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A COMPARATIVE STUDY ON BIOCHEMICAL AND PIGMENT PROFILE OF SIX RED SEAWEED SPECIES

M. Sathiyabama[1], G. Subha Devi[1], N. Senthilkumar*[1], P. Murugan[2], and S. Venkatesh[1]

[1]*Centre for Research and PG Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi - 626 124, Tamil Nadu (India)*
[2]*Department of Gunapadam-Maruthiyal, Sri Sairam Siddha Medical College & Research Centre, Chennai - 600 044, Tamil Nadu (India)*
*e-mail: *senthilkumarbotanyaj@gmail.com*

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ABSTRACT

This study evaluated the biochemical composition of six red seaweed species, collected from the Pamban Bridge area in Palk Strait, Tamil Nadu (India). Among the species, *Acanthophora spicifera* had highest carbohydrate content (48.18%) and lowest protein content (1.32%), while *Halymenia dilatata* had highest protein content (15.08%). *Hypnea valentiae* showed lowest carbohydrates (21.03%). Pigment analysis revealed that *Kappaphycus alvarezii* had highest concentration of chlorophyll a and lowest concentration of chlorophyll d. Notably, phycobiliproteins, a characteristic pigment in red seaweeds, were abundant in *H. dilatata*, especially R-phycoerythrin, while *A. spicifera* and *H. dilatata* consistently had R-phycocyanin, and *H. valentiae* had higher R-allophycocyanin. Moisture content in test red seaweeds ranged from 80.13 to 87.12%, and dry matter from 12.88 to 19.87%. *H. valentiae* possessed highest total phenolic content (3.17 mg gallic acid equivalents g^{-1}). The study depicts the potential of these test red seaweeds as source of bioactive compounds for their use in food, nutraceuticals, and pharmaceuticals.

Keywords: Biochemical compounds, phenols, pigments, red seaweeds

INTRODUCTION

Seaweeds, particularly the red seaweeds (Rhodophyta), have long been recognized as rich source of bioactive compounds with diverse use in food, nutraceutical, pharmaceutical, and environmental sectors. Their biochemical composition, which includes polysaccharides, proteins, phenolics, and pigments, have been widely studied for its health-promoting properties such as antioxidant, anti-inflammatory, and antimicrobial activities (Abomohra *et al*., 2016; Ponthier *et al*., 2020). Among red seaweeds, species like *Acanthophora spicifera*, *Gracilaria corticata*, *Grateloupia filicina*, *Halymenia dilatata*, *Hypnea valentiae*, and *Kappaphycus alvarezii* have demonstrated significant nutritional and therapeutic potential. The classification of seaweeds into phyla [brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta)] provides an essential framework for exploring their biochemical diversity and its relationship to specific environmental conditions (Cermeño *et al*., 2020).

Despite growing research, most studies have focused on red seaweeds from Southeast Asia, Europe, and other parts of the world, leaving a gap in the comprehensive evaluation of species found in the Palk Strait region of Tamil Nadu, India. Studies on biochemical composition of seaweeds from this region remains limited, particularly the detailed analysis of their pigment profiles, phenolic content, and overall bioactive potential. Earlier works on seaweeds have mostly overlooked the environmental influences on biochemical traits of these species, emphasizing a need for more region-specific research (Sudhakar *et al*., 2018; Ganesan *et al*., 2019). The present study was aimed

to fill these gaps by offering a detailed analysis of biochemical components of six red seaweed species collected from Pamban Bridge area in Palk Strait. The research focused on biochemical parameters, like carbohydrate, protein, pigment, moisture, and phenolic content, revealing their comprehensive nutritional and functional profiles. The pigment profiles i.e. chlorophyll and phycobiliproteins received special attention as they are crucial for understanding the unique bioactive properties of red seaweeds.

MATERIALS AND METHODS

Collection of red seaweeds and sample preparation

Fresh and healthy specimens of six red seaweed species were collected in 2019 from the Pamban Bridge area in the Palk Strait, Tamil Nadu, India. The collected species included *Acanthophora spicifera* (M. Vahl) Borgesen, *Gracilaria corticata* (J. Agardh) J. Agardh, *Grateloupia filicina* (J.V. Lamouroux) C. Agardh, *Halymenia dilatata* Zanardini, *Hypnea valentiae* (Turner) Montagne, and *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva. The seaweed samples were immediately transported to the laboratory and thoroughly washed under running tap water to remove salts, sand, and epiphytes. After rinsing with distilled water, the samples were air-dried at room temperature light. The dried seaweed was then milled into a fine powder and stored at 4°C until further analysis.

Total carbohydrate content

The 100 mg algal samples were ground thoroughly with acid-washed sand and mixed with 5 mL of sodium phosphate buffer at pH 6.8. The mixtures were centrifuged at 5000 rpm for 10 min (Remi, Mumbai, India), and the supernatant collected for carbohydrate estimation as per Dubois *et al*. (1956). For carbohydrate estimation, 1 mL sample was mixed with 1.0 mL of 5% phenol solution and 5.0 mL of sulphuric acid. After vigorous vortex, the mixture was left to stand at room temperature for 30 min. Optical density was read at 490 nm using a DU 40 spectrophotometer (Beckman, USA). A standard graph was constructed with various concentrations of D-galactose ranging from 10 to 100 µg mL^{-1}.

Total protein content

The algal samples (100 mg) were ground with acid-washed sand and mixed with 5 mL sodium phosphate buffer (pH 7.0). The mixtures were then centrifuged (Remi, Mumbai) at 5000 rpm for 10 min. The supernatant was collected for total protein estimation as per Bradford (1976). For this, 0.1 mL sample was combined with 0.9 mL double-distilled water and 5 mL Coomassie Brilliant Blue G-250 reagent. The mixture was thoroughly mixed, and the absorbance measured at 595 nm using a DU 40 Spectrophotometer (Beckman, USA), with a reagent blank as the reference. The total protein content was determined from a standard curve constructed with bovine serum albumin (BSA) concentrations ranging from 10 to 100 µg mL^{-1}.

Total lipid content

The algal samples (100 mg) were finely ground with acid-washed sand and mixed with 6.0 mL chloroform (2:1). The mixtures were transferred to the separating funnels and combined with 2.0 mL 0.9% NaCl solution, then thoroughly mixed. After allowing the mixture to separate overnight, 0.5 mL of lower chloroform phase which contain the lipids was collected in a vial. The chloroform was evaporated at room temperature, leaving the lipids behind. The total lipids were estimated as per Folch *et al*. (1956). The 0.5 mL conc. H_2SO_4 was added to algal samples and mixed thoroughly. The tubes were sealed with glass marbles, placed in a boiling water bath for 10 min, and cooled at room temperature. Then, 0.2 mL sample was mixed with 5 mL phosphovanillin reagent and allowed to stand for 30 min. The developed colour was measured at 520 nm using a DU 40 spectrophotometer. A standard curve was prepared using cholesterol concentrations ranging from 10 to 100 µg mL^{-1}.

Estimation of photosynthetic pigments

The algal samples (500 mg) were taken and finely ground in acid-washed sand with 5.0 mL diethyl ether using a pre-chilled pestle and mortar for 5 min. The resultant homogenates were stored at 4°C in a refrigerator overnight. Then the samples were centrifuged at 5000 rpm for 5 min. The supernatants were collected and their absorbance measured at 663 and 688 nm for chlorophyll a (Chl. a) and chlorophyll d (Chl. d), respectively, spectrophotometrically as per the method of Smith and Benitez (1955). The Chl. *a* and Chl. d contents were calculated using the following formulas:

$$\text{Chl. } a \ (\mu g mL^{-1}) = 9.92 \times A663 - 1.15 \times A688$$

$$\text{Chl. d} \ (\mu g mL^{-1}) = -0.166 \times A663 + 9.09 \times A688$$

Estimation of phycobiliproteins

One gram of The algal samples (1 g) were placed in sterile container were subjected to repeated freezing and thawing in 1 mM phosphate buffer at pH 7.2 for three cycles. Subsequently, the biomass was separated via centrifugation at 8,000 rpm for 15 min. The quantities of R-phycoerythrin (R-PE), phycocyanin (PC), and allophycocyanin (APC) pigments were determined as per Bennet and Bogorad (1973) by measuring the absorbance of supernatant at 562, 615, and 652 nm, respectively, spectrophotometrically. The concentrations of pigments were calculated by using the following formulas:

$$\text{PC (mg mL}^{-1}) = \frac{(\text{OD 615 nm} - 0.474 \times \text{OD 652 nm})}{5.34}$$

$$\text{APC (mg mL}^{-1}) = \frac{(\text{OD 652 nm} - 0.208 \times \text{OD 615 nm})}{5.09}$$

$$\text{PE (mg mL}^{-1}) = \frac{((\text{OD 562 nm} - 2.41\ (\text{PC}) - 0.849\ (\text{APC}))}{9.62}$$

Estimation of moisture, dry matter and ash content

The moisture content was assessed as per the methods of AOAC (2015). The algal samples, 2 g each, were taken in separate crucibles, oven-dried at 105°C till constant weight, and the moisture and dry matter contents calculated. The dried samples were incinerated in a muffle furnace (Carbolite, UK) overnight at 525°C to obtain ash content. The ash contents were determined as per AOAC (2015) with slight modifications.

Estimation of total phenols

Finely ground seaweeds were extracted using methanol and filtered to obtain clear extract. A reaction mixture was prepared by mixing 1 mL extract with 5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to react for 5 min in dark. Sodium carbonate solution (4 mL of 7%) was added to neutralize the reaction, and the mixture was incubated for 30 min in dark at room temperature. The absorbance was measured at 756 nm spectrophotometerically as per Makkar (2003). The total phenolic contents were quantified using a standard curve prepared with gallic acid, and the results expressed as mg gallic acid equivalents (GAE) g^{-1}dry weight.

Statistical analysis

The experiments were carried out in triplicate. The data generated was analysed by ANOVA and the results presented as mean values $\pm$ standard deviations, as well as percentage values.

RESULTS AND DISCUSSION

The carbohydrate content in six test red seaweeds ranged from 21.03 to 48.18%, with highest carbohydrate content (48.18%) in *Acanthophora spicifera,* followed by *Gracilaria corticata* (42.22%), and lowest in *Hypnea valentiae* (21.03%) [Table 1]. The findings are in close proximity to Murugaiyan (2020) who reported 42.05% carbohydrate in *G. corticata* (summer), while *A. spicifera* (post-monsoon) had a maximum of 67.25% carbohydrates.

In test seaweeds the total protein contents ranged from 4.18 to 15.08% of dry weight (Table 1). Amongst the test seaweeds *H. dilatata* had highest protein content (15.08%), followed by *G. corticata* (13.41%). The lowest protein content was found in *H. valentiae* (4.18%). These findings are consistent with those of Farghl *et al*. (2021), who reported 5 and 17% proteins in seaweeds. Rajaram *et al*. (2021) have reported that *K. alvarezii* had protein content of 9.59% during summer. Differences observed in protein content among the seaweeds, both across genera and within species, may be attributed to various factors like environmental conditions, nutrient availability, water quality, and geographical location (Fiset *et al*., 2017). Notably, the protein content in *G. corticata* (13.41% dry weight) closely resembled the value reported by Patel *et al*. (2020) for the same species (12.56 ± 0.03% of dry weight).

Table 1: Biochemical composition of six red seaweeds

Composition	*A. spicifera*	*G. corticata*	*G. filicina*	*H. dilatata*	*H. valentiae*	*K. alvarezii*
Carbohydrate (%)	48.18 ± 0.86	42.22 ± 0.90	38.35 ± 0.28	27.23 ± 0.26	21.03 ± 0.88	23.23 ± 0.48
Protein (%)	11.33 ± 0.16	13.41 ± 0.17	5.55 ± 0.28	15.08 ± 0.29	4.18 ± 0.25	9.34 ± 0.12
Lipid (%)	1.32 ± 0.10	3.08 ± 0.14	2.35 ± 0.21	2.72 ± 0.11	1.905 ± 0.09	3.79 ± 0.14
Ash (%)	8.05 ± 0.18	4.09 ± 0.25	7.98 ± 0.54	8.55 ± 0.22	15.24 ± 0.25	17.91 ± 0.52
Moisture (%)	86.18 ± 0.51	80.70 ± 0.56	82.57 ± 0.48	81.75 ± 0.61	87.12 ± 0.43	80.13 ± 0.44
Dry matter (%)	13.82 ± 0.51	19.30 ± 0.56	17.43 ± 0.48	18.25 ± 0.61	12.88 ± 0.43	19.87 ± 0.44
Total phenolics (mg GAE eq. g^{-1})	1.44 ± 0.29	1.29 ± 0.25	0.80 ± 0.25	2.58 ± 0.31	3.17 ± 0.20	0.54 ± 0.28

The values are expressed as mean ± standard deviation

The lipid content in test seaweeds varied from 1.32 to 3.79% (Table 1). *K. alvarezii* had highest lipid content of 3.79%, followed by *G. corticata* (3.08%); while *A. spicifera* possessed lowest lipid contentof1.32%. These findings are in agreement with Polat and Ozogul (2008), and Ganesan *et al*. (2020). Generally, seaweeds have low lipid content, typically <4% (Gosch*et al*., 2012; Khairy and El-Shafay, 2013). *G. filicina* had a lipid content of 0.21-1.0% (Sahu and Kumar, 2014). Chellamanimegalai *et al*. (2022) reported 0.89% lipids in seaweeds, both of which were lower than the values observed in present study. Patel *et al*. (2020) found a lipid content of 0.44% in *G. corticata*, which is notably lower than observed values in this study.

The moisture content in red seaweeds ranged from 80.13to 87.12%, with highest moisture content in *H. valentiae* and lowest in *K. alvarezii* (Table 1). Typically, the seaweeds have high moisture content ranging from 75 to 85% (Polat and Ozogul, 2008; Gupta *et al*., 2011). Sahu and Kumar (2014) reported a moisture content of 82.63% in *G. filicina* while Rode and Surekha (2017) observed moisture content of 95.99% in *A. spicifera*. The moisture content in *K. alvarezii* closely resembled with the values reported by Ahmad *et al*. (2016) for the same species. The dry matter in red seaweeds ranged from 12.88to 19.87% (Table 1). The values were similarly comparable with 13.17% reported by Polat and Ozogul (2008) for *A. nayadiformis*. The ash content in red seaweeds was in the range of 4.09 to 17.913% (Table 1). Contrarily, Osman *et al*. (2016) have reported higher ash content of 40.3% in *H. valentiae* while Patel *et al*. (2020) reported an ash content of 32.08% in *G. corticata*, which were higher than the value observed for the same species in present study.

In red seaweeds, the chlorophyll *a* (Chl. *a*) and chlorophyll d (Chl. d) are crucial photosynthetic pigments. *G. corticata* exhibited highest Chl. *a* content of 0.127%, while *K. alvarezii* had the lowest content of 0.058%. The Chl. d content ranged from 0.003 to 0.013% in seaweeds, with *A. spicifera* having maximum and *K. alvarezii* having minimum values. In contrast, Rajaram *et al*. (2021) have observed higher Chl. *a* level (0.68 to 0.87 mg g^{-1} fresh weight) in *K. alvarezii*, while Eswaran *et al*. (2001) reported even higher levels of 0.952 ± 0.47 mg g^{-1} fresh weight in these species.

The fluorescent pigmented proteins called phycobiliproteins, responsible for red hue colour of red seaweeds, reportedly comprise of50% of the total proteins (Niu *et al*., 2007). Among these proteins, R-phycoerythrin was most abundant in *H. dilatata* (0.64 mg g^{-1} fresh wt), while R-phycocyanin and R-allophycocyanin showed similar levels in *H. dilatata* and *A. spicifera* (0.138 mg

g^{-1}) [Table 2]. R-allophycocyanin was high in *H. valentiae* (0.222 mg g^{-1}) as compared to *K. alvarezii* (0.128 mg g^{-1}). These findings are in agreement with Senthilkumar *et al*. (2013). Francavilla *et al*. (2013) noted the phycocyanin in *G. gracilis* in the range of 3.0 to 0.7 mg g^{-1}, while Pina *et al*. (2014) found a phycocyanin content of 149 mg kg^{-1} in *Chondrus crispus.*

Table 2: Photosynthetic pigments and phycobiliproteins content in red seaweeds

Red seaweed species	Photosynthetic pigments (mg g^{-1}fresh wt)		Phycobiliproteins (mg g^{-1}fresh wt)		
	Chl. *A*	Chl. D	R-PE	R-PC	R-APC
Acanthophora spicifera	0.109 ± 0.005	0.013 ± 0.0005	0.281 ± 0.003	0.138 ± 0.013	0.192 ± 0.014
Gracilaria corticata	0.127 ± 0.004	0.010 ± 0.001	0.239 ± 0.009	0.055 ± 0.013	0.211 ± 0.006
Grateloupia filicina	0.082 ± 0.005	0.005 ± 0.001	0.187 ± 0.01	0.113 ± 0.017	0.164 ± 0.011
Halymenia dilatata	0.091 ± 0.001	0.008 ± 0.001	0.640 ± 0.011	0.138 ± 0.012	0.176 ± 0.009
Hypnea valentiae	0.081 ± 0.0004	0.007 ± 0.001	0.409 ± 0.008	0.030 ± 0.026	0.222 ± 0.037
Kappaphycus alvarezii	0.058 ± 0.0003	0.003 ± 0.0003	0.204 ± 0.011	0.056 ± 0.013	0.128 ± 0.012

Chl. A = Chlorophyll A, Chl. D = Chlorophyll D; R-PE = R-phycoerythrin, R-PC = R-phycocyanin, and R-APC = R-allophycocyanin; The values are expressed as mean ± standard deviation

In present study, the total phenolic content of red seaweeds,extracted by methanol extraction, ranged from 0.8 to 3.17 mg GAE g^{-1} [Table 1]. However, Arul Kumar *et al*. (2018) reported a higher phenolic content of 4.00 mg GAE g^{-1} dry weight in *G. corticata*. Chakraborty *et al*. (2015) observed a total phenolic content of 9.8 mg GAE g^{-1} in methanolic extract of *H. valentiae,* while Rajaram *et al*. (2021) reported 1.86 GAE in *K. alvarezii,* both of which were higher than the values observed inthe same species. The composition of seaweeds can differ based on various factors such as species, habitat, maturity level, water conditions, and environmental surroundings (Dhargalkar *et al*., 1980).

Conclusion: The bioactive compounds in the test red seaweed species depicted their substantial potential as a bio resource for food, cosmetics, pharmaceutical, and biotechnological industries. The biochemical composition and nutritional content emphasize their broad field applications related to human health and environmental problems. Further research is desired for sustainable management of red seaweeds in marine ecosystems.

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