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FULL LENGTH ARTICLE

Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria

Shimaa M. El Shafay, Samh S. Ali, Mostafa M. El-Sheekh *

Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

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Abstract This study evaluates the antibacterial activity of diethyl ether, methanol, ethanol and chloroform extracts of red algae *Ceramium rubrum* (Rhodophyta), *Sargassum vulgare*, *Sargassum fusiforme* and *Padina pavonia* (Phaeophyta) collected from Red sea, Egypt. The algal extracts were tested for their antibacterial activity against ten multidrug resistant clinical isolates of Gram positive and Gram negative bacteria. The highest inhibition activity among all extracts was obtained with 100 µl diethyl ether extract *S. fusiforme* against *Staphylococcus aureus* 2 and 50 µl ethanol extract of *S. vulgare* against *Klebsiella pneumoniae*. The algal extract of *S. fusiforme* and *S. vulgare* was characterized by Gas chromatography–mass spectrometry (GC–MS). The compounds with antimicrobial activity were identified, such as phenols, terpenes, acetogenins, indoles, fatty acids and volatile halogenated hydrocarbons. Transmission electron microscopy was applied for determining the morphological changes in *S. aureus* 2 and *K. pneumonia* treated with 100 µl diethyl ether extract of *S. fusiforme* and 50 µl ethanol extract of *S. vulgare*, respectively. Perforation of cell wall, leakage of cytoplasmic contents, severe distortion of outer cell shape, inner chromatin mild scattered cytoplasmic vacuolation, rupture of cell wall, and decreased cell size for both bacterial isolates treated with 100 µl diethyl ether of *S. fusiforme* extract and 50 µl *S. vulgare* ethanolic extract were recorded.

Introduction

Various natural antimicrobial compounds have been recorded in marine environment more than those in the terrestrial one (Ireland et al., 1988). Marine organisms such as marine algae are source material for structurally unique natural products with pharmacological and biological activities (Schwartsmann et al., 2001). Among the marine organisms, the macroalgae (seaweeds) occupy a special site as a source of biomedical compounds (Manilal et al., 2010). Seaweeds have been recognized as potential sources of the antibiotic substances. Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds (Chiheb et al., 2009). A wide range of bioactive compounds were derived from macro algae such as antibacterial active compounds (Lustigman and Brown, 1991). Seaweeds contain many different secondary metabolites which

* Corresponding author. Fax: +20 40 3350804.
E-mail address: mostafaelsheikh@science.tanta.edu.eg (M.M. El-Sheekh).

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have a wide spectrum of biological activities. It was observed, the presence of cytostatic, antiviral, anthelminthic, antifungal and antibacterial activities compounds in green, brown and red algae with cytostatic, antiviral, anthelminthic, antifungal and antibacterial activities (Lindequist and Schweder, 2001; Newman et al., 2003 and Chakraborthy et al., 2010). Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as antibiotics, antioxidant and anti-inflammatory (Tuney et al., 2006; Patra et al., 2008). Some macroalgae have bio-active components which affected the germination of some pathogenic bacteria (Kolanjinathan et al., 2009). Hornsey and Hide (1985) found that many species of marine algal crude extracts have inhibition activity against pathogenic bacteria. Seaweeds contain different substances which incorporated medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties (Gorban et al., 2003). Different diseases were treated with antibiotics, extracted from terrestrial sources that were used as therapeutic agents; new compounds were present in oceans and have commercial value (Smit, 2004).

Clinical and public health problem due to antibiotic resistance and multi-resistant bacteria are difficult and sometimes impossible to treat (Levy, 2002). Using antibiotics in different medicines has a significant role in the emergence of bacterial strains resistant to antibiotics (Bacon et al., 2000). Recently, new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug resistant (MDR) bacterial strains, some also known as "superbugs" (Sande-Bruinsma et al., 2008). The required number of new antimicrobial agents is higher than ever due to the rapid presence of new infections, emergence of multidrug resistance in common pathogens, and the potential for use of multidrug-resistant agents in bioweapons (Peters et al., 2008). Organisms resistant to more than one class of antimicrobial agents are identified as multidrug resistant organisms (MDROs) (Sameera et al., 2010).

This work aims to evaluate the antimicrobial activity of some seaweeds extracts from Red sea coast against some collected clinical multidrug resistant bacterial isolates in order to find alternative drugs and promising source of pharmaceutical agents.

Material and methods

Algal collection and preparation

Four seaweeds species, *Ceramium rubrum* (Rhodophyta), *Sargassum vulgare*, *Sargassum fusiforme* and *Padina pavonia* (Phaeophyta) were collected from Hurghada coastal along the Red sea, Egypt, and identified according to Aleem (1993). Different species of collected algae were cleaned with seawater to remove impurities. The seaweeds were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder.

Preparation of organic algae extracts

Different organic solvents (ethanol, methanol, chloroform and diethyl ether) were used for extraction. Five grams of each powdered sample were soaked in 40 ml of the solvent for three days. Remain extracts were filtered and concentrated in a rotatory evaporator at 35 °C. The residual water was removed with a vacuum pump. The weighted crude extracts were suspended in the dimethyl sulfoxide (DEMSO) to a final concentration of 50 mg/ml and stored in a refrigerator (Mohanta et al., 2007; Patra et al., 2008).

Collection of bacteria

Two isolates of *Pseudomonas aeruginosa* (PA1 and PA2) were recovered and identified by Khalil et al. (2015). Four isolates of *Staphylococcus aureus* (SA1, SA2, SA3 and SA4), *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Corynebacterium* sp. Bacterial species were brought from microbiology collection, Faculty of Pharmacy, Tanta University, Egypt. The morphological and biochemical tests were carried out continuously to ensure purity (Collee et al., 1996).

Antibiotic susceptibility testing

The antimicrobial susceptibility of the collected bacteria was assessed using the modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2012). The following concentrations of antibiotics were tested: Ampicillin (AMP, 10 μg), Amoxicillin (AX, 25 μg), Amoxycillin/Clavulanic acid (AMC, 20/10 μg), Oxacillin (OX, 1 μg), Piperacillin-tazobactam (TPZ, 100/10 μg), Ceftazidime (CAZ, 30), Cefepime (FEP, 30 μg), Ceftriaxone (CRO, 30 μg), Imipenem (IPM, 10 μg), Meropenem (MEM, 10 μg), Cefoperazone/sulbactam (CES, 75/30 μg), Aztreonam (ATM, 30 μg), Gentamicin (CN, 10 μg), Amikacin (AK, 30 μg), Neomycin (N, 30 μg), Streptomycin (S, 10 μg), Tobramycin (TOB, 10 μg), Kanamycin (K, 30 μg), Chloramphenicol (C, 30 μg), Colistin Sulfate (CT, 10 μg), Nalidixic acid (NA, 30 μg), Ciprofloxacin (CIP, 5 μg), Co-trimoxazole (SXT, 25 μg), Tetracycline (TE, 30 μg), Vancomycin (VA, 30 μg). The antibiotic disks were then applied to the prepared plates and incubated at 37 °C for 18 h then, the diameter of the growth inhibition zones was measured. The multiple antibiotic resistances (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Krumpernam, 1983; Olayinka et al., 2009; Jayaraman et al., 2012).

Ultra-structure of multiple drug resistant (MDR) bacterial isolates

The changes in ultra-structure of selected MDR, *S. aureus* 2 and *K. pneumoniae*, due to algal extract treatment were investigated by transmission electron microscope (JEOL-JEM-100SX, Japan). The samples were incubated by shaking at 37 °C for 18 h followed by centrifugation, and washing using saline solution (Richards and Cavill, 1976).

Minimum inhibitory concentration (MIC) of algal extracts

The MIC algal extract against the selected multi-drug resistant isolate was carried out using a modified procedure from Peng et al. (2010). Algal extracts were serially diluted with dimethyl sulfoxide (DMSO) to obtain diluted concentrations in the range of 50, 75 and 100 µl. Each plate was inoculated with 100 µl of standardized inoculums (10^6 cfu/ml) of the tested microorganisms separately, incubated at 37 °C for 18 h. The MIC was defined as the lowest concentration at which no visible growth was observed. The experiment was performed in triplicates.

Gas chromatography–mass spectrometry (GC–MS)

The composition of the promising algal extract was obtained using GC–MS (Agilent 6890 GC coupled to an Agilent 5975 quadrupole mass detector). The SPME fiber was desorbed in GC injector at 220 °C for 5 min in splitless mode and chromatographic separation was carried out on a 30 m × 0.25 mm × 0.25 µm film thickness HP-5MS (5% Phenyl Methyl Siloxane) capillary column. The GC oven temperature was programed from 60 °C to 250 °C at a rate of 2 °C min^{-1}. Helium was used as a carrier gas at a constant flow of 0.9 ml min^{-1}. Relative area values (as a percentage of total volatile composition) were directly obtained from total ion current (TIC).

Statistical analysis

Results are presented as the mean ± standard deviation (SD) of three replicates. The statistical analyses were carried out using SAS (v 6. 12). Data obtained were analyzed statistically to determine the degree of significance using a one way analysis of variance (ANOVA) at probability level $p \leqslant 0.05$.

Results and discussion

Resistance of bacterial isolates to different tested antibiotics is shown in Table 1. The incidence of resistance to different tested antibiotics ranged between 18.3% (tobramycin) and 100% (ampicillin, amoxicillin, cefepime and kanamycin). For, penicillins, the incidence of resistance to ampicillin, piperacillin and amoxicillin was 100%, 31.7% and 100%, respectively. Mixing clavulanate with amoxicillin and ticarcillin reduced resistance percentages to 99%.

In the case of cephalosporins, the incidence of resistance ranged between ceftizoxime (99%), cefotaxime (97.1%), ceftriaxone (96.1%), cefoperazone (86.5%) and ceftazidime (81.7%) among 3rd generation cephalosporins. On the other hand, high percentages (100%) of isolates showed resistance to cefepime (4th generation cephalosporin). Declined resistance percentage was detected for imipenem and meropenem to be 45.2% and 37.5% respectively. Resistance to aztreonam was observed in 76% of the tested isolates. The resistance of *Pseudomonads* to colistin sulfate (lipopeptide) was moderate (59.6%). The isolates also showed resistance to phenicol antibiotics, such as chloramphenicol (99%), 97.1% to co-trimoxazole (folate pathway inhibitor). In addition 93.3% of isolates were resistant to tetracycline (from tetracyclines). In case of ciprofloxacin (fluoroquinolone), the incidence of resistance was declined to 29.8%.

Regarding the aminoglycosides the incidence of resistance of tested isolates ranged between 18.3% and 100% in which most isolates showed resistance to kanamycin (100%), neomycin (99%), streptomycin (97.1%) followed by declined resistance to amikacin (35.6%), gentamicin (34.6%) and tobramycin (18.3%) which was found to be the most active antibiotic against the tested *P. aeruginosa* isolates.

The MAR (multi-antibiotic resistance) index analysis revealed that all tested isolates had a very high MAR index value (> 0.2) which indicated that the MAR values are an indication of the extent of microbial exposure to antibiotics used within the community (Olayinka et al., 2009). The resistance of the tested isolates ranged from 36% to 96% (Table 1). On observing the multidrug resistance (MDR), it was found that all the tested bacterial isolates showed high frequency multiple drug resistance (MDR) (9–24 agents). The widespread use of antibiotics has been identified as a main factor responsible for the increased incidence of antibiotics resistance (Khalil et al., 2015). Multiple antibiotic resistance index (MAR) was used in analyzing health risk and to test the resistance of antibiotic. Janda (2002) reported that many bacterial strains are known to harbor mobile elements that encode antibiotic resistance and can be transferred among themselves or to other bacterial species, to evolve multiple antibiotic resistances.

Agar well diffusion method was carried out to test the antibacterial activities of organic extracts of four collected marine species and the data are tabulated in Table 2. Diethyl ether extract of *S. vulgare* showed inhibition activity against *S. flexneri*, *E. coli* and *P. aeruginosa* 1 only. However, the methanol extract of *S. vulgare* did not show any a noticeable activity against the multidrug resistant bacteria tested except *K. pneumoniae* and *S. flexneri*. Ethanol extracts of *S. vulgare* show inhibition activity against *P. aeruginosa* 2 and high activity against *K. pneumoniae* while, chloroform extract of *S. vulgare* shows activity against *P. aeruginosa* 2, *K. pneumoniae* and *S. aureus* 1. Compared to water based methods organic solvent has a higher efficiency in extracting compounds for antibacterial activities (Tuney et al., 2006). Rajasulochana et al. (2009) concluded that chloroform: methanol is one of the main solutions for extracting the active antibacterial materials from the brown algae species. The methanol extracts of *S. vulgare* do not have antibacterial activity against *E. coli* and *S. aureus* growth as indicated by Ibtissam et al. (2009). Silva et al. (2013) found that *E. coli* and *P. aeruginosa* were affected only by the ethanolic extract of the brown seaweed *Padina gymnospora*. Antibacterial activities of algal extracts depend on algal species, the efficiency of the extraction method and concentration of the extract. For instance, diethyl ether extract of *S. fusiforme* shows high activity against most tested species specially *S. aureus* 2; however, the methanol extract of *S. fusiforme* shows inhibition activity against *P. aeruginosa* 1 and 2 (Table 2). Ethanol extract of *S. fusiforme* affected *S. flexneri*, *S. aureus* 3 and *P. aeruginosa* 1 and 2. In this investigation, chloroform extract of *S. fusiforme* showed high significant activity against *K. pneumonia*e and *S. aureus* 2.

Table 1 Overall antibiotic resistance profiles (ARPs) for the clinical bacterial isolates.

Code of antimicrobial agent	*Pseudomonas aeruginosa* 1	*Staphylococcus aureus* 1	*Staphylococcus aureus* 2	*Staphylococcus aureus* 3	*Pseudomonas aeruginosa* 2	*Klebsiella pneumonia*	*Shigella flexneri*	*Escherichia coli*	*Staphylococcus aureus* 4	*Corynebacterium* sp.
TZP	S	S	R	R	S	R	S	S	R	R
TOB	S	S	R	S	S	R	S	R	S	R
AM	R	R	R	R	R	R	R	R	R	R
NA	R	R	R	R	R	R	S	R	S	R
CES	R	S	R	R	R	R	R	R	R	R
S	R	S	R	S	R	S	S	R	R	R
K	R	S	R	S	R	R	S	R	R	R
FEP	R	R	R	R	R	R	R	R	R	R
CIP	S	S	S	S	S	R	S	R	R	R
VA	R	S	S	S	R	R	S	R	R	R
AX	R	S	R	R	R	R	R	R	R	R
OX	R	R	R	R	R	R	R	R	R	R
C	R	S	R	S	R	R	S	S	R	R
CN	S	S	S	S	S	R	S	S	R	R
CRO	R	R	R	R	R	R	R	R	R	R
CAZ	S	R	R	R	S	R	R	R	R	R
IPM	S	S	R	S	S	R	S	S	S	R
AK	R	S	R	S	S	R	S	S	S	R
MEM	S	S	R	S	S	R	S	S	S	R
AMC	R	S	R	S	R	R	R	R	S	R
TE	S	R	R	R	S	R	S	R	R	R
ATM	S	R	R	R	S	R	R	R	R	R
N	R	S	S	S	S	R	S	R	S	S
CT	R	R	R	R	R	R	R	R	R	R
SXT	R	S	R	S	R	R	R	R	S	R
MAR index	0.64	0.36	0.84	0.48	0.56	0.96	0.44	0.76	0.68	0.96

R = resistant, S = sensitive.

Table 2 Incidence of antimicrobial resistance among bacterial isolates ($n = 10$).

Antimicrobial agent	No. (%) of resistant isolates
β-Lactams	
Ampicillin (AMP)	10 (100%)
Amoxicillin (AX)	9 (90%)
Cefoperazone/sulbactam (CES)	9 (90%)
Amoxycillin/clavulanic acid (AMC)	7 (70%)
Piperacillin-tazobactam (TZP)	5 (50%)
Ceftazidime (CAZ)	8 (80%)
Cefepime (FEP)	10 (100%)
Ceftriaxone (CRO)	10 (100%)
Imipenem (IPM)	3 (30%)
Meropenem (MEM)	3 (30%)
Aztreonam (ATM)	8 (80%)
Aminoglycosides	
Gentamicin (CN)	3 (30%)
Amikacin (AK)	4 (40%)
Neomycin (N)	3 (30%)
Streptomycin (S)	6 (60%)
Tobramycin (TOB)	4 (40%)
Kanamycin (K)	7 (70%)
Fluoroquinolones	
Ciprofloxacin (CIP)	4 (40%)
Folate pathway inhibitors	
Co-trimoxazole (SXT)	7 (70%)
Tetracyclines	
Tetracycline (TE)	7 (70%)
Phenicols	
Chloramphenicol (C)	6 (60%)
Lipopeptides	
Colistin sulfate (CT)	10 (100%)
Glycopeptides	
Vancomycin (VA)	6 (60%)

In the present study, all extracts of *P. pavonia* show significant inhibition activity for most tested bacteria. The maximum inhibition activity was observed with ethanol extract of P. pavonia against *K. pneumonia* and *S. flexneri*. Diethyl ether extract of *C. rubrum* shows antibacterial activity against nearly all tested species. Data also revealed that Gram positive and Gram negative bacteria were affected by MICs ranged from 50 to 100 mg/ml of the tested algal extracts. Different concentrations of methanol extract of *C. rubrum* show activity against *S. flexneri*, *E. coli*, *P. aeruginosa* 2 and *K. pneumoniae*. As observed from Table 3 ethanol extract of *C. rubrum* showed inhibition activity against *P. aeruginosa* 1, *S. aureus* 1, *S. flexneri* and *S. aureus* 4. Chloroform extract of *C. rubrum* significantly affected *S. flexneri*, *P. aeruginosa* 2 and *K. pneumoniae*. El-Sheekh et al. (2014) reported that *Dictyota* sp. extract has low antibacterial activity against some Gram positive bacteria; the inhibition zone (17 mm) of *S. aureus* 1 followed by lower growth inhibition of *Staphylococcus pneumoniae*, *S. aureus* 2 and *Streptococcus viridians*. In this study, the antimicrobial activities of four algal extracts prepared by ethanol, diethyl ether, chloroform and methanol, were studied against some multidrug resistant bacteria. All algal extracts have different inhibition levels of the tested microbial growth. Among the algal samples screened for antibacterial activity, the organic extract of all algal species has high activity against *S. aureus* 4 at conc. 100 µl. These seaweeds act as potential bioactive compounds of interest for pharmaceutical applications (Solomon and Santhi, 2008). Hodgson (1984) reported antimicrobial activity of seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta. A number of marine algae from eastern Sicily like *Dictyota dichotoma*, *Cystoseira elegans* and *Laurencia obtusa* showed antibacterial activity (Caccamese and Azzolina, 1979).

As observed from Table 2, 100 µl diethyl ether extract of *S. fusiforme* showed the highest activity against *S. aureus* 2 than all tested antimicrobial agents. Data also showed that 50 µl ethanol extract of *S. vulgare* recorded the highest activity against *K. pneumonia*. In addition 100 µl diethyl ether extract of *S. fusiforme* and 50 µl ethanol extract of *S. vulgare* showed maximum inhibition activity. The presence of different antibacterial substances in the organic solvent extracts from the tested species may be the reason for the variation of antibacterial activity as reported by Lustigman and Brown (1991).

The GC–MS chromatograms showed various compounds present in diethyl ether extract from *S. fusiforme* and ethanol extract of *S. vulgare*. Names of these components and their amounts are listed in Tables 4 and 5; Figs. 1 and 2. The antibacterial activity of the 100 µl diethyl ether extract of *S. fusiforme* and 50 µl ethanol extract of *S. vulgare* might be attributed to a wide range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons. The two extracted included high percentage of Octadecadienoic acid, Eicosane, Dotriacontane, Tritetracontane, Docosane, Octatriacontyl, Heptacosane and Tetracosane. These compounds have been already proposed to have a certain antimicrobial activity (Alagic et al., 2006). Phlorotannins, phenolic compounds and diterpenediol (crinitol) are reported to be produced by brown algae *Sargassum critaefolium*, *Sargassum tortile*, *Ecklonia kurome*, *Eisenia bicyclis* and *Cystoseira crinite* exhibited antibacterial activity (Alam et al., 1994; Nagayama et al., 2002). Generally, the earlier studies found Gram positive was affected by algal extracts activity more than Gram negative bacteria; this may be due to the complex structure of the cell wall of Gram negative bacteria (Stirk et al., 2007). Many species of macro algae had foremost constituents like tetradecanoic acid, hexadecanoic acid, octadecanoic acid methyl esters etc. (Balamurugan et al., 2013) (see Table 5).

Photo (1 and 2) illustrated the intact cytoplasm and bacterial cell wall structures of none treated MDR *K. pneumoniae* and *S. aureus* 2 vegetative cells. Treated cells revealed high destructive effect of diethyl ether extract of *S. fusiforme* and ethanol extract of *S. vulgare*, as it caused perforation of cell wall, leakage of cytoplasmic contents and severe distortion of outer cell shape for both bacterial isolates. On the other hand, *S. aureus* 2 cells were less affected by the treatment with both 100 µl diethyl ether extract of *S. fusiforme*, as they cause slight shrinking of protoplasm cytoplasmic vacuolation and

Table 3 Antibacterial activity of some seaweeds against multidrug resistant bacteria.

Algal species	Organic solvents	*Pseudomonas aeruginosa* 1			*Staphylococcus aureus* 1			*Staphylococcus aureus* 2		
		50	75	100 µl	50	75	100 µl	50	75	100 µl
Sargassum vulgare	Diethyl ether	14.67 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Methanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	0^{a}	0^{a}	0^{a}	11.83 ± 0.76^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Sargassum fusiforme	Diethyl	23.33 ± 0.57^{c}	18.33 ± 0.57^{b}	0^{a}	14 ± 1^{c}	13 ± 1^{c}	0^{a}	15.33 ± 0.57^{b}	0^{a}	0^{a}
	Methanol	14.67 ± 0.57^{e}	9.33 ± 0.57d	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	11.67 ± 0.57^{f}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	15.5 ± 0.5^{b}	0^{a}	0^{a}
Padina pavonia	Diethyl	15.67 ± 0.57^{c}	13.33 ± 0.57^{b}	0^{a}	14.67 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Methanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	18.33 ± 0.57^{c}	11.33 ± 0.57^{b}	0^{a}
	Ethanol	15.67 ± 0.57^{c}	12.67 ± 0.57^{d}	0^{a}	20.33 ± 0.57^{e}	16.33 ± 0.57^{d}	11.67 ± 0.57^{c}	0^{a}	0^{a}	0^{a}
	Chloroform	16.33 ± 0.57^{c}	13.67 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	8.5 ± 0.5^{b}	0^{a}	0^{a}
Ceramium rubrum	Diethyl	15.67 ± 0.57^{c}	13.33 ± 0.57^{b}	0^{a}	16.33 ± 0.57^{c}	13.33 ± 0.57^{b}	11.33 ± 0.57^{a}	8.33 ± 0.28^{b}	0^{a}	0^{a}
	Methanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	13.33 ± 0.57^{b}	0^{a}	0^{a}	8.33 ± 0.57^{e}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	0^{a}	0^{a}	0^{a}	0^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
DMSO	DMSO	–	–	–	–	–	–	–	–	–

Algal species	Organic solvents	*Shigella flexneri*			*Escherichia coli*			*Staphylococcus aureus* 4			*Corynebacterium* sp.		
		50	75	100 µl	50	75	100 µl	50	75	100 µl	50	75	100 µl
Sargassum vulgare	Diethyl	14.67 ± 0.57^{c}	10.67 ± 0.57^{b}	0^{a}	13.67 ± 0.57^{c}	10.67 ± 0.57^{b}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Methanol	14.67 ± 0.57^{c}	13.7 ± 0.57^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
Sargassum fusiforme	Diethyl	12.67 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	9.67 ± 0.57^{b}	0^{a}	0^{a}
	Methanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	9.67 ± 0.57^{d}	8.67 ± 0.57^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	11.67 ± 0.57^{e}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	20.67 ± 0.57^{b}	0a	0^{a}	0^{a}	0^{a}	0^{a}
Padina pavonia	Diethyl	9.67 ± 0.57b	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	11.33 ± 0.57^{c}	8.67 ± 0.57^{b}	0^{a}	12.67 ± 0.5^{c}	9.67 ± 0.57^{b}	0^{a}
	Methanol	9.67 ± 0.57b	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	16.67 ± 0.57^{d}	0a	0^{a}	0a	0^{a}	0^{a}
	Ethanol	20.67 ± 0.57^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0a	0^{a}	0^{a}
	Chloroform	11.67 ± 0.57^{d}	0^{a}	0^{a}	15.67 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0a	0^{a}	11.67 ± 0.5^{d}	0^{a}	0^{a}
Ceramium rubrum	Diethyl	11.67 ± 0.57^{c}	9.67 ± 0.57^{b}	0^{a}	11.67 ± 0.57^{b}	0^{a}	0^{a}	15.67 ± 0.57^{b}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Methanol	11.67 ± 0.57^{c}	0^{a}	0^{a}	15.67 ± 0.57^{c}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Ethanol	17.67 ± 0.57^{e}	13.67 ± 0.57^{d}	0^{a}	0^{a}	0^{a}	0^{a}	15.67 ± 0.57^{b}	0a	0^{a}	0^{a}	0^{a}	0^{a}
	Chloroform	13.33 ± 0.57^{f}	10 ± 0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0a	0^{a}	0^{a}	0^{a}	0^{a}
DMSO	DMSO	–	–	–	–	–	–	–	–	–	–	–	–

Values with the same letter in each separate parameter are significant (one-way above).

Table 4 Composition of the *Sargassum fusiforme* diethyl ether extract as investigated by GC–MS chromatography.

No.	Compounds	R. time	Percentage (%)
1	10,13-Octadeadienoic acid	31.61	9.43
2	Tritriacontane	32.315	5.08
3	2-Butyloctanol	33.38	7.22
4	Pentacosane	33.672	4.54
5	Eicosane	35.733	15.18
6	Dotriacontane	36.866	10.38
7	Octatriacontyl pentafluoropropionate	37.25	2.13
8	Nonacosane	38.002	12.13
9	Hentriacontane	38.475	2.5
10	Tritriacontane	38.809	1.38
11	Eicosane	39.396	9.9
12	Tricosane	39.882	1.5
13	Dicosane	40.997	9.8
14	Pentatriacontane	45.268	5.3
15	Eicosane, 2-methyl	48.169	3.37

Table 5 Composition of the *Sargassum vulgare* ethanolic extract as investigated by GC–MS chromatography.

No.	Compounds	R. time	Percentage (%)
1	Cyclopentaneacetic acid	20.466	6.42
2	Tonalid	27.006	2.63
3	Tetratetracontane	35.734	5.02
4	Di-n-octyl phthalate	37.67	48.26
5	Octacosane	38.06	3.55
6	Heneicosane	39.4	9.61
7	Pentacosane	41.002	6.96
8	Docosane	42.946	6.69
9	Tetracosane	45.329	5.99
10	Heptacosane	48.247	4.83

distortion of outer cell boundary, 100 µl diethyl ether extract of *S. fusiforme* possessed increased cell size and protoplasmic agglutinations (Photo 2). While *K. pneumonia* showed cell wall dissolving with cytoplasmic leakage and alteration in cytoplasmic density, decrease in cell size and deformation in cell structure on the treatment with 50 µl ethanol extract of *S. vulgare* showed mild scattered cytoplasmic vacuolation, rupture of cell wall, and decreased cell size (Photo 1). Diethyl ether and ethanol extract from *S. fusiforme* and *S. vulgare*, respectively caused complete degradation and cell wall deformation in both treated MDR bacteria *S. aureus* 2 and in *K. pneumoniae* these variations might be due to genetic or biochemical composition of pathogenic bacteria (Caccamese et al., 1981). This result might be due to clinical bacterial cell wall that contains a high percentage of (90–95%) of peptidoglycan and has lipopolysaccharides and phospholipids (5–10) inside the G +ve cell and destroys the cell membrane or protein biosynthesis units (DNA and RNA) as compared to G −ve germs whose membrane consists of two layers (outer and inner membrane) separated by periplasmic space. The outer membrane consists of phospholipids, lipoprotein and muco-polysaccharides while the internal membrane consists of peptidoglycan (glycopeptide) (5–10%); it means the membrane of G −ve cell has high percentage of lipids (90–95%), which does not support and does not have an appropriate medium to interact with and enter the antimicrobial agents and then decreased its influence on the pathogenic bacteria (Al-Samary, 1999).

Conclusion

The solvent extracts of four different seaweeds used in the present study showed significant inhibitory action against MDR bacteria. Among the four seaweeds screened for their antibacterial activity, the 100 µl diethyl ether extract of brown alga *S. fusiforme* and 50 µl ethanol extract of *S. vulgare* showed more inhibitory activity against *S. aureus* 2 and *K. pneumoniae*, respectively. The phytochemical screening of seaweeds showed the presence of indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons in the selected species. The changes in ultrastructure of tested MDR bacteria, *S. aureus* 2 and *K. pneumoniae*, due to *S. fusiforme* and *S. vulgare* extract were investigated by transmission electron microscope which shows shrinking of protoplasm cytoplasmic vacuolation deformation in cell structure and distortion of outer cell boundary.

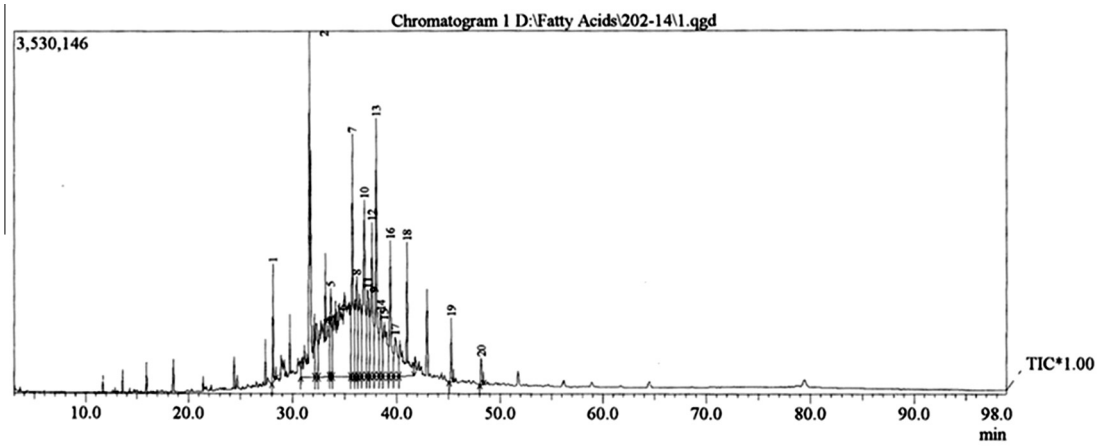

Figure 1 GC–MS chromatogram of *Sargassum fusiforme* diethyl ether extract.

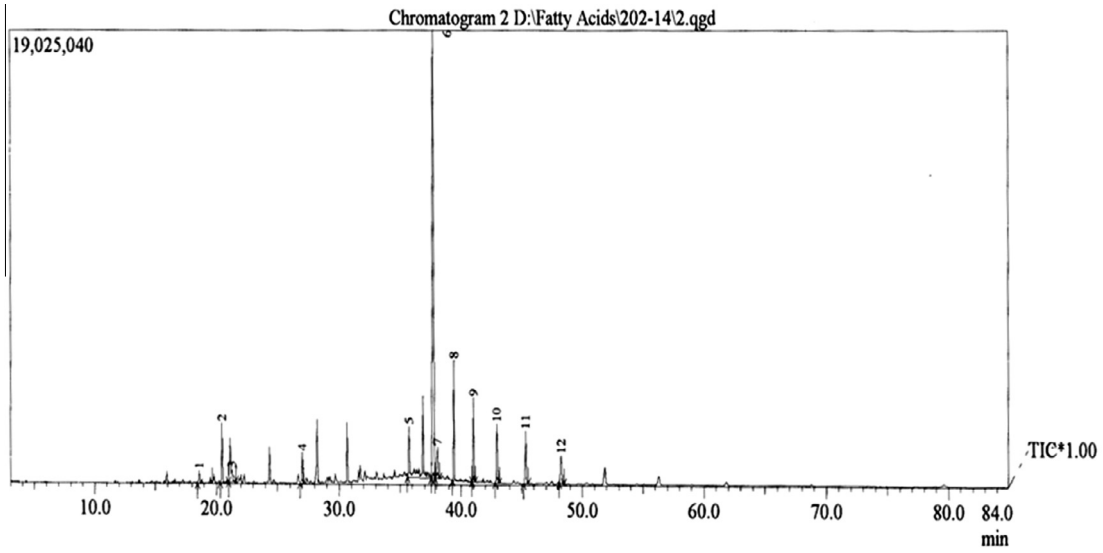

Figure 2 GC–MS chromatogram of *Sargassum vulgare* ethanolic extract.

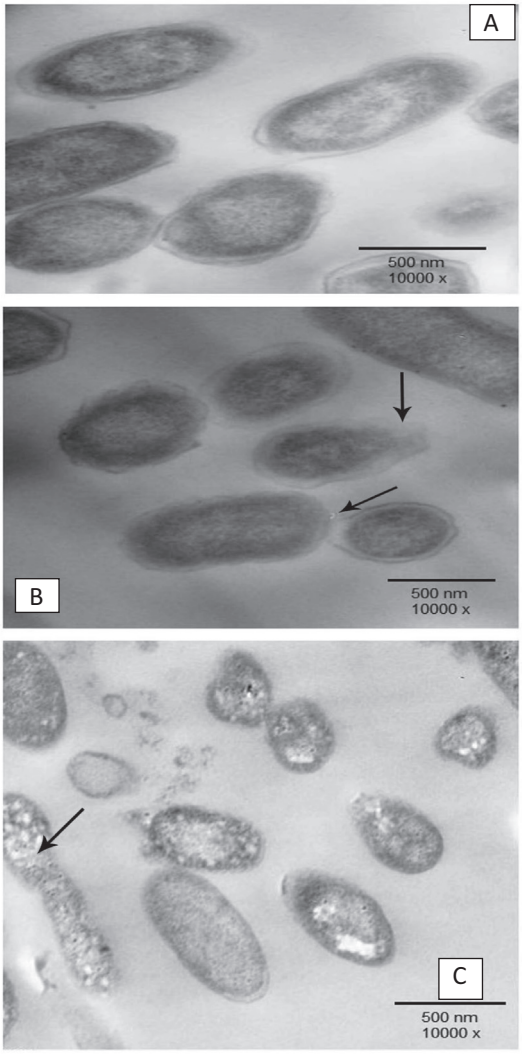

Photo 1 (A) *Klebsiella pneumonia* control, (B) and (C) damaged *K. pneumonia* treated 50 µl ethanol extract of *Sargassum vulgare*.

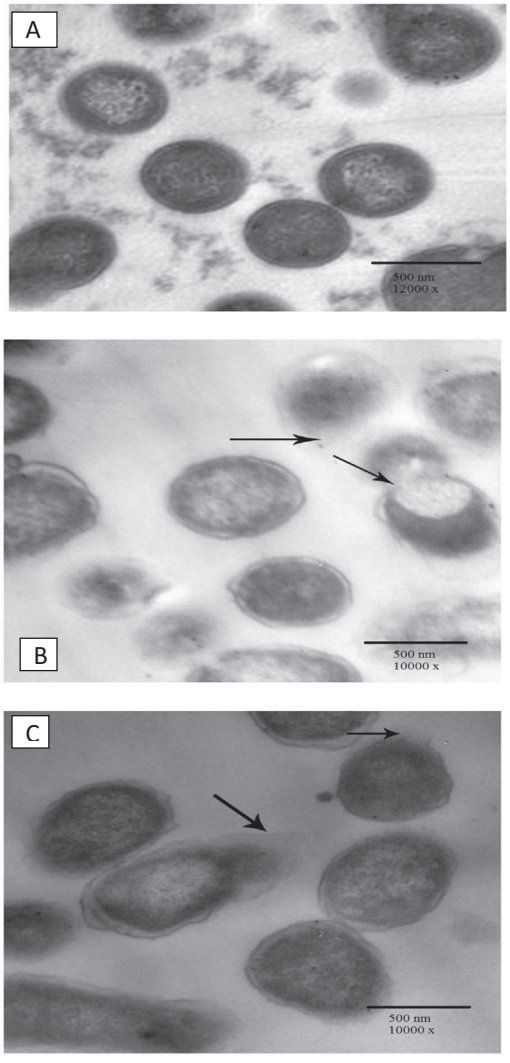

Photo 2 (A) *Staphylococcus aureus* 2 control, (B) and (C) damaged treated with 100 µl diethyl ether extract of *Sargassum fusiforme*.

Conflict of interest

The authors declare that there is no conflict of interest.

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