0.6	9.5 ± 0.5	25.5 ± 1.6	500	1000	250	500

a- Diameter of zone of inhibition (mm) including disc diameter of 6 mm; b- Mean of four assays; ± standard deviation; Me – Methicillin for Staphylococcus aureus (5 µg/disc); Cip – Ciprofloxacin for all the bacterial except S. aureus strains (10 µg/disc)

Table 3
Antibacterial activity of methanol extracts of Dictyota dichotoma and Gracilaria multipartite

S. No.	Name of the microorganisms	Mean zone of inhibition[a] (mm)[b]						Control (Me/ Cip)	*Dictyota dichotoma*		*Gracilaria multipartita*	
		Concentration of the disc (µg/disc)			Concentration of the disc (µg/disc)				MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
		Dictyota dichotoma			*Gracilaria multipartita*							
		1000	500	250	1000	500	250					
1	*Staphylococcus aureus*	18.3 ± 1.8	13.4 ± 1.5	7.3 ± 1.3	15.1 ± 1.6	12.0 ± 1.3	7.3 ± 1.0	8.6 ± 1.5	125	250	250	500
2	*Klebsiella pneumoniae*	13.0 ± 1.4	9.5 ± 1.0	7.1 ± 1.6	12.3 ± 1.5	10.0 ± 1.2	7.5 ± 0.6	27.3 ± 1.4	250	500	500	1000
3	*Pseudomonas aeruginosa*	13.3 ± 1.3	10.3 ± 1.2	8.5 ± 1.1	12.1 ± 1.3	9.3 ± 1.8	7.3 ± 1.5	29.3 ± 1.6	500	1000	250	500
4	*Escherichia coli*	13.0 ± 1.5	10.3 ± 1.2	8.7 ± 1.6	12.3 ± 1.2	9.0 ± 1.5	7.3 ± 1.6	28.0 ± 1.5	500	1000	500	1000
5	*Proteus mirabilis*	14.3 ± 1.4	11.0 ± 0.5	9.3 ± 0.2	12.0 ± 1.0	9.5 ± 0.9	7.3 ± 0.2	29.0 ± 1.8	250	500	250	500
6.	*Salmonella entrica*	16.5 ± 1.4	12.0 ± 0.7	10.0 ± 1.3	12.1 ± 1.0	9.5 ± 0.5	6.9 ± 0.5	27.5 ± 1.0	250	500	500	1000

a- Diameter of zone of inhibition (mm) including disc diameter of 6 mm; b- Mean of four assays; ± standard deviation; Me – Methicillin for Staphylococcus aureus (5 µg/disc); Cip – Ciprofloxacin for all the bacterial except S. aureus strains (10 µg/disc)

In the present study, methanol extracts of *U. lactuca, C. racemosa, C. sertularioides, P. gymnospora, D. dichotoma* and *G. multipartita* were studied against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis* and *S. enterica*. The highest mean zone of inhibition was observed by methanol extract of *Padina gymnospora* against *Staphylococcus aureus* (the mean zones of inhibition, 19.5 mm). All the extracts of marine macro algae tested possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. The mean values are presented in Tables 1, 2 & 3. When the different extracts were assayed against the test bacteria by agar disc diffusion assays, the mean zones of inhibition obtained were between 6.3 and 19.5 mm. Methicillin (10 µg/disc), antibacterial positive control produced mean zones of inhibition ranged from 7.5 to 9.5 mm. The Ciprofloxacin (10 µg/disc), antibacterial positive control produced mean zones of inhibition ranged from 24.3 to 30.5 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The MIC values of the different extracts of algal extracts ranged between 125 and 500µg/ml, while the MBC values were between 250 and 1000 µg/ml.

DISCUSSION

Marine macro algae are eukaryotic organisms that live in salty water in the ocean and is recognized as a potential source of bioactive natural products [19]. They contain compounds ranging from sterols, terpenoids to brominated phenol, which shows bioactivity against microorganisms [20]. Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations [21]. In the present investigation, methanol extracts of *U. lactuca, C. racemosa, C. sertularioides, P. gymnospora, D. dichotoma,* and *G. multipartita* showed the highest antibacterial activity against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis* and *S. enterica*. The highest mean zones of inhibition (19.5 mm) was observed in methanol extract of *P. gymnospora against S. aureus.* The MIC values of the methanol extracts of *P. gymnospora* ranged between 125 and 500µg/ml, while the MBC values were between

250 and 1000 µg/ml. Salem *et al.* [22] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *C. racemosa*, *Sargassum dentifolium, Padina gymnospora*; methanolic extracts of *Sargassum hystrix*, *C. racemosa*, *C. fragile*, *S. dentifolium* and *Cystoseria myrica* against *E. coli*, *S. aureus*, *E. feacalis*, *Salmonella sp., B. cereus* and *P. aeruginosa*. These results were in close agreement with those obtained by Patra *et al.* [23]. It was revealed that the chloroform and ethyl acetate extracts of *Enteromorpha compressa, Chaetomorpha linum* and *Polysiphonia subtilissima* were active against *Bacillus subtilis*, *B. brevis*, *E. coli*, *S. flexneri*, and *Vibrio cholerae*. Rangaiaha *et al.* [24] reported that the *Sargassum ilicifolium, Padina tetrastromatica* showed the highest inhibition was noticed with ethanol extracts and lowest with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. Antifungal activity of all the crude extractions of *G. corticata* showed highest activity against *Rhizopus stolonifer.* Mansuya *et al.* [25] reported the aqueous and methanolic extract of *U. lactuca, U. reticulata, Cladophora glomerata*, *G. corticata, Kappaphycus alvarezii* and *Sargassum wightii* against *E. coli, P. aeruginosa, S. typhi, Staphylococcus epidermis* and *S. pyogenes.* The antibacterial activity from methanol, ethanol, dichloromethane and hexane extracts of *Gracilaria fisheri* and *Ulva intestinalis* was tested against *S. aureus*, *Listeria monocytogenes*. Methicillin-resistant *S. aureus,* Enterobacter *faecalis, V. alginolyticus,V. parahaemolyticus, V. harveyi, E. coli, P. aeruginosa, K. pneumoniae, Salmonella typhi* and *P. mirabilis* [26]. Chandrasekaran *et al.* [27] showed the antibacterial activity of *U. fasciata* against multi-drug resistant bacterial strains of *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis* and *P. vulgaris.* In the present study, methanol extracts of *U. lactuca, C. racemosa, C. sertularioides,P. gymnospora, D. dichotoma,* and *G. multipartita* have possessed antibacterial activity against all the clinical and standard bacterial strains tested. Hediat *et al.* [28] reported that different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. In this present study also supported that optimizes their antibacterial activity by methanol extract the active compound from seaweeds. Seaweed extracts in different solvents exhibited different antimicrobial activities[24]. The high and low activity of organic extracts against microorganisms could be related to the presence of bioactive metabolites, which can be soluble in solvents [29]. In the present study, methanol extracts of marine macro algae possessed antibacterial activity against all the bacterial strains tested. Chandrasekaran *et al.* [12] methanol extracts of *Sargassum wightii* showed highest antibacterial activity against multi-drug resistant strains of *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, S. dysentriae, P. mirabilis* and *P. vulgaris.* The methanol crude extracts of *Sargassum longifolium* and *G. corticata* showed highest antibacterial activity against *Aeromonas hydrophilia, P. aeruginosa, V. cholerae, V. harveyi* and *V. parahaemolyticus* [30]. The methanol extracts of *G. verrucosa, G. ferugusoni, G. verrucosa* var *Hypnea musciformis, Enatiocladia prolifera,* and *Gelidium* species showed highest antibacterial activity against *B. substilis, E. coli, P. aeruginosa, S. typhi, Streptococcus aureus* and *Candida albicans* [31]. Radhika *et al.* [32] reported the antibacterial activity of seaweeds *Ulva fasciata, S. wightii* and *G. corticata* against *Bacillus cereus, Vibrio cholerae* classical, *V.cholerae* 0139, *E.coli, Pseudomonas aerogenosa*, *Aeromonas hydrophila, Salmonella typhii* and *Shigella flexneri.* Phytochemicals are compounds from food and medicine to protect and maintain human health. These have antioxidant or hormone-like effect which helps to fight against diseases like cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues. It is reported earlier that seaweeds are also rich in polysaccharides such as alginates, fucans, and laminarans which possess medicinal values[33]. Krishnaveni and Johnson[34] reported that phytochemical analysis of various solvents extracts revealed that the presence of alkaloids, glycosides, saponins, steroids, phenol and tannins in *G. corticata*. Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores[35]. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids are reported to have antimicrobial effects [36]. Tannins play a major role as antihaemorrhagic agent and showed to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties[37]. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities[38]. Steroids, saponins and triterpenoids showed the analgesic properties[39]. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [40]. In the present study, Gram-positive bacteria were found to be more susceptible than the Gram-negative bacteria. The resistance of Gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside [41]. However, the Gram positive bacteria do not possess such outer membrane and cell wall structures [41]. In the present study, almost all crude extracts tested have shown strong antibacterial potential against pathogenic bacteria.

CONCLUSION

Finally, it can be conclude that study proved the efficiency of antibacterial properties of seaweeds and advocates the potentiality of the plant as a source of alternative medicine. So, use of natural products, especially *P. gymnospora* and *D. dichotoma* may be considered as a new source of natural antibacterial agents.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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