EVALUATION OF PHYTOCHEMICAL, BIOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *Caulerpa peltata* Lamour

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ABSTRACT: The current study examines the biochemical, phytochemical, and antioxidant properties of the marine macroalgae *Caulerpa peltata* Lamour, which was found along the Thirumullavaram coast in Kerala. Physicochemical parameters were evaluated because hydrochemistry of an aquatic system affects its growth. Phytochemical and antioxidant analysis were done by using extracts of distilled water, acetone and chloroform. Secondary metabolites and antioxidants discovered in high concentrations in *C. peltata* include phenol, coumarin, cardiacglycosides, tannins, terpenoids, and saponins. As a result, it may have nutritional, neuroprotective, antioxidant, antibacterial, anticancer, anti-inflammatory and hemolytic actions.

INDEX TERMS: Seaweed, Antioxidant activity, Phytochemicals, Secondary metabolites

I. INTRODUCTION

Marine algae are the richest producers in the marine environment [1]. As early as 3000 BC, they were used in conventional treatments [2]. A variety of biological capabilities are shown by compounds obtained from marine algae, enhancing their usefulness as a source of novel bioactive molecules, according to research [3]. The presence of bioactive chemicals affects their ability to have anti-inflammatory, antioxidant and antibacterial properties [4]. In Asian countries including China, Japan, and Korea, seaweeds have a long tradition of use as food [5]. Due to their high nutritional content, edible green seaweeds have garnered a lot of interest. They include a wide range of nutrients, including minerals, essential amino acids, polyunsaturated fatty acids, and necessary carbohydrates [6]. Due to the abundance of many primary and secondary metabolites they contain, in addition to their nutritional value, they also have a variety of physiological impacts on health and disease [7][8]. Algal secondary metabolites have a financial impact on a number of businesses, including food, feed, aquaculture, biomedicine, veterinary medicine, the cosmetics industry, and health [9].

Among the valuable elements produced from marine algae, natural pigments have attracted a lot of attention. These natural pigments have antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic, and neuroprotective effects, among other biological advantages. Numerous natural colors obtained from marine algae as a result have drawn a lot of attention from the medicine, cosmetics, and food industries [10]. Seaweeds also contain a number of unique phytochemicals that are not found in terrestrial plants. It has been demonstrated that these substances safeguard both the plants and the people who consume them [11]. Studies show that seaweeds contain bioactive substances with strong antioxidant properties that can protect them against reactive oxygen species (ROS) [12]. Antioxidant-rich foods have recently gained popularity because of their capacity to protect against ROS and the associated health problems [13]. Phytochemicals may be the antioxidant components of seaweed [14].

Physicochemical investigations are necessary because the hydrochemistry of an aquatic system affects its biodiversity. Algal biomass, composition, and production are all impacted by temperature. Algal species, light, temperature, and different growth stages all affect the biochemical makeup of the species [15]. The hydrographic characteristic of temperature has a significant impact on virtually all chemical and biological interactions. Temperature has a variety of effects on algal development. The dispersion of biological communities is greatly influenced by salinity, one of the most crucial parameters that strongly control all other physical and chemical aspects of seawater [16]. The morphology of green algae is significantly influenced by varying salinities. Tropical and subtropical regions are home to the green seaweed *Caulerpa peltata* Lamour, which is a member of the Caulerpaceae family. These Caulerpaceae seaweeds are rich in proteins, lipids (especially polyunsaturated fatty acids), vitamins, pigments, and minerals, making them an excellent source of bioactive components [17] [18]. The objective of the current study is to evaluate the biochemical, phytochemical and antioxidant qualities of *C. peltata* .

II. MATERIALS AND METHODS

The algal samples were gathered in April 2019 along the Thirumullavaram Coast (8.90262540N 76.56110520E). The station contains a huge rocky promontory, a narrow bay of sand, and a very dense growth of algae. There is no influence of fresh water. The samples were immediately washed with sea water to remove the associated sand particles, and then delivered to the lab in plastic bags. The samples were gently removed from the epiphytes and properly cleaned with tap water to remove the remaining dirt particles. To get rid of all the sand and dirt, it was washed four or five more times. To remove extra water, it was drained off and spread out on blotting paper. Samples were powdered and stored at room temperature in airtight plastic bottles after being dried at room temperature and in a hot air oven at 40°C for two days.

The water samples collected from Thirumullavaram were kept under refrigerator for later analysis. Sea water of the study area was analysed for pH, dissolved oxygen, salinity phosphate and nitrate. pH of sea water were recorded using digital pH meter. Dissolved oxygen, salinity and nitrate [19] and inorganic phosphate [20] were also analyzed and recorded.

The biochemical analysis was carried out by using dried powder of algae. Total protein [21], total carbohydrate [22] and crude lipid [23] were estimated. Photosynthetic pigments like chlorophyll [24] and carotenoid [25] were measured. The same chlorophyll extract was measured at room 480nm in spectrophotometer to estimate the carotenoid content.

5gm dried crushed samples were extracted each using 100ml, solvents of distilled water, acetone and chloroform. The quantity of plant parts were then homogenized in different solvents and then properly covered with Aluminium foil and labelled. Each extract was filtered through Whatsman's filter paper No.1 separately. The extract was evaporated to dryness at 40°C on a dry heat incubator; extract was then kept in the refrigerator until the time of use for the following experiment. Afterwards a measured volume of solvent was used to dissolve the extract to required working concentrations. Qualitative phytochemical screening was carried out by using a standard procedure [26]. The amount of total phenol in the extract was determined with Folin-Ciocalteu reagent [27]. Calorimetric technique was used for flavonoid estimation [28]. Scavenging effects of samples for DDPH radical were monitored [29]. The mean and standard error for all analysis were calculated and reported. The data is the mean of three replicates.

III. RESULT AND DISCUSSION

Physicochemical studies are necessary because these characters largely influence the composition of seaweed. It is shown in Table 1. Atmospheric temperature was reported to 37^0c, which is greater than the surface water temperature. The surface water temperature was 36^0c. Temperature is an important hydrographical parameter which influences almost every chemical and biological interaction. It is considered as a vital environmental factor controlling growth and metabolic rates of marine organisms, especially metabolic processes of photosynthesis and respiration in macroalgae [30].

The dissolved oxygen (DO) of the sea water was 3.87±0.098 mg/l. It is less than the DO reported by Gourisahu *et al*., 2012 [16] which is fluctuated between 4.2mg/l - 6.1mg/l. The range of dissolved oxygen might be influenced by fresh water flow [31]. The concentration of DO varies as a result of photosynthesis and respiration by aquatic organisms [32]. According to Krishnamurthy, 1990, [33] the high DO in summer is due to high temperature and bright sunlight which accelerate photosynthesis. But in contrast the observed DO in the present study was low which was conducted in summer. The inverse relation between the temperature and DO is a natural process [34] which coincides with the present study. At higher temperature the capacity of water to hold oxygen decreases [35] and this might be also have contributed to the reduced oxygen.

The DO yielded a moderately negative correlation with salinity. Salinity level was recorded as 32.64± 0.07‰. The high value of salinity may be the reason behind low DO. According to Adam Lalata 1991,[36] different salinities have a considerable influence on the morphology of green algae. The pH of the sea water is reported 8.15±0.05. This shows that the sea water is slightly alkaline. The higher pH recorded during the summer season, which might be due to high biological activity [37]. This value agrees with James Anand, 2015 [38].The pH value is affected by the factors like removal of CO_2, dilution of seawater, primary productivity, salinity, temperature and decomposition of organic materials [39]. Nitrate and phosphate analyzed for nutrients were 0.49± 0.015µg at/l and 2.91±0.13µg at/l. In the present study the concentration of phosphate is greater than that of nitrate. The low N:P ratio denotes the bioavailability of nitrogen for algal productivity in this environment is less than the phosphorous hence the growth of algae can be considered as controlled. Boussiba and Vonshak, 1991,[40] have emphasized the need for sufficient quantity of nitrogen for continuous synthesis of protein responsible for supporting pigment formation.

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were quantitatively examined (Table 2). Carotenoid content was lesser in amount when compared to other pigments. The color in case of green seaweeds is due to the presence of chlorophyll a and b in the same proportions as the higher plants. In addition to their health advantages, algal pigments have significant commercial potential as natural colorants in the nutraceutical, cosmetics and pharmaceutical industries [41]. The biochemical compounds examined were protein, carbohydrate and lipid (Table 3). Carbohydrate and protein contents were in large amounts but lipid content was very low. Biochemical analysis of seaweeds is important for the assessment of nutritional value to marine herbivores and it is also important for the assessment of possible sources of protein, carbohydrate and lipid for human consumption or commercial use [42]. In general, storage compounds, such as protein, lipid and carbohydrate allow the algae to adjust their growth to the changing environmental conditions [43]. Carbohydrate content varies depending on the nutritional status of algal cells, seasonal and geographical variations and light in particular, generally with accumulation of carbohydrate under high light conditions and inverse correlation between nitrogen availability and carbohydrate content exists [44]. The increase in the carbohydrate content protect algae from salt harms [45].

Lipid accumulation in algae usually occurs during periods of environmental stress. Lipids serve as a source of energy, a crucial component of cell walls, and a storage substance. Lipids are abundantly dispersed in macro algae [46].

Algal protein is called complete protein since it contains essential amino acids [47]. Seaweed and microalgae have higher protein yield per unit area compared to terrestrial crops, such as soybean, pulse legumes and wheat [48].

Seaweeds have a challenging habitat because they are exposed to both light and high oxygen levels. Free radicals and other potent oxidizing agents may arise as a result of these conditions; however seaweed seldom sustains significant photodynamic damage during metabolism. This information suggests that the cells of seaweed have certain defense mechanisms and substances [49]. The synthesis of phytochemicals and secondary metabolites results in the development of these defense systems. Three solvents acetone, distilled water, and chloroform were used to analyze the phytochemicals (Table 4). In all three solvents, terpenoid and flavonoid concentrations were high. The distilled water extract was rich in coumarin, cardiacglycoside, tannins, terpenoids, and saponins. In contrast, the chloroform extract was devoid of phenol, coumarin, and saponin. It might be because water is highly polar while chloroform is non-polar.

The majority of the structurally distinct alkaloids found in marine algae belong to the phenylethylamine and indole families. When compared to alkaloids from terrestrial plants, marine algal alkaloids are very uncommon [50]. Numerous bodily processes are regulated with the use of steroids. A good source of unsaponifiable, non-toxic sterols with medicinal use has been found to be marine algae [51]. Tannins are employed as astringents and in the manufacture of pharmaceuticals. They also have anti-viral, anti-ulcer, anti-bacterial, anti-inflammatory, and antioxidant activities [52]. Biological events such as herbivore attack or pathogenic infection can affect plant saponin levels in a dynamic manner. Macroalgae and plants synthesize and combine saponins as one of their integrated defensive mechanisms [53]. Terpenoids, the biggest group of diverse phytochemicals, are frequently found in marine algae. These secondary metabolites have strong antioxidant characteristics and both in vivo and in vitro anticancer activity [54].

Edible seaweeds frequently contain phenolic compounds, and studies have linked these substances to the antioxidative properties of these plants [55]. The largest class of polyphenols, flavonoids are believed to have positive health effects due to their chelating and antioxidant capacities. They either works by preventing the synthesis of highly valent metal forms, scavenging free radicals, or stopping the chain events that lead to lipid peroxidation [56]. They have been shown to have antioxidant and antitumor effects [57].

The quantitative estimation of flavonoid and phenolic content was done in distilled water, acetone and chloroform extracts (Table 5). Distilled water extracts had the highest levels of phenol and flavonoid content, followed by acetone, while chloroform had the lowest levels. The least amount of phenol and flavonoid was found in the chloroform extract. Because chloroform is a wholly non-polar solvent, this is caused by solubility or polarity.

The current findings are in agreement with the result of Shyamala *et al*., 2014 [58]. Phenolic contents are more soluble in polar solvents. Different solvents have capacity to extract different phytochemicals depending upon their solubility or polarity in the solvent and abundance of the phytochemicals varies with their solubility or polarity in different extracts. Phenolic concentrations are more soluble in polar liquids [59]. Different phytochemicals can be extracted using different solvents depending on their solubility or polarity in the solvent, and the amount of the phytochemicals varies with their solubility or polarity in different extracts. Studies have shown a positive correlation between seaweed's total phenolic and flavonoid content with antioxidant activity [60].

Antioxidants act as reducing agents by scavenging free radicals and preventing or delaying the consequences of oxidation. The antioxidant activity of marine algae may be caused by the carotenoids and chlorophyll pigments, phenolic compounds, flavonoids, vitamins, terpenoids, and other pigments that either directly or indirectly contributes to the prevention of the oxidation process [61].

Different solvents were investigated for DPPH free radical scavenging activity (Table 6). It was found to be lowest in chloroform and highest in distilled water, followed by acetone. With increasing concentration (concentration ranges from100µg/l to 500µg/l), the DPPH scavenging activity increases. These results concurred with that of Leelavathi and Prasad, 2014 [62].

The research revealed that *C. peltata* is an excellent source of secondary metabolites, antioxidants, and storage nutrients like protein, carbohydrate and lipids. The phytochemicals in seaweed are used to make a variety of medications, cosmetics, immune system boosters, and other treatments. In commercial chemical compositions, this is also employed. This conclusion implies the importance of algae in the pharmaceutical, cosmetic and other industries.

Table 1. Physico-chemical analysis of seawater

Temperature(°c)		pH	Salinity(‰)	Dissolved Oxygen(mg/l)	Nutrients(µg at/l)	
Atmosphere	Surface water				Nitrates	Phosphate
37°c	36°c	8.15±0.05	32.64±0.07	3.87±0.098	0.49±0.015	2.91±0.13

The data expressed in mean ± S.D, n =3 in each group.

Table 2. Photosynthetic pigment analysis

Parameters	Value (mg/g dry weight)
Chlorophyll a	0.0495±0.0125
Chlorophyll b	0.0342±0.0077
Total Chlorophyll	0.0601±0.0232
Carotenoid	0.0229±0.0218

The data expressed in mean ± S.D, n= 3 in each group.

Table 3. Biochemical analysis

Parameters	Value (mg/g dry weight)
Protein	15.98±0.05
Carbohydrate	17.59±0.52
Lipid	1.98±0.02

The data expressed in mean± S.D, n=3 in each group.

Table 4. Qualitative phytochemical analysis

Phytochemicals	Solvents		
	Distilled water	Acetone	Chloroform
Alkaloid	++	+	+++
Steroid	+	++	+++
Flavonoid	++	+++	+++
Phenol	+++	+++	-
Coumarin	+++	+	-
Cardiac glycosides	+++	+++	+
Tannins	+++	+	+
Terpenoids	+++	+++	+++
Saponins	+++	-	-

(-) Absent, (+) Present, (++) Moderate, (+++) Abundant

Table 5.Quantitative analysis of Total phenolic and flavonoid content of various extracts

Solvents	Phenols(mg/g)	Flavonoid(mg/g)
Distilled water	10.86±0.23	15.78±0.24
Acetone	8.88±020	12.62±0.26
Chloroform	2.87±0.21	3.62±0.18

The data are expressed in mean± S.D, n = 3 in each group.

Table 6. DPPH Free radical scavenging activity

Sl. No.	Concentration(µg/ml)	% Activity(±SD) in Distilled water extract		% Activity(±SD) in Acetone extract		% Activity(±SD) in Chloroform extract	
		Standard(Ascorbic acid)		Standard(Ascorbic acid)		Standard(Ascorbic acid)	
1	100	66.66±0.57	34.76±0.23	53.33±0.46	26.60±0.52	90.51±0.26	23.71±0.26
2	200	65.59±0.52	35.16±0.29	52.32±0.57	28.50±0.47	94.52±0.45	26.51±0.45
3	300	67.67±2.08	38.50±0.50	55.48±0.48	30.58±0.52	96.57±0.50	27.52±0.50
4	400	71.66±0.57	42.59±0.52	56.42±0.51	31.53±0.46	97.34±0.34	28.46±0.34
5	500	73.62±0.54	45.52±0.46	58.47±0.45	32.32±0.51	98.54±0.51	30.15±0.51

The data are expressed in mean± S.D, n = 3 in each group.

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