Bioactivity Potential of *Gracilaria salicornia, Padina boergesenii, Polycladia myrica*: Antibacterial, Antioxidant and Total Phenol Assays

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Abstract

Antibacterial activities of MeOH and aqueous extracts of *Gracilaria salicornia* (C. Agardh) Dawson, *Padina boergesenii* Allander and Kraft, *Polycladia myrica* (S. G. Gmelin) Draisma, were examined against Gram-positive bacteria *Staphylococcus aureus* Rosenbach 1884, *Pseudomonas aeruginosa* (Schröter 1872) Migula 1900, and *Escherichia coli* (Migula 1895). Indeed, extracts of wet samples showed 5.3±0.58 to 34.3±0.6 mm antibacterial activity. Furthermore, antioxidant activities of algae evaluated using DPPH and ABTS radical scavenging methods. Whereas, in the DPPH method, aqueous extract of *Polycladia myrica* showed the highest antioxidant activity, MeOH and aqueous extracts of *Gracilaria salicornia* exhibited the lowest antioxidant activity. Beside, the antioxidant activity of extracts was higher using the ABTS method. Additionally, aqueous extracts showed the lowest IC_{50} values in comparison to MeOH extracts. Total phenolic content of aqueous extract was 5.07±0.08 to 46.73±0.24 mg gallic acid /100 g higher than the MeOH extract. The MeOH and aqueous extracts of *Padina boergesenii* demonstrated the highest TPC among others.

Keywords: Antibacterial; Antioxidant; Total phenol; Bioactive compounds; Seaweed.

Introduction

Seaweeds are marine macroalgae considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites (Bansemir et al., 2006; Yuan et al., 2005). Up to now almost 6000 species of seaweeds have been identified, and 2400 natural products have been isolated from macroalgae belonging to Rhodophyceae (red), Phaeophyceae (brown) and Chlorophyceae (green). Wijesinghe et al. (2012) reported that various groups of chemical compounds such as macrolides, peptides, proteins polyketides, sesquiterpenes, terpenes and fatty acids of seaweeds are effective in antibacterial activity. Wide variety of biological activities are attributed to marine macroalgae such as antimicrobial (Ibtissam et al., 2009; Rhimou et al., 2010;

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Tajbakhsh et al., 2011b), antiviral (Kim and Karadeniz, 2011), antifungal (de Felício et al., 2010), anti-allergic (Na et al., 2005), anticoagulant (Shi et al., 2010), anti-HIV (Thuy et al., 2015), anticancer (Kim et al., 2011), larvicidal (Bianco et al., 2012), antiplasmodial (Ravikumar et al., 2011), and antioxidant activities (Devi et al., 2011), and antithrombotic and cellular proliferation activities (Guerra Dore et al., 2013).

Seaweeds and plants contain diverse phenolic compounds, such as flavonoids, phenolic acids, lignins and tannins, which are recognized as compounds that contribute in antioxidant activity (Grassmann et al., 2004). Some studies improved a correlation between phenolic content and antioxidant activity (Jiménez et al., 1999).

Gracilaria species (Rhodophyceae) are one of the most valuable macroalgae and the main source of high-quality agar (Praiboon et al., 2006). Indeed, Gracilaria is a source of some food in human consumption and in pharmaceutical components (Torres et al., 2019). Fouladvand et al., (2011) reported anti-leishmanial activity of *Gracilaria corticata* (J. Agardh) J. Agardh, collected from the Persian Gulf. Furthermore, different aspects of bioactive compounds of *Gracilaria corticata* such as antimicrobial activity against human pathogens (Govindasamy et al., 2011), antiobesity (Kannan et al., 2014a), antioxidant (Guaratini et al., 2012, Kannan et al., 2014b), anti-inflammatory (Shu et al., 2013), anti-yeast (Sasidharan et al., 2011) and anti-proliferation (Murugan and Iyer, 2012) were studied

Likewise, brown algae are rich sources of bioactive compounds that possess different biological activities. For example, *Padina boergesenii* Allander and Kraft has been shown to have a hepatoprotective effect against CCl4-induced liver damage and also antioxidant activity (Karthikeyan et al., 2012).

The objective of the study was finding out the antibacterial potentials of some common algae growing in coastal waters of the Persian Gulf in Qeshm Island. Bacterial resistance to available antimicrobial drugs has become a challenge (Brooks et al., 2007). Thus, discovering new, economical and effective antibacterial agents and antioxidants with natural bases sounds very interesting to researchers.

Material and methods

Material preparation

Solvents, ascorbic acid (PubChem CID: 54670067) and Gallic acid (PubChem CID: 370) were purchased from Merck. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (PubChem CID: 74358), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (PubChem CID: 35687). Additionally, Folin-Ciocalteu's reagent supplied from Sigma and Aldrich companies. Biochemicals including nutrient agar and nutrient broth purchased from Merck. Finally, the absorption rate measured by spectrophotometer (Unico 2100, China). Indeed, freeze dry (Christ, Alpha 1-2 LD Plus) used for removing solvents.

Sample collection

Gracilaria salicornia (C. Agardh) Dawson, *Padina boergesenii* Allander and Kraft and *Polycladia myrica* (S.G.Gmelin) Draisma (formerly *Cystoseira myrica*) were collected from the Persian Gulf (55' 57 ºE and 26' 56 ºN) in Qeshm Island, Iran, from late October 2013 to December 2014. In addition, this region supports massive growth and distribution of these algae. *Gracilaria salicornia* and *Padina boergessenii* were identified by Ramezanpour (recorded in NCBI), and *Polycladia myrica* was identified by Dr. Michael Wynne. Then, the algae washed twice with seawater and tap water to remove epiphytes, freeze dried and stored in labeled plastic tubes at -19 °C.

Preparation of extracts

10 g of each fresh algal sample used for MeOH extraction (100 ml) by soxhlet extractor for 4 cycles. Then, the mixture was filtered through Whatman filter paper. Next, the extract was freeze dried and stored at −19 °C for further analysis. For aqueous extract, 10 g algal sample refluxed with deionized water (100 ml) for 2 hours. After that, the mixture was filtered through Whatman filter paper. Finally, the extract was freeze dried and stored at −19 °C for future analysis (Magaldi et al., 2004).

Antibacterial assay

The gram-positive bacteria *Staphylococcus aureus* Rosenbach 1884 and *Micrococcus luteus* Schroeter 1872 and gram-negative bacteria *Pseudomonas aeruginosa* (Schröter 1872)

Migula 1900 and *Escherichia coli* (Migula, 1895) Castellani and Chalmers 1919 were used as bacterial strains. The bacteria provided from microbiology laboratory of the University of Guilan. The antibacterial activities were evaluated by MeOH and aqueous extracts of *Gracilaria salicornia* (C. Agardh) Dawson, *Padina boergesenii* Allander and Kraft, and *Polycladia myrica* (SG. Gmelin) Draisma against both gram-positive and gram-negative bacterial strains. Additionally, the process performed using well-diffusion method (Balouiri et al., 2016). Then, a colony of each investigated organism was sub-cultured in order to obtain fresh bacteria on the nutrient agar plates at 37 ºC for 18 hours, and fresh suspensions of microorganisms (0.5 McFarland) were prepared. After that, a suspension of 30 µl bacteria added to each nutrient agar plate and was spread through plates by sterile spreader. Extract concentrations of 200, 100 and 50 $mg.ml^{-1}$ were prepared in dimethyl sulfoxide (DMSO). Next, each well received 30 µl of the corresponding concentration of extract. Later, plates incubated at 37 °C for 24 h. Finally, the inhibition zones were measured by coulisse and expressed in millimeters (mm). The experiments were performed in triplicate and the results are reported as mean± standard deviation (mean ± SD) of zone of inhibition. Tetracycline and Penicillin G were used as positive controls. DMSO and deionized water were used as negative controls.

Antioxidant assays

DPPH Radical scavenging activity

Antioxidant assay by DPPH assay was evaluated according to Jin et al.'s method (2012)

with some modifications. Extracts were prepared at the concentrations of 60, 30, 15, 7.5, 3.75 and 1.875 $mg.ml^{-1}$ in MeOH and in deionized water. 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) was dissolved in MeOH to achieve a concentration of 6.25×10^{-5} M. Then, 3.9 ml of DPPH solution added to 0.1 ml of extract solution at different concentrations. Next, the samples shaken vigorously and incubated at 25 ˚C for 30 min. Finally, the decrease of absorbance rate in resulting solution measured at 517 nm. In addition, MeOH used as a blank and 3.9 ml DPPH solution containing 0.1 ml of MeOH or deionized water used as the control. The experiments were conducted in triplicate.

Radical scavenging activity percentage calculated as follows:

DPPH radical scavenging rate (%) = $[(A_{control}-A_{sample})/ A_{control}] \times 100$ (1)

Were, $A_{control}$: the absorbance of all the reagents except the test extracts. Asample: the absorbance of the test extracts and all the reagents. IC_{50} of the samples was calculated by plotting the radical scavenging percentage against the concentration of extracts.

ABTS Radical Scavenging Activity

The ABTS assay was performed following the procedure described by Jean et al., (2012). ABTS (7.4 mM) in MeOH and potassium persulfate ($K_2S_2O_8$) (2.6 mM) as an oxidizing agent in MeOH were mixed in equal volumes, then the $ABTS^{\bullet+}$ radical cation was produced by reacting stock solution for 12 hours in the dark at room temperature. In order to give the absorbance of 1.1 ± 0.02 at 734 nm, the stock solution was diluted with MeOH. Then, extracts were prepared at concentrations of 60, 30, 15, 7.5, 3.75 and 1.875 $mg.ml^{-1}$ in MeOH for MeOH and aqueous extracts. Next, 150 µl of sample solution was added to 3.0 ml $ABTS^{\bullet+}$ solution and this mixture was shacked and incubated in the dark for 2 hours. Finally, the absorbance of each solution was recorded at 734 nm. Moreover, MeOH and water were used as blanks. Indeed, Radical scavenging activity was calculated by formula of DPPH radical-scavenging assay and the IC_{50} values of each compound for ABTS assay were calculated by plotting the inhibition percentage against concentration of the extracts.

Total phenol content

Evaluation of total phenolic content (TPC) was done by the Folin–Ciocalteu colorimetric method according to Skerget et al. (2005). Next, Folin-Ciocalteau reagent (2.5 ml) was diluted 1:10 with distilled water and added to 0.5 ml of extract (1 $mg.ml^{-1}$ of distilled water). After 2 minutes freshly prepared aqueous solution of Na_2CO_3 (2 ml, 20%) added and shacked vigorously, then, the mixture incubated at 25 ˚C for 30 minutes and the absorbance of solution was measured at 760 nm. In addition, blank solution contained 0.5 ml of distilled water instead of the extract. Furthermore, Gallic acid was used in 0.001 to 0.01 $mg.ml^{-1}$ concentrations as a standard. The results reported as equivalent to milligrams of gallic acid per 100 gram of dry weight extract (mg GAE/100 g). The experiments were performed in triplicate.

Statistical analysis

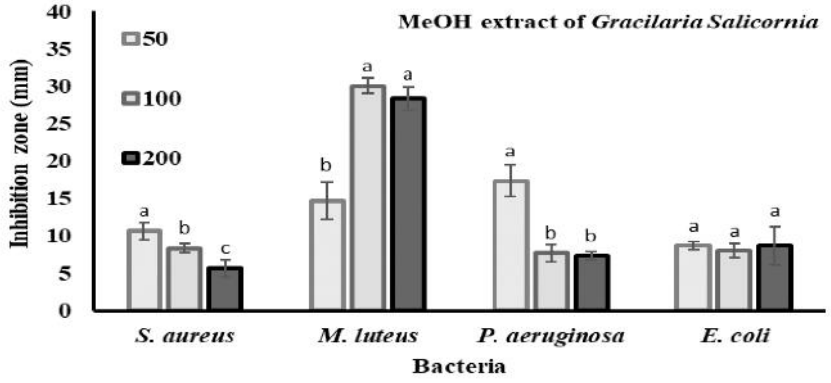
The statistical package SPSS 26 used for

analyses. Two-way ANOVA analysis was performed to compare the means. Values presented as mean ± SD of triplicate experiments.

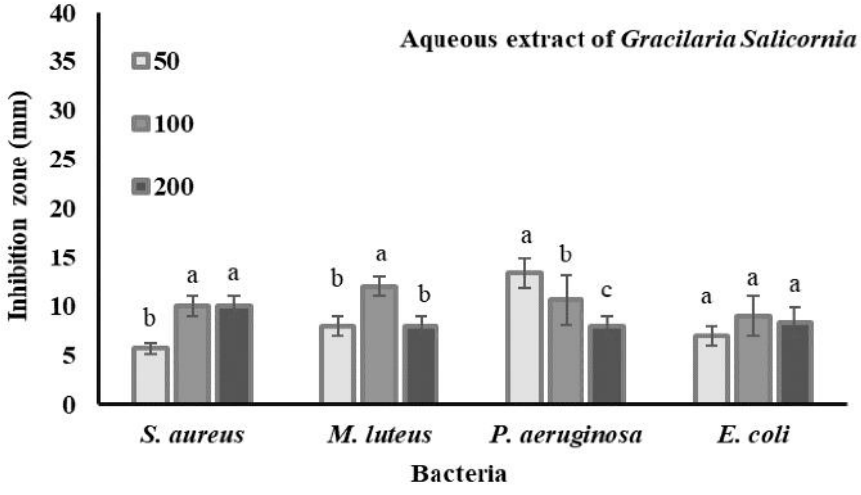
Results

Antibacterial assay

Antibacterial effect of MeOH and aqueous extract of *Gracilaria salicornia* is shown in Figure 1a, b. Indeed, antibacterial activity of MeOH extract of *Gracilaria salicornia* against all the tested bacteria was not significantly different at 100 and 200 mg.ml^{-1} concentrations except *Staphylococcus aureus*. Further, there was a significant difference between antibacterial activity of *Gracilaria salicornia* at 100 and 200 mg.ml^{-1} concentrations against *Micrococcus luteus* and *Pseudomonas aeruginosa*. There was no significant difference between any concentrations of aqueous extract against *Escherichia coli*. Additionally, the highest antibacterial activity of *Gracilaria salicornia* aqueous extract was at 50 mg.ml^{-1} concentration against *Pseudomonas aeruginosa*.



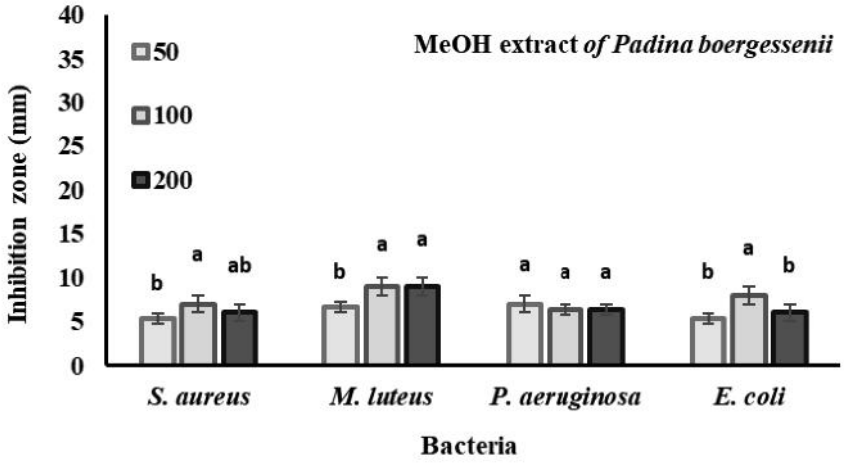
a



b

Fig. 1. (a) Antibacterial activity of MeOH and (b) aqueous extracts of *G. Salicornia*

Antibacterial activity assays of MeOH and aqueous extracts of *Padina boergesenii* against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia Coli* and *Pseudomonas aeruginosa* are presented in Figure 2a, b. The MeOH extract of *Padina boergesenii* exhibited no significant difference against tested bacteria including *Micrococcus luteus* and *Pseudomonas aeruginosa* at 100 and 200 mg.ml^{-1} concentrations. While Aqueous extract of *Padina boergesenii* showed significant difference only at 200 mg.ml^{-1} concentration against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, exhibited no significant difference against *Micrococcus luteus* and *Escherichia coli* at all concentrations.



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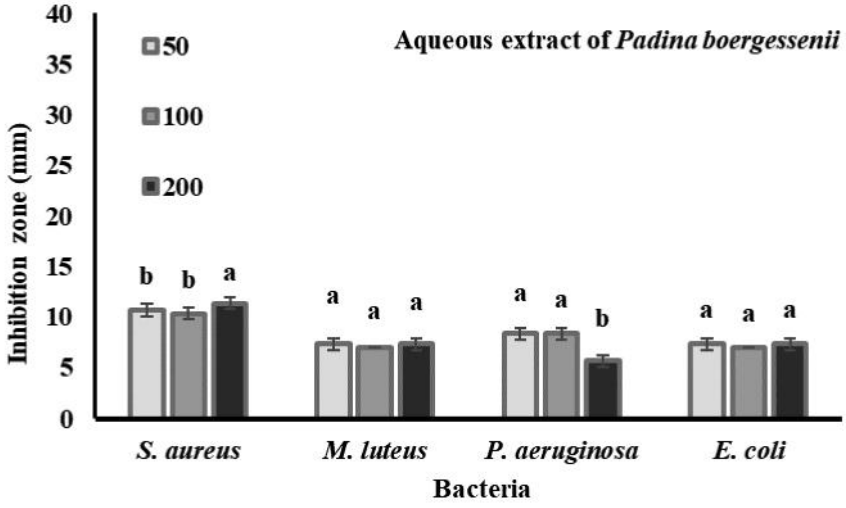

b

Fig. 2. (a) Antibacterial activity of MeOH and (b) aqueous extracts of *P. boergesenii*

Inhibition zone of MeOH and aqueous extracts of *Polycladia myrica* is represented in Figure 3. MeOH extract of *Polycladia myrica* inhibited most powerful at the concentration of 200 mg.ml^{-1} against *Micrococcus luteus*, then other concentrations in different tested bacteria. While, there are significant differences at the concentration of 100 and 50 mg.ml^{-1} against *Pseudomonas aeruginosa* and *Escherichia coli*, no considerable differences were observed between antibacterial activity of aqueous extracts of *Polycladia myrica* against *Staphylococcus aureus* and *Micrococcus luteus*.

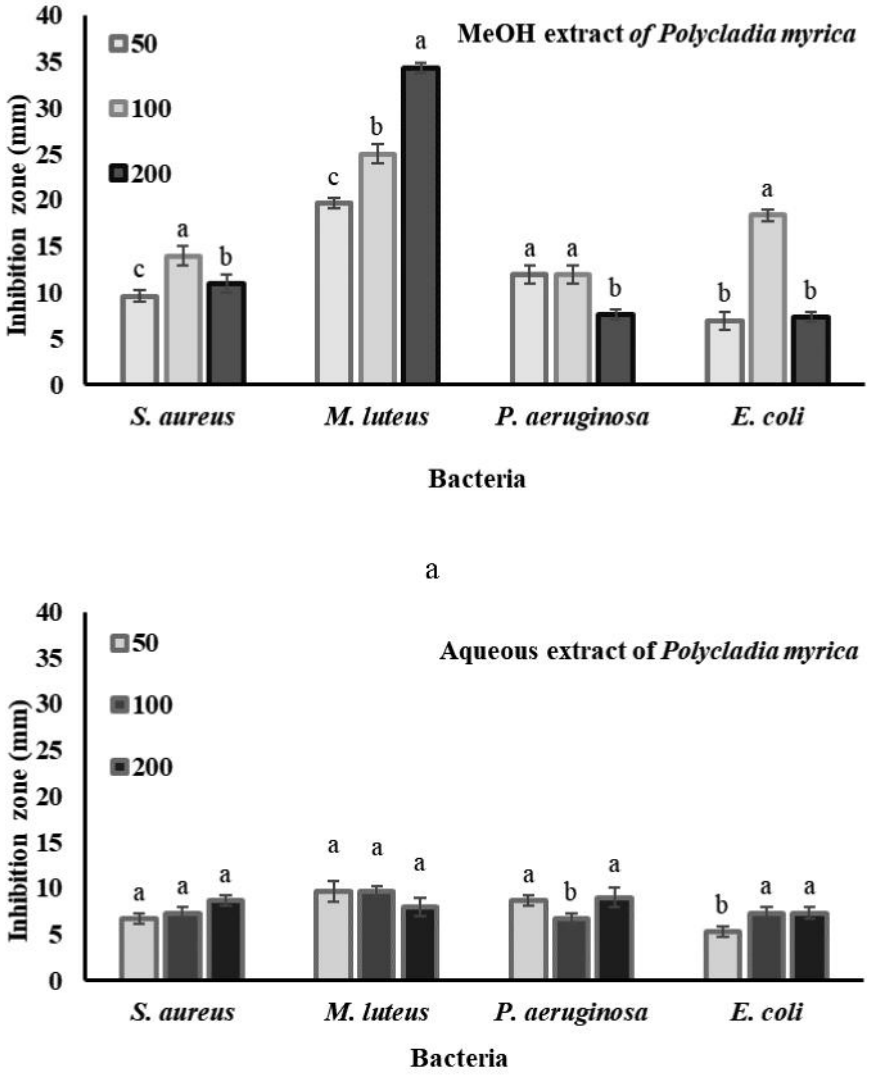

Fig. 2. (a) Antibacterial activity of MeOH and (b) aqueous extracts of *P. myrica*

The antibacterial activities of extracts were not comparable to penicillin and Cefixime as positive controls.

Antioxidant activity

The DPPH radical scavenging activities of MeOH and aqueous extracts of *Gracilaria salicornia*, *Padina boergesenii* and *Polycladia myrica* are depicted in Figure 4. Additionally, both extracts of *Gracilaria salicornia* possessed the lowest antioxidant activity. Furthermore, aqueous extract of *Polycladia myrica* possessed the highest antioxidant activity. In addition, the antioxidant activities of MeOH and aqueous extracts of *Gracilaria salicornia* were variable from 3.09 ± 0.22 to 27.71 ± 0.39 mg.ml^{-1}. Indeed, the antioxidant activities of MeOH and aqueous extracts of *Padina boergesenii* were from 21.39 ± 0.4 to 53 ± 0.39 mg.ml^{-1} and 37.14 ± 0.37-55.19 ± 0.39 mg.ml^{-1} for *Polycladia myrica*. Finally, the antioxidant activity of extracts is not comparable to ascorbic acid (100%) at similar concentrations. Although, there was a significant difference between all concentrations of aqueous extract of *Gracilaria salicornia*, there was no significant difference between some concentrations of MeOH extract. While, in the MeOH extract of *Padina boergesenii* there was no significant difference between concentrations of 30, 15, 7.5 mg.ml^{-1}, in aqueous extract there were significant differences between all concentrations except 7.5 and 3.75 mg.ml^{-1}. There was significant difference between concentrations of MeOH and aqueous extracts of *Polycladia myrica* except 7.5 and 3.75 mg.ml^{-1}. Finally, there was no significant difference between similar concentrations of MeOH and aqueous extracts of *Polycladia myrica*.

The results of ABTS and DPPH assays are depicted in Figures 4 and 5. Further, MeOH and aqueous extracts showed higher antioxidant activity using ABTS method in comparison with DPPH method. Furthermore, Minimum and maximum antioxidant activities according ABTS assay was observed in both MeOH and aqueous extraction, 15.24±0.57 mg.ml^{-1} of *Padina boergesenii* and *Gracilaria salicornia* 96.41±0.76 mg.ml^{-1}, respectively. Additionally, there was a significant difference between MeOH and aqueous extracts of *Polycladia myrica* in ABTS assays.

The IC_{50} values of MeOH and aqueous extracts for DPPH and ABTS assays are represented in Table 1. While, IC_{50} values of aqueous extracts in *Padina boergesenii* and *Polycladia myrica* are lower than MeOH extracts, in *Gracilaria salicornia* MeOH extract was lower than aqueous extract in ABTS method.

Total phenol content

TPC results of aqueous and MeOH extracts *Gracilaria salicornia*, *Padina boergesenii* and *P. myrica* are depicted in Table 2. TPC of MeOH and aqueous extracts of *Padina boergesenii* was higher than *Gracilaria salicornia* and *Polycladia myrica* and there was significant difference between TPC of MeOH and aqueous extracts of each alga.

Discussion

Bioactive components have already

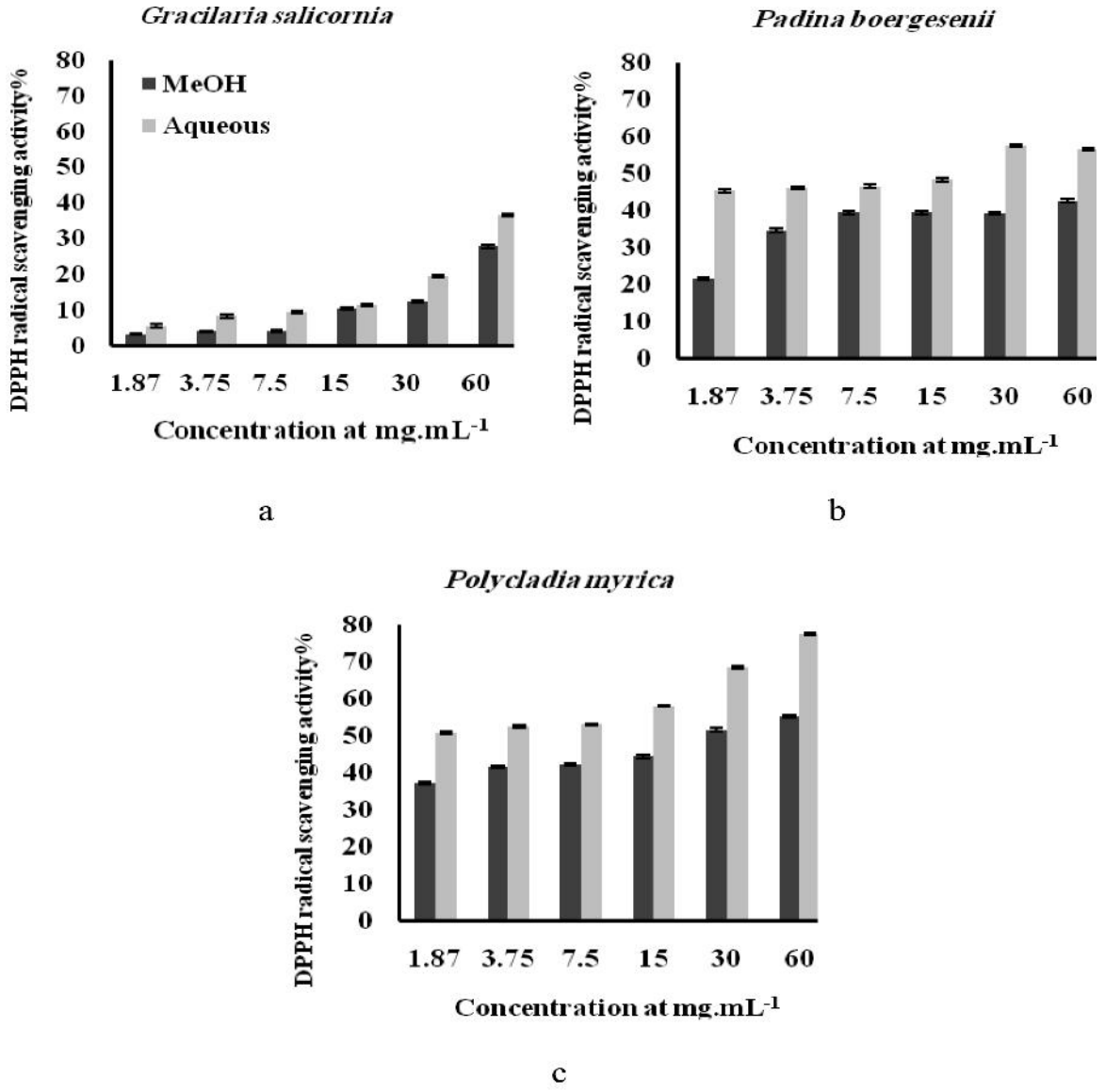

Fig. 4. DPPH radical scavenging activity of activities of MeOH and aqueous extracts of (a) *G. salicornia,* (b) *P. Boergesenii,* and (c) *P. myrica*

been known in seaweed such as sterols, terpenoids, phenols, flavonoids, halogenated components, fatty acid compounds (Holdt et al., 2011), and polysaccharides (Barros et al., 2013). Additionally, there are numerous records on antibacterial activities of different extracts of *Gracilaria* spp., *Padina* spp. and *Cystoseria* spp. against various bacterial species.

Sastry and Rao, (1994) reported antibacterial activity of MeOH and $CHCl_3$ *Gracilaria corticata* extracts against *Pseudomonas aeruginosa* and benzene extract of the same species against *Salmonella typhi* and *Escherichia coli*.

MeOH extract of *Gracilaria canaliculata* exhibited the highest antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria. Furthermore, Isoamyl alcohol and hexane extracts of *Graci-*

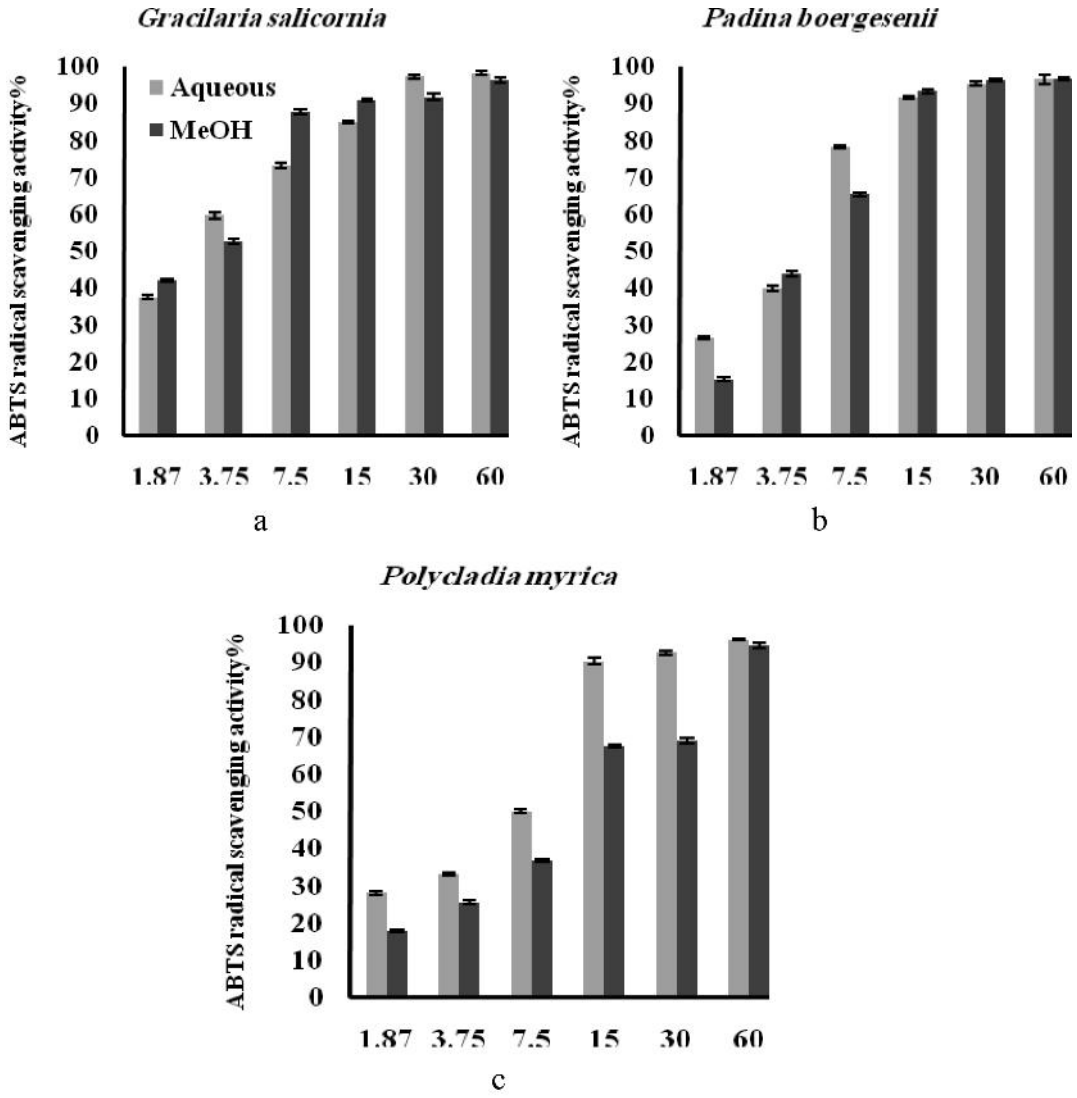

Fig. 5. ABTS radical scavenging activity of MeOH and aqueous extracts of (a) *G. salicornia*, (b) P. *boergesenii,* and (c) *P. myrica*

Table 1. IC_{50} values of antioxidant activity by DPPH and ABTS methods. Significant differences for each alga are indicated by different letters as determined by T-test ($p<0.05$)

Algae	Extract	IC_{50} ($mg.ml^{-1}$) by DPPH	IC_{50} ($mg.ml^{-1}$) by ABTS
Gracilaria salicornia	MeOH	119.7 ± 0.4^{a}	1.58 ± 0.30^{a}
	Aqueous	90.1 ± 0.7^{b}	2.78 ± 0.28^{b}
Padina boergesenii	MeOH	22.23 ± 0.52^{a}	6.53 ± 0.22^{a}
	Aqueous	17.41 ± 0.58^{b}	4.27 ± 0.12^{b}
Polycladia myrica	MeOH	35.07 ± 1.34^{a}	21.61 ± 0.32^{a}
	Aqueous	10.14 ± 0.31^{b}	10.31 ± 0.10^{b}
Ascorbic acid		0.038 ± 0.04	0.001 ± 0.02

Table 2. Total phenolic content of MeOH and aqueous extracts of *G. salicornia*, *P. boergesenii* and *P. myrica* (Data are expressed as mean ± standard deviation of triplicate samples).

Total phenolic content (mg GAE/100g)	Extraction solvent	Alga
G. salicornia	MeOH	5.07±0.08[a*]
	aqueous	7.34±0.07[b]
P. boergesenii	MeOH	20.22±0.11[a]
	aqueous	46.73±0.24[b]
P. myrica	MeOH	14.62±0.26[a]
	aqueous	29.22±0.21[b]

* Significant differences for each alga are indicated by different letters as determined by T- test($p<0.05$)

lariopsis longissima, *Gracilaria foliifera*, *Gracilaria corticata*, *Gracilaria canaliculata* and *Gracilaria edulis*, showed the highest and lowest antibacterial activity against human bacterial pathogens, respectively (Prabhahar et al., 2012).

Gracilaria edulis showed highest antibacterial activity against *Staphylococcus aureus* (Vallinayagam et al., 2009). EtOH extract of *Gracilaria cortica* resulted more antibacterial activity against *Pseudomonas aeruginosa* (Jeyanthi et al., 2013; De-Campos et al., 1988).

Adaikalaraj et al., (2012) recorded high antibacterial activity potential of *Gracilaria ferugosoni* and *Gracilaria verrucosa*. Additionally, in the present study all concentrations of MeOH and aqueous extracts of *Gracilaria salicornia* showed a low to high gradient antibacterial activity.

This study showed moderate antibacterial efficiency of aqueous and MeOH extracts of *Padina boergesenii* against all tested bacteria. El-Fatimy and Abdel-Moneim (2011) mentioned that while *Padina antillarum* showed no effective activity against the tested bacterial strains *Padina pavonica*, *Padina gymnospora*, and *Padina tetrastromatica* have antibacterial effect especially against *Staphylococcus aureus* (Rizvi, 2010).

Further, in this study MeOH extract of *Polycladia myrica* showed considerably higher antibacterial activity against *Micrococcus lutus* than other tested bacterial strains. *Cystoseira compressa* (Dulger and Dugler, 2014) and *Cystoseira barbata* showed moderate inhibitory activity against the *Staphylococcus aureus* and *Salmonella typhimurium* (Ertürk and Taş, 2011; Ozdemir et al., 2006).

Extraction of *Sirophysalis* (formerly Cystoseira) *trinodis* was respectively effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and

Pseudomonas aeruginosa (Tajbakhsh et al., 2011a). While, Diethyl ether and EtOH extracts of *Cystoseria mediterranea*, *Cystoseriata mariscifolia*, *Cystoseira sedoides* and *Cystoseira compressa* exhibited antibacterial activity on *Staphylococcus epidermidis aureus, Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Tüney et al., 2006; Ainane et al., 2014;), extractions of *Cystoseira crinita* were not effective against *Escherichia coli* (Berber et al., 2015).

In addition, there are obviously similarities between the results of present study and other authors. Furthermore, the reasons for high or moderate antibacterial activities could be due to innate differences in various algal species or strains. Additionally, Production of substances, geographical zones and habitats, seasonal variations, life phase also could be effective in antibacterial potentials of different extracts.

While, the IC_{50} of MeOH extract was higher. In this study MeOH extract showed lower antioxidant activity in comparison with aqueous extracts. The result of the research is in contrast with Ganesan et al. (2008) who reported higher antioxidant activity for MeOH than aqueous extract. However, the IC_{50} value is not comparable with ascorbic acid. Moreover, Vijayavel et al. (2010) reported higher antioxidant activity for MeOH extract of *Gracilaria salicornia* (Hawaiian marine algae) in comparison to present study, but EtOH and MeOH extracts of *Gracilaria birdiae* and *Gracilaria cornea* showed higher antioxidant activity (Souza et al., 2011). *Polycladia myrica* had higher antioxidant activity than *Gracilaria salicornia* and *Padina boergesenii*.

Widowati et al., (2014) reported low antioxidant activity for MeOH extract of *Gracilariopsis longissima*. According to Elalla and Shalaby, (2009) aqueous extract showed lower antioxidant activity in comparison to EtOAc and petroleum ether extracts. Antioxidant activities of non-polar and polar extracts of *Gracilaria manilaensis* showed that MeOH extract had the lowest antioxidant activity and is not comparable with ascorbic acid and vitamin E (Abdullah et al. 2013). *Stephanocystis* (formerly Cystoseira) *hakodatensis* was recognized as the best source for antioxidants based on its high phenolics and fucoxanthin content (Airanthi, et al. 2011).

Guner et al. (2013) reported *Gracilaria compressa* as a natural source of antioxidant. *Cystoseira tamariscifolia* and *Bifurcaria bifurcata* assayed by the β-carotene–linoleic acid system as high antioxidant activities (Zubia et al., 2009). Hydroalcoholic fraction of *Polycladia myrica* showed reasonable antioxidant activity (Sadati et al., 2011).

The content of phenolic compounds of *Gracilaria salicornia*, *Padina boergesenii*, and *Polycladia myrica* (*Polycladia myrica*) were determined by Folin-Ciocalteu reagent, and their values are expressed as mg gallic acid per 100g dry sample. Additionally, Phenolic compounds are very important constituents of natural compounds because of their scavenging ability due to their hydroxyl groups. Furthermore, some

studies showed a relationship between phenol content and antioxidant activity (Cao et al., 1997; Devi et al., 2008b). While, MeOH extract of *Gracilaria edulis* contained more phenol (Ganesan et al., 2008), *Gracilaria manilaensis* showed moderate phenol content in comparison with EtOAc and acetone extracts (Abdullah et al., 2013).,This results is in disagreement with aqueous extract of *Gracilaria salicornia* which showed more phenol than MeOH extract. Finally, a positive correlation between antioxidant activity and phenol content of *Gracilaria birdiae* and *Gracilaria cornea* has been reported (Souza et al., 2011).

In this research phenol content of MeOH extract of *Gracilaria salicornia* was higher than *Gracilaria edulis* (Murugan and Iyer, 2012). Moreover, aqueous extract of *Gracilaria salicornia* revealed higher phenol content in contrast with *Gracilariopsis longissima* (Elalla and Shalaby, 2009). Moreover, aqueous extract of *Gracilaria salicornia* showed higher antioxidant activity and more phenol content in comparison to the MeOH extract.

Aqueous extract of *Polycladia myrica* showed more phenol content compared with the MeOH extract in present study. Previous works proved high content of total phenol compounds (TPC) in *Stephanocystis hakodatensis* (Airanthi et al., 2011), *Cystoseira abies-marina* (Barreto et al., 2012), *Cystoseira tamariscifolia* (Zubia et al., 2009), *Polycladia myrica* (Sadati et al., 2011; Moein et al., 2015) and *Cystoseira tamariscifolia* possessed higher TPC (Zubia et al., 2009). TPC in *Padina tetrastromatica* (Chia et al. 2015) was measured 5 times more than *Padina Pavonica* (Husni et al., 2014). Moreover, TPC of aqueous extract of *Padina pavonica* was more than MeOH extract which is in consistency with our results. In addition, MeOH extract of *Padina boergesenii* from Gulf of Mannar Biosphere possessed higher TPC than diethyl ether extract (Karthikeyan et al., 2011). In accordance with our results, aqueous extract *Padina tetrastomatica* had higher TPC than MeOH extract (Chandini et al., 2008). Furthermore, *Padina antillarum* showed high raise TPC from 1240 to 2040 mg GAE. 100 g dried sample for different concentration of MeOH extracts. The result indicated that, aqueous extract of *Padina boergesenii* showed higher antioxidant activity and more phenol content in comparison with MeOH extract and other extracts.

Acknowledgments

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