

Phytochemical Screening and GC-MS Analysis of Bioactive Constituents in the Methanolic Extract of *Caulerpa racemosa* (Forssk.) J. Agardh and *Padina boergesenii* Allender & Kraft

Cholaraj Ragunath^{1*}, Yohannan Aron Santhosh Kumar², Iwar Kanivalan³ and Sruthi Radhakrishnan⁴

¹Unit of Aquatic Biotechnology and Live Feed Culture, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India

²Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India

³Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India

⁴Department of Biotechnology, Hindustan College of Arts and Science, Coimbatore, Tamil Nadu, India

Received : 22 July 2019, Revised: 8 November 2019, Accepted: 27 April 2020

Abstract

Marine macroalgae (seaweeds) are the potential renewable energy and promising resources of many biologically active compounds. The potential sources of commercial and therapeutic impact of seaweeds are still unaware. In this study, we aim to identify and evaluate the biological activity of *Caulerpa racemosa* (Forssk.) J. Agardh and *Padina boergesenii* Allender & Kraft. The seaweed samples were shade dried and ground. The coarse powdered material was subjected to Soxhlet apparatus and successively extracted with methanol. The phyto-constituents of the seaweeds were analyzed by standard methods and GC-MS. The GC-MS results of *C. racemosa* (Forssk.) J. Agardh and *P. boergesenii* Allender & Kraft revealed the presence of hexadecenoic acid, butanoic acid, arachidonic acid, octadecadienoic acid, cis-vaccenic acid, phytol and eicosatetraenoic acid. The seaweed extracts were subjected to antimicrobial assay by agar-well diffusion. *Padina boergesenii* Allender & Kraft showed the highest inhibition zones ($23.6 \pm 0.3\text{mm}$ and $22.7 \pm 0.5\text{mm}$) against Gram-positive bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. *Caulerpa racemosa* (Forssk.) J. Agardh showed the highest inhibition zone (22.0 ± 0.8) against Gram-positive bacteria *Staphylococcus aureus*. Extracts of both seaweeds, *C. racemosa* (Forssk.) J. Agardh and *P. boergesenii* Allender & Kraft, contain potential bioactive compounds with high antibacterial activity and could be deployed in the pharmaceutical applications.

Keywords: seaweeds, phytocompounds, antimicrobial activity
DOI 10.14456/cast.2020.24

*Corresponding author: Tel.: (+91) 96 77 87 42 20
E-mail: ragunath.chola@gmail.com

1. Introduction

Macroalgae are a group of autotrophic, holophytic and complex communities that live in the marine environment. Seaweeds are primitive and they are non-blooming plants, lack a true root, stem-leaves and jacket layer around reproductive structures. Moreover, seaweeds grow extensively in shallow marine water and estuaries and they are usually attached to the bottom or other solid structures by a holdfast, merely used for the attachment of thallus on the substratum and not for the absorption of nutrition [1]. Seaweeds or marine macroalgae are the potential renewable resources in a marine environment.

A total of ten thousand five hundred seaweed species are reported worldwide and 865 species of the seaweeds are found in India. Seaweeds are important for the coastal ecosystems as ocean producers. Seaweed cultivation is the third-largest source of aquaculture production worldwide. It has a portion of human foods exceeding a hundred years [1]. The worldwide seaweed production contributes approximately 20% of total global marine aquaculture production [2]. Seaweed farming signifies an essential component of aquaculture production and accounts for 27.3 million tons in 2014 [3].

Similar to terrestrial plants, macroalgae also synthesise their food by photosynthesis, in the presence of sunlight and seawater. Seaweeds are established in the coastline area, and live between high tide and low tide areas. They contain different types of pigments. Chlorophyta-green algae contains chlorophyll pigment, Phaeophyta-brown algae contains fucoxanthin, and Rhodophyta-red algae contains phycoerythrin and phycocyanin.

Seaweeds are excellent nutritional sources and contain various trace minerals, carbohydrates, vitamins, proteins and other bioactive compound [3]. They yield wide ranges of valuable natural metabolites that can be used in the arenas of the pharmaceuticals and food industries. Various bioactive compounds such as linoleic acid, stearic acid, oleic acid, fucoxanthin, fucophlorethol, loliolide and deschloroelatul have various biological activities [4]. Seaweeds are a rich source of numerous biologically active compounds and those compounds have been possibly exploited as active elements for humans and animal health uses. Although drastic labors have been made to isolate new-fangled compounds with important therapeutic activities, very few such compounds with high potential have been accessed. The bioactive compounds that have been most extensively researched include sulfated polysaccharides laminarin, fucoidan, β -glucans and phlorotannins, which have the ability to inhibit bacterial growth and also possess antioxidant properties [5].

The enormous importance is given to macroalgae due to their broad-spectrum of biological activity [5]. The bioactive compounds have been isolated from macroalgae include sulfated polysaccharides, peptides, sterols, brominates, aromatics, nitrogen-heterocyclic, nitro sulfuric heterocyclic, dibutanoids and proteins [6]. The polymeric carbohydrates are the most important constituents of marine macroalgae. They shows anticoagulant, antitumor and other activities [7]. When compared to telluric plants, macroalgae have innumerable inorganic and carbon-based constituents that have the advantage of the possible use in human food and pharmaceutical industries [8].

The study of the antimicrobial composites from seaweeds is vital as there is increasing demand for therapeutic drugs used for the treatment of pathogenic infection [9]. Recently, seaweeds show stimulatory effects on a wide range of pathogens and this characteristic feature of seaweeds has attracted many researchers to focus on the use of seaweed as an immunostimulant in health improvement and disease prevention in animals, thereby reducing the use of antibiotics and chemotherapeutics. Using these immunostimulants particularly for boosting the immune systems is effective against several opportunistic and secondary pathogens [10]. Immunostimulants can be introduced via the feed and the water. Water-soluble acidic polysaccharides taken from *Ulva rigida*

showed an immune stimulatory response in murine [10], whereas some seaweeds showed immune-suppressive effect [11]. Lipopolysaccharide extracted from seaweeds enhanced the proliferation of hemocytes in *Penaeus monodon* [10].

Extracts of seaweeds help to manage various of diseases in aquatic farm animals. Extracts obtained from the green alga fed to shrimp showed resistance to diseases [12]. Research work on the evaluation of the immunostimulant potential of seaweeds that inhabit the world's oceans and in particular those that live along the Indian coast have not been copious. In recent times, several works have revealed that numerous herbal products having immune-stimulant activities are recommended as herbal biomedicines that can be used in aquaculture as they are easily available, eco-friendly, cost-effective and can also be used in pharmaceutical industries because they can act against a wide range of pathogens [13]. Seaweeds are also used in the various fields of science, such as pharmaceuticals, medicine, tissue culturing, human genetics, microbiology and molecular biology. Although seaweeds are potential sources of compounds for many commercial and therapeutic sectors, the immense impacts of seaweeds are still unaware. Very little research has been carried out on the seaweeds *C. racemosa* and *P. boerghesii* from the region of Gulf of Mannar, Rameswaram, India. Therefore, the present study was carried out to assess the phytochemicals, biological activity and antibacterial activity of selected seaweeds.

2. Materials and Methods

2.1 Collection of seaweed sample

The well-grown species of brown and green algae are collected from the coastal area along the Mandapam, Rameshwaram (079° 20' E Long: 09° 25' N Lat) regions, the south-east coast of India during June 2018. After collecting, seaweed was thoroughly washed and cleaned with seawater followed by freshwater and distilled water to remove all epiphytes, sand and calcareous particles including other adhering detritus materials. After that, the seaweed was spread on blotting paper to remove excess water.

2.2 Identification of the seaweed species

The collected seaweed was authentically identified and certificated by the Botanical Survey of India, Southern Regional Center, Coimbatore. Identification was carried out based on morpho-anatomical features.

2.3 Preparation of seaweed extract by Soxhlet apparatus

The fresh materials were shade dried conditions and exposed to pulverization to get a coarse powder. The coarse powdered materials were subjected to Soxhlet extraction and successively extracted with methanol (14 g of algal powder per 250 ml of methanol) for 8 h. These extracts were collected and concentrated in a rotary evaporator (250-280 rpm, pressure 350 mmHg and 70°C) and the extracts were stored (-20°C) in the refrigerator in airtight containers for further use.

2.4 Qualitative phytochemical analysis

The phytochemical screening test was performed as described below by using standard methods [2, 14, 15].

2.4.1 Test of alkaloids by Wagner's test

Five ml of extract are added to 2ml of Wagner's mixture. The formation of magenta-brown precipitate indicates the occurrence of alkaloids.

2.4.2 Test for flavonoids by Shinoda test

A few drops of concentrated HCl are added to 1ml extract followed by addition of 0.5g of magnesium. A pink colouration points out the presence of flavonoids.

2.4.3 Test for saponins by Foam test

One ml of extract is shaken vigorously with 20 ml of distilled water for 10-15 min. The formation of a foam layer indicates the presence of saponins.

2.4.4 Test for phenolic compounds and tannins

One ml of extract is mixed with 2 ml of 5% ferric chloride. The genesis of dark blue or greenish-black colouration points out the occurrence of tannins or phenolic compounds.

2.4.5 Test for glycosides by Keller - Killiani test

An extract (0.5 ml) is added with 2 ml of glacial acetic acid, a few drops of 5% ferric chloride and 1ml of concentrated sulfuric acid. The appearance of a brown ring at the interface shows the presence of glycosides.

2.4.6 Identification of terpenoids by Salkoski test

One ml of the extract is mixed with 2 ml of chloroform along with concentrated sulfuric acid. A reddish-brown colouration indicates the presence of terpenoids.

2.4.7 Test for phlobatannins

One ml of extract is mixed with dilute HCl. The formation of a red precipitate indicates the presence of phlobatannins.

2.4.8 Test for protein and free amino acid by Ninhydrin test

For the identification of protein and amino acid, 2 ml of the extract is treated with 1ml of ninhydrin solution and kept in a hot water bath. The appearance of a purple colour indicates the presence of proteins and amino acid.

2.4.9 Test for carbohydrate by Molisch's test

Two ml of the extract is mixed with 1 ml of Molisch's reagent with a few drops of concentrated sulfuric acid along the sides of a test tube. A purple or reddish colour indicates the presence of carbohydrates.

2.5 Gas chromatography-mass spectroscopy analysis

Samples of the brown and green algae were extracted and the extracted fraction was subjected to GC-MS analysis. The phytoconstituents of the seaweed sample were analyzed using a Perkin Elmer Clarus Series Gas Chromatographic System with a capillary column. Identification of products was made by comparison of retention time and fragmentation patterns with known reference complexes as well as with mass spectra in the library search results kept in the software (TurboMass ver 5.4.2) of the GC-MS. The samples were analyzed under the following conditions: Elite-5MS (5% phenyl, 95% dimethylpolysiloxane) fused in a capillary column (30 in length x 250 µm in diameter x 30 µm in thickness of film). The spectroscopic detector for GC-MS involved an electron ionization system in 70 eV with helium as carrier gas flow at 1ml/min and the temperature programmed at 70°C and 6°C to 150 °C for 2 min, 6°C to 290°C for 5 min. The split ratio was 10:1, the injection sample volume was 1 µl. The injection and detector temperature were 250°C. Mass spectra were recorded and the range was m/z 40-500 amu. The GC-MS results were compared with the standard compounds library.

2.6 Collection of bacterial strains

The bacterial strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus mutans*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae* were obtained from PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu. The pathogens were maintained on agar slants at 4°C.

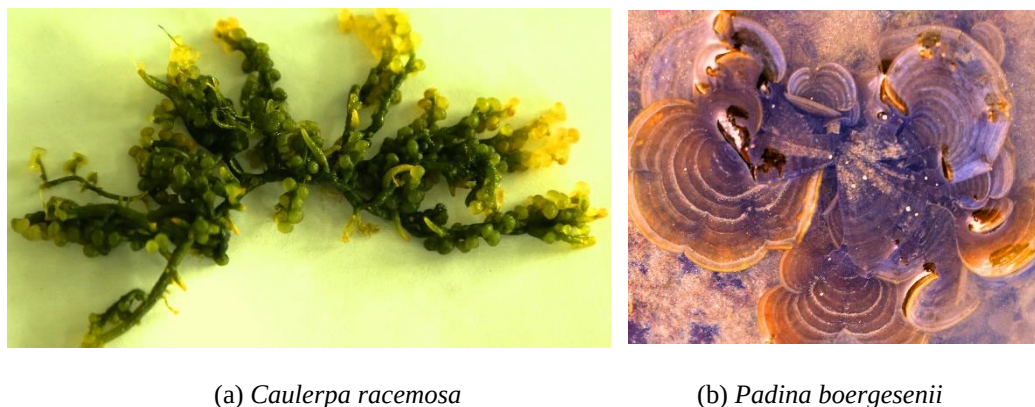
2.7 Antibacterial assays by well diffusion method

Antibacterial assays were performed using the agar well diffusion method. Muller Hinton agar (pH 7.4) was prepared and sterilized for 15 min. The sterilized medium was poured into sterile Petri dishes and the collected bacterial pathogens were swabbed on the plates. Different concentrations (2.0 to 50 mg/ml) of the algal extract was loaded into the wells (8 mm diameter). Distilled water was used as a negative control [16]. The bacterial plates were then incubated at 37°C for 24 h and the antibacterial activity was determined by measuring inhibition zone in mm [17-19].

3. Results and Discussion

3.1 Seaweed identification

The collected seaweed samples were identified as green algae: *Caulerpa racemosa* (Forssk.) J. Agardh (Figure 1a) and brown algae: *Padina boergesenii* Allender & Kraft (Figure1b). Identification was based on the morphological and anatomical characteristics of the seaweeds.

**Figure 2.** Green and brown algae

3.2 Qualitative phytochemical analysis

Secondary metabolites are characterized by a broad spectrum of biological activities. In this study, we observed positive results for flavonoids, saponins, a phenolic compound, terpenoids, phlobatannins and amino acids (Table 1). The identified compounds had cytostatic, antiviral, anthelmintic, antifungal and antibacterial properties [20].

Table 1. Qualitative phytochemical analysis, inference of primary phyto-compound found in *C. racemosa* and *P. boergesenii*.

Phytochemical	Inference	
	<i>C. racemosa</i>	<i>P. boergesenii</i>
Alkaloids	-	-
Flavonoids	+	+
Saponins	+	+
Phenolic compound and tannins	+	+
Glycosides	-	-
Terpenoids	-	+
Phlobatannins	+	-
Amino acid and protein	+	+
Carbohydrate	+	-

(+) presence of the compound, (-) absence of the compound

3.3 Gas chromatography-mass spectroscopy analysis

The extracted seaweed (*C. racemosa* and *P. boergesenii*) samples were analyzed with GC-MS to detect phyto-components, various fatty acids and other compounds. GC-MS technique provides the identification and quantification of chemical compounds based on their characteristic fragmentation patterns at specific retention times. The results of GC-MS analysis revealed that a wide range of compounds was identified and these are listed on Table 2 (*C. racemosa*) and Table 3 (*P. boergesenii*). Tables 2 and 3 show the variety of compounds found and also shows their molecular weight and biological activity.

Table 2. The GC-MS analysis of *C. racemosa*, biological activity, molecular formula and molecular weights

Retention time (min)	Peak area (%)	Compound	Molecular weight	Molecular formula	Biological activity
9.766	44.798	Chloroxyletol	156.609	C ₈ H ₉ C ₁₀	Anti-bacterial [21]
17.349	0.476	6-Hydroxy-4, 4,7a-trimethyl-5, 6, 7,7a-tetrahydro-1-benzofuran-2(4H)-one	196.242	C ₁₇ H ₃₂ O ₂	Antipyretic [22, 23]
20.600	2.097	Hexadecanoic acid, methyl ester	270.457	C ₁₇ H ₃₂ O ₂	Anti- fungal and antibacterial [24]
21.256	8.634	n-Hexadecanoic acid	256.430	C ₁₇ H ₃₄ O ₂	Antioxidant [24, 25]
21.811	0.887	Palmitic anhydride	494.845	C ₁₇ H ₂₄ O ₅	Anticancer [24]
22.091	0.308	Rhodovibrin	584.914	C ₄ H ₇ ClO ₂	Anticancer [15]
24.027	1.580	Phytol	296.539	C ₂₀ H ₃₂ O ₂	Anticancer and Anti-inflammatory [26-28]
24.397	1.826	9,12-Octadecadienoic acid (Z, Z)	280.445	C ₁₉ H ₃₄ O ₂	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthma and diuretic [29]
24.522	6.069	Oleic acid	282.470	C ₁₉ H ₃₆ O ₂	Antioxidant [30]
25.342	0.363	Strychane, 1-acetyl-20 α -hydroxy-16-methylene	338.443	C ₂₀ H ₄₀ O	Not reported
27.373	0.607	Methyclothiazide	360.224	C ₁₇ H ₃₂ O	Not reported
27.428	0.493	Glycidyl palmitate	312.494	C ₁₈ H ₃₄ O ₂	Not reported
29.754	0.410	2-cis,cis-9,12-Octadecadienyloxy ethanol	310.514	C ₂₀ H ₃₄ O ₂	Not reported
29.844	1.083	Glycidyl oleate	338.532	C ₁₉ H ₃₆ O ₃	Not reported
30.084	0.753	1H-Indene, 1-hexadecyl-2,3-dihydro	342.601	C ₂₈ H ₃₈ O ₁₀	Not reported
30.304	0.802	Glycerol 1-palmitate	330.509	C ₃₉ H ₇₈ O ₃	Anticancer [31]
30.373	0.467	1H-cyclopropal	534.600	C ₂₁ H ₃₈ O ₃	Not reported
30.579	0.563	Diisooctyl phthalate	390.564	C ₂₅ H ₄₂	Not reported
32.745	0.399	17-Octadecynoic acid, TMS derivative	352.626	C ₂₇ H ₅₆ O ₄ Si ₂	antioxidant, hepatoprotective, antihistaminic, hypocholesterolemic and anti-eczematic activities [32]

Table 2. (cont.)

32.747	0.398	Linoleic acid ethyl ester	308.498	C ₂₆ H ₅₀	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Anti-androgenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Anti-eczemic and Anti-acne [19, 22, 33, 34]
32.835	0.623	Glucobrassicin	448.461	C ₁₆ H ₂₀ N ₂ O ₉ S ₂	Antioxidant and antimicrobial [17]
33.295	0.410	Oleic acid, eicosyl ester	563.008	C ₈ H ₉ C ₁₀	Antiinflammatory, Cancer preventive, Hypocholesterolemic and Anemia gentic Insectifuge [35]
33.380	0.496	5(α)Pregane-3 α ,20 α diol	489.600	C ₁₇ H ₃₂ O ₂	Antioxidant and antimicrobial [36]

Table 3. GC-MS analysis of *P. boergesenii*, biological activity and molecular weight and formula

Retention Time (min)	Peak area (%)	Name of the compound	Molecular Weight	Molecular formula	Biological activity
9.731	10.07	Chloroxyletol	156.609	C ₈ H ₉ C ₁₀	Anti-bacterial [21]
20.330	0.35	9-Hexadecenoic acid, methyl ester (Z)	268.434	C ₁₇ H ₃₂ O ₂	Not reported
20.570	3.878	Hexadecanoic acid, methyl ester	270.457	C ₁₇ H ₃₂ O ₂	Anti- fungal and antibacterial [24]
21.111	10.617	n-Hexadecanoic acid	256.430	C ₁₇ H ₃₄ O ₂	Antioxidant [24]
21.776	0.370	Pyrano [4,3-b] benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro	308.374	C ₁₇ H ₂₄ O ₅	Antitoxidant [24]
21.921	0.362	Butanoic acid, 4-chloro	122.548	C ₄ H ₇ ClO ₂	Not reported

Table 3. (cont.)

22.651	0.592	Arachidonic acid	304.474	C ₂₀ H ₃₂ O ₂	Antimicrobial activity [24]
23.722	1.416	11,14-Octadecadienoic acid, methyl ester	294.479	C ₁₉ H ₃₄ O ₂	Anticancer [24]
23.862	3.204	9-Octadecenoic acid (Z)-, methyl ester	296.500	C ₁₉ H ₃₆ O ₂	Anticancer and antimicrobial [1, 37]
24.017	0.905	Phytol	296.539	C ₂₀ H ₄₀ O	Anticancer, anti-inflammatory, antimicrobial, antispasmodic activity and vitamins E, K1 [26, 27]
24.177	1.128	13-Heptadecyn-1-ol	252.442	C ₁₇ H ₃₂ O	Not reported
25.507	12.631	cis-Vaccenic acid	282.468	C ₁₈ H ₃₄ O ₂	Anticancer [38]
26.678	0.596	5,8,11,14 - Eicosatetraenoic acid, methyl ester, (all-Z)	318.493	C ₂₁ H ₃₄ O ₂	Not reported
27.408	3.511	Glycidyl palmitate,	312.494	C ₁₉ H ₃₆ O ₃	Not reported
28.864	0.369	1H-Cyclopropa [3,4] benz [1,2-e] azulene-4a,5,7b,9,9a (1aH)	534.600	C ₂₈ H ₃₈ O ₁₀	Not reported
28.969	0.448	Stearic acid, 3-(octadecyloxy) propyl	595.050	C ₃₉ H ₇₈ O ₃	Antifungal [39]
29.819	2.799	Glycidyl oleate	338.532	C ₂₁ H ₃₈ O ₃	Not reported
30.069	1.506	(1S)-1-Hexadecyl-2,3-dihydro-1H-indene	342.601	C ₂₅ H ₄₂	Not reported
32.720	0.992	2-Oleoylglycerol, 2TMS derivative	500.902	C ₂₇ H ₅₆ O ₄ Si ₂	Not reported
33.265	0.596	Cyclohexane, 1,1-dodecylidenebis [4-methyl]	362.686	C ₂₆ H ₅₀	Not reported
33.340	0.867	Glucobrassicin	448.461	C ₁₆ H ₂₀ N ₂ O ₉ S ₂	Not reported

3.4 Antibacterial assays by agar-well diffusion method

The antibacterial activity of seaweed extracts is presented in Table 4. It was found that the *C. racemosa* extract showed the highest inhibition zone ($22.0 \pm 0.8\text{mm}$) against Gram-positive bacterium *S. aureus* whereas *P. boergesenii* showed the highest inhibition zone ($23.6 \pm 0.3\text{mm}$) against the Gram-positive bacterium *Ps. aeruginosa*. Both *P. boergesenii* and *C. racemosa* showed the highest antibacterial activity at 50 mg/ml concentration.

Table 4. Antibacterial activity of seaweed extracts at 50 mg/ml concentration

Bacterial strain	Zone of inhibition (mm)	
	<i>C. racemosa</i>	<i>P. boergesenii</i>
<i>Ps. aeruginosa</i>	21.0 ± 0.2	23.6 ± 0.3
<i>S. aureus</i>	22.0 ± 0.8	22.7 ± 0.5
<i>S. saprophyticus</i>	19.8 ± 0.3	21.8 ± 0.5
<i>S. epidermidis</i>	10.5 ± 0.4	13.5 ± 0.8
<i>E. faecalis</i>	No activity	10.1 ± 0.1
<i>E. coli</i>	20.9 ± 0.2	22.2 ± 0.1
<i>B. cereus</i>	12.6 ± 0.1	15.4 ± 0.2
<i>B. subtilis</i>	17.5 ± 0.3	19.7 ± 0.2
<i>S. mutans</i>	17.8 ± 0.9	20.3 ± 0.3
<i>S. pyogenes</i>	14.2 ± 0.5	16.9 ± 0.9
<i>S. pneumoniae</i>	No activity	11.2 ± 0.9
<i>K. pneumoniae</i>	12.2 ± 0.7	18.2 ± 0.3

3.5 General discussion

In this study, the antibacterial activity of the two macro-algae and their bioactive abilities were assessed. Previous researchers reported that macroalgae are a tremendous source of compounds such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, carotenoids and these compounds were responsible for different biological activities [35, 40-42]. In this study the antibacterial activity of extracts of *P. boergesenii* and *C. racemosa* were evaluated. From the results, a higher antibacterial activity was obtained in brown algae *P. boergesenii* compared to green algae *C. racemosa*. Both algae have previously reported higher activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* pathogens [33, 43].

The outcome of the present study is that preeminent antibacterial activity was exhibited in *C. racemosa* and *P. boergesenii* extracts. Methanol has high antibacterial activity when compared to other organic solvents [20, 44]. Methanolic extracts of macroalgae contains phenolics, alkaloids and amino acids, which may account for the antibacterial activity [43, 45].

Previous reports showed that seaweeds contain momentous amounts of proteins, vitamins, minerals and trace elements. Macroalgae contains phenolic complexes and polysaccharides, which have various biological activities [16]. In the present study, a total of twenty-three chemical constituents from green seaweed and twenty-one chemical constituents from brown seaweed were extracted and identified in methanolic extracts of *C. racemosa* and *P. boergesenii* and included were flavonoids, saponins, phenolic compounds, tannins, amino acids and proteins. While *C. racemosa* showed positive result for phlobatannins and carbohydrates, *P. boergesenii* showed positive result for terpenoids. Both seaweeds showed negative results for glycosides and alkaloids.

4. Conclusions

Collectively, the present data offers great insight and it is the first report of the biological activity of *C. racemosa* and *P. Boergesenii*. Various fatty acids and bioactive compounds present in *C. racemosa* and *P. boergesenii* (Hexadecenoic acid, Hexadecanoic, Butanoic acid, Arachidonic, Octadecadienoic, cis-Vaccenic, Octadecenoic, Phytol and Eicosatetraenoic acid) exhibited effective inhibition against a wide range of pathogens. The research clearly shows that *C. racemosa* and *P. boergesenii* are rich potential sources of many bioactive compounds. From the results, we suggest that *C. racemosa* and *P. boergesenii* could be very beneficial to pharmaceutical and therapeutics applications in future.

References

- [1] Bjerregaard, R., Valderrama, D., Radulovich, R., Diana, J., Capron, M., Cedric Amir, M., Cedric, M., Hopkins, K., Charles, Y., Goudey, C. and Forster, J., 2016. *Seaweed Aquaculture for Food Security, Income Generation and Environmental Health in Tropical Developing Countries (English)*. [online] Available at: <http://documents.worldbank.org/curated/en/947831469090666344/Seaweed-aquaculture-for-food-security-income-generation-and-environmental-health-in-Tropical-Developing-Countries>
- [2] Divyagnaneswari, M., Christyapita, D. and Michael, R.D., 2007. Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. *Fish & Shellfish Immunology*, 23(2), 249-259.
- [3] Ghosh, P., Adhikari, U., Ghosal, P., Pujol, C.A., Carlucci, M.J. and Damonte, E.B., 2004. In vitro anti-herpetic activity of sulfated polysaccharide fractions from *Caulerpa racemosa*. *Phytochemistry*, 65(23), 3151-3157.
- [4] Bouhla, R., Haslin, C., Chermann, J.C., Collic-Jouault, S., Sinquin, C., Somin, G., Cerantola, S., Riadi, H. and Bourgougnon, N., 2011. Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophyta, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). *Marine Drugs*, 9(7), 1187-209.
- [5] Priyadharshini, S., Bragadeeswaran, S., Prabhu, K. and Ran, S., 2011. Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. *Asian Pacific Journal of Tropical Biomedicine*, 1(1), S38-S39.

- [6] Kuda, T., Taniguchi, E., Nishizawa, M. and Araki, Y., 2002. Fate of water-soluble polysaccharides in dried *Chorda filuma* brown algae during water washing. *Journal of Food Composition and Analysis*, 15(1), 3-9.
- [7] Lavanya, R. and Veerappan, N., 2011. Antibacterial potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. *Advances in Biological Research*, 5, 38-44.
- [8] Kolanjinathan, K. and Stella, D., 2009. Antibacterial activity of marine macro-algae against human pathogens. *Recent Research in Science and Technology*, 1(1), 20-22.
- [9] Holdt, S. and Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 23(3), 543-597.
- [10] Leiro J.M., Castro R., Arran, J.A. and Lamas, J., 2007. Immunomodulation activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. *International Immunopharmacology*, 7 (7), 879-888.
- [11] Felix, S., Robins, P. and Rajeev, A., 2004. Immune enhancement assessment of dietary incorporated marine algae *Sargassum wightii* (Phaeophyceae/Punctariales) in tiger shrimp *Penaeus monodon* (Crustacea/penaeidae) through prophenoloxidase (pro PO) systems. *Indian Journal of Marine Science*, 33(4), 361-364.
- [12] Kolanjinathan, K., Ganesh, P. and Govindarajan, M., 2009. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. *European Review for Medical and Pharmacological Sciences*, 13, 173-7.
- [13] Yadav, S., Bajagai, Athol, V., Klieve, Peter, J., Dart., Wayne, L. and Bryden, 2016. Probiotics in animal nutrition. Production, impact and regulation. In: Harinder P.S Makkar, ed. *FAO Animal Production and Health Paper No. 179*, Rome: FAO, pp. 1-170.
- [14] Duke, 2016. *Dr. Duke's Phytochemical and Ethnobotanical Databases*. [online] Available at: <https://phytochem.nal.usda.gov/phytochem/search/list>
- [15] El-Baz, F.K, Hassan, A.Z., Abd-Alla, H.I., Aly, H.F. and Mahmoud, K. 2017. Phytochemical analysis, assessment of antiproliferative and free radical scavenging activity of *Morus alba* and *Morus rubra* fruits. *Asian Journal of Pharmaceutical and Clinical Research*, 10(6), 189-199.
- [16] Kumar, K.S., Ganesan, K. and Subba Rao, P.B., 2008. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty-edible seaweed. *Food Chemistry*, 107(1), 289-295.
- [17] Bischoff, K.L., 2016. Chapter 40-Glucosinolates. [online] Available at : <https://doi.org/10.1016/B978-0-12-802147-7.00040-1>
- [18] Priyadharshini, S., Subramanian, B., Prabhu, K. and Ran, S., 2011. Antimicrobial and hemolytic activity of seaweed extract *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. *Asian Pacific Journal of Tropical Biomedicine*, 1, S38-S39.
- [19] Ragunath, C., Komathi, S. and Ragunathan, R., 2018. Molecular Characterization of *Leptospira interrogans*, isolated from animal samples. *International Journal of Pure and Applied Bioscience*, 6(2), 995-1004.
- [20] Mozhi, S., Muthuvel, A., Gnanambigai, D. and Thangavel, B., 2009. Total flavanoid and in vitro antioxidant activity of two seaweeds from Rameshwaram Coast. *Global Journal of Pharmacology* 3, 59-62.
- [21] Mcdonell, G.E. and Ruseell, A.D., 1999. Antiseptics and disinfectants activity, action and resistance. *Clinical Microbiology Reviews*, 12(1), 147-179.
- [22] Igwe, O. and Onwu, F.K., 2015. Leaf essential oil of *Senna alata* Linn from South East Nigeria and its antimicrobial activity. *International Journal of Research in Pharmacy and Chemistry*, 5(1), 27-33.
- [23] Lahaye, M., 1991. Marine algae as a source of dietary fibres: determination of soluble and insoluble dietary fibre contents in some sea vegetable. *Journal of the Science of Food and Agriculture*, 54 (4), 587-594.

- [24] Chandrasekaran, M., Senthilkumar, A. and Venkatesalu, V., 2011. Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences*, 15, 775-780.
- [25] Shahidi, F. Janitha, P.K. and Wanasundara, P.D., 1992. Phenolic antioxidants. *Critical Reviews in Food Sciences and Nutrition*, 32(1), 67-103.
- [26] Lee, A.C., Baula, I.U., Miranda, L.N. and Min, S.T. 2015. *A Photographic Guide to the Marine Algae of Singapore*. Singapore: Tropical Marine Science Institute.
- [27] Lee, K.L., Lee, S.H. and Park K.Y., 1999. Anticancer activity of phytol and Eicosatrienoic acid identified from *Perilla* leaves. *Journal of the Korean Society of Food Science and Nutrition*, 28, 1107-1112.
- [28] Gideon, V.A., 2015. GC-MS analysis of phytochemical components of *Pseudoglochidion anamalayanum* Gamble: An endangered medicinal tree. *Asian Journal of Plant Science & Research*, 5(12), 36-41.
- [29] Tyagi, T. and Agarwal, M., 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanol extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 195-206.
- [30] Yu, F., Lian, X., Guo, H., McGuire, P.M., Li, R., Wang, R.B. and Yu, F., 2005. Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *Journal of Pharmacy & Pharmaceutical Sciences*, 8 (3), 528-535.
- [31] Pascual, G., Avgustinova, A., Mejetta, S., Martín, M., Castellanos, A., Attolini, C.S, Berenguer, A., Prats, N., Toll, A., Hueto, J.A., Bescos, C., Di Croce, L. and Aznar Benitah, S.A, 2017. Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature*, 541, 41-45.
- [32] Fadeyi, O.E., Olatunji, G.A. and Ogundele, V.A., 2015. Isolation and characterization of the chemical constituents of *Anacardium occidentale* cracked bark. *Natural Products Chemistry Research*, 3(5), <https://doi.org/10.4172/2329-6836.1000192>
- [33] Choi, J.S., Park, N.H., Hwang, S.Y., Sohn, J.S., Kwak, I., Cho, K.K. and Choi, I.S., 2013. The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. *Journal of Environmental Biology*, 34(4), 673-676.
- [34] Ogidi, O.C., Oyetayo, V.O. and Akinyele, B.J., 2015. In vitro evaluation of antimicrobial efficacy of extracts obtained from raw and fermented wild macrofungus, *Lenzites quercina*. *International Journal of Microbiology*, 2015, <https://www.hindawi.com/journals/ijmicro/2015/106308>
- [35] Sheela, D. and Uthayakumari, F., 2013. GC-MS analysis of bioactive constituents from coastal sand dune taxon - *Sesuvium portulacastrum* (L.). *Bioscience Discovery*, 4(1), 47- 53.
- [36] Altameme, H.J., Hameed, I.M. and Kareem, M.A., 2015. Analysis of alkaloid phytochemical compounds in the ethanolic extract of *Datura stramonium* and evaluation of antimicrobial activity. *African Journal of Biotechnology*, 4(19), 1668-1674.
- [37] Bhakuni, D.S. and Rawat, D.S., 2006. Bioactive marine natural products. *Journal of the American Chemical Society*, 128 (13), 4494, <https://doi.org/10.1021/ja059852v>
- [38] Pongprayoon, U., Baeckstrom, P., Jacobsson, U., Lindstrom, M. and Bohlin, L., 1992. Antispasmodic activity of beta-damascenone and e-phytol isolated from *Ipomoea pes-caprae*. *Planta Medica*, 58(1), 19-21.
- [39] Abubacker, M.N. and Devi, P.K., 2014. In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence. *Asian Pacific Journal of Tropical Biomedicine*, 7(1), S190-S193.

- [40] Mahabaleshwara, K., Chandrasekhar, N. and Govindappa, M., 2016. Phytochemical investigations of methanol leaf extracts of *Randia spinosa* using column chromatography, HPTLC and GC-MS. *Natural Products Chemistry & Research*, 4 (2), <https://doi:10.4172/2329-6836.1000202>
- [41] Zaha, A.E., Rajashri, R.N., Ashok, K.S. and Sanaa K.B., 2016. Fatty acid analysis, antioxidant and biological activity of fixed oil of *Annona muricata* L. seeds. *Journal of Chemistry*, 2016, 1-6.
- [42] Rodriguez, D., Carro, A.M., Cela, R. and Lorenzo, R.A., 2010. Microwave-assisted extraction and large-volume injection gas chromatography-tandem mass spectrometry determination of multi-residue pesticides in edible seaweed. *Analytical and Bioanalytical Chemistry*, 398, 1005-1016.
- [43] Kavitha, M. and Vidhya, I., 2014. Antioxidant and antiproliferative activities of extracts of selected red and brown seaweeds from the Mandapam Coast of Tamil Nadu. *Journal of Food Biochemistry*, 38(1), 92-101.
- [44] Seenivasan, R., Indu, H., Archana, G. and Geetha, S., 2010. The antibacterial activity of some marine algae from South East Coast of India. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 9(5), 480-489.
- [45] Wikfors, G.H. and Ohno, M., 2001. Impact of algal research in aquaculture. *Journal of Phycology*, 37(6), 968-974.