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Seaweed Extracts Effectiveness against Selected Gram-negative Bacterial Isolates

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Abstract. Aqueous and six solvent extracts of four seaweeds *Codium tomentosum* (Chlorophyceae); *Corallina mediterranea, Hypnea musciformis* (Rhodophyceae), and *Sargassum vulgare* (Phaeophyceae) were screened for their antibacterial activity against 10 gram-negative bacterial isolates. Seaweeds crude extracts potent antibacterial activity have been evaluated based on zone of inhibition (ZI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values as reported in many researches. Overall, aqueous algal extracts were non active against all tested isolates regardless examined seaweed species. It was noticed that ZIs were in the following order: *S. vulgare* (17 mm) against *Acinetobacter baumannii* and *C. mediteranea* (17 mm) against *Salmonella typhimurium* > *H. musciformis* (13 mm) against *Escherichia coli* O:157 > *C. tomentosum* (11 mm) against *S. typhimurium.* Data revealed that the *S. vulgare* extracts showed the most inhibitory activity by showing the lowest MIC_{50} value of 0.08 mg/mL (methanolic extract against *Shigella flexneri* and hexane extract against *E. coli* O:157 isolate) and also the lowest MBC value of 1.00 mg/mL (methanolic extract against *S. typhimurium, Serratia marcescens, E. coli* O:157 and *Brucella melitensis* isolates; and also with ethanolic extract against *S. marcescens* and *E. coli* O:157 isolates). Future studies on the *S. vulgare* extracts are required due to their importance as a potent, promising and cheap source of bioactive compounds for antibacterial pretreatment.

Keywords: seaweed, antibacterial activity, zone of inhibition, minimum inhibitory concentration, minimum bactericidal concentration

Introduction

Bacterial pathogens caused loss of living organisms with different rate *e.g.* mastitis, abortion and upper respiratory complications diseases yielded by *Escherichia coli* and *Pseudomonas aeruginosa* pathogen, whereas, *Salmonella* sp. infection lead to diarrhoea and typhoid fever diseases (Leven, 1987; Jawetz *et al*., 1985; Boyd, 1955). Moreover, *Acinetobacter baumannii* multidrug resistance (MDR) has been reported worldwide causing human skin flora. It has been demonstrated that their occurrence in human skin and mucous membrane occur into over 43% of human population, where, it displayed different infection forms *e.g.* bacteremia, urinary tract infection, meningitis, wound and burn infections, and most importantly nosocomial pneumoniae, particularly in ventilated patients (Saleh *et al*., 2015; King *et al*., 2009). *A. baumannii* resistance to antibiotics and disinfectants and the capacity to survive desiccation, makes it as an important persistence pathogen (Srinivasan *et al*., 2009).

The emergence of resistant bacterial strain worldwide as a common phenomenon caused antibacterial therapeutic failure. Thereby, great challenge achieved to augment antibacterial efficacy based on natural sources such as plants and algae. These living organisms became efficient, cheap, and potent agent for inhibition of bacterial growth due to their richness in different bioactive compounds.

Macro and microalgae displayed efficient activity against bacterial isolates due to their richness in bioactive compounds and their occurrence worldwide encouraged the scientists to introduce them in pharmacological research as antimicrobial agents. Many reports indicated potential use of seaweeds for their antibacterial activity (Boujaber *et al*., 2016; Kausalya and Rao, 2015; Karthick *et al*., 2015; Sushanth and Rajashekhar, 2015; Chandrasekaran *et al*., 2014; Kavita *et al*., 2014; Elnabris *et al*., 2013; Jeyaseelan *et al*., 2012; Tajbakhsh *et al*., 2011; Rhimou *et al*., 2010; Ibtisam *et al*., 2009; Kandhasamy and Arunachalam, 2008).

Moreover, seaweed crude extracts or their purified compounds make them a promising source not only as antibacterial, but also as an antioxidant, anticoagulant, anticancer, antiviral, and anti-inflammatory effects (Perez *et al*., 2016).

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Bacterial isolates could be divided into gram positive and negative, among them, gram-negative bacteria as reported in literature were more resistant to algal crude extracts due to their cell wall structure (thickness) and their composition, preventing entry of inhibitory agents into the cell (Chandrasekaran *et al*., 2014; Kandhasamy and Arunachalam, 2008). For this reason, antibacterial activity of 4 seaweed species crude extracts collected from Syrian coast has been evaluated against 10 gram-negative bacterial isolates. Thereby, the most potent seaweed will be handled in the future as antibacterial agent for performance studies.

Materials and Methods

Seaweed sampling. Four seaweed samples of *Codium tomentosum* (Chlorophyceae); *Corelline mediterranea* and *Hyphea musciformis* (Rhodophyceae), and *Sagassum vulgare* (Phaeophyceae) were collected from the Syrian coast of the Mediterranean Sea at 5 km North Lattakia – Syria (Latitude 35°33'786"N and Longitude 38°29'766"E). Seaweed identification was done by taxonomical study in the Division of Plant Biotechnology at the AECS in Damascus-Syria. Seaweed samples were harvested by hand with disposable gloves and washed with seawater followed by two successive washings with double-distilled water. Samples were transfered to Whatman filter papers for elimination attached of water and acceleration their drying. Seaweed samples were shade dried for two weeks, powdered by special electric mill and stored separately in polyethylene bags until analysis.

Seaweed crude extracts preparation. Crude extracts of the *C. tomentosum, C. mediterranea, H. musciformis* and *S. vulgare* seaweeds were prepared using aqueous and six solvents (methanol, ethanol, chloroform, acetone, ethyl acetate and hexane) as previously reported in many investigations. One gram of shade-dried powdered seaweed materials were subjected to extraction in 100 mL solvent, until complete solubility. Then, the extracts were filtered with Whatman filter papers. Extracts were kept at laboratory temperature for 2 h to evaporate the solvent. All extracts were then kept in tightly fitting stopper bottles and stored in 4°C. The concentration of extract was considered 10 mg/mL.

Tested microorganisms and growth conditions. Ten pure gram-negative bacteria clinical isolates of *Salmonella typhimurium*, *Serratia marcescens, Escherichia coli:* O: 157, *Proteus vulgaris, Acinetobacter baumannii*, *Brucella melitensis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri* and *Vibrio cholerae* were obtained from the Microbiology and Immunology Division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus - Syria. Culture was maintained at 37°C on 2YT agar (peptone, 16 g/L; yeast extract, 10 g/L; NaCl, 5 g/L; agar, 13 g/L [Difco, BD, Spars, MD]); and incubated for 24-48 h. Prior to antimicrobial sensitivity test, 0.2 mL of overnight culture of each organism was dispensed into 20 mL of sterile Mueller Hinton Broth (Hi-media Laboratory Pvt. Ltd., Mumbai, India) and then incubated for about 18-24 h. The bacterial microorganisms were suspended in a sterile Phosphate-Buffered Saline (PBS). Bacterial abundance in PBS was screened by recording the optical density (OD) at 590 nm, to standardize the cultures to approximately 106 CFU/mL (Saleh *et al*., 2015). The exact counts were assessed retrospectively by viable counts on 2YT agar plates.

Antibacterial activity assay. *The disc-diffusion test*. The disc-diffusion test was carried out for monitoring seaweed antibacterial inhibitory effect and Ciprofloxacin (10 mg/L) (Bayer, Istambul, Turkey) antibiotic was used as a standard drug control for seaweed antibacterial effect (Saleh *et al*., 2015; Bauer *et al*., 1966). The sterilized discs filter paper (Whatman no.1 of 6 mm diameter) were inoculated with 100 µL of extract dilutions (10 mg/mL) and reconstituted in minimum amount of solvent were applied over each of the culture plates previously cultivated with the 106 CFU/mL cultures of bacteria. Bacterial cultures were then incubated at 37 °C for 18 h, whereas, paper discs were inoculated with 20 µL of a solution of 10 mg/L of Ciprofloxacin were used as standard drug. Negative control was achieved using solvents (final concentration of the solvent in the highest concentration of seaweed extracts was tested). Antibacterial inhibitory effect was determined by measuring the zone of inhibition (mm) appeared around each paper disc. For each extract, duplicate trials were conducted against each microorganism.

Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) Determination. Microdilution broth susceptibility assay was performed as previously described by Rios-Duenas *et al*. (2011). Three replicates of serial dilutions of seaweed extracts (50 mg/mL) or of antibiotic (128 mg/L) were prepared in LB broth medium in 96-well microtiter plates, using a range of concentrations for

aqueous and six solvents extracts of each examined seaweed species from 0.166 to 40 µL per well. One hundred microlitres of freshly grown bacteria standardized at 106 CFU/mL in LB broth were added to each well. Positive control was prepared with the same conditions but without extract. As for negative control, it was also made with the same conditions but without adding the bacteria. The plate was then incubated with shaking for 24 h at 37°C. The lowest concentration that completely inhibited visual growth was recorded and interpreted as the MIC_{50}.

Whereas, MBC was determined by plating 0.010 mL from the wells showing no visible growth on Mueller-Hinton agar plates (Oxoid) and incubating for 18-24 h at 37°C. The MBC was defined as the concentration at which there was a 99.9% reduction in CFU compared with the original inoculum.

Statistical analysis. All statistical analysis were performed using Graphpad Prism6 programming at the 5% level of significance (p=0.05). All tests were performed in triplicates and mean values are presented as mean± SD.

Results and Discussion

Zone of inhibition (ZI) assay. Antibacterial activity of 4 seaweed crude extracts has been investigated based on estimated ZI (Table 1) using aqueous and 6 different solvents. Overall, aqueous seaweed extracts were not active against all tested bacteria, whereas, seaweed extracts using the six solvents showed adverse antibacterial effect as expressed as ZI. For *C. tomentosum,* ZI value ranged between 3 (hexane against *K. pneumoniae* and *V. cholerae*) and 11 mm (methanol and acetone against *S. typhimurium*). whereas, this value ranged between 10 mm (hexane extracts against *S. marcescens* and *P. vulgaris*) and 17 mm (acetone against *S. typhimurium*) for *C. mediterranea.* As for *H. musciformis*, this value ranged between 5 mm with ethyl acetate against *A. baumannii* and *V. cholerae,* and 13 mm with chloroform against *E. coli* O:157. while, for *S. vulgare,* estimated ZI varied between 10 mm (ethyl acetate against *P. vulgaris* and *V. cholerae*) and 17 mm (methanol against *A. baumannii*). (Table 1). Seaweed crude extracts showed adverse effect against tested pathogens with different manner, according to the examined seaweed and solvent.

It is worth noting that the seaweed inhibitory effect against all tested gram negative bacteria was in the order of *S. vulgare* (17 mm) and *C. mediterranea* (17 mm) > *H. musciformis* (13 mm) > *C. tomentosum* (11 mm) (Table 1). In global, methanolic extracts of *C. mediterranea* and *S. vulgare* followed by ethanolic extracts showed relatively the strongest inhibitory effect against all tested microorganisms compared to acetonic one. This observation could be related to the synergetic effect of alkaloids and flavonoids components as bioactive compounds whereas, the same extract did not show similar effect in other examined algal species due to the absence of this synergetic effect.

Statistical variance analysis revealed that the effect of different extracts of seaweed species using different solvents was significantly different. In this respect, *H. musciformis* was more significant (p <0.0005) vs *C. mediterranea* and *S. vulgare* for all microorganisms except *P. vulgaris* and *E. coli* O:157 isolates. Moreover, *C. tomentosum* was more significant (p <0.0005) vs *C. mediterranea*, *S. vulgare* and *H. musciformis* for all microorganisms except *S. marcescens*, *A bumanii* and *P. aeroginose* for *H. musciformis*. In this respect, acetone extract of *C. mediterranea* was the best vs *S. typhimurium* and methanol extract of *S. vulgare* was the best vs *A. bumanii* (Table 1).

Previously, Kandhasamy and Arunachalam (2008) reported antibacterial activity of *H. muciformis* and *S. myricocystum* against *K. pneumoniae*, *Enterobacter aerogenes*, *E. coli* and *P. aeruginosa*, gram-negative bacteria. This study revealed that ZIs were found to be 13, 12, 0 and 12 mm with *H. muciformis* against *K. pneumoniae*, *E. aerogenes*, *E. coli* and *P. aeruginosa,* respectively, whereas, these were 13, 13, 0 and 12 mm with *S. myricocystum* against the same pathogens, respectively. Moreover, Chiheb *et al*. (2009) reported the biological activity of 32 macroalgae (13 Chlorophyta and 19 Phaeophyta) using methanol solvent, against 2 gram-negative bacteria (*E. coli* and *K. pnomeuniae*). This study showed that *Cystoseira mediterranea* among the Phaeophyceae displayed the highest ZI of 16 mm against *E. coli* whereas, *Dictyota linearis* (C. Agardh) Greville and *Padina pavonica* (Linnaeus) were potent against *K. Pneumoniae* with ZI of 15 mm. Similarly, *Ulva lactuca* among the Chlorophyceae was the most active against *E. coli* with ZI of 16 mm. Other investigations, however described the methalonic antibacterial activity of 20 species of marine benthic algae belonged to different algae members (9 Chlorophyceae, 3 Phaeophyceae and 8 Rhodophyceae), collected from the Mediterranean Moroccan coasts

Table 1. Inhibitory effect of the four seaweed extracts using disc-diffusion method expressed as ZIs (mm)

Microorganisms	Zone of inhibitions (ZIs) (mm)						
	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Ciprofloxacin (mg/L)
C. tomentosum							
S. typhimurium	11 ± 0.25	10 ± 0.29	9 ± 0.18	11 ± 0.14	10± 0.26	9± 0.18	27±0.18
S. marcescens	9± 0.11	10± 0.09	8± 0.16	7± 0.2	8± 0.19	7± 0.17	24±0.16
E. coli O:157	9± 0.15	7± 0.17	9± 0.19	8± 0.17	6± 0.09	6± 0.19	23±0.35
P. vulgaris	9± 0.15	9± 0.14	6± 0.08	6± 0.25	7± 0.09	6± 0.11	24±0.4
A. baumannii	8± 0.2	8± 0.27	6± 0.15	5± 0.19	5± 0.17	4± 0.12	15±0.17
B. melitensis	7± 0.16	6± 0.18	5± 0.06	8± 0.2	8± 0.22	4± 0.07	20±0.32
P. aeruginosa	10± 0.25	9± 0.11	10± 0.27	8± 0.09	8± 0.22	6± 0.19	26±0.21
K. pneumoniaee	9± 0.2	9± 0.07	7± 0.24	6± 0.15	8 ± 0.2	3 ± 0.06	23±0.16
S. flexneri	9± 0.11	7± 0.24	7± 0.15	8± 0.3	9± 0.25	4± 0.12	31±0.2
V. cholerae	8± 0.15	6± 0.09	6± 0.17	5± 0.18	7± 0.18	3± 0.07	19±0.15
C. mediterranea							
S. typhimurium	16 ± 0.2[2,5]	14 ± 0.17	13 ± 0.37	17 ± 0.28	14± 0.22	11± 0.4	27±0.18
S. marcescens	14± 0.2[2,5]	14± 0.33	12± 0.29	15± 0.37	13± 0.27	10± 0.15	24±0.16
E. coli O:157	16± 0.5[2,5]	13± 0.18	12± 0.27	16± 0.45	13± 0.2	11± 0.09	23±0.35
P. vulgaris	13± 0.45[5]	14± 0.19	11± 0.18	15± 0.2	14± 0.26	10± 0.11	24±0.4
A. baumannii	13± 0.21[2,5]	12± 0.19	13± 0.33	14± 0.29	13± 0.2	12± 0.0	15±0.17
B. melitensis	15± 0.25[2,5]	13± 0.35	13± 0.44	15± 0.5	14± 0.24	11± 0.27	20±0.32
P. aeruginosa	13± 0.29[2,5]	13± 0.49	11± 0.37	15± 0.4	13± 0.24	11± 0.36	26±0.21
K. pneumoniaee	14± 0.33[2,5]	13± 0.19	12± 0.27	15± 0.4	13 ± 0.25	12 ± 0.38	23±0.16
S. flexneri	14± 0.2[2,5]	12± 0.29	11± 0.3	14± 0.26	13± 0.18	13 ± 0.55	31±0.2
V. cholerae	15± 0.13[2,5]	13± 0.29	12± 0.39	14± 0.26	13± 0.24	12± 0.26	19±0.15
H. ***musciformis***							
S. typhimurium	9 ± 0.35[6]	6 ± 0.2	11 ± 0.45	10± 0.31	8± 0.8	9± 0.5	27±0.18
S. marcescens	9± 0.33	6± 0.29	7± 0.3	9± 0.35	9± 0.4	7± 0.6	24±0.16
E. coli O:157	12± 0.58[6]	11± 0.45	13± 0.35	11± 0.27	9± 0.6	12± 0.33	23±0.35
P. vulgaris	12± 0.3[4]	7± 0.19	11± 0.4	9± 0.22	7± 0.1	10± 0.5	24±0.4
A. baumannii	9± 0.19	7± 0.33	9± 0.35	8± 0.14	5± 0.15	10± 0.44	15±0.17
B. melitensis	9± 0.35[6]	6± 0.22	11± 0.32	10± 0.43	7± 0.18	10± 0.4	20±0.32
P. aeruginosa	9± 0.4	10± 0.32	11± 0.54	8± 0.45	7± 0.26	9± 0.65	26±0.21
K. pneumoniaee	11± 0.35[6]	9± 0.45	12± 0.40	9± 0.27	10 ± 0.55	10 ± 0.5	23±0.16
S. flexneri	12± 0.55[6]	10± 0.29	12± 0.45	9± 0.3	7± 0.22	11 ± 0.48	31±0.2
V. cholerae	10± 0.19[6]	9± 0.34	11± 0.27	7± 0.37	5± 0.25	7 ± 0.17	19±0.15
S. vulgare							
S. typhimurium	15± 0.27[1,5]	15 ± 0.32	12 ± 0.34	13 ± 0.36	12± 0.25	12± 0.34	27±0.18
S. marcescens	14± 0.4[1,5]	13± 0.33	13± 0.52	13± 0.49	12± 0.38	11± 0.22	24±0.16
E. coli O:157	15± 0.16[3,5]	15± 0.4	13± 0.28	12± 0.35	12± 0.5	11± 0.45	23±0.35
P. vulgaris	16± 0.55[1,5]	14± 0.42	12± 0.22	13± 0.35	10± 0.29	11± 0.34	24±0.4
A. baumannii	17± 0.55[1,5]	15± 0.42	12± 0.33	13± 0.28	13± 0.45	12± 0.45	15±0.17
B. melitensis	16± 0.35[1,5]	14± 0.3	11± 0.29	13± 0.4	11± 0.42	12± 0.55	20±0.32
P. aeruginosa	15± 0.44[1,5]	14± 0.36	12± 0.46	14± 0.44	11± 0.24	11± 0.4	26±0.21
K. pneumoniaee	16± 0.45[1,5]	15± 0.54	13± 0.27	13± 0.44	12± 0.3	11 ± 0.35	23±0.16
S. flexneri	15± 0.53[1,5]	15± 0.5	11± 0.44	13± 0.29	11± 0.33	12 ± 0.22	31±0.2
V. cholerae	13± 0.26[1,5]	14± 0.44	11± 0.38	13± 0.34	10± 0.23	12± 0.37	19±0.15

Comparing the effect of the same solvent by using extracts from different seaweed, methanol is the best solvent; - Using *H. musciformis*; 1p<0.0005 vs *S. vulgare* for all microorganisms except *E. coli* O:157; 2p<0.0005 vs *C. mediterranea* for all microorganisms except *P. vulgaris;* - Using *C. mediterranea*;3p<0.05 vs *S. vulgare* for *E. coli* O:157; 4p<0.05 vs *H. musciformis* for *P. vulgaris*; - Using *C. tomentosum*; 5p<0.0005 vs *S. vulgare* and *C. mediterranea* for all microorganisms; 6p<0.0005 vs *H. musciformis* for all microorganisms except *S. marcescens*, *A. baumannii* and *P. aeruginosa* microorganisms.

(Zbakh *et al*., 2012). This investigation revealed that the Rhodophyceae among the 3 examined algae groups were the most potent by showing the highest ZIs varied between 20-24 mm. Rhimou *et al*. (2010) also reviewed inhibitory effects of 26 red seaweed collected from Morocco using methanolic crude extracts against two gram negative bacterial isolates. This study showed that out of the 26 seaweeds, *H. musciformis* extracts displayed the highest inhibition zone of 20.67 mm and 19.00 mm against *E. coli* and *K. pneumoniae,* respectively.

Jeyaseelan *et al*. (2012) studied ethanol extracts of 5 algal (*S. polycystum*, *S. tenerrimum, Turbinaria ornata*, *Gracilaria crassa* and *Codium fragile*) species collected from different coastal regions of Sri Lanka; against *E. coli* as a gram-negative bacteria and the highest activity was recorded with sequentially extracted ethanol extract of *C. fragile* (12.2 mm). Other investigation however, showed the utility of *H. musciformis* as a promising source for antimicrobial effect against *E. coli, S. typhi* and *P. aeruginosa* (Shareef *et al*., 2012). Similarly, Ramalingami and Amutha (2013) investigated the antibacterial activity of 4 algae species collected from Thondi Coast, Tamilnadu – India against 5 gram-negative bacteria using acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and water. This study revealed that the ZIs were found to be < 8 mm for all examined algal extracts. Moreover the chloroform extract displayed the highest activity and *Acanthophora spicifera* extracts were the most active whereas, *S. wightii* were the lowest ones. Elnabris *et al*. (2013) reported the inhibitory effect of 4 methanolic extracts of algae species *Enteromorpha compressa* and *Ulva lactuca* (Chlorophyta); *Jania rubens* (Rhodophyta) and *P. pavonica* (Phaeophyta) collected from Palestine for their antibacterial activity against 4 gram-negative bacterial isolates (*P. aeruginosa, E. coli, K. pneumoniae* and *P. vulgaris*). This study showed that the highest ZI was obtained with green *U. lactuca* algae with 9.8 and 5.8 mm against *K. pneumoniae* and *P. vulgaris* isolates, respectively. However, *J. rubens* (red) and *P. pavonica* (brown) displayed the lowest activity. Chandrasekaran *et al*. (2014) studied *S. wightii* antibacterial activity against 8 gram-negative bacterial isolates. This investigation showed that ZIs varied between 10.1 mm (methanol) - 12.6 mm (ethyl acetate) against *E. coli;* between 10.5 mm (acetone and methanol) -12.8 mm (ethyl acetate) against *K. pneumoniae;* between 9.8 mm (hexane) - 12.8 mm (ethyl acetate) against *P. vulgaris,* whereas, it ranged between 10.1 mm (hexane) - 13.1 mm (ethyl acetate) against *P. aeruginosa;* between 10.6 mm (acetone and methanol) – 13.3 mm (ethyl acetate) against *S. typhimurium;* and between 10.8 (methanol) - 13.1 mm (ethyl acetate) against *S. flexneri.* Kavita *et al*. (2014) also showed that the methanolic extract of *Laurencia papillosa* (Rhodophyceae) extract among 11 algae species exhibited the strongest inhibitory effect with ZIS of 12.33 and 11.66 mm against *E.coli* and *P. aeruginosa* bacteria, respectively.

Recently, Kausalya and Rao (2015) investigated antimicrobial activity of *S. polycystum* and *S. tenerrimum* collected from India against 6 gram-negative bacteria using chloroform, ethanol, methanol and water solvents. This study revealed that the highest ZI was observed in the case of methanol against *P. vulgaris* and *K. pneumoniae* (18 mm). Sushanth and Rajashekhar (2015) investigated the antimicrobial activity of ethanol, methanol and hexane extracts for *Chaetoceros calcitrans*, *Skeletonema costatum*, *Chroococcuc turgidus* and *Nannochloropsis oceanica* microalgae collected from Arabian Sea of Karnataka Coast, against 4 gram-negative bacterial strains. This investigation revealed that, ZI values ranged between 6.1 mm (with ethanol extract of *S. costatum* against *K. pneumoniae*) and 21.4 mm (with hexane extract of *C. turgidus* against *E. coli*). Karthick *et al*. (2015) investigated the antibacterial activity of methanolic extracts from 5 algae (2 green 2 red and 1 brown species collected from South Andaman, India) against 4 gram-negative (*E.coli, S. typhi, P. aeruginosa* and *K. pneumoniae*) bacterial isolates, the highest ZI was recorded with methanolic extracts of *Dictyosphaeria cavernosa* (16 mm) against *K. pneumoniae* and for *Galaxura marginata* (16 mm) against *E.coli,* whereas, *Acetabularia calyculus* extract exhibited the lowest ZI value (11 mm) against *E.coli;* with no inhibitory activity against the 3 other tested isolates. Moreover, the same study showed that *Corallina* sp. extract was not active against all examined pathogens. More recently, Boujaber *et al*. (2016) reported *Gelidium sesquipedale* (red) and *Laminaria ochroleuca* (brown) collected from the Mediterranean Moroccan coasts inhibitory effects against two gram-negative bacterial isolates (*E. coli* and *Pseudomonas* sp.) using hexane, dichloromethane, dichloromethane/methanol (50:50), methanol and water as solvents. This study demonstrated that the dichloromethane/methanol showed the strongest antibacterial activity with ZI of 14 mm for *G. sesquipedale,* whereas, it was recorded to be 13 and 16 mm in the case of *L. ochroleuca* against *E. coli* and

Pseudomonas sp., respectively while aqueous, hexane and dichloromethane extracts of both algae showed no activity against all examined bacterial pathogens.

Recently Alves *et al*. (2016) reviewed antimicrobial activity of five seaweeds (2 Chlorophyta, 1 Rhodophyta and 2 Phaeophyta) collected from Brazil, using hexane, chloroform, ethyl acetate and methanol solvents against *E. coli* and *K. pneumoniae* isolates and revealed that methanolic extract of *S. polyceratium* (Phaeophyta) displayed ZIs of 15 and 8.67 mm against *E. coli* and *K. pneumoniae* isolates, respectively.

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assay. To investigate seaweed inhibition activity, MIC_{50} values were also estimated (Table 2). In this regards, for *C. tomentosum,* this value varied between 3.3 mg/mL (methanol against *E. coli* O:157; acetone against *A. baumanni, P. aeruginosa* and *S. flexneri*) and >10 mg/mL with hexane against all isolates except *S. typhimurium* (10.0 mg/mL). As for *C. mediterranea,* this value varied between 1.0 mg/mL with methanol against *S. marcescens,* and *E. coli* O:157*;* and 10.0 mg/mL with hexane against *P. vulgaris, B. melitensis, P. aeruginosa* and *K. pneumoniae* isolates whereas, in the case of *H. musciformis* this value ranged between 13.3 mg/mL (methanol against *S. typhimurium, S. marcescens, A. baumanni, P. aeruginosa* and *K. pneumoniae*) and 80.0 mg/mL with hexane against *S. flexneri.* while, for *S. vulgare*, this value varied between 0.08 mg/mL (hexane against *E. coli* O:157 and methanol against *S. flexneri*) and 0.32 mg/mL with ethyl acetate against *P. vulgaris, B. melitensis* and *K. pneumoniae* isolates (Table 2).

Statistical variance analysis revealed that comparing the effect of the same solvent by using extracts from different seaweeds, methanolic extracts were the best. This effect was significant ($p = 0.05$) vs *C. mediterranea* and *C. tomentosum* for *S. flexneri* and also ($p = 0.0005$) vs all other algae for all organisms except *S. flexneri* (Table 2).

Moreover, seaweed antibacterial potency to kill tested isolates has been evaluated by determination of MBC values (Table 3). Overall, MBC values followed similar tendency of MIC_{50}. In this respect, the lowest MBC values (1.00 mg/mL) were recorded for *S. vulgare* with methanol against *S. typhimurium, S. marcescens, E. coli* O:157 and *B. melitensis* isolates and ethanol against *S. mancesces* and *E.coli* 0.157.(Table 3).

Variance analysis in comparing the effect of the same solvent by using extracts from different seaweed showed that methanolic extracts of *S. vulgare* were the most potent. This inhibitory effect was significant ($p = 0.005$) vs *C. tomentosum* against *K. pneumoniae* (Table 3). It is worth noting that the highest relative phenolic content observed in *S. vulgare* seaweed as well as the presence of the other bioactive components lead to their better and potent effect against tested bacterial isolates.

In this regards, methanol, ethanol and hexane extracts of *S. vulgare* showed the most potent activity against examined pathogens by showing lowest MIC_{50}/MBC values of 0.08/1.00 mg/mL against *E. coli* O:157 isolate as mentioned in Tables 2-3. The observed highest antimicrobial activity of *S. vulgare* extracts could be related to their bioactive compounds such as flavonoids, terpenoids, tannins, carbohydrates and highly phenolic compounds present in methanolic extracts; whereas, terpenoids, phenols and highly saponins compounds were found in hexane extracts. These compounds constitute an important class as secondary metabolites reported to display strong antimicrobial activity.

More recently, Perez *et al*. (2016) reviewed the antimicrobial potential effect of seaweed related to their different bioactive constituents such as polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids and halogenated compounds. Tajbakhsh *et al*. (2011) reported the antibacterial activity of *S. oligocystum* (collected from south west of Iran) against 2 gram-negative bacterial pathogens (*P. aeruginosa* and *E. coli*). This study showed that hot water displayed an inhibitory activity with MIC value recorded to be 9.556 mg/mL against *P. aeruginosa* isolate. Whereas, Inhibitory effect of *S. polycystum* and *P. australis* (brown) seaweed against 3 gram- negative bacteria isolates was reported using methanol, dichloromethane and *n*-hexane solvents, showed that *S. polycystum* seaweed was more potent as compared to *P. australis.* In this regards, the lowest MIC of *S. polycystum* extracts against *E. coli* were 0.521 mg/mL (methanol) and 0.417 mg/mL (n-hexane) and were 0.104 mg/mL (*n*-Hexane) and 0.208 mg/mL (methanol and dichloromethane), respectively against *P. aeruginosa*. As for bactericidal effect (MBC), the two examined seaweed extracts were non active against the tested isolates regardless of examined solvent (Chiao-Wei *et al*., 2011)

Moreover, Chandrasekaran *et al*. (2014) also reported inhibitory activity of *S. wightii* expressed as MIC and MBC values. In this respect, MIC values ranged between

Table 2. Minimum inhibition concentrations (MIC_{50}) values of the four seaweed extracts against the tested isolates

Microorganisms	Minimum inhibitory concentration (MIC_{50}) (mg/mL)						
	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Ciprofloxacin (mg/L)
C. tomentosum							
S. typhimurium	6.7±2.9**	6.7±2.9	8.3±2.9	6.7±2.9	6.7±2.9	10.0±0.0	0.25±0.13
S. marcescens	4.2±1.4**	5.8±3.8	5.8±3.8	5.0±0.0	4.2±1.4	>10.0±0.0	0.5±0.11
E. coli O:157	3.3±1.4**	5.8±3.8	8.3±2.9	5.8±3.8	4.2±1.4	>10.0±0.0	4.0±0.5
P. vulgaris	5.0±0.0**	5.8±3.8	6.7±2.9	4.2±1.4	5.8±3.8	>10.0±0.0	1.0±0.13
A. baumannii	4.2±1.4**	4.2±1.4	5.8±3.8	3.3±1.4	5.0±0.0	>10.0±0.0	64±0.26
B. melitensis	5.0±0.0**	6.7±2.9	8.3±2.9	5.0±0.0	5.8±3.8	>10.0±0.0	0.5±0.09
P. aeruginosa	4.2±1.4**	5.8±3.8	6.7±2.9	3.3±1.4	4.2±1.4	>10.0±0.0	1.0±0.2
K. pneumoniaee	5.8±3.8**	8.3±2.9	6.7±2.9	5.8±3.8	6.7±2.9	>10.0±0.0	6.0±0.15
S. flexneri	4.2±1.4*	4.2±1.4	5.0±0.0	3.3±1.4	4.2±1.4	>10.0±0.0	0.38±0.11
V. cholerae	5.0±0.0**	6.7±2.9	8.3±2.9	5.8±3.8	5.8±3.8	>10.0±0.0	0.75±0.07
C. mediterranea							
S. typhimurium	1.3±0.0**	1.7±0.7	2.1±0.7	1.7±0.7	2.1±0.7	6.7±2.9	0.25±0.13
S. marcescens	1.0±0.4**	1.7±0.7	3.3±1.4	1.3±0.0	2.1±0.7	6.7±2.9	0.5±0.11
E. coli O:157	1.0±0.4**	1.7±0.7	2.1±0.7	1.3±0.0	1.7±0.7	8.3±2.9	4.0±0.5
P. vulgaris	1.7±0.7**	2.1±0.7	2.9±1.9	1.7±0.7	2.1±0.7	10.0±0.0	1.0±0.13
A. baumannii	4.2±1.4**	4.2±1.4	6.7±2.9	8.3±2.9	3.3±1.4	ND	64±0.26
B. melitensis	1.4±0.4**	1.4±0.7	2.9±0.7	2.9±0.7	1.4±0.7	10.0±0.0	0.5±0.09
P. aeruginosa	2.1±0.7**	2.5±0.0	2.9±1.9	1.7±0.7	2.5±0.0	10.0±0.0	1.0±0.2
K. pneumoniaee	2.1±0.7**	3.3±1.4	3.3±1.4	2.1±0.7	3.3±1.4	10.0±0.0	6.0±0.15
S. flexneri	1.7±0.7*	2.1±0.7	3.3±1.4	1.3±2.2	3.3±1.9	ND	0.38±0.11
V. cholerae	2.1±0.7**	2.1±0.7	3.3±1.4	2.5±2.2	2.9±1.9	ND	0.75±0.07
H. musciformis							
S. typhimurium	13.3±5.8**	20.0±0.0	23.3±15.3	26.7±11.5	33.3±11.5	40.0±0.0	0.25±0.13
S. marcescens	13.3±5.8**	26.6±11.5	30.0±17.3	26.7±11.5	33.3±11.5	26.7±11.5	0.5±0.11
E. coli O:157	16.7± 5.8**	16.7± 5.8	23.3±15.3	23.3±15.3	33.3±11.5	33.3±11.5	4.0±0.5
P. vulgaris	16.7± 5.8**	23.3±15.3	26.7±11.5	40.0±0.0	46.7±30.5	53.3±23.1	1.0±0.13
A. baumannii	13.3±5.8**	23.3±15.3	33.3±11.5	33.3±11.5	53.3±23.1	53.3±23.1	64±0.26
B. melitensis	20.0±0.0**	33.3±11.5	23.3±15.3	40.0±0.0	46.7±30.6	66.7±23.1	0.5±0.09
P. aeruginosa	13.3±5.8**	23.3±15.3	26.7±11.5	33.3±11.5	33.3±11.5	53.3±23.1	1.0±0.2
K. pneumoniaee	13.3±5.9**	26.7±11.5	26.7±11.5	46.7±30.6	46.7±30.6	53.3±23.1	6.0±0.15
S. flexneri	23.3±15.3	30.0±17.3	33.3±11.5	40.0±0.0	66.7±23.1	80.0±0.0	0.38±0.11
V. cholerae	16.7±5.8**	26.7±11.5	30.0±17.3	60.0±34.6	53.3±23.1	66.7±23.1	0.75±0.07
S. vulgare							
S. typhimurium	0.13±0.1	0.21±0.1	0.11±0.0	0.13±0.1	0.27±0.1	0.11±0.1	0.25±0.13
S. marcescens	0.16±0.1	0.21±0.1	0.13±0.1	0.19±0.1	0.21±0.1	0.13±0.1	0.5±0.11
E. coli O:157	0.11±0.1	0.16±0.1	0.11±0.0	0.13±0.0	0.27±0.0	0.08±0.1	4.0±0.5
P. vulgaris	0.13±0.1	0.19±0.0	0.13±0.1	0.19±0.1	0.32±0.1	0.27±0.1	1.0±0.13
A. baumannii	0.11±0.1	0.21±0.1	0.11±0.1	0.19±0.1	0.27±0.1	0.13±0.1	64±0.26
B. melitensis	0.11±0.1	0.31±0.1	0.13±0.1	0.13±0.1	0.32±0.1	0.11±0.2	0.5±0.09
P. aeruginosa	0.13±0.2	0.19±0.0	0.16±0.1	0.21±0.1	0.27±0.1	0.27±0.1	1.0±0.2
K. pneumoniaee	0.16±0.1	0.21±0.1	0.21±0.1	0.21±0.1	0.32±0.1	0.11±0.1	6.0±0.15
S. flexneri	0.08±0.0	0.13±0.1	0.11±0.1	0.16±0.1	0.27±0.1	0.11±0.1	0.38±0.11
V. cholerae	0.11±0.1	0.21±0.1	0.13±0.1	0.21±0.1	0.27±0.1	0.11±0.0	0.75±0.07

ND: not determined; Comparing the effect of the same solvent by using extracts from different seaweed, methanol is the best solvent; - using *S. vulgare*; *p<0.05 vs *C. mediterranea* and *C. tomentosum* for *S. flexneri*; **p<0.0005 vs all other seaweed for all microorganisms except *S. flexneri*.

0.250 mg/mL (ethyl acetate) and 0.5 mg/mL (hexane, chloroform, acetone and methanol). Whereas, for MBC these varied between 0.5 mg/mL (ethyl acetate) and 1 mg/mL (hexane, chloroform, acetone and methanol). An other study revealed that the methanolic extract of *L. papillosa* (Rhodophyceae) among 11 algae species

Table 3. Minimum bactericidal concentrations (MBC) values of the four seaweed extracts against the tested isolates

Microorganisms	Minimum bactericidal concentration (MBC) (mg/mL)						
	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane	Ciprofloxacin (mg/L)
C. tomentosum							
S. typhimurium	8.3±2.9	10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	0.5±0.18
S. marcescens	6.7±2.9	8.3±2.9	>10.0±0.0	8.3±2.9	>10.0±0.0	>10.0±0.0	1.0±0.15
E. coli O:157	8.3±2.9e	>10.0±0.0	>10.0±0.0	8.3±2.9	>10.0±0.0	>10.0±0.0	8.0±0.4
P. vulgaris	8.3±2.9e	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	1.75±0.32
A. baumannii	8.3±2.9	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	128±0.48
B. melitensis	8.3±2.9e	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	1.0±0.24
P. aeruginosa	8.3±2.9	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	2.0±0.15
K. pneumoniaee	>10.0±0.0a	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	12.0±0.19
S. flexneri	8.3±2.9f	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	0.75±0.09
V. cholerae	10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	>10.0±0.0	1.5±0.14
C. mediterranea							
S. typhimurium	2.1±0.7d	2.9±1.9	3.3±1.4	2.1±0.7	3.3±1.4	8.3±2.9	0.5±0.18
S. marcescens	1.7±0.7d	2.9±1.9	4.2±1.4	2.1±0.7	3.3±1.4	8.3±2.9	1.0±0.15
E. coli O:157	1.7±0.7d	1.7±0.7	2.1±0.7	2.1±0.7	2.9±1.9	8.3±2.9	8.0±0.4
P. vulgaris	2.1±0.7d	3.3±1.4	4.2±1.4	2.5±0.0	3.3±1.4	10.0±0.0	1.75±0.32
A. baumannii	5.0±0.0c	5.0±0.0	8.3±2.9	10.0±0.0	6.7±2.9	>10.0±0.0	128±0.48
B. melitensis	2.1±0.7d	2.5±0.0	3.3±1.4	3.3±1.4	3.3±1.4	10.0±0.0	1.0±0.24
P. aeruginosa	3.3±1.4d	4.2±1.4	4.2±1.4	2.5±0.0	4.2±1.4	>10.0±0.0	2.0±0.15
K. pneumoniaee	3.3±1.4d	4.2±1.4	4.2±1.4	3.3±1.4	5.0±0.0	>10.0±0.0	12.0±0.19
S. flexneri	2.5±0.0d	3.3±1.4	4.2±1.4	2.1±0.7	4.2±1.4	>10.0±0.0	0.75±0.09
V. cholerae	3.3±1.4d	3.3±1.4	4.2±1.4	3.3±1.4	4.2±1.4	>10.0±0.0	1.5±0.14
H. musciformis							
S. typhimurium	16.7±5.8	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	0.5±0.18
S. marcescens	16.7±5.8	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	1.0±0.15
E. coli O:157	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	8.0±0.4
P. vulgaris	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	1.75±0.32
A. baumannii	13.3±5.8	16.7±5.8	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	128±0.48
B. melitensis	20.0±0.0	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	1.0±0.24
P. aeruginosa	16.7±5.8	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	2.0±0.15
K. pneumoniaee	20.0±0.0	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	12.0±0.19
S. flexneri	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	0.75±0.09
V. cholerae	20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	>20.0±0.0	1.5±0.14
S. vulgare							
S. typhimurium	1.0±0.4b	1.5±1.0	2.1±0.7	1.7±0.7	2.1±0.7	6.7±2.9	0.5±0.18
S. marcescens	1.0±0.4b	1.0±0.4	2.1±0.7	1.5±1.0	2.9±1.9	4.2±1.4	1.0±0.15
E. coli O:157	1.0±0.4b	1.0±0.4	1.7±0.7	1.5±1.0	3.3±1.4	6.7±2.9	8.0±0.4
P. vulgaris	1.5±1.0b	2.1±0.7	3.3±1.4	1.9±1.1	2.1±0.7	8.3±2.9	1.75±0.32
A. baumannii	1.7±0.7b	2.1±0.7	2.9±1.9	2.1±0.7	4.2±1.4	8.3±2.9	128±0.48
B. melitensis	1.0±0.4b	1.7±0.7	3.3±1.4	2.9±1.9	4.2±1.4	10.0±0.0	1.0±0.24
P. aeruginosa	1.5±1.0b	2.1±0.7	3.3±1.4	2.1±0.7	4.2±1.4	10.0±0.0	2.0±0.15
K. pneumoniaee	1.5±1.0b	2.1±0.7	3.3±1.4	2.1±0.7	5.0±0.0	10.0±0.0	12.0±0.19
S. flexneri	1.7±0.7b	2.1±0.7	3.3±1.4	2.1±0.7	3.3±1.4	10.0±0.0	0.75±0.09
V. cholerae	1.5±1.0b	2.1±0.7	3.3±1.4	2.1±0.7	4.2±1.4	10.0±0.0	1.5±0.14

Comparing the effect of the same solvent by using extracts from different seaweed, methanol is the best solvent:
- using *S. vulgare*; ap<0.005 vs *C. tomentosum* for *K. pneumoniae* microorganisms; - using *H. musciformis*; bp<0.0005 vs *S. vulgare* for all microorganisms; cp<0.005 vs *C. mediterranea* for *A. baumannii* microorganisms; dp<0.0005 vs *C. mediterranea* for all microorganisms except *A. baumannii*; ep<0.005 vs *C. tomentosum* for *E. coli* O:157, *P. vulgaris* and *B. melitensis* microorganisms; fp<0.0005 vs *C. tomentosum* for *S. flexneri* microorganisms.

exhibited the strongest inhibitory effect with MIC_{50} of 0.00079, 0.00158 mg/mL against *E. coli* and *P. aerugenosa* bacteria, respectively (Kavita *et al*., 2014).

Shanmughapriya *et al*. (2008) investigated inhibitory effects of 14 seaweeds (5 Chlorophyta , 5 Rhodophyta and 4 Phaeophyta) collected from India against 4 gram negative bacterial isolates using methanol:toluene (3:1) and ethanolic extracts. This study revealed that methanol:toluene (3:1) was the most potent, whereas, ethanolic extracts showed no activity against examined isolates. Among the studied seaweeds, *Acrosiphonia orientalis* (Chlorophyta) and *Stocheospermum marginatum* (Phaeophyta) were the most potent by showing the lowest MIC/MBC values 50/10 and 10/0.5 mg/mL, respectively against *P. aeruginosa* isolate.

Recently, Sushanth and Rajashekhar (2015) reported the ethanol, methanol and hexane extracts antimicrobial activity of 4 microalgae against 4 gram-negative bacterial strains. This investigation revealed that the ethanol *Skeletonema costatum* extract displayed the lowest MIC value of 0.5 mg/mL against *K. pneumoniae.* Whereas, the highest MIC was recorded to be 2 mg/mL (this value varied according to the tested solvent, bacteria isolate and microalgae species) while, hexane extracts of *S. costatum* exhibited a moderate activity with MIC of 1 mg/mL against *S. typhimurium* isolate.

More recently, Alves *et al*. (2016) reported that the seaweed inhibitory effect as expressed by MIC were 12.5 mg/mL with methanolic *S. polyceratium* extracts against both *E. coli* and *K. pneumoniae* isolates that estimated MBC values were recorded to be 50 and 12.5 mg/mL against *E. coli* and *K. pneumoniae* isolates, respectively.

Conclusion

Antibacterial activities of seaweeds were screened based on ZI, MIC_{50} and MBC values using water and six solvents. Aqueous seaweed extracts showed no activity against all isolates regardless examined seaweed species. Data presented here depicted that the methanolic and hexane extracts of *S.valgare* were the most potent by showing the lowest MIC_{50} values of 0.08/1 mg/mL (against *S. flexneri* and *E. coli* O:157 isolate) and the lowest MBC value of 1.00/mL (methanolic extract *S. typhimurium*, *S. maccens*, *E. coli* O: 157 and *B. melitensis* isolates and also with ethanolic extract against *S.maccens* and *E. coli* O: 157 isolates. Overall, standard antibiotic was more potent than seaweeds crude extracts against tested bacterial isolates regardless examined solvents. Based upon data presented herein, it is important to investigate the inhibitory effect of purified bioactive constituents of *S. vulgare* extracts as a potential source for antibacterial pretreatment.

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