ijlpr 2022; doi http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.5.L88-102

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Bioactive Compounds of Marine Algae

Comparative Assessment of Bioactive Compounds, Nutritive, Mineral Composition and *In-Vitro* Bioactivity of Marine Macro Algae *Valoniopsis Pachynema* and *Dictyota Ciliolata*

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Abstract: Marine-based nutraceuticals have proved their extensive applications in functional foods and its bioactive molecules play a crucial role as therapeutic agents. The aim of the study is to elucidate in-depth understanding of the nutritional properties and biological activities of the two marine algae. The main objectives of the current study is to comparatively analyse the phytochemicals, nutritional composition and its pharmacological activities of the green and the brown marine macro algae named *V.pachynema* and *D.ciliolata* respectively using three different solvents. Additionally, the total chlorophyll content, phenolic content, nutritional, mineral composition, antioxidative and antibacterial activities of the methanolic extract were evaluated. Results showed elemental distribution of *V.pachynema* in the decreasing order Na > Ca> k > Mg > Fe > Ni> Mn >Co> As > Pb > Cu> Zn > Cr > Cd> Se> V while *D.ciliolata* in the order of Na > Ca> k > Mg >Ni >Fe> As> Co >Pb> Mn > Zn> Se>Cr> Cd> V>Cu. The relative nutritional composition showed high ash and carbohydrate for both the algae. The percentage composition of ash content was 68.42 ± 0.43% , 37.45 ± 0.26 % (g $100g^{-1}$ DW) while carbohydrate content was 50.17± 0.60%, 45.78 ± 0.69% (g $100g^{-1}$ DW) for *V.pachynema* and *D.ciliolata* respectively. The crude methanolic extract of both the algae were investigated for antioxidant activity with two *in vitro* antioxidant assays including 1,1-diphenyl-2- picrylhydrazyl radical (DPPH), Phosphomolybdenum (PMB) assays. In addition, Folin–Ciocalteu was used to measure phenolic content (TPC) to determine the antioxidant components. Total phenolic content was found to be highest in *V.pachynema* 51 GAE 10µg/ml and the methanolic extract of *V.pachynema* showed higher levels of phenolic compounds and antioxidant activities when compared with *D.ciliolata*. The tested marine algal extracts exhibited radical scavenging activity that is comparatively dose-dependent to its phenolic concentration. Methanolic extract of the marine algae *V.pachynema* exhibited better inhibitory activity against gram positive bacterium that are resistant to drugs when compared to the brown algae *D.ciliolata*. This study enabled a detailed understanding of the nutritional and biochemical composition, free radical scavenging activities of two marine macroalgae. This algal biomass could be potent resources of anti-oxidants and could act as dietary food supplements owing to their rich diversified bioactivities.

Keywords: Marine Macro Algae, *Valoniopsis Pachynema*, *Dictyota Ciliolata*, Antioxidant Activity, DPPH, PMB, Proximate Nutritional Analysis, Mineral Composition.

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Received On 2 May, 2022

Revised On 4 July, 2022

Accepted On 11 July, 2022

Published On 01 Sept, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Bhuvaneshwari.J and Dr.Thirumalaivasan.P , Comparative Assessment of Bioactive Compounds, Nutritive, Mineral Composition and In-Vitro Bioactivity of Marine Macro Algae Valoniopsis Pachynema and Dictyota Ciliolata.(2022).Int. J. Life Sci. Pharma Res.12(5), L88-102 http://dx.doi.org/http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.5.L88-102

1. INTRODUCTION

Marine ecosystem is generally considered as the home for almost 80% of the world's plant and animal species. It is estimated that relatively 150,000 species of seaweeds are found along the intertidal zone. These seaweeds are known to be a rich source of a wide variety of natural products and hence these find an important place in the pharmaceutical, cosmeceutical and drug developmental industries.[1] The diverse range of these seaweeds that are most commonly present in abundance in the submerged oceanic waters, acts as their inhabitant area. These seaweeds are broadly classified into red algae (Rhodophyta), brown algae (Phaeophyta) and green algae (Chlorophyta) based on their chemical constitution and structure.[2] The aim of the current research is to perform comparative analysis of green and brown marine algae in terms of nutritional, biochemical composition and free radical scavenging activities. The methanol extracts of green and brown seaweeds exhibited far better radical scavenging activity, reducing power ability with greater phenolic content present in the seaweeds [3]. Algae are generally described as a heterogeneous group of organisms with significant metabolic diversities. These include compounds such as isoprenoid, terpenoids, steroid, sterols, phenolic compounds, acrylic acid, fatty acids, and alkaloids. Exceptional sources act as antimicrobials, antioxidants, anticancer and antiviral, anti-inflammatory, wound healing and neuroprotective compounds.[4] Hence, the objectives of the study were to evaluate the presence of phytochemicals, nutritional and mineral composition, its antibacterial and antioxidant activities of the two different macro algae. In India, especially the southern part of India, seaweeds occur in areas of seashore such as coastal areas of Mandapam, Ramanathapuram district. These seaweeds possess distinct features that promote their survival in the salty marine ecosystem. The osmolarity in their cytoplasm is well adjusted with respect to the osmolarity of the salty sea water. Owing to this adaptation, desiccation does not take place. The seaweeds often lack true leaves, stems and roots. They have modified stems, roots and leaves to sustain in the marine ecosystem.[5] The biochemical compositions of seaweeds have also been greatly influenced by geographical location, seasonal changes, physico-chemical parameters of water, local environmental condition and the land effluents.[6] Seaweed extracts can play an efficient role for inhibiting uncontrollable cell growth of human cancer cell lines.. The ubiquitous nature of marine macroalgae might kindle the interest of the researchers worldwide to scrutinize for its antioxidantand antimicrobial activity in different geographical regions.[8] Cyclic polysulfides and halogenated compounds are essentially used against disease causing pathogens. Brown and green macroalgae varieties display significant antibacterial activity in the methanolic extract whereas the red algae require acetone for providing best results [9]. Antioxidants found in many algae are important bioactive compounds that play a major role against many pathogenic diseases and anti-ageing processes via protection of cells from oxidative damage. Seaweeds that act as antioxidants have a plausible role as health promoting constituents in the pharmaceutical and functional food industries.[10] Macro algae have been known to contain high amounts of ash, thereby indicating a consequential concentration of macro and micro minerals. These minerals comprise an altogether summation of up to sixty more trace elements that are a much higher amount in comparison to other edible terrestrial plants, hence making these organisms potential dietary food supplements to regulate human nutrition, sustenance and health [11]. Minerals that are most abundantly found in the seaweeds include iodine, magnesium, calcium, phosphorus, iron, potassium and copper. These seaweeds usually contain the non-starch polysaccharides, minerals and vitamins that acts as dietary fibres and food ingredients which, when ingested will prevent the occurrence of chronic disorders [12]. Therefore, the present study is mainly focused on the proximate nutrient, mineral analysis and also to elucidate the algae extracts for its various bioactive properties. These findings could possibly help us to progress for the next level of scientific and biomedical research.

1.1 Novelty of the work

Comparative studies were accomplished between the two marine macro algae used in the study and these studies could be very beneficial for the utilization of marine algae as a source of dietary food supplement since the algae contains high nutrition and antioxidants. In addition to these, the anti-oxidant activity in these marine algae furthermore suggests that these could be evaluated for the treatment of various chronic ailments.

2. MATERIAL AND METHODS

2.1 *Collection of Algae*

The two different marine macro algae such as *Dictyota ciliolata* and *Valoniopsis pachynema* were collected from the intertidal shallow zone of Mandapam, Ramanathapuram district. The algae were obtained from the coast of Mandapam, Rameshwaram (Lat.: 9°16'32.56" N and Lon.: 79°7'25.03" E) along the southern regions of Tamil Nadu. The harvested algae were washed with water to remove the sand particles and epiphytes. They were then packed in a polythene bag and were brought to the laboratory. The algae were further washed with distilled water to remove traces of salts and other contaminants, shade dried and then stored for further analysis.

2.2 *Authentication of Algae*

The collected algae were authenticated by Dr. V. Veeragurunathan, Senior Scientist, CSIR- Central Marine Algal Research Station (CSIR-CSMCRI), Mandapam, Ramanathapuram district, Tamilnadu, India. The algae identified as *Dictyota ciliolata* and *Valoniopsis pachynema* were used for the research work.

Fig 1(a) Green marine macro algae - *Valoniopsis pachynema*

Fig 1(b) Brown marine macro algae -*Dictyota ciliolata*

2.3 *Extract Preparation*

Seaweeds were shade dried for over 10 days. The dried seaweeds were finally pulverized in the commercial grinder and the seaweed samples were powdered, weighed and stored. Direct extraction method was used in the preparation of the algal extracts. These extracts were kept for 24 hrs at room temperature and mixed at regular intervals. The algal samples were dissolved and soaked into various solvents such as methanol, ethanol and acetone for preparing a goodquality of extracts. After 24hrs, the dissolved samples were filtered using Whatman filter paper. Some unwanted residues were removed through this filtration process and extracts were kept under refrigerated conditions and preserved until further analysis was conducted.

2.4 *Screening of Bioactive Compounds*

Three different solvent extracts methanol, ethanol and acetone of *Dictyota ciliolata* and *Valoniopsis pachynema* were analyzed for the presence of Carbohydrates, Proteins, Glycosides, Tannins, Saponins, Steroids, Terpenoids, Alkaloids, Phenols and Flavonoids according to the standard methods.[13].

2.5 *Determination of Total Chlorophyll Content*

Chlorophyll content was determined spectrophotometrically according to the method of the AOAC (2000)[14]. Methanolic extract of seaweed without chlorophyll removal prepared were dehydrated with anhydrous sodium sulfate. Immediately, the pigments were quantified spectrophotometrically at 660 and 642 nm. For the blank, ethanol was used instead of extract. [15]

2.6 *Determination of Total Polyphenolic Content*

Total phenolic content was estimated with Folin and Ciocalteau reagent and by using gallic acid as standard. [16] The concentration range 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml of the sample was mixed with 9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent and 10 ml of stock of 7% sodium carbonate each tube 2ml was added. After 90 min of incubation the absorbance was determined at 760 nm. The phenolic content was expressed as GAE (Gallic acid equivalent) in mg/ml. It is calculated in %.

2.7 *Nutritional Characterization*

The composition of the macroalgae (protein, ash, fiber, moisture and lipid contents) was determined according to standard methods (Association of Official Analytical Chemists, 2011)[17]. The carbohydrate contents were calculated using the formula: Carbohydrates = [100% - (%protein + %lipid + %ash + %water)], according to [15]. The results were expressed as a percentage of the component by dry weight.

2.8 *Moisture Content*

The moisture content of the dried algal biomass was measured by drying a 2 g sample at 100°C for 20 hrs or continued till constant weight was attained and the weight difference was calculated. The moisture content (%) was calculated using the following formula[18],

$$\text{Moisture Content (\%)} = \frac{\text{weight before drying} - \text{weight after drying}}{\text{weight before drying}} \times 100$$

2.9 *Ash Content*

Ash was determined by incineration of a representative 1 g of algal sample at 450°C for 5 hrs. in a pre-weighed silica crucible. The residue was weighed in the crucible and the difference in weights was calculated as the ash content[19].

2.10 *Estimation of Total Lipid, Protein, Carbohydrate Content*

The composition of the macroalgae (protein, ash, fiber, moisture and lipid contents) was determined according to standard methods (Association of Official Analytical Chemists, 2011). The carbohydrates were estimated using the formula:

$$\text{Carbohydrates} = [100\% - (\%\text{protein} + \%\text{lipid} + \%\text{ash} + \%\text{water})]$$

According to [20] The results were calculated as a percentage of the component by dry weight.

2.11. Elemental Analysis

The dried powder of *Valoniopsis pachynema* and *Dictyota ciliolata* were subjected to elemental composition analysis following standard procedures (AOAC, 2011) for the detection of both macro and micro elements such as Sodium, potassium, Calcium, Magnesium, Iron, Copper, Zinc, Manganese, Vanadium, Chromium , cobalt, nickel, arsenic, selenium, molybdenum, lithium, vanadium, chromium, ruthenium, rhodium, palladium, cadmium, barium and lead using by inductively coupled plasma mass spectrometry (ICP-MS).

2.12 *Antioxidant Activity*

Free radical scavenging activity of the algal extracts were determined by the two various methods such as 2,2 Diphenyl-1-Picryl-Hydroxyl assay and Phosphomolybdenum assay.

2.13 DPPH Radical Scavenging Assay

DPPH activity was followed by the method that was proposed by Ribeiro *et al*., 2008 with some modifications. Different concentrations of 50µg, 100µg, 150µg, 200µg, 250µg, of the methanol extract of the two algae were mixed with varying concentrations of DPPH solution followed by adding 2 ml of ethanol. The solution mixture was kept in a dark place for 30 minutes and ethanol used as a blank. The absorbance of each sample was read at 517nm in the spectrophotometer with some modification from standard methods. [21].

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

Where, A = Optical density of Sample,
B = Optical density of Blank

2.14 Phosphomolybdenum Assay

The antioxidant activity was determined by the Phosphomolybdenum method. Reagent was synthesized by the mixture of 0.6M H2 SO4, 28mM Sodium phosphate and 4mM ammonium molybdate dissolved into distilled water with known concentration. The methanolic extract of *Dictyota ciliolata* and *Valoniopsis pachynema* were taken in each test tube containing 3ml of distilled water and 1ml of Phosphomolybdate solution. Each sample mixture was kept in incubation at 95°C for 90 min resulting in a samplecooled at room temperature. After 30 min, absorbance is measured at 695nm in spectrophotometer. [22]

2.15 Antibacterial Activity

2.16 *Microbial Culture Preparation*

The antibacterial activity determination was performed using gram positive bacteria *Staphylococcus aureus* and gram negative bacteria such as *Escherchia coli*, *Pseudomonas aeruginosa* and *Klebesiella pneumoniae*. The bacterial strains were collected from The Government Hospital, Srirangam, Tiruchirappalli, Tamil Nadu. They were sub-cultured on their selective media. Disc diffusion method [23] was followed to determine the antibacterial activity of the isolated strain. Log phase petri plates containing 20 mL of Muller Hinton agar (Hi Media) were seeded with 4 h old fresh culture of pathogens. 10, 20, 30 and 40 µl of methanol extract of *Valoniopsis pachynema* and *Dictyota ciliolata* was loaded on the discs which were then dispensed on the solidified Muller Hinton agar with test organisms and incubated at 37 °C for 24 h in an incubator. The zone of inhibition was measured using an antibiotic zone scale (Hi Media).

3. STATISTICAL ANALYSIS

Statistical analysis has been carried out using SPSS Software version 15 statistical analysis packages (SPSS Inc., Chicago, IL, USA). All data has been presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was carried out to analyze the mean values. Considerable differencesin the total chlorophyll content, total phenolic content, antioxidant activities, nutritional and mineral composition of the extract of the two algal species were performed. One way ANOVA was

executed followed by Duncan's multiple range test with the data showing $p < 0.05$ was significant statistically. [24]

4. RESULTS AND DISCUSSION

Bioactive compounds analysis of the two marine macro algae *Valoniopsis pachynema* and *Dictyota ciliolata* in three different solvents methanol, ethanol acetone extracts were done. Methanolic extract of *V.pachynema* showed the presence of carbohydrates, flavonoids, alkaloids, glycosides, saponins, tannins and phenolic compounds whereas *D.ciliolata* showed the presence of carbohydrates, saponins, tannins, flavonoids and phenols. (Table 1 a and b). Both these algae exhibited a significant presence of bioactive compounds in this solvent compared to the other two solvents. Free radical scavenging capacities of living cells are most commonly induced by the presence of phenolic compounds in medicinal plants. There are plenty of bioactive compounds found in nature. Among these polyphenols, flavonoids showed an excellent antibacterial, antioxidant and anti-inflammatory activity[25]. The methanolic extract exhibited more similarities of phenolic compounds which possess antibacterial, anti-inflammatory and antioxidant characteristic features. Methanolic extract have been found to contain tannin compounds which are primarily essential for inhibiting bacterial growth. These help in scavenging hydroxyl compounds and reducing inflammation of allergic consequences [26].

TABLE 1 (a):Screening of Bioactive compounds of *Valoniopsis pachynema* in three different solvents

COMPOUND	ACETONE	ETHANOL	METHANOL
CARBOHYDRATES	+	-	+
TANNINS	+	-	+
SAPONINS	+	+	+
FLAVONOIDS	-	+	+
ALKALOIDS	-	-	+
QUINONES	-	-	-
GLYCOSIDES	-	-	+
CARDIAC GLYCOSIDES	-	-	-
TERPENOIDS	-	-	-
PHENOLS	-	+	+
STEROIDS	-	+	+
ANTHRA-QUINONES	-	-	-

Table 1 Qualitative analysis of Bioactive compounds were performed for Valoniopsis pachynema in methanol, ethanol and acetone extracts. The methanolic extract were found to contain abundance of phytochemicals.

TABLE 1(b):Bioactive compounds Screening of *Dictyota ciliolata* in three different solvents.

COMPOUND	ACETONE	ETHANOL	METHANOL
CARBOHYDRATES	-	+	+
TANNINS	+	-	+
SAPONINS	+	+	+
FLAVONOIDS	-	-	+
ALKALOIDS	-	+	-
QUINONES	-	-	-
GLYCOSIDES	+	-	-
CARDIAC GLYCOSIDES	-	-	-
TERPENOIDS	-	-	-
PHENOLS	-	-	+
STEROIDS	-	+	-
ANTHRA-QUINONES	-	-	-

Table 1b: Qualitative analysis ofBioactive compounds were performed for Dictyota ciliolata in methanol, ethanol and acetone extracts. The methanolic extract were found to contain abundance of phytochemicals.

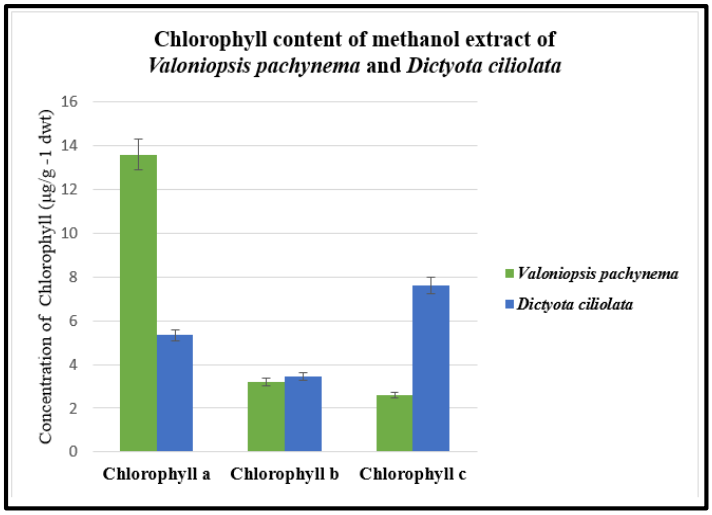
4.1 Total Cholorophyll Content

The major chlorophyll pigment concentrations were conducted for both the marine macro algae *V.pachynema* and *D.ciliolata.* (Table 2). The chorophyll content significantly varied in both the green algae *V.pachynema* (Chlorophyceae) and the brown algae *D.ciliolata* (Phaeophyceae). While Chlorophyll A content was higher in *V.pachynema* 13.6 $\mu g/g^{-1}$ DW, Chlorophyll B and C was higher in *D.ciliolata*. 3.45 $\mu g/g^{-1}$ DW and 7.6 $\mu g/g^{-1}$ DW respectively.

TABLE 2: Chlorophyll content of *Valoniopsis pachynema* and *Dictyota ciliolata*

Chlorophyll (µg/g -1 dwt)	*Valoniopsis pachynema*	*Dictyota ciliolata*
Chlorophyll a	13.6 ± 0.10^{b}	5.34 ± 0.06^{a}
Chlorophyll b	3.2 ± 0.01^{c}	3.45 ± 0.07^{b}
Chlorophyll c	2.6 ± 0.03^{b}	7.6 ± 0.02^{a}

**** Mean ± SD (n = 3) All determinations were carried out in triplicates. Different letters in the same column for each seaweed indicate significant differences between the two macroalgae (p <0.05)***



The results were expressed in Mean±SD. All the values in the histogram are statistically significant at p < 0.05. The chlorophyll content was found to be high for the methanolic extract of Valoniopsis pachynema compared to Dictyota ciliolata.

Fig 2: Chlorophyll content of methanol extract of *Valoniopsis pachynema* and*Dictyota ciliolata*

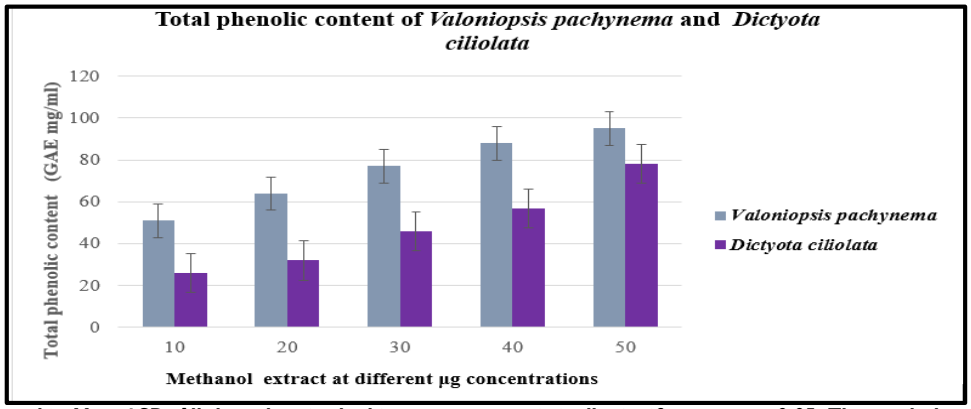
4.2 Total Phenolic Content

TPC activity is the process that helps in figuring out the amount of phenolic content in the samples. Phenolic compounds that are present in the plants are known to have redox properties, and these properties allow them to act as potent antioxidants [27]. Research findings have shown that the radical-scavenging properties along with the antibacterial efficacy of the marine macro algal species have been reported to be associated with the phenolic compounds present within them. [28]. The absorbance was recorded at 760 nm. The result shows the comparative analysis of the total phenolic count in the algal extracts of *V.pachynema* and *D.ciliolata*. It was observed that the methanolic extract of the green algae *Valoniopsis pachynema* exhibited higher TPC (51 GAE 10µg/ml) as compared to that of the brown algae *Dictyota ciliolata* (26 GAE 10µg/ml). (Table 3). Higher phenolic content in the methanolic extract is majorly responsible for bioactivity; therefore, this extract is expected to exhibit good result in antioxidant and antimicrobial activities. Hence it was proved that *V.pachynema* showed the maximum activity here.

TABLE 3: Total phenolic content of Valoniopsis pachynema and *Dictyota ciliolata*

S.No	Concentration of extract	Volume of Folin Ciocalteau reagent	Volume of Na2CO3	Optical density at 760nm GAE mg/ml	
				Valoniopsis pachynema	Dictyota *ciliolata*
1	10 µg/ml	1 ml	2 ml	51 ± 0.03	26 ± 0.01
2	20 µg/ml	1 ml	2 ml	64 ± 0.04	32 ± 0.02
3	30 µg/ml	1 ml	2 ml	77 ± 0.03	46 ± 0.01
4	40 µg/ml	1 ml	2 ml	88 ± 0.03	57 ± 0.02
5	50 µg/ml	1 ml	2 ml	95 ± 0.04	78 ±0.03

Table 3: The results were expressed in Mean±SD. All determinations were carried out in triplicates. The total phenolic content was found to be high for the methanolic extract of Valoniopsis pachynema compared to Dictyota ciliolata. All the values in the table are statistically significant at p < 0.05.



The results were expressed in Mean±SD. All the values in the histogram are statistically significant at p < 0.05. The total phenolic content was found to be high for the methanolic extract of Valoniopsis pachynema in comparison with Dictyota ciliolata.

Fig 3: Total phenolic content of methanol extract of *Valoniopsis pachynema* and*Dictyota ciliolata*

4.3 Nutritional composition of marine macro algae

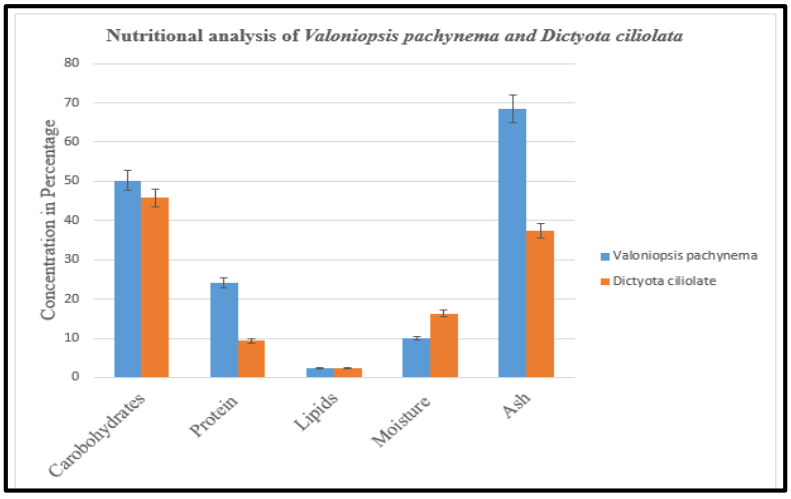
Macroalgae significantly differ from their terrestrial plants in terms of their chemical composition and their physiological and morphological features [29]. They are a potent source of protein, fiber, vitamins, polyunsaturated fatty acids, macro and trace elements, as well as important bioactive compounds [30]. In addition, the biochemical composition of seaweeds varies and it is generally affected by the species, nutrition and environment [31]. Biochemical composition of *V.pachynema* and *D. ciliolata* collected from Mandapam region in Rameshwaram, showed that the biochemical composition of these seaweeds displayed a good nutritional profile. The protein, lipid, carbohydrate, fiber, ash and moisture contents of the two marine macroalgae *Valoniopsis pachynema* and *Dictyota ciliolata* were hereby determined. Protein has crucial functions in all algal biological processes; the activity of the protein can be described by enzymatic catalysis, transport and storage, and mechanical sustentative control [32]. The moisture, ash, protein, lipid, carbohydrate, and mineral content of different macro algal species are shown in Table 4. Generally, the total protein content of green, red, and brown seaweeds ranged from 7 to 32% DW, 4 to 50% DW, and 3 to 22% DW, respectively. The mean protein content values obtained in the green algae *Valoniopsis pachynema* was higher when compared to that of the brown algae *Dictyota ciliolata*. High protein accumulation was found in the green algae, *Valoniopsis pachynema* (24.17%) which was higher than that of the brown algae *Dictyota ciliolata* (9.32%) (g $100g^{-1}$ DW) . These results show that these species growing in different levels of the ocean and hence, can have varying amounts of nutrition. In general, green seaweeds contain higher protein contents (10–30%) than brown seaweeds (5–15%) according to [33]

Table 4: Nutritional Composition of the marine algae *Valoniopsis pachynema* and *Dictyota ciliolata*

Proximate Composition	Percent Composition (%)* (g $100g^{-1}$ DW)	
	Valoniopsis pachynema	*Dictyotaciliolata*
Moisture Content	10 ± 0.56	16.28 ± 0.67
Ash Content	68.42 ± 0.43	37.45 ± 0.26
Crude Protein	24.17 ± 0.21	9.32 ± 0.29
Lipid	2.45 ± 0.20	1.82 ± 0.33
Carbohydrate	50.17 ± 0.60	45.78 ± 0.69

Table 4 The results were note asMean ± SD (n = 3)

The experiment was carried out in triplicates. All the values in the table are statistically significant at $p < 0.05$. The percentage representation of algal dry weight was observed. The percentage of proximate nutritional composition holds high for Ash content, crude protein and lipid content in *Valoniopsis pachynema* whereas *Dictyota ciliolata* exhibited high moisture content. Seaweeds possess relatively low contents of lipid, ranging from 0.5 to 6 % DW [34]. Lipids tend to have a far greater efficacy in oxidation processes than other biological compounds and they constitute a convenient storage material for living organisms.In macroalgae, these lipids are widely distributed, especially in several resistance stages . Most macroalgae have a low lipid content and it only provides a very low amount of energy and is low in calories [35]. In the present study, the lipid content ranged in the low values of 2.45±0.20 % and 1.82 ± 0.33 % in both species . The lowest lipid content was found in *Dictyota ciliolata* (1.82 ± 0.33%) (g $100g^{-1}$ DW).



The results were note as Mean ± SD and the experiment were performed in triplicates. All the values in the histogram are statistically significant at p < 0.05. The nutritional composition holds good for Valoniopsis pachynema compared to Dictyotaciliolata

Fig 4: Nutritional Composition of *V.pachynema* and *D.ciliolata*

Carbohydrate is considered one of the most important components for metabolism and it supplies the energy needed for respiration. Since, these serve as a primary source of energy it is mostly regarded as the most significant biochemical component in seaweeds to execute various important metabolic processes.[36]. The carbohydrate fraction was the major component in the proximate composition of both the seaweeds. It was noted to be significantly (50.17± 0.60% in *V.pachynema* and 45.78 ± 0.69% (g $100g^{-1}$ DW) in *D.ciliolata)*. The carbohydrate content is higher compared to some of the

seaweeds obtained from the region such as *Hypnea Valentiae*, *Enteromorpha intestinalis* but is lower than the red algae *Gracilaria edulis*, *Gelidium acerosa* and *Laurencia papillosa*.[37] Moisture content gives information with respect to the storage/shelf life of food products. The moisture content for commercially dried seaweeds should be maintained between 15 to 35% and must remain stable even below 15% [38]. The result of the proximate analysis for moisture content of *V.pachynema* (10±0.56%) and *D.ciliolata* (16.28 ± 0.67%) (g $100g^{-1}$ DW) suggests that it can be stabilized and stored under appropriate conditions for commercial marketing. In this study, Ash content was observed to be the highest in both the marine algae *V.pachynema* and *D.ciliolata* at 68.42 ± 0.43% and 37.45 ± 0.26% (g $100g^{-1}$ DW) proportionately. Ash content was found to be higher than some algae *G. lithophila* 18.00% followed by *G. corticata* 20.86% but found to be lower than *J.rubens* 86.66 %. Ash is generally associated with the durability and the longevity of the product. Analysis of the ash content of the two marine algal samples were carried out by burning the organic content, leaving behind the inorganic minerals in the sample. This study will direct the way to better understand the type and the amount of minerals present in each sample and their physiochemical properties and how well it retards the growth of microorganisms. [39]

4.4 Mineral composition

Algae have been the most extensively studied among marine sources, yielding a huge number of novel anti-inflammatory chemicals. Marine algae, also known as seaweed or macroalgae, are photosynthetic eukaryotic organisms that are typically found in coastal environments and have a prolonged durability.[40] These seaweeds contain quite a high concentration of minerals because of the diverse kinds of substances that they tend to absorb from the marine habitat where they grow and proliferate [41]. Differences in these biosorption of metals by seaweeds may be best elucidated by differences in the amount and composition of polysaccharides in seaweed cell walls [42]. In addition to these, there are certain factors, such as the environmental features of each region (water, temperature, pH and salinity) that may affect metal accumulation [43,44]. The investigated algal species were analyzed for its various minerals. (Table 5). In the present study, it was found that the mineral content of seaweed samples was high and this in turn could be quite beneficial as a nutrient supply for the human body. The macro-nutrients such as (Na, Ca, K and Mg) as well as trace elements such as (Fe, Mn, Cr, Cu and Zn) were found higher in the seaweeds. The average concentration of some important minerals of *Valoniopsis pachynema* and *Dictyota ciliolata* are hereby presented. The results of the elemental analysis of seaweed samples are summarized in Tables. Mineral content of *Valoniopsis pachynema* (Chlorophyceae) and *Dictyota ciliolata* (Phaeophyceae) differed significantly ($p < 0.05$). There has been a wide variation in mineral concentration in different marine algae species, even when differences in environmental factors were minimized.[45] The elemental distribution in *V.pachynema* was observed to be in decreasing order of Na > Ca> k > Mg > Fe > Ni> Mn >Co> As > Pb > Cu> Zn > Cr > Cd> Se> V while that of *D.ciliolata* was observed as Na > Ca> k > Mg >Ni >Fe> As> Co >Pb> Mn > Zn> Se>Cr> Cd> V>Cu. *V. pachynema* in its specificity, contained higher concentrations of both the macro and micro minerals analyzed than that of its counterpart *D.ciliolata.* Both the green seaweed *V.pachynema* and brown seaweed *D.ciliolata* had higher concentrations of sodium at 164.76 ppm and 171.43 ppm followed by calcium at 135.86 ppm and 82.21ppm, potassium at 10.58 and 8.37 ppm and magnesium at 1.34 and 1.12 ppm in their algal biomass respectively. *D. Ciliolata* on the other hand contained higher levels of As, Co and Se. This showed that phaeophyceae as content is relatively more than that of Chlorophyceae and Rhodophyceae[46]. Sodium, Calcium, potassium and magnesium were the most abundant macro elements. High levels of sodium in both these algal biomass is similar to the findings reported in seaweeds such as *Fucus vesiculosus*, *Halimeda opuntia*, *Gracilaria corticata*, and *Turbinaria artriquetra* [47,48]. At present, in India, the average consumption of sodium in some of the local and processed foods is known to be far beyond than that of the recommended concentration levels. Regular consumption of these edible seaweeds that has been known to have a high sodium concentration can further lead to a greater intake of sodium, thus causing health-associated diseases such as hypertension and high blood pressure. However, this can be instead used alternatively in place of common salt in various processed foods with its mineral contents thereby helping in lowering the blood pressure levels and also maintaining the taste of foods as well. [49]. Latest findings show the formulation of processed meat products fortified with seaweeds (such as *Porphyra umbilicalis, Himanthalia elongata or Undaria pinnatifid*a) targets the decrease of the use of traditional salt (NaCl) and further enhancing its mineral composition and content [50]. The second most abundant element in these algal biomasses is calcium. Calcium acts as a cofactor for several extracellular enzymes and aids in bone strengthening, tooth structure, and muscle contraction. A few of the major advantages of calcium obtained from these seaweeds makes them commercially more important and valuable. Calcium from seaweeds generally help in enhancing the bone density irrespective of any age. The main drawback of the conventionally derived calcium through calcium supplements would result in certain side effects such as bloating, nausea and constipation. Marine algal calcium also possesses 12 essential bone supporting minerals (magnesium, boron, copper, manganese, silicon, nickel, selenium, strontium, phosphorus, potassium, vanadium and zinc) besides vitamins like C, D3 and K2, thereby resulting in the adequate supply of nutrients to the bones. [51] Potassium is the third most abundant element found in both the algal biomass Both potassium and magnesium which are the next copious minerals found are essentially required for striking a healthy balance of different body functionalities as they play a crucial role in accordance with calcium for regulation of the muscular system.[52] Magnesium supports the proper functioning of the immune, muscular, and the nervous system. This mineral mainly fortifies the bones, controls blood pressure and also helps in curbing sugar levels. Also, studies show that high magnesium intake can cause a lowering of blood pressure, reduced kidney function, diarrhoea and cardiac arrest .[53] Potassium and magnesium were found to be present in appreciable quantities in *V. pachynema* and *D.ciliolata* respectively. These data are quite fascinating from the nutritional perspective, as a diet with an enriched Na, Ca, k level ratio is crucial for those people who consume diuretic drugs to control hypertension and suffer from excessive loss of potassium. A high K: Na ratio can help in reducing blood lipid level, obesity, and also lower the risk of coronary heart disease.

Table 5: Elemental Composition of Marine Macro Algae *V.pachynema* and *D.ciliolata*

PARAMETERS	MARINE MACRO ALGAL SAMPLES	
Macroelements (ppm)	***Valoniopsis pachynema***	***Dictyota ciliolata***
Sodium	164.76 ± 1.09	171.43 ±1.52
Potassium	10.58 ± 0.21	8.37 ± 0.09
Calcium	135.86 ± 0.04	82.21 ± 0.03
Magnesium	1.34 ± 0.003	1.12 ± 0.02

Parameters	MARINE MACRO ALGAE	
Micro elements (ppm)	***Valoniopsis pachynema***	***Dictyota ciliolata***
Iron	13.42 ± 0.02	5.45 ± 0.04
Copper	1.24 ± 0.004	0.58 ± 0.003
Zinc	0.96 ± 0.013	1.02 ± 0.021
Manganese	3.71 ± 0.006	1.61 ± 0. 007
Cobalt	2.07 ± 0.004	2.31 ± 0.009
Nickel	5.32 ± 0.04	5.54 ± 0.06
Arsenic	1.81 ± 0.003	2.58± 0.005
Selenium	0.62 ± 0.001	0.99 ± 0.003
Vanadium	0.44 ± 0.003	0.59 ± 0.004
Chromium	0.82.01 ± 0.001	0.64 ± 0.002
Cadmium	0.75 ± 0.006	0.62 ± 0.007
Lead	1.64 ± 0.004	2.14 ± 0.003

Table 5 All results are indicative of triplicate values expressed as Mean ± SD. All the values in the table are statistically significant at p < 0.05. Table 5 demonstrates the various elemental composition of both the marine algae.

Trace elements or micro minerals are also important for the proper metabolism in the human body. Although they are present in minimal quantity, they are required to perform various physiological activities. For instance, manganese plays a role in balancing the sugar levels in the blood, boosting the absorption of calcium and for the proper functioning of connective tissues and bone. Trace microelements are the metal constituents that are classified into two main subclasses. The first group includes cobalt, copper, iron, manganese, and zinc, which are proven vital for the human body as they aid in proper functioning of various biochemical processes but can otherwise be quite toxic at very high concentrations. On the other hand, there is the second group that comprises mercury, cadmium, chromium, and lead, which are those metals that do not necessarily have any biological role but rather include certain critical metallic chemical contaminants in the marine ecosystem. [54] Manganese was noted in the biomass of *V.pachynema* as 3.71ppm and in *D. ciliolata* 1.61 ppm in considerable amounts . Although manganese is noted in these biomass, it is lower than that of *Cladphora glomerata* which is approximately 4.30 ppm. Based on these results, these edible seaweeds can also be used to supplement manganese in food which is important in controlling blood sugar levels, formation of bone and tissues as well as prevention of diseases (diabetes, osteoporosis, arthritis and epilepsy).[55] Iron is another crucial mineral that plays a pivotal role in the metabolic activities of the human body. Iron deficiency can be seen as quite a common health issue irrespective of any nation worldwide. From numerous research studies conducted, it is a global concern and also a commonly known fact that deficiency in iron will pave the way for anaemic conditions in the human body. Limitation of iron can result in chronic infections and bleeding, as well as a deficiency in folic acid, vitamin A and vitamin B12. Higher concentration of iron is observed in *V.pachynema* 13.42 ppm followed by *D.ciliolata* 5.45 ppm. This is in compliance with the study that suggests that these edible seaweeds can serve as an excellent alternative source of dietary iron to address iron deficiency [56]. Zinc and copper are important metallic chemical elements that are required at most in minimal concentration by humans for the proper immune function of some important biochemical processes in the body. Zn and Cu, act as essential cofactors for enzyme systems (cytochrome C-oxidase) that stabilize membranes, hormones, and nucleic acids. Since Zinc is involved in the repairing of the cells, a deficiency in the intake of zinc will result in the delayed wound healing. Deficiency of zinc is also associated with symptoms like hair loss and diarrhoea. Copper is critical in the synthesis of phospholipids and hemoglobin. It also boosts the production of melanin pigment for the skin [57]. Both the marine algae *V.pachynema* and *D.ciliolata* contains these microelements in minimal amounts (Table 5) and thus serves as a potential natural source of these important nutrients The amount of copper and zinc obtained in this study is lower than that obtained from that of *F. vesiculosum* with copper and zinc concentration of 12.7 ppm and 89 ppm, respectively. [58] Concentration of copper for *V.pachynema* and *D.ciliolata* are found to be 1.24 and 0.58 ppm, whereas the concentration of zinc was observed in trace amounts 0.96 and 1.02 ppm respectively. Chromium, cobalt, and copper are three essential microelements that support important physiological processes in the human body. Chromium is a significant mineral that helps in the regulation of blood sugar and pressure levels as well as the normal development of body muscles.[59] On the other hand, cobalt is an important microelement that is needed for vitamin C and vitamin B12 synthesis. This mineral helps in the normal cardiac functioning of the human body and facilitates iron absorption within the body. [60] Toxic microelements such as cadmium, mercury and lead, if present at minimal concentrations, can be detrimental to human health. These elements are generally not needed in the proper functioning of the body. Thus, non-inhalation and non-ingestion of these microelements are encouraged for the reason that serious toxic poisoning can result in death. [61] Though cadmium, vanadium are generally not considered that essential, still these do help in the proper functioning of important internal organs such as kidney and liver. Selenium is

considered another essential trace element for thyroid function and other metabolic processes. However, these trace elements are also considered toxic in high amounts, causing damage to important organs (kidneys, liver, and lungs), and also bring about adverse effects such as chronic diarrhoea, cardiovascular problems, anaemia, and severe damage to the brain. [62] Contrastingly, lead has been noted for its significance in the normal functioning of the brain, bone, and muscles but higher dose has also been known to cause seizures, kidney failure, coma, anaemia, and heart attacks. [63] *V.pachynema* and *D.ciliolata* have revealed the presence of these microelements but in amounts that are not considered toxic and confirms the nutritional potential and benefit of these edible seaweeds. The results of the present study suggests that both the seaweeds *V.pachynema* and *D.ciliolata* are potentially a good alternative source of macro and micro elements that are required for the overall human health and nutrition. Differences in the concentration of trace element contents between *V.pachynema* and *D.ciliolata* in comparison to other seaweed species are highly influenced by several environmental and cultural factors including: pH, interactions, salinity and interactions among elements, light intensity, amount of trace elements in water, and other metabolic influences like dilution of elemental composition caused by growth and development of seaweed. [64,65] The result of this study provides baseline information on the nutritional value of *V.pachynema* and *D.ciliolata* that can be used as a dietary food supplement for human consumption to tackle nutrient deficiency in the world.

4.5 Anti-oxidant activity - DPPH method

The DPPH assay is the furthermost widely functional technique for viewing natural product's antioxidant activity since they can provide accommodation for a lot of models in a given period of time and spot ingredients (active) at low concentrations. The declining absorbance of DPPH radicals was mainly due to the occurrence of an antioxidant and is because of hunting of radicals by hydrogen contribution.[66] Table 6 shows the free radical scavenging activity of the crude methanolic extract of the marine algae in *Valoniopsis pachynema* and *Dictyota ciliolata* using the DPPH method. The absorbance was measured at 517nm. The extracts were taken as 50µg, 100µg, 150µg, 200µg concentrations. Figure 6 shows the comparative analysis of *Valoniopsis pachynema* and *Dictyota ciliolata* in the DPPH method. Maximum activity was seen in *Valoniopsis pachynema* extract which was noted as 24.53 ± 0.01, 32.17 ± 0.03, 56.71 ± 0.02, 72.63 ± 0.04, 92.45 ± 0.02 whereas in *Dictyota ciliolata* it was 20.46 ± 0.05, 31.20 ± 0.03, 49.31 ± 0.04, 64.11 ± 0.03, 80.15 ± 0.04 respectively.

Table 6: Free Radical Scavenging Activity by DPPH of *V.pachynema* and *D.ciliolata*

Concentration of extract in µg/ml	Optical density at 517nm % Inhibition (I %)	
	Valoniopsis pachynema	*Dictyota ciliolata*
50 µg	24.53 ± 0.01	20.46 ±0.05
100 µg	32.17±0.03	31.20±0.03
150 µg	56.71±0.02	49.31±0.04
200µg	72.63 ±0.04	64.11±0.03
250 µg	92.45±0.02	87.15±0.04

Table 6 The results were expressed inMean ± SD (n = 3) All determinations were carried out in triplicates. All the values in the table are statistically significant at p < 0.05.

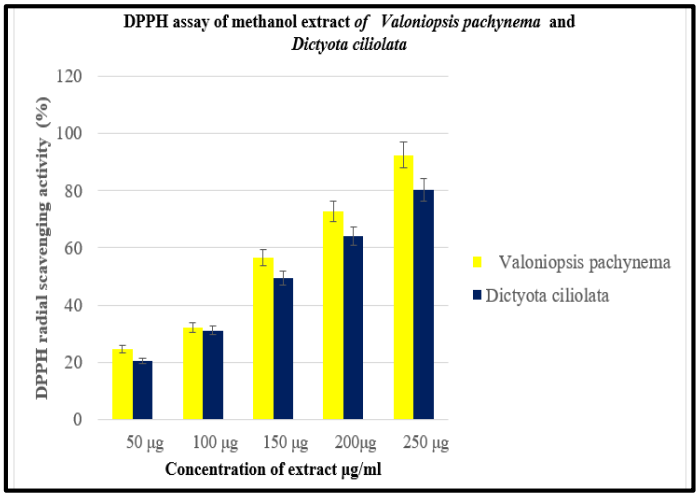


Fig 6: Free radical scavenging activity by DPPH assay

Table 6 and Figure 6 illustrates the free radical scavenging activity was assessed using DPPH assay and it showed more than 90% of antioxidant activity for *Valoniopsis panchynema* and Dictyota ciliolata exhibited 80% of radical scavenging activity. All the values in the histogram are statistically significant at $p < 0.05$.

4.6 Phosphomolybdenum method

Comparative analysis of the phosphomolybdenum assay has to a certain extent proved that the methanolic extract of *Valoniopsis pachynema* exhibited higher free radical scavenging capacity than that of *Dictyota ciliolata*. Phosphomolybdenum assay in this degree, proves than the previous method that

methanol extract of *Valoniopsis pachynema* could exhibit a potent anti-oxidant activity with the values as 54.24 ± 0.02, 63.28 ± 0.01, 77.39 ± 0.05, 85.14± 0.02, 93.01±0.03 compared to that of *Dictyota ciliolata* 32.46 ± 0.01, 45.17 ± 0.03, 53.32 ± 0.02, 62.44 ± 0.03, 71.58 ± 0.05 in varying concentrations. (Table 7). Although various reports have suggested that ethanol extract has yielded far better anti-oxidant activities, this study mainly focuses on the methanol extract of both these algae. In the pharmaceutical industry, some medicinal plants are used for scavenging free radicals and preventing tissue injury which plays a vital role in therapeutic purposes. [67] In the present study, its suggested that the green algae *Valoniopsis pachynema* of methanol extract indicates highest free radical scavenging capacity compared to that of the brown algae *Dictyota ciliolata.*

Table 7: Phosphomolybdenum assay of methanol extract *of Valoniopsis pachynema* and *Dictyota ciliolata*

Concentration of extract in µg/ml	Optical density at 695nm % Inhibition (I %)	
	Valoniopsis pachynema	*Dictyota ciliolata*
100 µg	54.24 ± 0.02	32.46 ± 0.01
150 µg	63.28 ± 0.01	45.17 ± 0.03
200 µg	77.39 ± 0.05	53.32 ± 0.02
250µg	85.14 ± 0.02	62.44 ± 0.03
300 µg	93.01 ± 0.03	71.58 ± 0.05

Mean ± SD (n = 3) All determinations were carried out in triplicates. All the values in the table are statistically significant at p < 0.05.

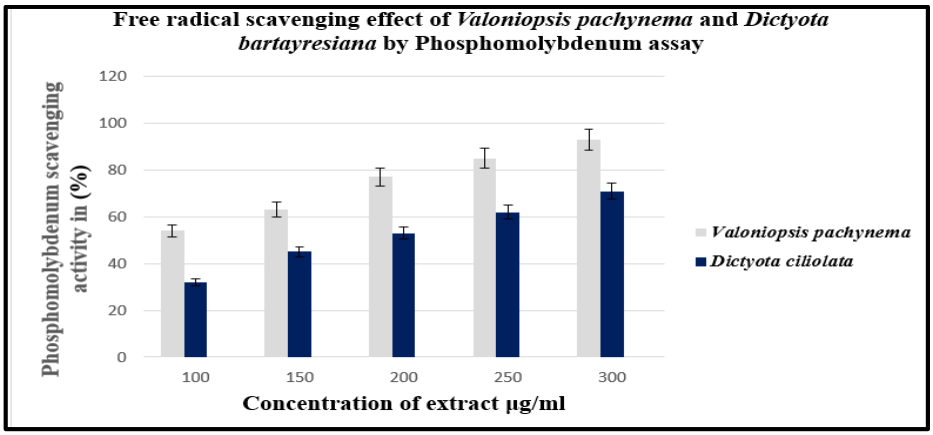


Fig 7: Free radical scavenging activity by PMB Assay

Table 7 and Figure 7 demonstrates the free radical scavenging activity was assessed using PMB assay and it showed more than 90% of antioxidant activity for *Valoniopsis panchynema* and Dictyota *ciliolata* exhibited 70% of radical scavenging activity. All the values in the histogram are statistically significant at p < 0.05.

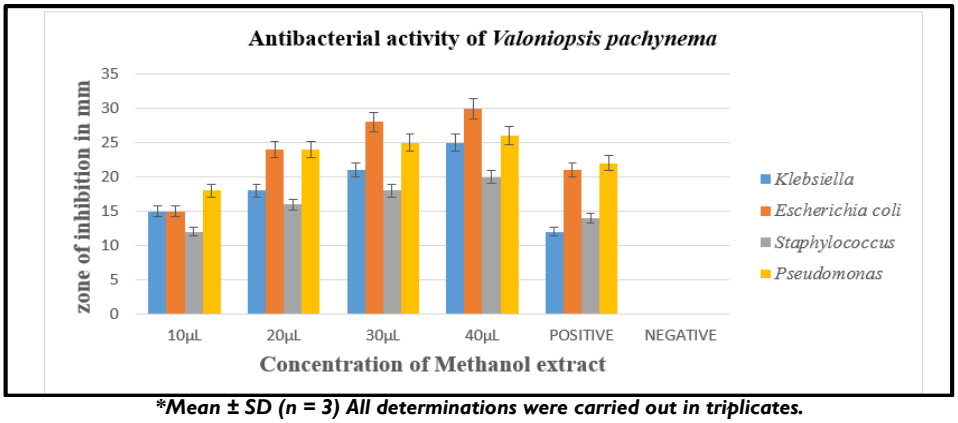
4.7 Anti-bacterial activity

Agar disc diffusion method was performed for describing the antibacterial activities of seaweeds. This disc diffusion assay is a convenient method as it produces predominantly qualitative results. In order to screen the qualitative antibacterial activity of methanolic extracts of seaweed species, the disc diffusion method was quite useful to obtain preliminary information. [68] Certain pathogenic bacteria (*Staphylococcus,E. coli, Pseudomonas spp andKlebsiella sp*) were used to determine the antibacterial activities of the seaweed crude methanolic extract and synthetic compounds by the disk diffusion assay. *Staphylococcus sp, Escherichia coli, Klebsiella sp,* and *Pseudomonas sp* were used to check the antibacterial activity of *Valoniopsis pachynema* and *Dictyota ciliolata* (Fig.8 a). While all the strains exhibited varying degrees of antibacterial activity, Valoniopsis *pachynema* exhibited the maximum antibacterial activity against *E.coli (*31mm ± 0.12), followed by the rest that includes *Staphylococcus* (21mm ± 0.31), *Klebsiella* (27mm ± 0.12) *and Pseudomonas* (26mm ± 0.10). The minimum activity was observed for *Staphylococcus aureus* (21 ± 0.11). Ampicillin was used as the positive control for all the strains. The findings are in accordance with a previous report [69] , where the authors demonstrated that the antimicrobial activity of *Valoniopsis pachynema* activity was due to the availability of various bioactive metabolites in the extracts. The bioactive compounds are alkaloids, flavonoids, phenols which are responsible for having various pharmacological activities.[70] In the present study conducted, it was found that the crude methanolic extract of the marine macro green algae *V.pachynema* exhibited far better antibacterial activity than the brown algae *D.ciliolata.*

Table 8 (a): Anti-bacterial activity of *Valoniopsis pachynema*

S.no	Organism	10µL	20µL	30µL	40µL	Positive	Negative
1	*Klebsiella*	15	18	21	25	12	-
2	*E.coli*	15	24	28	30	21	-
3	*Staphylococcus*	12	16	18	20	14	-
4	*Pseudomonas*	18	24	25	26	22	-

The antibacterial activity of the green marine algae V.pachynema was assessed using Agar well diffusion method and it is found to be susceptible to the gram positive and gram negative microorganism having zone of inhibition greater than brown marine algae D,ciliolata



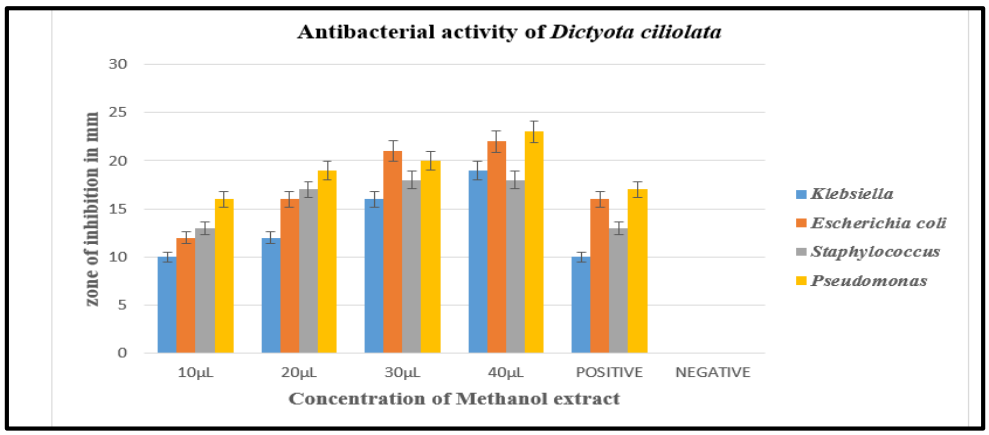
**Mean ± SD (n = 3) All determinations were carried out in triplicates.*

Fig 8(a): Antibacterial activity of *Valoniopsis pachynema*

Table 8 (b) : Anti-bacterial activity of *Dictyota ciliolata*

S.no	Organism	10µL	20µL	30µL	40µL	Positive	Negative
1	*Klebsiella*	10	12	16	19	10	-
2	*E.coli*	12	16	21	22	16	-
3	*Staphylococcus*	13	17	18	18	13	-
4	*Pseudomonas*	16	19	20	23	17	-

The antibacterial activity of the brown marine algae D.ciliolata was assessed using Agar well diffusion method and it is found to be susceptible to the gram positive and gram negative microorganism having zone of inhibition lesser than green marine algae V.pachynema .



**Mean ± SD (n = 3) All determinations were carried out in triplicates.*

Fig 8 (b): Antibacterial activity of *Dictyota ciliolata*

5 CONCLUSIONS

Seaweeds are considered as potential sustainable resources that have been known to exhibit numerous health benefits. The elemental composition and proximate composition of the marine macro algae suggest that these algae have far greater potential as dietary supplements in the food industry. The present study throws light on the various therapeutic possibilities of marine algae and gives substantial evidence that marine macro algae could act as beneficial nutrient food supplements thereby creating global health awareness. Although this study concludes that the marine algae has natural anti-oxidants, further research is needed to deeply understand the digestibility, toxicological effects and bioavailability of the biologically active substances that are present in these algal biomass.

6 ACKNOWLEDGEMENT

The Authors would like to sincerely thank the Algae Research Centre for their assistance in securing the permit to collect seaweeds. The first author would like to thank the Corresponding author Dr.Thirumalai Vasan.P for his technical assistance throughout the conduct of the study. Also, the authors would like to express their heartfelt gratitude to the Management of Srimad Andavan Arts and Science College, Tiruchirappalli, Tamil Nadu for their kind co-operation throughout the course of the research.

7 AUTHOR CONTRIBUTION STATEMENT

Ms.Bhuvaneshwari.J conceptualized and gathered the data with regards to this research work. Dr.Thirumalai Vasan.P discussed the methodology and analyzed all the data. Necessary inputs were given towards designing of the manuscript. Both the authors read and discussed the methodology and contributed to the final version of the manuscript.

8 CONFLICT OF INTEREST

Conflict of interest declared none

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