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Physico-Phytochemical Analysis of Gracilaria salicornia found along Pudupattinam Coast, India

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Abstract

Marine algae can be a rich source of nutrients and bioactive chemicals with pharmacological potential. They are renewable, economically important, polysaccharides rich high-yielding marine source including wide groups of algae among which red algae are predominant and primitive group. *Gracilaria salicornia* a dominant species along Indian coast are commcercially used for agar extraction. In this study marine red algae *G Salicornia* of Pudhupattinam coast was collected and proximate composition, mineral content, pigment levels, phytochemical screening was carried out, further in powder studies FTIR, fluorescence test, was also studied. Proximal analysis showed higher amounts of protein, carbohydrates, non-significant levels of amino acids, less amount of fibers, ash, lipids, minerals and dissolved pigments. further phytochemicals such as alkaloids, tannins, saponins were found to be abundant in *G. salicornia*.

Introduction

Macroalgae, sometimes referred to as seaweed, is one of the most important and renewable marine resources with rich nutrients, trace elements, and bioactive chemicals. They are now subject to greater interest due to their economic significance and abundance of functional food constituents such as polyunsaturated fatty acids (PUFA), essential amino acids, microelements and soluble dietary fibers. They are also reservoirs of bioactive compounds such as polyphenols, phycosterols, sulfated polysaccharides and pigments making them a potential natural resource with a variety of pharmacological advantages like antioxidant (Pangestuti and Kim 2011) neuroprotective (Hanna *et al.,* 2020) and anti-cancerous (Khalifa *et al.,* 2019).

Current study involves study of nutritional value, phytochemical profile in seaweed powder of *Gracilaria salicornia*, one of the most prevalent seaweed species along the coast of Tamil Nadu, India. Proximal compositions such as crude carbohydrate, protein, lipid, fiber, ash. minerals like potassium, zinc, calcium, iron, and sodium. Pigment levels of chlorophyll, carotenoid, fuxoxanthin, phycoerythin, phycocyanin were noted. Phytochemical profiles of alkaloid, flavonoid, tannin, saponin, triterpenes, steroids, glycoside, phenols were screened and powder studies such as fluorescence test, FTIR screening were all performed and datas were recorded. Comparative analysis assists in selecting the finest seaweed as an alternative to existing commercial sources in the food industry, pharmaceutical, and cosmetic industries thus limiting their over-exploitation.

Materials And Methods

Collection and preparation of seaweed sample

Gracilaria salicornia species was collected in the month of December from Pudupattinam coast, Ramanathapuram district, Tamil Nadu, India. Samples were properly cleansed with seawater, then with fresh water and then sealed in black polybags to prevent degradation of compounds by UV radiation. The samples were allowed to shade dry for a week, then blended in a kitchen appliance called an analytical mill, to produce a uniform fine powder. The sample was sieved in 60-mesh filter and the seaweed powder was stored in sample bags at 20◦C until further study.

Fig: Pudupattinam Coast

Proximate composition profiling

Estimation of nutrient profiles such as Crude carbohydrate by Phenol sulphuric acid method (Nielsen et al., 2010, Murugaiyan 2020), Biuret Method for crude protein (Lalitha and Dhandapani 2018, Mahesha 2017), Standard Ninhydrin method for total amino acid and crude fat was extracted by three different methods, 1.Soxhlet method using two separate extractions, one extraction involved sample and hexane solvent, another extraction contained sample with combination of Hexane and Ethanol solvent. 2.Bligh and dyer method 3.Folch method; Acid-Base hydrolysis method (AOAC 2015, Adharini et al., 2019) for crude fiber analysis; Ash content was determined by placing 100mg sample in crucible inside the muffle furnace for 3 hours at 600 ◦C. The sample was left undisturbed for 2days and weighed for changes. Moisture content was determined subtracting fresh weight and dry weight of the sample (Hossain *et al.,* 2021 & Horwitz 2000).

Pigment levels

Pigment extraction was done by grinding 500 mg of seaweed in a pestle and mortar with 10 ml of 80% acetone, the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was collected. The pellet was re-extracted by repeated washing with 5 ml of 80 % acetone until the pellet became colourless. All the extracts were pooled and utilized for pigment quantification. Chlorophyll levels such as chlorophyll a, chlorophyll b, and total chlorophyll were read at 645 and 663 nm in a UV-spectrophotometer. Carotenoid was measured at 480nm, Fucoxanthin (470, 631, 581, 664 nm), Phycocyanin (618, 645, 592, and 645 nm), Phycoerythin (564, 592, and 455nm) (Vimala & Poonghuzhali 2015, Arnon 1949, Kirk and Allen 1965)

By formula:

Carotenoid (μg/g.fr.wt) = ΔA480 + (0.114 × ΔA663) – (0.638 × ΔA645)

Fucoxanthin (mg g-1) = [A470 −1.239 (A631+A581−0.3 ×A664) − 0.0275(A664)] /141

Phycocyanin (mg g-1) = [A618- A 645) – A 592 – A 645) 0.15] 0.15

Phycoerythin (mg g-1) = [(A 564 – A 592) – (A 455 – A 592) 0.20] 0.12.

Where, ΔA = Absorbance at particular wavelength,

Phytochemical screening

Phytochemical screening was performed in two non-polar, two-polar, and one aqueous solvent extract. Each extract was tested for phytochemical compounds (Hossain et al., 2021; Putri et al., 2019, Rout et al., 2020).

Presence of alkaloids

To The algal sample of 0.5ml 2% sulphuric acid was added and heated for two minutes then filtered, five drops of dragendorff's reagent was added to the filtrate, a crimson precipitation indicated the presence of alkaloids.

Presence of flavonoids

The algal extract of 0.5ml was heated with 10ml ethyl acetate for five minutes, to this dilute 1 ml ammonium solution was added and shaken. The appearance of yellow colour indicates the presence of flavonoids.

Presence of tannin

The extract was heated with 10 ml double distilled water and filtered. To the filtrate 0.1% of ferric chloride was added, a blue-black or brownish-green colour indicated the presence of tannin.

Presence of saponin

A stable foam formation was formed when 10 ml water was added to the algal extract, heated and shaken which denoted saponin content in the sample.

Triterpenoid and Steroid Tests

Sample was dissolved in 0.5ml chloroform, 0.5ml anhydrate acetate acid and slightly evaporated. Further few drops of sulphuric acid is added to the walls of the test tube, a blue-green ring formation indicates tri-terpenoid and steroid presence.

Glycoside Test

Appearance of Blue colour on addition of 10 ml conc sulfuric acid and 5 ml acetic acid anhydrate to the extract indicates the presence of glycoside.

Tests for Phenols

10% of freshly prepared ferric chloride solution with distilled water was added to 1 ml of algal extract, appearance of blue-black colour indicates the phenol content.

Seaweed powder analysis

FTIR

FTIR was analyzed using Shimbazu 1R affinity 1. The spectra were scanned at room temperature in absorption mode at the wavelength of 582– 4020 cm−1.

Fluorescence analysis

A pinch of dried algal powder was placed over separate microscopic slide and 1-2 drops of freshly prepared solution like conc HNO_3, conc HCl, conc H_2SO_4, Ammonia solution, $FeCl_3$, Acetic acid, NaOH, distilled water, chloroform and Iodine were added to each slide. The colour change on reaction to solution was observed under both daylight and UV illuminator.

Statistical analysis

One-way analysis of Variance (ANOVA) was used for analyzing data and expressed as mean± standard deviation with at least triplicate analysis with a significant difference at $p<0.05$ and $p<0.01$ respectively.

Results and discussion

Proximate composition profiling

Proximate nutrient content such as crude carbohydrate and protein content was observed to be significant at 1% levels (22.7274±0.001 and 60.9749±0.001) according to F value with $P<0.05$. However amino acid contents were not very significant in the sample (1.0010±0.451) (Table 1). Mean was expressed as percentage for ash, fiber, and lipids (Table 2).The seaweed contained good levels of fiber and average levels of ash, while lipids were analyzed under three different methods, Hexane and ethanol composition yield was high compared to hexane solvent while Bligh and Folch method yielded more lipid than Soxhlet extraction method.

The study analysed the nutritional values, phytochemical, and powder analysis of *Gracilaria salicornia* growing along the coast of Pudupattinam of Ramanathapuram district, Tamil Nadu, India. The proximate profiles showed that seaweed possessed a significant amount of protein, carbohydrate, fiber, ash, and medium levels of lipids and minerals. In the current study the carbohydrate level found was 22.7274±0.001 in the seaweed collected post-monsoon, which on comparison with other Rhodophyceae members collected from Gulf of mannar during post monsoon season showed lower carbohydrate content in *Hynea valentiae* (16.73±0.96) and almost equivalent content in *Solieria robusta* (23.08±1.04) and *Amphiroa fragilissima* (23.78±2.81) (Murugaiyan 2020), West Algerian Coast red macroalgae *Corallina elongata* protein content (Ouzif *et al.,* 2019) was almost similar to *G.salicornia* content. Similarly the fiber and protein values observed in the present study was significantly higher than the six red algae species collected from central and southern regions of chile (Ortiz Viedma *et al.,* 2021)

Proximate Characterisation of red seaweed of Southern Peru was performed by Vilcanqui *et al.,* 2021, the determined mean of ash (13.34) , protein (12.16), and fat (0.16) was lower than the observation made in this study that is ash (13), protein (60.97), fat (6.20) respectively. Penalver *et al.,* 2020 reported nutritional profiles of five cultivated red seaweeds where their protein and fat values were observed insignificant to current values, Like insignificant proximate content of protein (8.55, 11.72); fiber (8.80, 2.49) provided by Hidayah *et al.,* 2022 in two Red algae *Eucheuma cottoni* and *Gracilaria* sps, stating the effectiveness of *G.salicornia* as supplement.

Table 1: Proximate Composition Profiles of *Gracilaria salicornia*

PARAMETER	CARBOHYDRATE (490 nm)	PROTEIN (590 nm)	AMINO ACID (570nm)
F Value P<0.05	22.7274 0.001**	60.9749 0.001**	1.0010 0.451^{NS}

Values are mean ± SD of three samples in each group

** - Significant at 1% level

NS – Not Significant

Table 2: Proximate Composition Profiles of *Gracilaria salicornia*

PARAMETER	ASH	FIBRE	LIPID			
			Soxhlet Method - n-hexane	Soxhlet method- n hexane &ethanol	Bligh & Dyer Method	Folch Method
Mean (%)	13%	29%	2.73%	5.34%	6.20%	6.20%
Standard Deviation	0.01	0.01	0.001	0.001	0.01	0.003

Mineral content analysis

Mineral analysis of *Gracilaria salicornia* showed higher ppm concentration of Iron (4.46±0.13) and lower levels of Calcium (0.16±0.21) as shown in Table 3.

Mineral percentage observed by Penalver *et al.,* 2020 (0.781±0.015) was in accordance with current finding. Mineral concentration of red macroalgae *Corallina elongata* along Algerian coast (Ouzif *et al.,* 2019) the Sodium levels mentioned was higher but almost equivalent to their observation (0.286±0.013 in G.salicornia) while Zinc content observed in *Gracilaria tenuistipitata* (0.789±0.016) and *Hypnea boergesenii* (1.140±0.014) collected along coast of Cox's Bazar, Bangladesh (Hossain *et al.,* 2021) was lesser than G.salicornia Mineral content in G.salicornia of Pudupattinam coast possessed 7% of mineral value which is average but possessed predominant levels of iron.

Table 3: Mineral concentration of *Gracilaria salicornia*

Mineral	Wavelength (nm)	Concentration (Ppm)	Mean ±SD
Potassium	766.5	1.7419	1.75±0.01
Zinc	213.9	1.5552	1.65±0.09
Calcium	422.7	0.0395	0.16±0.21
Iron	248.3	4.3319	4.46±0.13
Sodium	589.0	1.4086	1.51±0.09

Pigment levels

Five different pigment concentration in the sample was determined under various wavelengths using two extraction method. Comparatively, all pigment levels were higher in acetone extract than in Dimethyl sulfoxide (DMSO). Fucoxanthin content was high in acetone extract while carotenoid in DMSO extract showed lower contents of Phycocyanin in both extracts with significance at 1 % levels (Table: 4)

Chinnadurai *et al.,* 2013 accessed algal pigments such as chlorophyll, and carotenoid in *Grateloupia lithophila* and *Hypnea valentiae* two red algae members, the chlorophyll content of both the members 0.52±0.03; 0.61±0.03 respectively was almost in accordance to the chlorophyll content of *G.salicornia* while the carotenoid of both members observed were slightly lower. The carotenoid content reported by Hossain *et al.,* 2021 from *Hynea muciformis*

(31.59±0.3) and *Gellidium pusilium* (52.74±2.18) along the coast of Cox's Bazar Bangladesh compared to the P value of *G.salicornia* seems less insignificant under acetone based extraction. Vimala and Poonghuzhali 2014 estimated Chlorophyll, carotenoids, and fucoxanthin. Total chlorophyll of acetone extract yielded (4.83±0.94; 11.12±1.03; 8.48±1.22; 15.59±3.02) and in DMSO extract (3.12±0.63; 4.56±0.62; 8.59±1.11; 7.74±2.02) similarly for Carotenoid and Fucoxanthin certain values are in accordance with G.salicornia F values (2118.20 and 18869.76)

Table 4: Various Pigment levels of *Gracilaria Salicornia*

PARAMETER	Chlorophyll 645 and 663 nm	Carotenoid 480, 663, 645nm	Fucoxanthin 470, 631, 581, 664, 615 nm	Phycocyanin 615, 280, 620, 652 nm	Phycoerythrin 564, 592, 455nm
F value P<0.01 (80% Acetone)	7106.4500 0.001**	2118.2093 0.001**	18869.7652 0.001**	339.3088 0.001**	4198.2494 0.001**
F value P<0.05 (DMSO)	0.9969 0.375^{NS}	400.3772 0.001**	19.2500 0.001**	0.6108 0.627^{NS}	0.8222 0.484^{NS}

Values are mean ± SD of three samples in each group

** - Significant at 1% level;

NS – Not Significant

Qualitative phytochemical screening

The Red seaweed *Gracilaria salicornia* was screened for phytochemical in a series of non-polar, polar, and aqueous extracts indicated maximum compound solubilisation in Benzene extract (non-polar) and least in aqueous extract. The overall phytochemical analysis in indicated in Table 5. The seaweed contained a rich amount of alkaloids, tannins, saponins and fewer phenols, glycosides, and steroids.

Phytochemical screening of marine red algae *Haymenia palamata* (Deepak *et al*., 2019) their profile shows the absence of tannins and the presence of flavonoids, saponins, glycosides,

alkaloids in the methanol extract of a sample was in accordance with present findings with exception to tannins. Alghazeer *et al.,* 2022 performed phytochemical screening in eight red seaweed growing along the Western coast of Libya the phytochemicals screened are mostly alike with compounds of the present observation.

Table 5: Phytochemical compounds screened in G.salicornia

Solvent Compounds	Benzene	Petroleum Ether	Ethanol	Acetone	Water
Polarity	Non Polar	Non Polar	Polar	Polar	Aqueous
Alkaloid	+	+	+	+	+
Flavonoid	+	-	+	+	-
Tannin	+	+	+	+	+
Saponins	+	+	+	+	+
Triterpenoid & Steroids	+	+	-	-	-
Glycosides	+	+	-	-	-
Phenols	-	-	+	+	-

Seaweed powder analysis

Crude seaweed powder was subjected FTIR analysis, the spectra were scanned at room temperature in absorption mode at the wavelength of 582– 4020 cm−1. Spectral studies showed presence of strong aliphatic, anhydride, aldehyde, alcohol, and ether groups (Table 7).

Fluorescence analysis under UV illuminator showed significant color change of brownish yellow (Table 8) on reaction to certain chemicals was noted to provide datas for identifying adulterants in the crude seaweed sample on comparison with pre-existing data. Since adulterants play a major role within the food industry as adulterants might also cause severe allergies on consumers.

Table 7: FTIR analysis of Red algae *Gracilaria salicornia*

Wavenumber(cm)	Functionalgroup	Type of Vibration	Intensity
582.5	Aliphatic iodo	C–I stretch	Strong
634.58 - 650.01	Aliphatic bromo	C–Br stretch	Strong
1031.92	Anhydride	CO-O-CO stretch	Strong, broad
1222.87	Vinyl ether	C-O stretching	Strong
1373.32- 1444.68	Methyl	C-H asym./sym. Bend	Medium
1653	Alkene	C = C stretch	Medium
1730.15	Aldehyde		Strong
1975.11	Allene	C=C=C stretching	Medium
2524.82	Thiols	S-H stretch	Weak
3273.2	Alcohol(free)	O-H stretching	Strong, broad
3597.24 -3705.26	Alcohol	O-H stretching	Medium

Table 8 represents pure crude seaweed powder reaction to chemical under UV and normal light.

Treatment	Visible/day light	UV light (254 nm)
Powder as such	Light brown	Brown
Powder + Concentrated HNO3	Dark yellow	Yellowish brown
Powder + Concentrated HCl	Brown	Reddish brown

Powder + Concentrated H2SO4	Dark brown	Dark brown
Powder + FeCl3	Greenish yellow	Greenish Yellow
Powder + Acetic acid	Brown	Light brown
powder + NaOH	Bark brown	Brown

Conclusion

Current research indicated that *G.salicornia* contains a good amount of protein, carbohydrate, fiber (13%), ash (13%), lipids (6%) and minerals (7%), and phytochemicals such as alkaloids, flavonoids, tannins and saponins, suggesting that seaweed to be a is promising alternative dietary supplement or functional food for human diet. However, more research is required to determine the fatty acid composition, isolate bioactive compounds, assess additional biological activities like anti-oxidant, anti-cancerous, etc, and to formulate value-added products.

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Disclosure statement

No potential conflict of interest was reported by the authors

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