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Original paper

Antibacterial efficacy and phytochemical characterization of some marine brown algal extracts from the red sea, Egypt

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Abstract

This study was carried out to evaluate the antibacterial activity of ethanol, methanol, acetone, and ethyl acetate extracts of the brown seaweeds, *Cystoseira myrica*, *Padina boergesenii* and *Sargassum cinereum* (Phaeophyta), as well as to identify the phytochemical constituents of the most effective algal extracts. Antibacterial activities were expressed as inhibition zones and minimum inhibitory concentrations (MICs) of the algal extracts. All seaweed extracts tested exhibited a broad spectrum of antibacterial activity. The maximum inhibition activities were recorded for methanolic extracts of *P. boergesenii* and ethyl acetate extracts of *C. myrica* and *S. cinereum* against *Shigella flexneri*, *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus*, respectively. The MIC values of the marine algal extracts tested for inhibiting pathogenic bacteria ranged from 3.13 to 300 mg/ml. GC-MS and FTIR analyses of algal extracts revealed the chemical components and their functional constituents in the brown seaweeds that might have potent antimicrobial activities. These components include fatty acids esters, alcohols, phenols, amines-containing compounds and others. The results indicated that brown seaweeds may be main sources of phytoconstituents which exhibited antibacterial properties and will be helpful in diminishing the adverse effects of synthetic drugs.

Keywords

Red sea Seaweeds, solvent extracts, antibacterial efficiency, pathogenic bacteria.

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Introduction

Many types of seaweeds could synthesize bioactive secondary metabolites which have antimicrobial activities. Marine macroalgae represent main sources for biomedical compounds (MANILAL & al [1]). Several antimicrobial agents were identified from marine macroalgae such as chlrorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulfur-containing heterocyclic compounds, and phenolic inhibitors which showed a high ability to inhibit the growth of microorganisms (LAVANYA & VEERAPPAN [2]).

Seaweeds contain many different secondary metabolites which have a wide spectrum of biological activities. Compounds with cytostatic, antifungal, antiviral, antibacterial, antioxidant and anthelmintic activities have been recorded in brown, red and green algae (NEWMAN & al [3]; CHINGIZOVA & al [4]). Seaweeds contain numerous substances such as carrageenan, alginate, and agar as phycocollids which have bacteriostatic and bactericidal properties (GORBAN & al [5]; EL-SHEEKH & al [6]). The marine algal extracts have many phytochemicals, such as alkaloids, flavonoids steroids, terpenes, glycosides, tannins and phenolic compounds (CARDOSO & al [7]; SUMAYYA & MURUGAN [8]). Furthermore, halogenated ketone, fatty acids, alkalines, and cyclic polysulphides were recorded (CHEUNG & al [9]; KHELIL-RADJI & al [10]). These compounds serve as a source of medicinal and ornamental purposes, flavoring, food additives, and preservatives. Recently, several studies reported the phytochemistry of seaweeds worldwide (LALITHA & al [11]; GARCIA-DAVIS & al [12]).

The revolutionized treatment of infectious diseases using antimicrobial medicine has shown limitations because

of the altering patterns of resistance in pathogens and side effects they made. These limitations require searching for new antimicrobial composites with improved pharmacokinetic properties (AL-HAJ & al [13]). Consequently, pharmacological industries should give a high importance to the composite derivatives from traditional sources (plants & soil) and ancient sources such as marine organisms (SOLOMON & SANTHI [14]). Hence, the interest in marine organisms as a potential and promising supply of pharmacological agents has been recently improved (RAJKUMAR & al [15]).

The present work aimed to investigate the antibacterial efficacy of different extracts of brown seaweeds and to select the most active species against the most common pathogenic bacteria. Furthermore, FTIR and GC-MS analyses were conducted to detect and characterize the active constituents in algal crude extracts.

Materials and Methods

1. Collection of algal samples

Three seaweeds species, including *Cystoseira myrica*, *Padina boergesenii* and *Sargassum cinereum* (Figure 1) were collected from the Red Sea, kept at the national institute of oceanography and fisheries (NIOF, 5 km north to Hurgada, 27° 17' 03" N and 33° 46' 21" E) in Egypt, and were then identified according to ALEEM [16]. The collected algal samples were cleaned using seawater to remove impurities. The samples were transported to the laboratory in sterilized polyethylene bags and put into an icebox. In the laboratory, algal samples were rinsed with tap water and then shade dried, cut into small pieces and powdered in a mixer grinder.

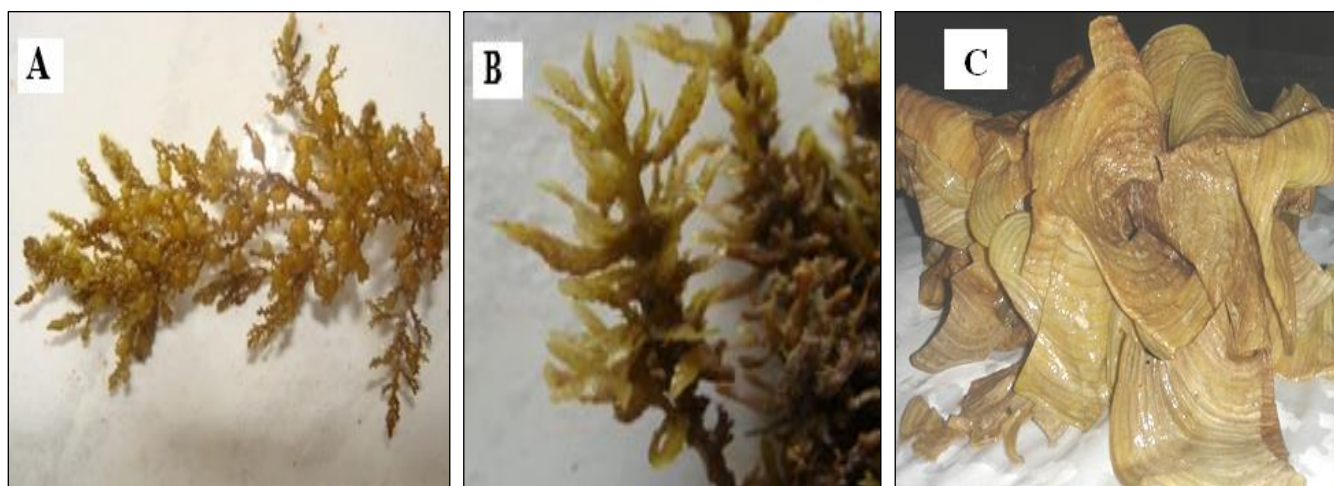


Figure 1. Marine brown algae collected from Red sea: *C. myrica* (A), *S. cinereum* (B) and *P. boergesenii* (C).

2. Preparation of algal extracts

The different seaweeds were extracted by soaking in different organic solvents of 85% (acetone, ethanol, methanol and ethyl acetate) in a ratio of (1:10 w/v) for 7 days on a rotary shaker at 120 rpm for 24 hrs. The extracts

were filtrated then solvents were evaporated using rotary evaporator at 45°C. The thick deposits produced were dissolved in dimethylsulfoxide (DMSO) at concentrations of 1 g/ml and stored in a refrigerator (PATRA & al [17]). The algal extracts were tested in three levels of 300, 200 and 100 mg/ml.

3. Bacteria and culture conditions

The bacteria *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalli* ATCC 29212, *Streptococcus pyogens* ATCC 19615 and *Staphylococcus aureus* (MRSA) ATCC 4330 as gram-positive species and *Pseudomonas aeruginosa* ATCC 278223, *Shigella flexneri* ATCC 12022, *Enterobacter aerogenos* ATCC 13048 and *Salmonella typhimurium* ATCC 14028 as gram-negative species were used. These bacterial strains were maintained on suitable media at 4°C and subcultured on Mueller Hinton Broth and Mueller Hinton agar at 37°C for 18 h before testing (MUELLER & HINTON [18]).

4. Antibacterial activity assay

4.1. Disc diffusion method

The antibacterial activity of the seaweeds was determined by the paper disc assay method (EL-MASRY & al [19]). Whatman No. 1 filter paper disc of 6-mm diameter was sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with different concentrations of algal extracts. Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2×10^8 CFU ml⁻¹. The impregnated discs were placed on the Muller Hinton medium suitably spaced apart and the plates were incubated at 37°C for 24 h. Ethanol, methanol, acetone and ethyl acetate were used as negative control while commercial antibiotic discs (chloramphenicol, 10 mg/disc) were used as a positive control. Diameters of the growth inhibition zones were measured by centimeters. All the assays were prepared in triplicate.

4.2. Minimum inhibitory concentrations (MICS) of seaweed extracts

The MICs of the algal extracts were determined with a tube dilution technique according to COLLINS & al [20]. The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of algal extract inhibiting visible growth. The experiment was performed in triplicates.

GC/MS analysis

The GC-MS analysis was used to determine the chemical composition of the antibacterial materials of seaweeds. The acquisition parameters were conducted with column (30 m x 250 µm x 0.25 µm film thickness) using helium as carrier gas (0.8 ml/min) with Perkin Elmer: Clarus 580/560 S model system. The GC oven temperature was programmed from 60°C to 250°C at a rate of 2°C/min. Relative area values (as a percentage of total volatile composition) were directly obtained from total ion current (TIC).

5. FTIR analysis

FTIR analysis was performed using spectrophotometer (Perkin Elmer 1430), which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. FT-IR spectra were recorded in the range of 4000-400 cm⁻¹ (JANAKIRAMAN & al [21]).

6. Data analysis

Results are presented as the mean ± standard deviation (SD) of three replicates. Data obtained were analyzed statistically to determine the degree of significance using a one-way analysis of variance test using statistical computer program SPSS (version 20).

Results and Discussion

1. Disc diffusion test

Agar disc diffusion method was carried out to test the antibacterial activities of different organic extracts of three marine species at different concentrations, and the data are recorded in Tables 1-3. Methanolic extract of *P. boergesenii* showed the highest significant inhibition activity (indicated as inhibition zone) against *Shigella flexneri* (2.03 cm, 49% increase compared to chloramphenicol, Figure 2) at c. 300 mg/ml, followed by ethanol extract against the same strain (1.70 cm) by 25 % increase. On the other hand, methanolic extract of *P. boergesenii* did not show any inhibition zones up to 200 & 100 mg/ml (0 cm) when tested against *Enterobacter aerogenosa*, *E. coli*, and *Streptococcus pyogenes* respectively (Table 1).

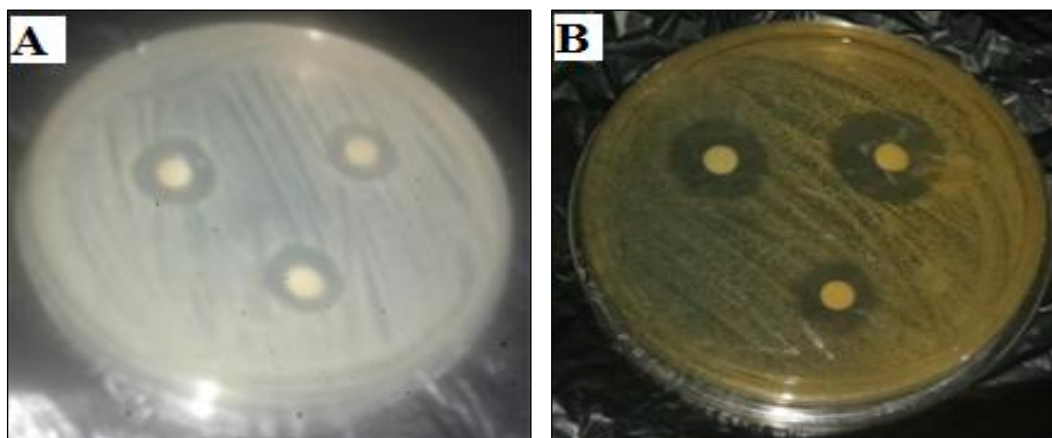


Figure 2. Inhibition zone of methanol extract of *P. boergesenii* against *Staphylococcus aureus* (A) and *Shigella flexnerii* (B).

Table 1. Antibacterial activity of *P. boergeresii* extracts against human pathogenic bacteria

Bacterial strains		<i>Pseudomonas aeruginosa</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Enterobacter aerogenes</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i> (MRSA)	<i>Streptococcus pyogenes</i>
Inhibition zone (cm)										
Control / Chloramphenicol		0.9 ±0.00	1.36 ±0.20	1.35 ±0.04	1.06 ±0.20	1.03 ±0.10	1.1 ±0.20	1.06 ±0.06	1.23 ±0.02	1.2 ±0.1
Organic solvent	Conc. mg/ml									
Ethanol	100	0.86 ±0.057	1.03 ±0.10	1.13** ±0.11	0.80* ±0.10	0.80** ±0.00	0.80* ±0.10	0.76** ±0.11	1.26 ±0.05	0.83** ±0.05
	200	0.93 ±0.057	1.5 ±0.20	1.33 ±0.15	1.06 ±0.05	0.83* ±0.05	0.90 ±0.00	0.90 ±0.17	1.50** ±0.00	0.96** ±0.05
	300	1.06 ±0.152	1.70** ±0.10	1.43 ±0.05	1.20 ±0.17	1.03 ±0.15	1.10 ±0.20	1.0 ±0.00	1.53** ±0.05	1.23 ±0.05
Methanol	100	0.86 ±0.15	1.0** ±0.10	1.06** ±0.15	0.83** ±0.05	0.00	0.76* ±0.05	0.00	1.3 ±0.10	0.00
	200	0.93 ±0.05	1.96** ±0.11	1.46 ±0.15	0.93 ±0.11	0.00	0.90 ±0.20	0.70** ±0.00	1.40* ±0.10	0.73** ±0.05
	300	0.93 ±0.05	2.03** ±0.05	1.63 ±0.05	1.13 ±0.05	1.0 ±0.10	0.96 ±0.05	1.26** ±0.05	1.50** ±0.10	1.26 ±0.15
Acetone	100	0.086	1.0** ±0.17	1.06** ±0.15	0.73** ±0.05	0.70** ±0.00	0.00	0.73* ±0.05	1.03 ±0.11	0.73** ±0.05
	200	0.93 ±0.10	1.23 ±0.05	1.40 ±0.1	1.06 ±0.05	0.70** ±0.00	0.70** ±0.00	1.03 ±0.11	1.13 ±0.20	0.90** ±0.10
	300	1.20** ±0.05	1.23 ±0.05	1.63* ±0.05	1.06 ±0.05	1.03 ±0.05	0.86* ±0.05	1.2 ±0.26	1.66** ±0.05	0.96** ±0.05
Ethyl acetate	100	0.83 ±0.15	0.86** ±0.1	1.0** ±0.1	0.90* ±0.10	0.80** ±0.10	0.73** ±0.05	0.70** ±0.00	0.86** ±0.11	0.70** ±0.00
	200	0.90 ±0.10	0.93** ±0.05	1.40 ±0.1	1.03 ±0.05	0.83** ±0.05	0.76** ±0.05	0.80** ±0.10	1.53** ±0.05	0.90* ±0.17
	300	0.96 ±0.05	0.96** ±0.05	1.66** ±0.05	1.36** ±0.05	0.96 ±0.05	0.93 ±0.05	1.0 ±0.10	1.46** ±0.05	1.13 ±0.11

Mean ± SD, n=3, * significant value, ** high significant. The mean difference is significant at the 0.05 level.

Table 2. Antibacterial activity of *C. myrica* extracts against human pathogenic bacteria

Bacterial strains		<i>Pseudomonas aeruginosa</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Enterobacter aerogenes</i>	<i>Salmonella typhimurium</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i> (MRSA)	<i>Streptococcus pyogenes</i>
Inhibition zone (cm)										
Control /Chloramphenicol		0.9 ±0.00	1.36 ±0.20	1.35 ±0.04	1.06 ±0.20	1.03 ±0.10	1.1 ±0.20	1.06 ±0.06	1.23 ±0.02	1.2 ±0.1
Organic solvent	Conc. mg/ml									
Ethanol	100	0.86 ±0.11	0.86** ±0.05	1.0** ±0.1	0.83* ±0.05	0.96 ±0.05	0.76** ±0.11	0.90* ±0.10	1.20 ±0.10	0.86* ±0.05
	200	0.93 ±0.11	1.10** ±0.17	1.13** ±0.11	1.0 ±0.10	1.13 ±0.11	0.80** ±0.00	0.90* ±0.10	1.26 ±0.05	1.13 ±0.20
	300	1.20* ±0.17	1.33 ±0.05	1.30 ±0.26	1.23 ±0.15	1.30* ±0.26	0.90 ±0.10	0.96 ±0.05	1.33 ±0.05	1.30 ±0.26
Methanol	100	0.70** ±0.11	0.80** ±0.10	0.96** ±0.15	0.73** ±0.05	0.80** ±0.00	0.76** ±0.05	0.00	1.23 ±0.11	0.83** ±0.05
	200	0.90 ±0.00	1.03** ±0.15	1.06** ±0.20	0.83** ±0.05	0.90* ±0.10	0.86* ±0.05	0.70** ±0.00	1.23 ±0.20	0.90** ±0.17
	300	1.13** ±0.11	1.03** ±0.05	1.36 ±0.05	0.93* ±0.05	1.20** ±0.00	1.06 ±0.15	1.03 ±0.15	1.40 ±0.10	0.93** ±0.05
Acetone	100	0.80* ±0.15	0.76 ±0.05	0.93** ±0.05	0.00	0.00	0.00	0.00	1.10 ±0.10	0.70** ±0.00
	200	0.83* ±0.05	0.86** ±0.05	1.03** ±0.05	0.80** ±0.13	0.00	0.83** ±0.05	0.80** ±0.11	1.26 ±0.05	0.83** ±0.05
	300	0.93 ±0.05	1.23** ±0.05	1.53 ±0.05	0.96 ±0.05	0.83** ±0.05	1.03 ±0.05	1.06 ±0.11	1.36 ±0.11	0.86** ±0.05
Ethyl acetate	100	0.83 ±0.05	1.23 ±0.05	1.23* ±0.15	0.96 ±0.05	0.90 ±0.17	0.73** ±0.05	0.00	1.23 ±0.15	0.83** ±0.10
	200	0.90 ±0.10	1.30 ±0.10	1.50 ±0.00	1.20 ±0.10	0.90 ±0.10	0.83* ±0.05	0.76** ±0.05	1.46* ±0.05	1.23 ±0.11
	300	1.1* ±0.10	1.36 ±0.15	1.56 ±0.05	1.36** ±0.05	1.06 ±0.11	1.03 ±0.11	0.96* ±0.05	1.60** ±0.10	1.23 ±0.05

Mean ± SD, n=3, * significant value, ** high significant. The mean difference is significant at the 0.05 level.

Table 3. Antibacterial activity of *S. cinereum* extracts against human pathogenic bacteria

Bacterial strains		<i>Pseudomonas aeruginosa</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Enterobacter aerogenes</i>	<i>Salmonella typhimurim</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i> (MRSA)	<i>Streptococcus pyogenes</i>
Control / Chloramphenicol		0.9 ±0.00	1.36 ±0.20	1.35 ±0.04	1.06 ±0.20	1.03 ±0.10	1.1 ±0.20	1.06 ±0.06	1.23 ±0.02	1.2 ±0.1
Organic solvent	Conc. mg/ml	Inhibition zone (cm)								
Ethanol	100	0.70** ±0.00	0.90** ±0.10	0.80** ±0.00	0.83** ±0.05	0.80* ±0.10	0.93 ±0.05	0.90* ±0.10	0.93** ±0.05	0.83** ±0.05
	200	0.90 ±0.00	1.06* ±0.15	1.06** ±0.11	0.86** ±0.05	0.93 ±0.15	0.96 ±0.05	0.96 ±0.05	0.93** ±0.05	0.96* ±0.15
	300	0.90 ±0.10	1.10* ±0.17	1.50 ±0.17	0.93* ±0.05	1.06 ±0.11	1.03 ±0.05	1.20 ±0.10	1.26 ±0.05	1.0* ±0.10
Methanol	100	0.90 ±0.00	0.73** ±0.05	0.73** ±0.05	0.86* ±0.05	0.83** ±0.05	0.93 ±0.05	0.80** ±0.00	0.83** ±0.05	1.0 ±0.10
	200	1.0 ±0.10	1.0** ±0.17	0.86** ±0.11	0.90* ±0.10	0.86** ±0.05	1.23 ±0.05	0.93 ±0.05	1.03 ±0.20	1.20 ±0.26
	300	1.06* ±0.11	1.10** ±0.10	1.30 ±0.20	1.0 ±0.10	0.90* ±0.10	1.33* ±0.15	1.06 ±0.15	1.16 ±0.15	1.4 ±0.17
Acetone	100	0.80 ±0.00	0.83** ±0.05	0.83** ±0.05	0.83** ±0.05	1.0 ±0.10	0.96* ±0.05	1.03 ±0.11	0.80** ±0.00	0.00
	200	0.86 ±0.05	1.0** ±0.10	1.03** ±0.11	0.90* ±0.10	1.03 ±0.05	1.20 ±0.10	1.06 ±0.05	0.80** ±0.00	0.93** ±0.15
	300	1.03 ±0.05	1.06** ±0.15	1.46 ±0.05	1.03 ±0.05	1.16 ±0.15	1.36 ±0.05	1.13 ±0.11	1.0** ±0.10	1.23 ±0.05
Ethyl acetate	100	0.73* ±0.05	1.0* ±0.20	1.20* ±0.10	0.93 ±0.05	0.80** ±0.00	0.90 ±0.10	0.83** ±0.05	1.10 ±0.20	0.90** ±0.10
	200	1.03 ±0.05	1.0* ±0.10	1.33 ±0.05	1.0 ±0.10	0.83** ±0.05	1.03 ±0.11	1.0 ±0.10	1.30 ±0.20	1.23 ±0.05
	300	1.16** ±0.15	1.03* ±0.20	1.63* ±0.11	1.20 ±0.10	0.96 ±0.05	1.16 ±0.05	1.13 ±0.05	1.50* ±0.10	1.26 ±0.05

Mean ± SD, n=3, * significant value, ** high significant. The mean difference is significant at the 0.05 level.

With regard to the extracts from *C. myrica*, ethyl acetate extract induced the maximum inhibition activity against *S. aureus* and *S. aureus* (1.60 and 1.56 cm by 30 and 15%, increase, respectively, Figure 3), followed by acetone and methanolic extracts against *S. aureus* (1.53 cm, increase 13%) and *S. aureus* (MRSA) (1.40 cm, increase 11%), compared to chloramphenicol (Table 2).

In case of the extracts from *S. cinereum*, 300 mg/ ml ethyl acetate extract of *S. cinereum* showed the highest activity against *S. aureus* and *S. aureus* (MRSA) (1.63 and 1.50 cm, respectively, Figure 4), followed by ethanolic extracts against the same strains (1.50 and 1.26 cm) more than chloramphenicol (1.35 and 1.23 cm). On the other hand, methanolic extract induced little activity against all tested bacteria.

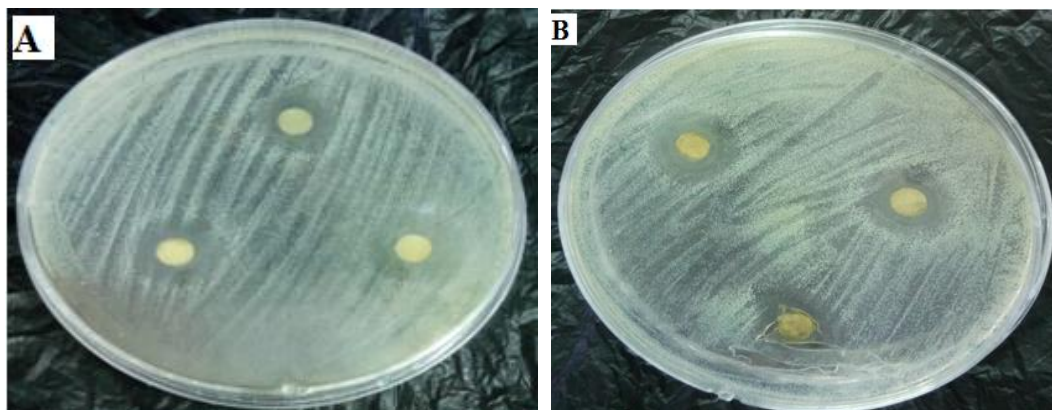


Figure 3. Inhibition zone of ethyl acetate extract of *C. myrica* extracts against *Staphylococcus aureus* (A) and *Staphylococcus aureus* (MRSA) (B).

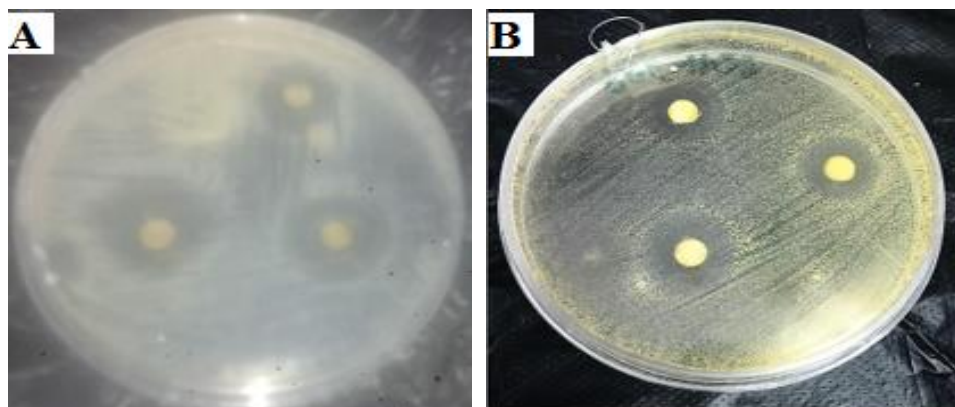


Figure 4. Inhibition zone of ethyl acetate extract of *S. cinereum* against *Staphylococcus aureus* (A) and *Staphylococcus aureus* (MRSA) (B).

These results were in agreement with those obtained by PUSHPARAJ & al [22] who reported that *P. tetrastromatica* extract of different concentrations has an activity against human pathogenic bacteria. Methanol extraction yielded higher antimicrobial activity than ethyl acetate and n-hexane (RANGAIAH & al [23]). MOREAU & al [24] found that *C. mediterranea* and *C. usneoides* were strongly active against *S. aureus*, which may be due to the active components present in seaweed extracts.

Similar results were obtained by CHIAO-WEI & al [25] who reported that *S. polycystum* extracts exhibited higher bacteriostatic activity against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa* when compared to other algal extracts. Our results indicated that the inhibitory effect increased with the increasing concentration of algal extract. In agreement with current results, DARAH & al [26] reported that the high concentration of the algal extract is necessary to kill the microorganism's cells.

In contrast to our results, EL SHAFAY & al [27] reported a non-efficiency of the methanolic extract of

S. vulgare, which did not show a noticeable activity against *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*.

2. Minimum inhibitory concentrations (MICs)

The MIC values of the algal extract tested for inhibiting Gram-positive and Gram-negative bacteria ranged from 3.13 to 300 mg/ml (compared to chloramphenicol 10 mg/ml against all bacterial strains). The lowest MIC value was recorded for the ethyl acetate extract of *P. boergesenii* and acetone extract of *S. cinereum* against *Staphylococcus aureus* (3.13 mg/ml), followed by ethyl acetate extracts of *S. cinereum* (4.16 mg/ml), and methanolic and acetone extracts of *P. boergesenii* (4.33 & 5.16 mg/ml) against *Shigella flexneri* and *S. aureus*, respectively. Consequently, ethyl acetate extract of *P. boergesenii* was considered as the ideal algal extract (inhibition zone 1.66 and MIC 3.13 mg/ml). Gram-positive bacteria especially *S. aureus* were more susceptible to the seaweed extracts (MIC=3.13 to 50 mg/ml, Table 4).

Table 4. Minimum inhibitory concentrations (MICs mg/ml) of seaweed extracts against human pathogenic bacteria

Bacterial strains		<i>Pseudomonas aeruginosa</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Enterobacter aerogenes</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i> (MRSA)	<i>Streptococcus pyogenes</i>
Algal species	Organic solvent									
<i>P. boergesenii</i>	Ethanol	100	50	50	50	41.6	100	100	25	27
	Methanol	100	4.33	6.25	75	300	75	200	25	200
	Acetone	50	25	5.16	41.6	75	200	100	50	83.3
	Ethyl acetate	100	25	3.13	50	50	100	75	12.5	50
<i>C. myrica</i>	Ethanol	41.6	75	10.41	75	41.6	75	75	25	50
	Methanol	83.3	75	50	75	50	100	200	50	25
	Acetone	25	41.6	12.5	200	233	200	200	50	75
	Ethyl acetate	83.3	50	50	83.3	50	100	200	50	50
<i>S. cinereum</i>	Ethanol	75	25	16.6	66.6	50	50	25	33.3	50
	Methanol	75	50	33.3	50	50	50	50	50	50
	Acetone	12.5	25	3.13	50	75	75	83.3	50	50
	Ethyl acetate	25	25	4.16	20.8	66.6	50	50	50	25

In this concern, CHIAO- WEI & al [25] showed that Gram-positive bacteria especially *B. cereus* was more susceptible to the seaweed extracts (MIC=0.130 to 0.065 mg/ml). They also found that *Sargassum polycystum* extracts exhibited higher bacteriostatic activity (lower MICs) against all the tested bacterial strains as compared to *Padina australis*.

Our results indicated that Gram-positive bacteria were more sensitive to the marine extracts than Gram-negative bacteria, which may be due to the alterations in their cell wall structure and composition (Taskin & al [28]). In Gram-negative bacterium, the outer membrane acts as a barrier to several environmental substances as well as antibiotics (TORTORA & al [29]). The presence of thick murine layer within the plasma membrane prevents the entry of the inhibitors (KANDHASAMY & ARUNACHALAM [30]).

Data also indicated that the antibacterial activities of marine algal extracts depend on algal species, the efficiency of the extraction method, and concentration of algal extract. For instance, methanolic extract of *P. boergesenii* showed a high activity against most tested species, particularly *Shigella flexneri* and *S. aureus*. However, ethyl acetate extract of *C. myrica* and ethyl acetate and ethanol extracts of *S. cinereum* induced a significant inhibition activity against *S. aureus* (MRSA) and *S. aureus*. The high and low effect of the organic extract against microorganisms could be related to the presence of bioactive metabolites, which can be soluble in the solvent (KOLANJINATHAN & STELLA [31]).

3. GC/MS analysis

The GC–MS chromatograms showed various compounds present in the ethyl acetate extracts of *P. boergesenii*, *S. cinereum* and *C. myrica*. The bioactive principles with its retention time (RT) and peak area (%) are listed in Table (5) and Figure 5.

A total of 23, 22 and 17 compounds were identified in the ethyl acetate extracts of *C. myrica*, *P. boergesenii*, and *S. cinereum*, respectively. The major compounds detected in *C. myrica*, *P. boergesenii* and *S. cinereum* extracts are n-Hexadecanoic acid (63.14, 62.3 & 54.7%), Tetradecanoic acid (8.6, 9.7 & 8.5%), Octadecanoic acid (5.12, 6.9 & 2.02%) and 9-Octadecenamide, (Z) (3.9, 5.4 & 8.13%), respectively (Table 5). The GC-MS analysis of the volatile compounds of the marine algal extracts showed the

presence of amide-containing compounds, long-chain hydrocarbons, aldehyde, alcohols, fatty acids esters, phenols and amines-containing compounds. Therefore, the antibacterial activity of these extracts might be attributed to the presence of these compounds, which have been already proposed to have a certain antimicrobial activity (OKUNOWO & al [32]). MANILAL & al [1] reported that fatty acids and their derivatives have antimicrobial and antifouling activity. Citral has also been reported to have antimicrobial activity by GITERU & al [33] who studied physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films.

Following the present study, many terpenes are active against various microorganisms, including gram-negative and gram-positive bacteria. In general, gram-positive bacteria are most sensitive to terpenes (NIKITINA & al [34]).

Natural sources of drugs extracted from seaweeds are broadly used, even when its biologically active composites are unknown, because of their effectiveness, minimal side effects in clinical experience and relatively low cost (LALITHA & al [11]).

4. FTIR analysis

The FTIR spectra were used to identify the functional group of the active components responsible for the antibacterial activity based on the peak value in the region of infrared radiation.

Components were separated for ethyl acetate extract of *P. boergesenii* at 2918 cm^{-1} (Lipid (C-H) stretching), 2850.27 cm^{-1} (Alkanes C-H stretch), 1714.41 cm^{-1} (Ketones (C=O) stretching) and 1464.67 cm^{-1} (Nitro groups N=O Stretch /N=O bend (Fig. 6A). In ethyl acetate extract of *S. cinereum*, the functional groups of the components were separated at 3430 cm^{-1} (phenols & alcohols (O-H) stretching), 2923 cm^{-1} (Lipid (C-H) stretching, 1738 cm^{-1} (Fatty Acids (C=O) stretching of esters) and 1172.51 cm^{-1} (carbohydrate (O-H) stretching) (Fig. 6B).

FTIR spectrum of ethyl acetate extract of *C. myrica* showed only three peaks with three different functional groups such as 3423 cm^{-1} (phenols & alcohols (O-H stretching), protein $\nu(\text{N-H})$ stretching (amide A), 2925 cm^{-1} (Lipid (C-H) stretching) and 1732 cm^{-1} (Carboxylic acids C=O stretching) (Fig. 6C).

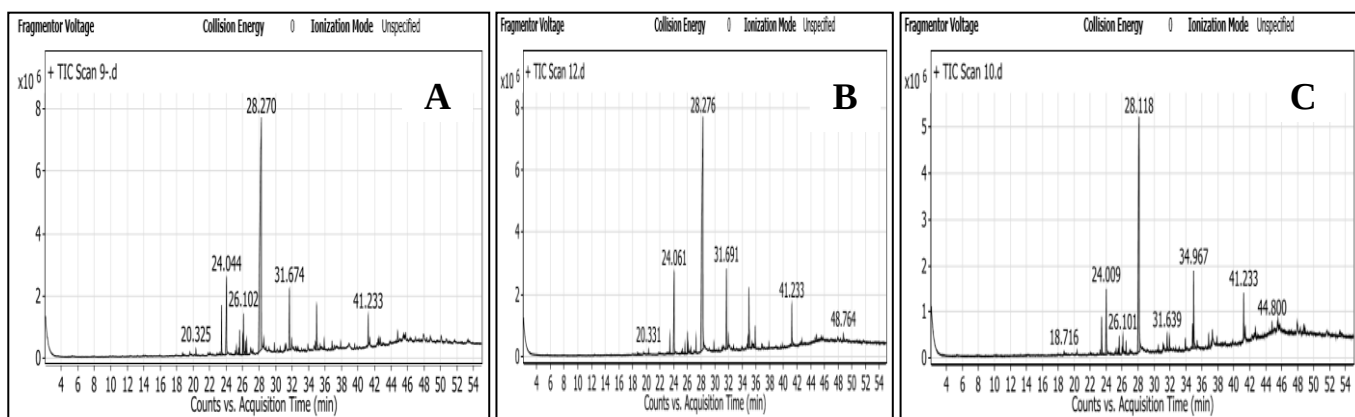
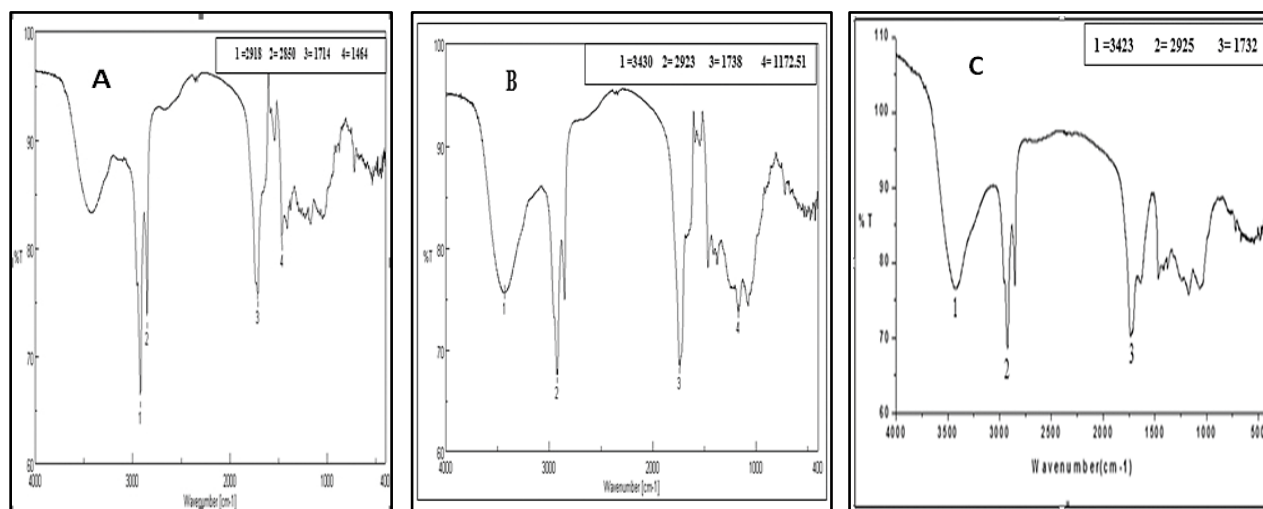


Figure 5. Chromatogram obtained from the GC/MS with the ethyl acetate extracts of *C. myrica* (A) *P. boergesenii* (B) and *S. cinereum* (C)

Table 5. Composition of ethyl acetate extracts of *C. myrica*, *P. boergesenii* and *S. cinereum* as investigated by GC-MS chromatography

S.NO	Compounds	<i>C. myrica</i>		<i>P. boergesenii</i>		<i>S. cinereum</i>	
		R. time (min)	Peak area %	R. time (min)	Peak area %	R. time (min)	Peak area %
1	Bicyclo [3.2.2] nonane-1,5-dicarboxylic acid, 5-ethyl ester	18.693	0.39	18.711	0.65	18.716	1.04
2	5-Isopropenyloxymethylene-3,3-dimethyl-cyclohexanone	20.325	0.44	20.325	0.38	-----	-----
3	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	23.438	3.46	23.438	1.32	23.432	3.89
4	Tetradecanoic acid	24.044	8.61	24.044	9.73	24.009	8.46
5	Estra-1,3,5(10)-trien-17. beta. -ol	25.111	0.29	25.111	0.34	----	----
6	2-Pentadecanone, 6,10,14-trimethyl	25.629	1.42	25.629	0.53	25.635	1.4
7	Pentadecanoic acid	26.002	1.26	26.002	1.39	----	----
8	Phthalic acid, butyl tetradecyl ester	26.102	2.84	26.102	0.51	26.101	1.52
9	1-Hexadecanol, 2-methyl	26.329	0.96	---	-----	---	---
10	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	26.498	1.01	26.492	0.37	26.498	1.06
11	n-Hexadecanoic acid	28.27	63.14	28.276	62.29	28.118	54.78
12	Heptadecanoic acid	29.855	0.62	29.861	0.66	-----	-----
13	Oleic Acid	30.549	0.35	30.549	0.21	-----	-----
14	trans-2-Hexadecenoic acid	31.126	0.36	----	----	---	----
15	Octadecanoic acid	31.674	5.12	31.691	6.94	31.639	2.02
16	1-Heptatriacotanol	32.507	0.13	----	-----	----	----
17	Oxirane octanoic acid, 3-octyl-, cis	32.717	0.33	32.723	0.38	---	----
18	7-Methyl-Z-tetradecen-1-ol acetate	34.827	0.56	34.827	0.63	30.543	0.6
19	9-Octadecenamide, (Z)	34.973	3.92	34.979	5.45	34.967	8.13
20	Phenol, 2,2'-methylenbis[6-(1,1-dimethylethyl)-4-methyl Ionization	35.888	0.75	35.888	1.53	-----	-----
21	Hexadecanoic acid, 2,3-dihydroxypropyl ester	36.838	0.75	-----	-----	36.844	0.36
22	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	37.887	0.34	37.887	0.35	37.887	5.12
23	13-Docosenamide, (Z)	41.233	2	41.233	3.31	41.233	0.13
24	Hexadecanoic acid, methyl ester	-----	-----	27.244	0.85	----	----
25	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	----	-----	33.918	0.17	33.912	1.35
26	cis-9,10-Epoxyoctadecanamide	---	---	----	-----	31.936	1.65
27	4,8,12,16-Tetramethylheptadecan-4-olide	----	-----	-----	-----	34.821	2.55
28	Spirost-8-en-11-one, 3-hydroxy-, (3. beta., 5. alpha., 14. beta., 20. beta., 22. beta., 25R)	-----	-----	-----	----	44.8	0.33
29	Palustric acid	---	-----	35.684	0.23	----	-----

R.time: Retention time ---: absent

Figure 6. Infrared absorption spectra from ethyl acetate extracts of *P. boergesenii* (A) *S. cinereum* (B) and *C. myrica* (C).

The IR spectra of the different extracts confirmed the presence of amines, alkane, alkene, esters, carboxylic acid, cyanides, lipids, carbohydrates of polysaccharides, and a boron compound. The antibacterial activities could, therefore, be attributed to the presence of these compounds which have a role in the overall defense against pathogenic bacteria (AL-SAIF & al [35]). Thus the present study of phytochemical analysis of three different seaweeds can help the manufacturers for identification and selection of raw materials for drug production. Many studies suggested that phenol compounds are the major chemical components of the marine macroalgal cell and could have an activating or inhibitory effect on bacterial growth based on their constitution and concentration (REGUANT & al [36]; MOUBAYED & al [37]). This is indicated by the highest absorption of hydroxyl amide groups, relative to phenol compounds with ranges of 3430 cm^{-1} (Fig. 6A), 3423 cm^{-1} (Fig. 6B) and 3409 cm^{-1} (Fig. 6C) for *P. boergesenii*, *S. cinereum* and *C. myrica*, respectively. In our study, it was observed that the presence of different antibacterial substances in the organic solvent extracts of the tested species might be the reason for the variation of antibacterial activity as reported by LUSTIGMAN & BROWN [38].

These results indicated that ethyl acetate extracts were the most effective solvent for the extraction of the bioactive compounds from marine algae, followed by ethanol and acetone extracts. Data also indicate that the antibacterial agents from the seaweeds can be used in control of bacterial infections. Organic solvents, including ethanol, methanol, acetone and ethyl acetate showed higher efficiency for the extraction of antibacterial natural products from marine macroalgae, and confirmed the broad antimicrobial effect of marine algae.

Conclusions

The organic solvent extracts of the tested marine algal species showed significant inhibitory actions against pathogenic bacteria. Among the solvent extracts screened for their antibacterial activity, ethyl acetate extracts of *C. myrica* and *S. cinereum*, and methanolic extract of *P. boergesenii* comprised useful bioactive components with strong antibacterial activities. As a result, they may be explored for the improvement of anti-pathogenic drugs in the pharmaceutical industries. However, further research is needed to identify the effect of each bioactive component on tested isolates.

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