



# EVALUATION OF BIOACTIVE SUBSTANCES AND BIOCHEMICAL COMPOSITION OF *Valoniopsis pachynema* (Martns) Boergs AND *Stoechospermum marginatum* (Ag.) Kutz

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## ABSTRACT

Marine resources have an unrivalled supply of biologically active natural compounds, many of which have structural characteristics not seen in terrestrial creatures. Algal biomass comprises lipids, proteins, and carbohydrates that can be chemically, biochemically, or thermochemically converted into fuels or other important co-products. The goal of this research is to look at the physicochemical, antioxidant, biochemical, and phytochemical properties of *Valoniopsis pachynema* and *Stoechospermum marginatum*, which were gathered from the Thikkodi shore.

Key words: Phytochemical compounds, Algae, *Valoniopsis pachynema*, *Stoechospermum marginatum*, Antioxidant

## 1.INTRODUCTION

The most productive vegetation in the cosmos is marine vegetation. Algae are thought to make up around 90% of marine plant species, and algae contribute roughly 50% of world photosynthesis. Around 8000 varieties of seaweed live throughout the world's coasts, and they can reach depths of up to 270 metres [1]. In the entire sea area, there are 25 species of green seaweeds, 90 species of brown seaweeds, and 350 species of red seaweeds that are commercially important due to their protein, amino acids, and mineral content [2]. Algae can be found in a variety of forms in both the pelagic and benthic regions of the hydrosphere [3]. Marine resources provide an unrivalled supply of biologically active natural compounds, many of which have structural characteristics not seen in terrestrial creatures [4]. Sea vegetation has been used as a food source and a medication by people all over the world for ages. Macro and micro algae, primarily kelp forests, make up marine vegetation. Planktons, which are the principal food source for the marine ecology, are formed by microalgae. Algae have been divided into a variety of categories based on coloration, reserve food materials, and reproductive characteristics. They include the chlorophyceae, phaeophyceae, and rhodophyceae families, among others.

Seaweeds are considered a source of bioactive compounds because they may produce a wide range of secondary metabolites and are characterised by a broad spectrum of biological activities. Brown, red, and green algae have all been found to produce bioactive chemicals [5,6,7]. Vitamins A, B1, B12, C, D, and E are abundant in seaweeds. Secondary metabolites found in seaweeds, such as alkaloids, glycosides, flavonoids, saponins, tannins, and steroids-related active metabolites, have a high therapeutic potential and have been widely exploited in the drug and pharmaceutical industries [8]. Seaweeds are now used as a raw

material in the manufacturing of agar, algin, and carrageenin, although they are still widely consumed as food in Asian countries [9].

Scavenging activity against superoxide and hydroxide radicals, chelating ability, quenching singlets and triplet oxygen, and reducing power are all factors that contribute to these chemicals' antioxidant properties [10,11]. The cell wall composition and fibre content of seaweeds are connected to their physicochemical qualities. Antioxidant, antidiabetic, anticarcinogenic, antibacterial, antiallergic, antimutagenic, and antiinflammatory effects are all found in phenolic phytochemicals [12,13]. In vitro studies have shown that algal polysaccharides act as free-radical scavengers and antioxidants in the prevention of oxidative damage in live organisms [14].

Marine ecosystem diversity and productivity are critical for maintaining the health of the aquatic and terrestrial environments, as well as providing essential sources of food for humans and animals, food additives in the food and cosmetics sector, and pharmaceuticals. Macroscopic sea algae has been closely associated with human life from ancient times, and has been extensively employed in different ways as a source of food, feed, fertiliser, and medicine, with phycocolloids being the most economically important.

Physicochemical investigations are required since the hydrochemistry of that aquatic system has a significant impact on its biodiversity. The quality of the water determines the growth and survival of aquatic species. Lipids, protein, and carbohydrates found in algae biomass can be converted into fuels or other important co-products via chemical, biochemical, or thermochemical methods. In terms of lipid production, algae outperforms other biomass sources, with some estimates claiming that under perfect conditions, algae may produce up to 30 times as much oil per unit area of land as conventional oilseed crops [15].

## 2. MATERIALS AND METHODS

### 2.1 Study area

During December 2020, algal samples were obtained from Thikkodi (11° 29' N lat & 75° 37' E long). The station includes a large rocky promontory with small sand bays and abundant algae flora. There is no influence from fresh water.

### 2.2 Collection of seaweeds

*Valliniopsis pachynema* and *Stoechospermum marginatum*, two seaweed species, were collected from Thikkodi. The samples were promptly washed in seawater to remove the adhering sand and transported to the laboratory in plastic bags. To eliminate connected epiphytes and adherent dirt particles, the samples were thoroughly rinsed with tapwater. The seaweeds were then properly cleaned three to four times with water to remove the dirt and sand particles.

### 2.3 Preparation of seaweed powder

The surplus water was drained away, and the seaweeds were spread out on blotting paper to absorb it. All of the samples were dried at room temperature before being baked for two days at 40°C in a hot air oven. They were then pulverised and stored at room temperature in airtight plastic bottles.

### 2.4 Physicochemical analysis of water

Thikkodi water samples were kept in the refrigerator for analysis. pH, dissolved oxygen, salinity, nitrate, and phosphate levels in the research area's seawater were all measured. A digital pH metre was used to record the pH of the ocean region. Inorganic phosphate [16], dissolved oxygen, salinity, and nitrate [17], as well as dissolved oxygen, salinity, and nitrate [17], were also measured and recorded.

### 2.5 Biochemical composition of seaweeds

The biochemical composition was determined using dried algae powder.

### 2.6 Estimation of moisture

With minor modifications, the moisture content of seaweeds was determined using the method given by [18]. 2 g samples were placed in a crucible and dried at 105°C until consistent weights were obtained.

## 2.7 Qualitative phytochemical screening

Phytochemical screening was carried out by using standard procedure described by [19]

## 2.8 Total phenolic content

The total phenolic content of the extract was evaluated using the Folin-Ciocalteu reagent according to [20].

## 2.9 Total flavonoid content

Flavonoid estimation was done using the calorimetric method [21].

## 2.10 Antioxidant assay

The DPPH radical scavenging effects of samples were measured using the method [22].

## 3.RESULT AND DISCUSSION

Physicochemical properties such as dissolved oxygen, salinity, pH, nitrate, and phosphate were evaluated in a saltwater sample taken from the Thikkodi coast. The temperature of the atmosphere was higher than the surface water temperature. The pH of the water sample was  $7.44 \pm 0.13^\circ\text{C}$ .  $34.70 \pm 0.28$  ‰ was the salinity level. The dissolved oxygen content was measured to be  $4.82 \pm 0.11$  mg/l. Nitrate ( $1.08 \pm 0.02$  µg at/l) and phosphate ( $3.55 \pm 0.49$  µg at/l) were employed in the nutritional analyses.

Biochemical components such as glucose, proteins, lipids, fibre, and ash were found in various extracts (ethyl acetate+ethanol, hexane) of two algae species, *Valoniopsis pachynema* and *Stoechospermum marginatum*.

*Stoechospermum marginatum* had the highest moisture content ( $86.81 \pm 0.24\%$ ) and *Valoniopsis pachynema* had the lowest ( $71.55 \pm 0.049\%$ ). *Valoniopsis pachynema* ( $81.45 \pm 0.63\%$ ) had the highest total ash content, whereas *Stoechospermum marginatum* had the lowest. The highest part content was found in *Stoechospermum marginatum* hexane extract ( $14.78 \pm 0.28\%$ ) and the lowest in *Valoniopsis pachynema* hexane extract ( $9.61 \pm 0.54\%$ ). Carbohydrate content was highest in *Valoniopsis pachynema* ethyl acetate+ ethanol extract ( $35.63 \pm 0.49\%$ ) and lowest in *Stoechospermum marginatum* ethyl acetate + ethanol extract ( $6.68 \pm 0.48\%$ ). In terms of lipids, the highest value was found in an ethyl acetate+ ethanol extract of *Valoniopsis pachynema* ( $7.66 \pm 0.47\%$ ), while the lowest was found in *Stoechospermum marginatum* ( $1.65 \pm 0.42\%$ ). When the photosynthetic pigments were examined, the highest chlorophyll content was found in *Valoniopsis pachynema* ( $4.73 \pm 0.35$  mg/g) and the lowest in *Stoechospermum marginatum* ( $3.66 \pm 0.46$  mg/g). *Stoechospermum marginatum* had the highest carotenoid value ( $6.84 \pm 0.20$  mg/g) and *Valoniopsis pachynema* had the lowest ( $1.81 \pm 0.24$  mg/g).

Alkaloids, steroids, flavonoids, phenols, coumarins, cardiac glycosides, tannins, terpenoids, and saponins were found in several extracts (ethyl acetate+ ethanol, hexane) of two different algae species.

*Valoniopsis pachynema* ethyl acetate+ ethanol extract contains a high concentration of alkaloids, but *Stoechospermum marginatum* ethyl acetate+ ethanol extract and *Valoniopsis pachynema* hexane extract have none. Steroids are plentiful in *Stoechospermum marginatum* ethyl acetate+ ethanol extract but missing in *Valoniopsis pachynema* ethyl acetate+ ethanol extract. Flavonoids are plentiful in both algae's ethyl acetate+ ethanol extracts and are found in trace amounts in their hexane extracts. In ethyl acetate+ ethanol, phenols are prevalent, but hexane has just a minor amount. Coumarins are found in large quantities in hexane and ethyl acetate+ ethanol. *Stoechospermum marginatum* has copious cardiac glycosides in ethyl acetate+ ethanol extract and hexane, however *Valoniopsis pachynema* has abundant cardiac glycosides in hexane extract. Tannins are plentiful in both, but missing in *Stoechospermum marginatum* hexane extract and present in minimal amounts in *Valoniopsis pachynema* hexane. Both extracts have a high concentration of terpenoids. Saponin is prevalent in hexane from *Stoechospermum marginatum* but absent in ethyl acetate+ ethanol.

The concentration of phenol and flavonoids in different extracts of *Valoniopsis pachynema* and *Stoechospermum marginatum* is shown in Table 5. Phenol content was highest in *Stoechospermum marginatum* hexane extract ( $8.90 \pm 0.15$ ) and lowest in *Valoniopsis pachynema* ethyl acetate+ ethanol extract ( $2.41 \pm 0.35$ ). The largest amount of flavonoid was found in an ethyl acetate+ ethanol extract of

*Valoniopsis pachynema*(28.81±0.21), while the lowest value was found in a hexane extract of *Stoechospermum marginatum*(8.88±0.07).

The maximal DPPH free radical scavenging activity of ethyl acetate+ ethanol in *Valoniopsis pachynema* was 79.72±0.37 at a concentration of 200g/ml, while the minimum value was 70.83±0.08 at a concentration of 300g/ml. The highest DPPH free radical scavenging activity of ethyl acetate+ ethanol in *Stoechospermum marginatum* was 4.46±0.49 at a concentration of 500g/ml, while the minimum was 34.86±0.17 at a concentration of 100g/ml

The greatest value for DPPH free radical scavenging activity of *Valoniopsis pachynema* hexane seaweed extract was 42.86±0.042 at a concentration of 500g/ml, and the lowest value was 35.97±0.05 at 100g/ml. The highest value was 33.24±0.14 at a concentration of 500g/ml and the lowest was 27.19±0.014 at a concentration of 100g/ml in *Stoechospermum marginatum*. A linear association was found between the concentration of solvent and DPPH inhibition in *Valoniopsis pachynema*, with correlation coefficient values of VP-EA+E,VP-H of 0.426,0.997, and *Stoechospermum marginatum*, with correlation coefficient values of SM-EA+E,SM-H of 0.968,0.975, respectively. The Beta-coefficients for VP-EA+E are -0.016 in this case, which is negative and significant at the 0.01 level. It implies that the concentration of each solvent has a considerable impact on the prediction of DPPH. As a result, the null hypothesis that no substantial individual contribution of solvent concentration in predicting DPPH is rejected is rejected. VP-H, SM-EA+E, and SM-H have Beta coefficients of 0.021,0.015, and 0.014, respectively, which are significant at the 0.05 level. As a result, the null hypothesis that each of the three solvent concentrations has a large individual contribution to predicting DPPH is accepted. As a result, the three solvents were discovered to be the best predictor of DPPH.

**Table 1. Physico-chemical parameters of seawater**

Temperature(°C)		pH	Salinity(‰)	Dissolved Oxygen (mg/l)	Nutrients(g at /l)	
Atmosphere	S.W				Nitrate	Phosphate
3.07±0.28	28.80±0.35	7.44±0.13	34.70±0.28	4.82±0.11	1.08±0.02	3.55±0.49

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

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

Parameters	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>
Chlorophyll a	3.68±0.45	2.76±0.32
Chlorophyll b	1.056±0.06	0.886±0.13
Total chlorophyll	4.73±0.35	3.66±0.46
Carotenoid	1.81±0.24	6.84±0.20

**Table 3. Biochemical composition of two seaweeds**

Parameters	Solvents	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>
Moisture		71.55±0.049	86.81±0.24
Ash		81.45±0.63	48.00±1.41
Protein	Ethyl acetate+ethanol	10.75±0.28	12.60±0.49
	Hexane	9.61±0.54	14.78±0.28
Carbohydrates	Ethyl acetate + ethanol	35.63±0.49	6.68±0.42
	Hexane	29.72±0.28	7.72±0.28
Lipid	Ethyl acetate+ ethanol	7.66±0.49	1.65±0.42
	Hexane	5.29±0.30	2.80±0.26

**Table 4. Qualitative phytochemical analysis of various extracts of *Valoniopsis pachynema* and *Stoechospermum marginatum*.**

Solvents	Ethyl acetate+ethanol		Hexane	
	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>
<b>Phytochemicals</b>				
Alkaloids	++++	-	-	++
Steroids	-	+++	++	+
Flavonoids	+++	+++	+	+
Phenols	+++	+++	+	+
Coumarins	++	++	+++	+++
Cardiac glycosides	+++	+++	++	+++
Tannins	+++	+++	+	-
Terpenoids	+++	+++	+++	+++
Saponins	-	-	+	+++



**Table 5. Total phenol and flavonoid content of various extracts of *Valoniopsis pachynema* and *Stoechospermum marginatum***

Algae	Solvent	Phenol(mg/g)	Flavonoid(mg/g)
<i>Valoniopsis pachynema</i>	Ethyl acetate+ethanol	2.41±0.35	28.81±0.21
	Hexane	7.8±0.21	28.80±0.23
<i>Stoechospermum marginatum</i>	Ethyl acetate+ethanol	8.87±0.16	15.69±0.20
	Hexane	8.90±0.15	8.88±0.07

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

No.	Concentration g/ml	% of activity(±S.D)		
		Standard	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>
1	100	89.95±0.07	77.41±0.35	34.86±0.17
2	200	25.00±1.41	79.72±0.37	35.70±0.23
3	300	97.00±1.41	70.83±0.08	37.87±0.16
4	400	98.66±1.15	71.87±0.16	38.57±0.42
5	500	99.50±0.70	73.56±0.47	40.46±0.49

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

No.	Concentration g/ml	%of activity (±S.D)		
		Standard	<i>Valoniopsis pachynema</i>	<i>Stoechospermum marginatum</i>
1	100	65.61±0.28	35.97±0.05	27.19±0.014
2	200	65.86±0.04	36.56±0.49	28.90±0.12
3	300	67.92±0.09	38.49±0.21	30.92±0.07
4	400	67.60±0.42	40.97±0.035	32.23±0.07
5	500	71.72±0.35	42.86±0.042	33.24±0.14

**Table 8 student t test and Anova for DPPH scavenging & concentration of the extracts of *Valoniopsis pachynema* and *Stoechospermum marginatum***

parameters	Multiple R	R-square	dF	slope	Y-intercept	t-value	P-value	95% confidence level	
								lower	upper
DPPH & EA+VP	0.652	0.426	5	0.016	79.34	2.22	0.23	-0.049	0.018
DPPH& H-VP	0.999	0.997	5	0.021	32.24	699.15	0.001	0.017	0.024
DPPH&EA+E-SM	0.984	0.968	5	0.015	32.91	60.09	0.016	0.007	0.023
DPPH&H-SM	0.987	0.975	5	0.014	26.31	77.95	0.013	0.007	0.021

H- Hexane, EA- Ethylacetate, E- Ethanol, VP-*Valoniopsis pachynema*, SM- *Stoechospermum marginatum*.

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

#### 4.CONCLUSION

Sea vegetation is said to be the most primitive and productive of all types of vegetation. Many phytochemicals and biochemical substances have been extracted from marine macroalgae and have been employed in medical and industrial applications. Antioxidant activity is also found in marine macroalgae, which has to be investigated further to determine its exact ingredients.

The current study looks at the physicochemical, biochemical, phytochemical, and antioxidant properties of *Valoniopsis pachynema* and *Stoechospermum marginatum*, two maritime macroalgae. Temperature, dissolved oxygen, pH, salinity, and nutrients are among the physicochemical characteristics examined. Ash, photosynthetic pigments, carbohydrates, lipids, protein, and other biochemical characteristics are among them. Alkaloids, steroids, flavonoids, phenols, saponins, cardioglycerides, terpenoids, tannins, and coumarins are among the phytochemical elements studied. The ability of both macroalgae to scavenge DPPH free radicals was investigated.

According to the current study, the sample seawater contains a moderate amount of parameters, and both algae include all phytochemicals and biochemicals. However, the rate at which they are present varies depending on the solvent. *Valoniopsis pachynema* has the most photosynthetic pigments. *Valoniopsis pachynema* has more lipids, carbs, and ash content, although *Stoechospermum marginatum* has higher moisture and protein content.

#### REFERENCES

- [1]Luning,K.1990.Seaweeds,their environment,biogeography and ecophysiology.Willey Interscience Publication.pp.3-370
- [2] Santhanam, R. N., Remanathan, N and Jagathusan, G. 1990. Coastal aquaculture in India. C.B.S Publishers and distributors, 159- 162.
- [3]Ghazala,B and Shameel M 2005,Phytochemistry and bioactivity of some fresh water green algae from Pakistan.Pharmaceutical biology.43(4):358-369
- [4]Saritha,K.,Mani,A.E.,Priyalaxmi,M and Patterson,J,2013.Antibacterial activity and biochemical constituents of seaweed U.lactuca.Glob.col.,7 : 276-282
- [5]Yuan ,Y.V.,Bone,D.E and Carrington, M.F.2005 Antioxidant activity of dulce( *Palmaria palmata*) extract evaluated in vitro. Food Chemistry, 91: 485-494.
- [6]Bansemir,A., Blume,M.,Schroder,S and Lindequist,U.2006.Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252: 79-84.
- [7]Chew,Y.L.,Lima,Y.Y.,Omar,M.,Khoo,K.S.2008.Antioxidant activity of three edible seaweeds from two areas in South East Asia.LWT Food Sci.Technology.41: 1067-1072.

- [8]Elavukal, T.Sivakumar,S.R and Arunkumar,K.2010.fucoidan in some Indian seaweeds found along the coast of Gulf of Mannar. *International journal of Botany*. 6(2): 176- 181.
- [9]Mishra, V.K., Temeli, F., Ooraikul Shacklock, P. F and Craigie, J. S. 1993. Lipids of the red algae *Palmaria palmate*. *Botanica Marina*. 36(2): 169- 176.
- [10]Ruberto, G., Baratta, M. T., Biondi, D.M and Amico, V.2001. Antioxident activity of the marine algal genus *Cystoseria* in a micellar model system. *Journal of Applied Phycology*,13: 403- 407.
- [11]Athukorala, Y., Kim, N and Jeon, Y. J. 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown algae, *Ecklonia cava*. *Food and Chemical Toxicology*. 44: 1065-1074.
- [12]Art,I.C. and Hollman,P.C. 2005.Polyphenols and disease risk in epidemiological studies.American Journal of Clinical Nutrition. 81: 317-325.
- [13]Scalbert,A.,Manach,C.,Remesy,C and Jimenez,L.2005.Dietary polyphenols and prevention of diseases. *Cri Rev Food Sci Nutr.*, 45: 287-306.
- [14]Zhang, Q., Li, N., Liu, X., Zhao, Z., Li, Z and Xu, Z. 2004. The structure of a sulfated galactan from *porphyra haitanensis* and its in vivo antioxidant activity. *Carbohydr. Res.*, 105- 111.
- [15]Sheehan,J.,Dunahay,T.,Benemann,J and Roessler,P. 1998.Alook back at the U.S. Department of Energy's Aquatic Species Program- Biodiesel from algae.Golden, Co.: National Renewable Energy Laboratory,Golden,CO:Report NREL/TP-580-24190.
- [16]Murphy,J. and Riley,J.P.1962. A modified single solution method for the determination of phosphate in natural waters. *Anal.Chem.Acta*. 27: 31- 36.
- [17]Strickland,J.D.H. and Parson,T.R.1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Canada. No. 167*: 1- 811.
- [18]AOAC International .2000.Association of official Analytical Chemists.Official Methods of Analysis of AOAC . Washington DC:AOAC International.
- [19]Harbone, J. B. 1998. Phytochemical methods- A guide to modern technologies of plant analysis. *Third Edn. Chapman and London*. 1- 295.
- [20]Singleton, V. L and Rossi, J. A. 1965. Calorimetry of total phenolics with the Phosphomolybdic, phosphotungstate acid reagent. *American Journal of Enology Viticulture*. 6: 144-158.
- [21]Chang,C.C., Yang,M.H., Ucen,H.M. and Chen,J.C. 2002. Estimation of total flavanoid content in Propolis by complimentary colorimetric method. *Journal of ood and Drug Analysis*. 10: 178- 182.
- [22]Yen,G.H. and Chen,H.Y. 1995. Antioxidant activity of various tea extracts in relation to their anti-mutagenic activity. *Journal of Agriculture and Food Chemistry*. 43: 27- 32.