



ANALYSIS OF PHYTOCHEMICAL, BIOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *Sargassum tenerrimum* J.Agardh

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ABSTRACT

The current study looks at the biochemical, phytochemical, and antioxidant properties of *Sargassum tenerrimum* J.Agardh., a marine macroalgae collected from the Thirumullavaram coast in Kerala. Extracts of distilled water, acetone, and chloroform were used for phytochemical and antioxidant analyses. Antioxidants and secondary metabolites identified in *S. tenerrimum* include phenol, coumarin, cardiacglycosides, tannins, terpinoids, and saponins. This indicates that it may have nutritional, neuroprotective, antioxidant, antibacterial, anticancer, anti-inflammatory, anti-feedant and hemolytic properties.

Keywords: Seaweed, Antioxidant, Phytochemicals, Secondary metabolites

1. INTRODUCTION

Ecologically and commercially, marine and freshwater macro algae are important in many parts of the world, particularly in Asian countries like China, Japan, and Korea. They are nutritious foods that are low in calories and high in vitamins, minerals, polysaccharides, steroid hormones, and dietary fibers. They have been regarded traditional treatments since 3000 BC [1]. They are nutritious as fresh or dried vegetables, or as ingredients in a range of prepared dishes [2]. Certain edible seaweeds are high in protein, fat, mineral, and vitamin content [3]. They are utilized as fertilizer, manure, and fodder in agriculture, as well as in aquaculture, medical, textile, paper, and paint industries. They are also employed as thickening agents, gelling agents, and stabilizing agents [4]. Algae also play a role in soil binding agents, which help to prevent

erosion and can be used to treat waste water [5]. Carotenoids, terpenoids, xanthophylls, chlorophylls, vitamins, saturated and poly saturated fatty acids, amino acids, acetogenins, antioxidants like polyphenols, alkaloids, halogenated compounds, and polysaccharides like agar, carrageenans, alginate laminarin, rhamna sulphate, galactocyl glycerol, and fucoidan [6]. They are thought to be a source of bioactive chemicals because they can create a wide range of secondary metabolites with a wide range of biological activity [7]. They have been widely used in the pharmaceutical and drug industries [8]. Tannins and flavonoids are phenolic molecules that have a wide range of chemical and biological actions, including antioxidant, anti-inflammatory, and free radical scavenging characteristics [9]. As a result, they are used in medical, pharmaceuticals, and cosmetics [10]. Seaweed biochemical and phytochemical screening could aid in the creation of novel medications to combat severe diseases. As a result, the current study aims to assess the biochemical, phytochemical, and antioxidant properties of *Sargassum tenerrimum* J.Agardh.

2. MATERIALS AND METHODS

During April 2021, algal samples were obtained from the Thirumullavaram Coast (8.9026254°N 76.5611052°E). The station includes a large rocky promontory with a little sand bay and abundant algal flora. There was no influence from fresh water. The samples were promptly rinsed with sea water to remove the adherent sand particles and then transported to the laboratory in plastic bags. The samples were thoroughly cleaned with tap water to remove any adherent dirt particles, and the epiphytes were carefully removed. The samples were drained of water and then blotted dry with blotting paper to remove any remaining moisture. The samples were pulverized and stored in airtight plastic bottles at room temperature after being dried at room temperature and in a hot air oven at 40°C for two days. The biochemical composition was determined using dried algae powder. The methods of [11,12,13] were used to calculate total protein, total carbohydrate, and crude lipid, respectively. The quantity of chlorophyll in the seaweeds was calculated using the method described in [14]. The amount of carotenoid was calculated using the method described in [15]. To determine the carotenoid concentration, the same chlorophyll extract was quantified in a spectrophotometer at room 480nm.

Each of the 5gm dry crushed samples was extracted with 250ml of distilled water, acetone, and chloroform. The plant components were then homogenised in various solvents before being neatly wrapped in aluminium foil and labelled. Each extract was filtered individually using Whatman's filter paper No. 1. On a dry heat incubator, the extract was evaporated to dryness at 40°C; the extract was then preserved in the refrigerator until needed for the next experiment. The extract was then dissolved in a known volume of solvent to reach the requisite working concentrations. The screening of phytochemicals was done according to the conventional approach outlined by [16]. The total phenol content of the extract was measured using the Folin-Ciocalteu reagent, as described in [17]. Flavonoid estimation was done using the calorimetric method [18]. The DPPH radical scavenging assay was monitored using the method described in [19]. For

each analysis, the mean and standard errors were determined and reported. This information is based on the average of three replicates.

3. RESULT AND DISCUSSION

Natural pigments are found to create a lot of interest among the useful compounds discovered in marine algae. Antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic, and neuroprotective actions are among the many biological properties of these natural pigments [20]. The contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were quantitatively investigated and found in this study (Table 1). Brown seaweeds have a high concentration of carotenoid pigment (0.51100.3103 mg/g DW), which is responsible for their color. Other pigments such as chlorophyll a and c, as well as other xanthophylls, are obscured by this molecule. In a variety of ways, carotenoids have been proved to be potent antioxidants. For starters, they can reduce the creation of singlet oxygen (1O_2) by directly absorbing excess excitation energy from chlorophyll. Second, they can immediately quench 1O_2 . Finally, they can use the xanthophylls cycle to dissipate excess excitation energy, reducing the severity of oxidative damage [21].

Seaweed biochemical analysis is vital for determining the nutritional value of seaweeds to marine herbivores, as well as determining potential supplies of protein, carbohydrate, and fat for human diet or commercial use [22]. There was a significant amount of protein (16.510.50 mg/g dry weight DW), as well as a significant amount of carbohydrate (11.800.32 mg/g DW), but just a trace amount of fat (0.470.03 mg/g DW) (Table 2.). Seaweeds have a low lipid content in general [23,24]. *Sargassum* sp. lipid content contains 50% PUFAs, and the biomass is particularly rich in docosahexaenoic acid, according to [25]. DHA is utilised in newborn dietary formulae and as a nutritional supplement for adults. It is necessary for adult brain function as well as in the development of the nervous system and visual capacities throughout the first six months of an infant's life. This is because it includes all of the necessary amino acids and hence algal protein is referred to as complete protein [26]. Polysaccharides make up the majority of algal cell walls. Kelp typically contains roughly 60% carbs by dry weight. The present study has a lower carbohydrate content than the previous report. This is most likely due to the thallus's broad expansion [27]. Seasonal changes in carbohydrate content are also significant [28]. Phytochemicals are the secondary metabolites produced by seaweeds. In distilled water, acetone, and chloroform, qualitative phytochemical screening was performed (Table 3.). Phenol, coumarin, cardiacglycoside, tannins, terpenoids, and saponins were prevalent in the distilled water extract. All extracts were high in saponin and cardiacglycoside. Only distilled water extract and chloroform extract contained alkaloid and steroid, respectively. The presence of phenol and coumarin in chloroform was confirmed, as was their abundance in the other two solvents.

Seaweeds grow in a hostile environment where they are exposed to a combination of light and high oxygen concentrations. Although these conditions can result in the generation of free radicals and other powerful oxidizing agents, seaweeds rarely experience substantial photodynamic damage during metabolism. This

suggests that seaweed cells have some kind of defense mechanism and chemicals [29]. Alkaloids are chemical substances that, despite their reputation as a deadly material, protect the plant from stress. Antimicrobial properties against both gram positive and gram negative microorganisms have been discovered frequently [30]. They are employed in medication research and can be used to treat a variety of disorders. Tannins are also utilised as astringents and in the creation of medications. They are also known for their anti-inflammatory, antibacterial, antioxidant, antiviral, and anti-ulcer activities.[31]. Saponins have a wide range of biological activities, including antibacterial, anti-inflammatory, anti-feedent, and hemolytic properties [32]. Coumarins are used to treat asthma and lymphedema as anticoagulants. Because they have a wide variety of biochemical effects on cell growth and development, cardiacglycosides are used in the treatment of cardiac failure as well as cancer. Terpenoids are found in a variety of industrially important chemicals such as medications, insecticides, and flavors [33]. Steroids aid in the control of a variety of bodily functions. It imitates the masculinizing effects of testosterone, the male sex hormone. Steroids have an anti-inflammatory effect on the body. It lowers the risk of cancer and cholesterol, as well as acting as an immunity booster. Marine algae have been found to be a good source of non-toxic, unsaponifiable sterols with therapeutic significance [34].

Flavonoids, phenolic acids, and tannins are thought to be the primary contributors to antioxidant activity [35,36]. Edible brown, green, and red seaweeds contain phenolic chemicals, and their antioxidative properties have been linked to their phenolic concentration [37]. The largest group of polyphenols, flavonoids, have been shown to have anticancer and antioxidant characteristics [38]. Flavonoid and phenolic content were measured quantitatively in distilled water, acetone, and chloroform extracts (Table 4.). The distilled water extract had the highest phenol and flavonoid levels, followed by acetone, and finally chloroform. The amount of phenol and flavonoid in chloroform extract was the lowest. This is because chloroform's polarity makes it a non-polar solvent. The recent findings corroborate the conclusions of [39]. In polar solvents, phenolic content is more soluble [40]. The solubility of phytochemicals and the polarity of the solvents influence their existence and abundance in different solvents.

There is a positive association between phenol and flavonoid concentration and antioxidant activity, according to many studies [41]. Antioxidants scavenge free radicals and act as reducing agents, preventing or delaying oxidative changes. Antioxidant activity in marine algae can be attributed to pigments such as carotenoids and chlorophyll, as well as phenolic compounds, flavonoids, vitamins, terpenoids, and other chemicals that contribute directly or indirectly to the prevention of oxidation [42]. The ability of DPPH to scavenge free radicals was investigated in a variety of solvents (Table 5.). In distilled water, it was found to be higher than acetone, and lowest in chloroform. All of the seaweed extracts tested in this study have strong DPPH scavenging activity, which rises with concentration (concentration ranges from 100g/l to 500g/l). These findings corroborated the findings of [43].

In *S. tenerrimum*, (Table 6.) a linear association between solvent concentration and DPPH inhibition was discovered, with correlation coefficient values (R^2) of 0.930, 0.983, and 0.979, respectively. The coefficients for DPPH-W, DPPH-A, and DPPH-C are 0.964, 0.992, and 0.979, respectively, and are significant at the 0.008, 0.009, and 0.021 levels. Three P-values are below the 0.05 level in this situation. The higher the P-values, the more likely it is. ST-W ($y=0.025x+8.091$, $R^2=0.93$), ST-A ($y=0.0216x+3.84$, $R^2=1$), and ST-C ($y=0.0176x+1.982$, $R^2=1$) were the rectilinear regression coefficients obtained, respectively. ST-W, ST-A and ST-C had beta coefficients of 0.025, 0.022, and 0.017, respectively, which are significant at the 0.05 level. Positive contributions are made by DPPH-W, DPPH-A, and DPPH-C. It means that each of these three factors plays a substantial role in predicting solvent concentrations. As a result, the null hypothesis that each of the three solvent concentrations has a large individual contribution to predicting DPPH is accepted. Because the concentration beta coefficients of ST-W, ST-A, and ST-C are positive. As a result, the three solvents were discovered to be the best predictor of DPPH.

Table 1. Photosynthetic pigment analysis of *S. tenerrimum* (in mg/g of dry weight DW)

Parameters	(mg/g DW)
Chlorophyll a	0.0268±0.0234
Chlorophyll b	0.0067±0.0013
Total Chlorophyll	0.0178±0.0005
Carotenoid	0.5110±0.3103

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

Table 2. Biochemical analysis of *S. tenerrimum* (in mg/g of dry weight DW)

Parameters	(mg/g DW)
Protein	16.51±0.50
Carbohydrate	11.80±0.32
Lipid	0.47±0.03

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

Table 3. Qualitative phytochemical analysis of *S. tenerrimum*

Phytochemicals	Solvents		
	Distilled water	Acetone	Chloroform
Alkaloid	+	-	-
Steroid	-	-	+
Flavonoid	+	+++	+++
Phenol	+++	+++	+
Coumarin	+++	+++	+
Cardiacglycosides	+++	+++	+++
Tannins	+++	+	+
Terpinoids	+++	+	+++
Saponins	+++	+++	+++

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

Table 4. Quantitative analysis of total phenolic and flavonoid content of *S. tenerrimum*

Solvents	Phenols(mg/g)	Flavonoid(mg/g)
Distilled water	8.76±0.29	7.77±0.32
Acetone	6.92±0.23	6.91±0.14
Chloroform	1.89±0.15	2.79±0.21

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

Table 5. DPPH Free radical scavenging activity of *S. tenerrimum*

Sl. No.	Conc extrac tion(μ g/ml)	% Activity(±SD) in Distilled water extract		% Activity(±SD) in Acetone extract		% Activity(±SD) in Chloroform extract	
		Standard(A scorbic acid)	<i>Sargassum tenerrimum</i>	Standard(A scorbic acid)	<i>Sargassum tenerrimum</i>	Standard(A scorbic acid)	<i>Sargassum tenerrimum</i>
1	100	66.66±0.57	9.47±0.41	53.33±0.46	6.42±0.43	90.51±0.26	4.67±0.30
2	200	65.59±0.52	14.87±0.21	52.32±0.57	7.42±0.43	94.52±0.45	5.64±0.24
3	300	67.67±2.08	15.41±0.51	55.48±0.48	10.54±0.47	96.57±0.50	6.77±0.16
4	400	71.66±0.57	17.58±0.50	56.42±0.51	12.58±0.52	97.34±0.34	9.64±0.13
5	500	73.62±0.54	20.61±0.53	58.47±0.45	14.64±0.56	98.54±0.51	10.56±0.14

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

Table 6. Student t-test and Anova for DPPH scavenging and concentration of the extracts of *S. tenerrimum*

Parameters	Multiple R	R-square	LF	Slope	Y-intercept	t-value	P-value	95% confidence level	
								Lower	Upper
DPPH & W	0.964	0.930	4	0.025	8.091	6.15	0.008	0.012	0.037
DPPH & A	0.992	0.983	4	0.022	3.84	13.37	0.0009	0.016	0.027
DPPH & C	0.979	0.957	4	0.017	1.982	6.70	0.021	0.028	0.006

W- distilled water, A- acetone, C- chloroform

One way Anova (concentration of extracts and DPPH variations. Significant (P<0.05).

4. CONCLUSION

According to the findings, *S. tenerrimum* is a good source of storage for molecules such as protein, carbohydrate, and lipids, as well as secondary metabolites and antioxidants. Phytochemicals are commercially important in chemical preparations and contribute in the production of drugs, cosmetics, immune boosters, fertilizers, and a variety of other medications. As a result, this research suggests that algae are important in pharmaceutical, nutraceutical, cosmetic, agricultural, and other industries.

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