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EVALUATION OF BIOACTIVE AND BIOCHEMICAL CONSTITUENTS OF *Ulva lactuca* (Linn.)Le Jolis AND *Padina tetrastromatica* Hauck.

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Abstract: Marine vegetation is relatively primitive when compared to terrestrial vegetation. The most prevalent type of plant found in the water is seaweed. Seaweeds are a type of marine macroalgae that can be found in shallow waterways, estuaries, and backwaters. Since ancient times, people have recognised the value of seaweeds. Lipids, proteins, carbohydrates, and other useful compounds can be found in seaweed. *Ulva lactuca* and *Padina tetrasromatica* were taken from the Thikkodi coast and their physicochemical, antioxidant, biochemical, and phytochemical properties were investigated.

Key words: phytochemicals, seaweed, *Ulva lactuca* , *Padina tetrastromatica*,DPPH

I. INTRODUCTION

Marine environments are made up of various types of habitats that support marine life. Marine ecosystems, unlike terrestrial environments, are constantly changing and transient. The presence of sea water is common to all marine habitats. Seaweeds cover practically all of the marine environments.Seaweeds are classified into three groups based on their pigmentation: brown, red, and green [1]. Brown algae are known as phaephyceaea, whereas red algae are known as rhodophyceae and green algae are known as chlorophyceae.

Edible seaweeds offer the highest nutritional value, containing proteins, vitamins, and minerals [2]. Seaweeds are consumed as food in Asian countries and serve as the raw material for the commercial manufacturing of agar, algin, and caragneenin [3].Seaweeds provide an alternate source of dietary fibres, having a high proportion of soluble to total dietary fibres [4]. Seaweeds have a major role in the pharmaceutical, food, and cosmetic industries in Western Europe as a source of hydrocolloids [5,6].Seaweeds play an important function in pharmacology, particularly for anti-aging and anti-inflammatory actions [7]. Proteins are macromolecules that are necessary for the normal growth and survival of humans [8]. Proteins serve a range of activities in biological systems, including molecular transportation and storage, repair and maintenance, and energy generation.All aminiacids are abundant in seaweeds[9]. The lipid extracts from edible seaweeds demonstrated antioxidant activity and a synergistic impact with tacopherol [10].

Alkaloids, flavonoids, terpenes, amino acids, tannins, phenols, and sterols are some of the most important phytochemicals found in seaweeds. It has a vital part in human health.Flavanoids are a type of chemical found in macroalgae that has anti-inflammatory, anti-hepatotoxic, and anti-ulcer properties, as well as significant anti-allergic, antiviral, and free radical scavenging properties [11]. Seaweed extract is thought to be a good source of phenolic compounds as well. Terpenes make up the majority of them, but fatty acids and nitrogenous substances are also common [12].

II MATERIALS AND METHODS

2.1 Study area

During December 2020, algal samples were obtained from Thikkodi (11°29'N latitude, 75°37'E longitude). The station includes a large rocky promontory with a little sand bay and abundant algal flora. There is no influence from fresh water.

2.2 Collection of seaweeds

Fresh plants of two seaweed species, *Ulva lactuca* (Chlorophyceae) and *Padina tetrasromatica* (Phaeophyceae), were collected at Thikkodi beach.Samples were immediately washed in seawater and brought to the laboratory in plastic bags to remove the

adhering sand. To remove adhering dirt particles and epiphytes, the samples were thoroughly rinsed with tap water, then washed four to five times more to remove all debris and sand particles.

2.3 Preparation of seaweed powder

Water was drained from the sample, which was then spread on blotting paper to absorb any remaining moisture. The samples were pulverised 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.

2.4 Physicochemical analysis of water

The water samples collected from Thikkodi were preserved in the refrigerator to be analysed later. pH, dissolved oxygen, salinity, nitrate, and phosphate were all measured in the research area's seawater. A digital pH metre was used to record the pH of seawater. Inorganic phosphate [13], dissolved oxygen, salinity, and nitrate [14], and dissolved oxygen, salinity, and nitrate [14] were also measured and reported.

2.5 Biochemical composition of seaweeds

Dried algal powder was used to determine the biochemical composition.

2.6 Estimation of moisture

The APAC method(2000) was used to determine the moisture content of seaweeds, with minor modifications .For consistent weights, 2 g samples were put in a crucible and dried at 105°C.[15] .

2.7Total phenolic content

The total phenolic content of the extract was evaluated using the Folin-Ciocalteu reagent [16]

2.8 Total flavonoid content

Flavonoid estimation was done using the calorimetric method [17].

2.9 Antioxidant assay

DPPH radical scavenging assay

The DPPH radical scavenging effects of samples were measured using [18]

III.RESULT AND DISCUSSION

3.1 Physico-chemical analysis of sea water

Hydrographic features

It is the study of measuring and describing physical aspects of the ocean, seas, coastal areas, and lakes, as well as predicting their change through time, for the primary objective of ensuring the safety of navigation and supporting all other maritime operations. Temperature, salinity, pH, and dissolved oxygen of saltwater are important hydrographic parameters that are described here. (Table 1).

The temperature (°C) of the atmosphere was 3.07± 0.28°C, the temperature (°C) of surface water was 28.80±0.35°C, and the salinity (‰) of the saltwater was 34.70±0.28 percent, according to physico-chemical examination of seawater. The pH of seawater was measured, and the pH of seawater was estimated to be 7.440±0.13. The dissolved oxygen of seawater was measured and calculated to be 4.82± 0.11 mg/l. A physico-chemical examination of seawater was undertaken, and nutrients such as nitrate in seawater were calculated to be 1.080±0.02gμ at/l. A physico-chemical examination of seawater was undertaken, and nutrients such as phosphate were determined to be 3.55±0.49g μ at/l.

3.2 Biochemical features

Ulva lactuca and *Padina tetrastromatica* were biochemically analysed, and several biochemical investigations were estimated. Moisture, ash, protein, carbohydrate, and fat were the major biochemical characteristics mentioned here (Table 3). The moisture content of *Ulva lactuca* was determined to be 81.61±0.42% FW based on biochemical study of two seaweed species (fresh weight). *Padina tetrastromatica* has a moisture content of 84.55±0.49% FW. The ash content of *Ulva lactuca* and *Padina tetrastromatica* was measured and found to be 35.73±0.3 percent dry wt and 51.25±0.35% dry wt, respectively (Table 2).

The combination extract (Ethyl acetate+ Ethanol) in *Ulva lactuca* has a high protein content (8.530.16%), whereas hexane extracts have a lower protein content (6.81±0.11%). Biochemical analysis of *Padina tetrastromatica* revealed that the combination extract has a greater protein content (14.85±0.21%), but the hexane extract has a lower protein content (10.61±0.56%). In this biochemical investigation, protein concentration was highest in the combined extract of brown algae *Padina tetrastromatica* and lowest in the hexane extract of green algae *Ulva lactuca*. This finding was consistent with [19]

The carbohydrate content of *Ulva lactuca* was highest in the combination extract (54.91±0.07%) and lowest in the hexane extract (46.61±0.49%). The carbohydrate content of *Padina tetrastromatica* was higher in the combination extract (18.66±0.18%) and lower in the hexane extract (12.71±0.35%). Carbohydrates, which are abundant in *Ulva lactuca* and *Padina tetrastromatica*, are biological compounds. [20] back up the current findings. Carbohydrate content is highest in green seaweed and lowest in brown

seaweed in his data, which is similar to the above result. Carbohydrate deficiency can lead to unhealthful weight loss, hypoglycemia, weariness, and unhappiness.

Ulva lactuca biochemical study revealed that the combination extract has a substantially higher lipid content (6.78±0.32%) than the hexane extract (4.86±0.71%).*Padina tetrastromatica* was studied biochemically, and it was discovered that the combination extract has a high lipid content (2.28±0.31%), whereas the hexane extract has a lower lipid content (1.96±0.06%). The photosynthetic pigments of two seaweeds were studied, and it was discovered that chlorophyll a (2.08±0.02mg/g dry wt) content in *Ulva lactuca* is substantially higher than chlorophyll b (1.66±0.42mg/g dry wt) level in *Ulva lactuca* . Chlorophyll a (1.99±0.01) is considerably higher in *Padina tetrastromatica* than chlorophyll b (0.008±0.002mg/g dry wt). In comparison to *Padina tetrastromatica* (1.66±0.45mg/g dry wt), *Ulva lactuca* has a substantially higher total chlorophyll content . Ulva lactuca (2.71±0.35mg/g dry wt) has a greater carotenoid concentration than *Padina tetrastromatica* (1.81±0.24mg/g dry wt) (Table 2).

Ulva lactuca extracts were analysed phytochemically, and it was discovered that alkaloids and steroids were plentiful in the mixed extract (Ethyl acetate + Ethanol) but only trace amounts in the Hexane extract. Flavonoids and phenols were also found in high concentrations in the combination extract and in low amounts in the hexane extract. Cardiac glycosides and terpenoids were prevalent in the combination extract, but not so much in hexane. Tannins were not found in hexane extract, however they were abundant in mixed extract. Saponin was not found in the mixed extract but was found in abundance in the hexane extract. Phytochemical investigation of *Padina tetrastromatica* extracts was carried out.

Alkaloids and steroids were found in abundance in the combination extract and in trace amounts in the hexane extract. Flavonoids and phenols were plentiful in the mixed extract, while flavonoids were copious and phenols were trace in the hexane extract. Coumarin, cardiac glycosides, tannins, and terpenoids were all plentiful in the mixed extract. Coumarins and cardiac glycosides were prevalent in hexane extract, tannin was only negligible amounts, and terpenoids were significant. Saponins were not found in the mixed extract, but they were abundant in the hexane extract (Table 4).

The phenolic content of *Ulva lactuca* is highest in the combination extract (9.71±0.39mg/g) and lowest in the hexane extract (4.80±0.25mg/g). The combination extract (20.79±0.27mg/g) had a high flavonoid concentration, while the hexane extract (18.83±0.22mg/g) had a lower flavonoid level. *Padina tetrastromatica's* mixed extract has a high phenolic content (8.73±0.38mg/g) and flavonoid content (15.89±0.11mg/g). The phenolic content (2.79±0.28mg/g) and flavonoid content (10.80±0.29mg/g) were the lowest in the hexane extract.(Table 5)

The DPPH free radical scavenging activity of *Ulva lactuca* mixed extract was found to be greater at 500g/ml (66.92±0.08%) and lowest at 100g/ml (59.65±0.14%). *Padina tetrastromatica* had higher activity in 500g/ml (51.73±0.21%) and lower activity in 100g/ml (43.16±0.05%). (Table 6) The DPPH radical scavenging activity of *Ulva lactuca* hexane extract was found to be higher at 500g/ml (60.07±0.021%) and lower at 100g/ml (50.72±0.20%). In *Padina tetrastromatica*, more activity was seen in 500g/ml (40.61±0.40%), whereas less activity was seen in 100g/ml (32.68±0.42%). (Table 7). In Ulva lactuca, with corelation coefficient values of EA+E-UA,H-UA of 0.862.0.992, and Padina tetrastromatica, with corelation coefficient values of EA+E-PT,H-PT of 0.942,0.998, respectively, a linear relationship between solvent concentration and DPPH inhibition was discovered. EA+E-UA,H-UA, EA+E-PT,H-PT had beta-coefficiets of 0.0232, 0.0228,0.0209, 0.0194, respectively, which are significant at the 0.05 level. As a result, the null hypothesis that each of the three solvent concentrations contributes a significant amount to the prediction of DPPH is accepted. As a result, it was observed that the three solvents were the strongest predictors of DPPH.(Table 8)+

Table 1.Physico-chemical analysis of sea water

Temperature (°C)		pH	Salinity (‰)	Dissolved oxygen(mg/l)	Nutrients (µg at/l)	
					Nitrate	Phosphate
Atmos	S.W.	7.44±0.13.	34.70±0.28	4.82±0.11	1.08±0.02	3.55±0.49
3.07±0.28	28.80±.35					

Atmos- Atmosphere, S.W- surface water ,The data are expressed in mean±SE; n=3 in each group.

Table 2.Photosynthetic pigments of two seaweed species(mg/g dry wt)

Parameters	*Ulva lactuca*	*Padina tetrastromatica*
Chlorophyll a	2.08±0.02	1.99±0.01
Chlorophyll b	1.66±0.21	0.008±0002
Total chlorophyll	3.76±0.21	1.66±0.45
Carotenoids	2.71±035	1.81±0.24

Values are mean SD; Sample size (n) =3

Table 3.Biochemicalcomposition of seaweeds

Solvents	Parameters	*Ulva lactuca*	*Padina tetrastromatica*
	Moisture(% FW)	81.61±0.42	84.55±0.49
	Ash(% DW)	35.73±0.32	51.25±0.35
Ethyl acetate+ Ethanol	Protein(% DW)	8.53±0.16	14.85±0.21
Hexane	Protein(%DW)	6.81±0.11	16.61±0.56
Ethyl acetate+ Ethanol	Carbohydrate(%DW)	54.91±0.07	18.66±0.18
Hexane	Carbohydrate(%DW)	Y46.61±0.49	12.71±0.35
Ethyl acetate+ Ethanol	Lipid(% DW)	6.78±0.32	2.28±0.31
Hexane	Lipid(% DW)	4.86±0.71	1.96±0.06

Values are mean SD± Sample size (n)=3

Table 4.Qualitative analysis of various extracts of *Ulva lactuca* and *Padina tetrastromatica*

Solvents	Ethyl acetate + Ethanol		Hexane	
Seaweeds	UEAE	PEAE	UH	PH
Phytochemicals				
Alkaloids	+++	+++	+	+
Steroids	+++	+++	+	+
Flavonoids	+++	+++	+	++
Phenols	+++	+++	+	+
Caumarins	++	++	+++	++
Cardiac glycosides	+++	+++	+++	++
Tannins	+++	+++	—	+
Terpenoids	+++	+++	+++	+++
Saponins	—	—	+++	+++

U-*Ulva lactuca* , P-*Padina tetrastromatica*, (+) – Present (trace amount), (++) – Abundant,(+++) – Very abundant

(-) - Absent

Table 5.Total phenol and flavonoid content of various extract *Ulva lactuca* and *Padina tetrastromatica*

Algae	Solvent	Phenol(mg/g)	Flavonoids(mg/g)
Ulva lactuca	Ethyl acetate+ Ethanol	9.71±0.39	20.79±0.27
	Hexane	4.80±0.25	18.83±0.22
Padina tetrastromatica	Ethyl acetate+ Ethanol	8.73± 0.38	15.89±0.11
	Hexane	2.79±0.28	10.80±0.29

Values are Mean ± SD; Sample size (n)=3

Table 6. DPPH free radical scavenging activity of ethyl acetate + ethanol seaweed extract

No.	Concentration µg/ml	% Activity (±SD)		
		Standard (Ascorbic acid)	*Ulva lactuca*	*Padina tetrastromatica*
1	100	89.95±0.07	59.65±0.14	43.16±0.05
2	200	95.00±1.41	62.54±0.40	46.45±0.06
3	300	97.00±1.41	64.30±0.14	49.39±0.35
4	400	98.66±1.15	65.15±0.042	50.24±0.14
5	500	99.50±0.70	66.92±0.08	51.73±0.21

Values are Mean ± SD; Sample size (n)=3

Table 7.DPPH free radical scavenging activity of hexane seaweed extract

no.	concentration µg/ml	%activity(±sd)		
		Standard ascorbic acid	*Ulva lactuca*	*Padina tetrastromatica*
1	100	65.61±0.28	50.72±0.20	32.68±0.42
2	200	65.86±0.04	53.90±2.27	34.93±0.09
3	300	67.92±0.09	55.66±0.45	36.54±0.65
4	400	67.60±0.42	57.98±0.04	38.47±0.49
5	500	71.72±0.35	60.07±0.021	40.61±0.40

Values are Mean ± SD; Sample size (n)=3

Table 8 student t test and Anova for DPPH scavenging & concentration of the extracts of *Ulva fasciata* and *Padina tetrasromatica*

parameters	Multiple R	R-square	dF	Slope	Y-intercept	t-value	P-value	95% Confidence level	
								lower	upper
DPPH & EA+E-UL	0.929	0.862	5	0.023	56.17	31.70	0.022	0.0061	0.040
DPPH& H-UL	0.996	0.992	5	0.023	48.83	121.76	0.0003	0.0189	0.0266
DPPH&EA+E-PT	0.971	0.942	5	0.021	41.92	42.15	0.006	0.0114	0.0305
DPPH&H-PT	0.998	0.998	5	0.019	30.83	170.33	4.89E-05	0.0176	0.0211

H- Hexane, EA- Ethyl acetate, E- Ethanol, UL-*Ulva fasciata*, PT- *Padina tetrasromatica*

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

IV CONCLUSION

The present work deals with the study of physico-chemical properties of seawater. The parameters such as temperature, pH, salinity, nutrients, ash, moisture and also the photosynthetic pigments of two seaweeds were studied. The present study also provide the information about the phytochemical constituents present in the seaweeds such as alkaloids, phenols, steroids, flavonoids, coumarins, cardiac glycosides, tannins, terpenoids, Saponins and biochemical constituents such as proteins, carbohydrates and lipids, mainly concentrated on the DPPH free radical activity of selected seaweeds collected from Thikkodi coast.

The present study confirmed that in both seaweeds like *Ulva lactuca* and *Padina tetrastromatic*a may be a rich sources of phytoconstituents. It shows variations in this two solvents such as Ethanol+ Ethyl acetate (mixture extract) and hexane because of it's differential solubility. Most of the phytochemicals were found in the mixture extract. Compared to *Ulva lactuca*, *Padina tetrastromatica* showed high amount of proteins. But the carbohydrate and lipid content was high in *Ulva lactuca* when compared to *Padina tetrastromatica*. Both the algae has antioxidant activity and both of them were used as antioxidants in the field of medicinal science.

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