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SHORT COMMUNICATION

ASSESSMENT OF PHYTOCHEMICALS IN SOME RED SEAWEEDS GROWING ALONG THE GULF OF MANNAR COASTAL REGION OF INDIA

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ABSTRACT

Seaweeds are potential renewable resources in marine environment. This study explored the biochemical composition of red seaweeds collected from the Gulf of Mannar, Tamil Nadu, India focussing on the species like *Hypnea musciformis*, *Gracilaria salicornia*, *G. folifera, G. crassa* and *Jania rubens*. The results showed that amongst the tested red seaweeds *H. musciformis* had highest carbohydrate content (28.21%), while *G. salicornia* had highest lipid content (3.89%). *J. rubens* contained the most protein (14.47%), whereas *G. folifera* had highest levels of chlorophyll *a* and flavonoids (20.1%). *G. crassa* had highest phenol content (7.91%) while ash content was higher in *J. rubens* (39.1%) and lower in *G. salicornia* (18.2%). Phycocyanin and allophycocyanin were maximum in *G. crassa*; whereas highest phycoerythrin was noticed in *H. musciformis*. Conversely, minimum phycobiliprotein pigments were found in *G. salicornia* and *G. crassa.* The findings highlighted the potential of these seaweeds for medicinal and industrial applications.

Keywords: Gulf of Mannar, phytochemical analysis; red seaweeds

INTRODUCTION

Seaweeds, depending on their thallus pigmentation and chemical composition, are classified into three groups *viz*., Chlorophyceae (green algae), Rhodophyceae (red algae) and Phaeophyceae (brown algae) (Manzelat *et al.,* 2018). Phytochemical analysis of marine red seaweed *Gracilaria crassa* has revealed the presence of carbohydrates, protein, lipids, phenols, flavonoids, alkaloids, and chlorogenic acid in its extract (Parvathi *et al.,* 2021). Red seaweeds contain high protein content as compared to the brown species while protein content of macroalgae varies significantly from species to species and also fluctuates within the species with season (Harnedy *et al.,* 2011). Seaweeds being rich in various phytochemicals and antioxidants show potential benefits for human health. The phytochemicals in macro-algae and their antioxidant activities are sometimes correlated (Widowati *et al.,* 2021). Phycobiliproteins are water-soluble non-toxic proteins mainly found in red seaweeds and cyanobacteria. These red pigmented proteins have strong fluorescence properties and absorbance. Moreover, it is widely used in cosmetics, food, biomedical and pharmaceutical industries (Sonani *et al.,* 2016). The present study was aimed to assess the biochemicals like carbohydrate, protein, lipids, chlorophyll (chl *a* and chl d), ash, phenol and flavonoids as well as phycobiliporteins in some red seaweeds (*G. salicornia, G. folifera, G. crassa, J. rubens*, and *H. musciformis)* collected from the coastal area of Gulf of Mannar, Tamil Nadu (India).

MATERIALS AND METHODS

Fresh and healthy specimens of five seaweed species were collected in February-March 2019 along the Mandapam coast in the Gulf of Mannar, Tamil Nadu, India, during low tide at the depth of 1-5 m. The seaweeds were handpicked, washed with seawater to remove debris, and placed in an ice box for transport to the laboratory. The seaweeds were identified as *Hypnea musciformis* (Wulfen), *Jania rubens* (Linnaeus), *Gracilaria salicornia* (C. Agarth), *G. folifera* ((Forsskål), and *G. crassa* Harvey ex (J. Agardh). After thorough washing with tap water, these seaweeds were dried on blotting paper, and representative samples shade-dried and finely powdered for proximate analysis of carbohydrates, proteins, total lipids, ash, phenols, flavonoids and phycobiliproteins. For proximate analysis, 100 mg algal samples were ground well in acid-washed sand along with 5 mL sodium phosphate buffer (pH 6.8) and centrifuged (Remi, Mumbai) at 5000 rpm for 10 min. The supernatants were used for the estimation of total carbohydrates and proteins.

Estimation of total carbohydrates

The total carbohydrates were extracted as per phenol-sulphuric acid method (Dubois *et al.*, 1956). For this, 1.0 mL sample was mixed with 1.0 mL of 5% phenol and 5.0 mL of H_2SO_4. After vortexing, the solution was left at room temperature for 30 min, and optical density measured at 490 nm in a DU 40 spectrophotometer (Beckman, USA). The total carbohydrates calculated with the help of a standard curve prepared from D-galactose (10-100 μg mL^{-1}).

Estimation of total protein

The total protein was estimated as per Bradford **(**1976**)**. For this, 0.1 mL extracted sample was mixed with 0.9 mL double distilled water and 5 mL Coomassie Brilliant Blue G-250 reagent. The absorbance was measured spectrophotomerically at 595 nm, and the amount of total protein calculated using a standard graph prepared with bovine serum albumin (10-100 μg mL^{-1}).

E*stimation of total lipid*

The lipid extraction was done by chloroform-methanol mixture method (Folch *et al.*, 1956). For this, 100 mg of each algal sample was ground with 6.0 mL chloroform: methanol (2:1) and transferred to a separating funnel, to which 2.0 mL of 9.0% NaCl solution was added. The mixture was left undisturbed overnight, and 0.5 mL of lower chloroform phase containing lipids was collected and allowed to evaporate at room temperature. For lipid estimation, 0.5 mL conc. sulphuric acid was added to the sample, mixed, and heated in a boiling water bath for 10 min. After cooling, 0.2 mL sample was mixed with 5 mL phosphovanillin reagent and left to stand for 30 min. The developed colour was measured spectrophotometrically at 520 nm using cholesterol ((10-100 μg mL^{-1}) as standard curve.

Estimation of photosynthetic pigments

For determination of photosynthetic pigments, as per Smith and Benitez (1955), 500 mg algal sample was finely ground along with 5 mL diethyl ether for 5 min and the homogenate kept overnight at 4°C in a refrigerator. Then the mixture was centrifuged at 5000 rpm for 5 min to collect the supernatant. Chlorophyll a (Chl.a) and chlorophyll d (Chl.d) were measured at 663 nm and 688 nm, respectively, using a DU 40 Spectrophotometer. The chlorophyll *a* and d were calculated as follows:

$$\text{Chl } a \text{ } (\mu g\ mL^{-1}) = 9.92 \times A663 - 1.15 \times A688$$
$$\text{Chl d } (\mu g\ mL^{-1}) = -0.166 \times A663 + 9.09 \times A688$$

Moisture and ash content in selected seaweeds

The moisture content in algal samples was recorded in a moisture analyser (Mettlerr Toledo GMBH, German). The ash content was estimated by first ashing the well-ground known quantity of dried samples in a crucible in a muffle furnace for 3 h at 550°C and then weighing it again to calculate the ash content.

Estimation of total phenolic and flavonoid content

Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent method (Singleton and Rossi, 1965). For TPC estimation, dried seaweed powders were extracted with non-boiled distilled water at 40°C for 3 h, and then with boiled water for 10 min. The extracts were mixed with Folin-Ciocalteau reagent and incubated for 120 min. The absorbance was measured at 765 nm and TPC expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight of sample.

For flavonoids, the aluminium chloride calorimetry assay of Zhishen *et al*. (1999) was followed. Quercetin was used for preparing the calibration curve and the absorbance of samples was read at 510 nm.

Extraction and estimation of phycobiliproteins (Bennet and Bogard, 1973)

Algal samples (1 g each) were taken in sterile containers and kept in 1 mM phosphate buffer at pH 7.2 for freezing and thawing. The biomass was separated by centrifugation at 8000 rpm for 15 min. The quantity of phycocyanin, allophycocyanin and and phycoerythrin pigments were recorded by reading the supernatants at 562, 615 and 652 nm, spectrophotometerically using the following formulae:

$$\text{Phycocyanin [PC] (mg mL}^{-1}) = \frac{\text{A615 nm} - 0.474 \times \text{A652 nm}}{5.34}$$

$$\text{Allophycocyanin [APC] (mg mL}^{-1}) = \frac{\text{A652 nm} - 0.208 \times \text{A615 nm}}{5.09}$$

$$\text{Phycoerythrin (mg mL}^{-1}) = \frac{\text{A562} - 2.41\,(\text{PC}) - 0.849\,(\text{APC})}{9.62}$$

All the experiments were conducted in completely randomized design and each treatment replicated three times. The data were statistically analysed and reported as mean ± standard deviation.

RESULTS AND DISCUSSION

In present study, five red seaweeds namely *Hypnea musciformis*, *Jania rubens*, *Gracilaria salicornia*, *G. folifera*, and *G. crassa*, collected along the Gulf of Mannar coast of South Tamil Nadu (India) at a depth of 1 to 5 m during tide for proximate composition evaluation. The carbohydrate content in test red seaweeds varied from 19.57 to 28.21%, with maximum content in *H. musciformis*, followed by *J. rubens* and *G. folifera* (Table 1). Siddique *et al.* (2013) also reported similar results in *H. musciformis* and *H. pannosa* (22.86 and 20.60%). While Khairy and EI-Shafay (2013) reported 42.2% carbohydrates in *Jania rubens.* Conversely, some red seaweeds are reportedly high in carbohydrates *viz*., *G. edulis* (97.69 mg g^{-1}), and *G. acerosa* (96.29 mg g^{-1}) (Roy and Anantharaman, 2017).

The protein content in red seaweed samples fluctuated from 7.98 to 14.47% with maximum in *J. rubens* and minimum in *G. folifera* (7.98%) (Table 1). Similar findings were reported in *J. rubens* (Nazni and Deepa, 2015; Ismail *et al.*, 2017). Red seaweeds possess high amounts of proteins as compared to other seaweeds (Mwalugha *et al.*, 2015). *H. musciformis* isolated from Kenyan coast had high protein content (19.8%) as compared to other seaweed species (Carneiro *et al.*, 2014).

Table 1: Proximal phytochemical analysis (% DW) of five seaweeds in Gulf of Mannar coastal regions of Tamil Nadu, India [mean ± SD, n = 3)

Proximal analysis	*G. salicornia*	*J. rubens*	*H. musciformis*	*G. folifera*	*G. crassa*
Carbohydrates	19.57 ± 0.40	24.58 ± 0.15	28.21 ± 0.33	22.23 ± 1.20	20.24 ± 0.10
Proteins	13.14 ± 0.19	14.47 ± 0.16	11.15 ± 1.24	7.98 ± 0.09	9.24 ± 0.60
Lipids	3.89 ± 0.34	1.89 ± 0.20	0.88 ± 0.31	3.23 ± 0.14	0.97 ± 0.18
Chl *a*	0.87 ± 0.18	0.69 ± 0.19	0.85 ± 0.17	2.80 ± 0.30	0.66 ± 0.25
Chl d	3.02 ± 0.13	1.92 ± 0.11	3.58 ± 0.82	4.80 ± 0.40	2.80 ± 0.30
Ash	18.20 ± 0.62	39.10 ± 1.90	21.89 ± 1.80	28.22 ± 1.16	34.18 ± 1.15
Phenol (GAE g^{-1})	5.09 ± 0.09	0.64 ± 0.04	1.07 ± 0.03	2.46 ± 0.22	7.91 ± 0.32
Flavonoid (GAE g^{-1})	2.23 ± 0.11	0.39 ± 0.06	2.39 ± 0.20	20.10 ± 1.10	4.45 ± 0.34

The lipid content in test red seaweeds ranged from 0.88 to 3.89%, with maximum lipids in *G. salicornia* (Table 1). Comparatively, lower lipid contents have been reported in *G. salicornia*, and *J. rubens* (Mwalugha *et al.*, 2015; Freitas *et al.*, 2022). Notably *Gracilaria folifera* from Red sea contained high amount of lipids as compared to Indian species (Bhaskar *et al.*, 2004).

The photosynthetic pigments were highest in *G. folifera* (Chl. *a* 2.8%, Chl d 4.8%) and lowest in *G. crassa* and *J. rubens* (Table 1). Previous studies reported that *L. obtusa* had chlorophyll *a* content of 0.24 mg g^{-1}, much higher than chlorophyll d (0.0008 mg g^{-1}) (Hegazi, 2002). Almost similar value of Chl *a* was reported in *C. officinalis* and *C. elongate* (Abou Gabal *et al.*, 2021).

The ash contents in red seaweeds ranged from 18.2 to 39.1% with maximum in *J. rubens* and minimum in *G. salicornia* (Table 1). Conversely, higher amount of ash has been reported in *G. crassa* (43.18%) and *G. foliifera* (39.33%) (Baghel *et al.*, 2014), in *G. folifera* (39.33%) (Osman *et al.*, 2017). Comparatively lower level has been reported in *Jania rubens* (25.53%) (Ismail *et al.* 2017).

Total phenolic content (TPC) analysis showed the values between 0.64 and 7.91 mg GAE g^{-1}, with higher content in *G. crassa* and minimum in *J. rubens* (Table 1). However, Ismail *et al.* (2017) have reported maximum phenol content in *J. rubens* (29.55 mg g^{-1}) and *G. salicornia* (23.37 mg) [Nursid *et al.*, 2020].

Flavonoid content in red seaweeds was in the range of 0.39-20.1% with maximum in *G. folifera* and minimum in *J. rubens* (Table 1). Similar results were reported in *J. rubens* (Dixit and Reddy, 2017) and in *G. folifera* (Osman *et al.*, 2017). Comparatively, low flavonoid content in *Hypnea musciformis* and *J. rubens* was reported by Alghazeer *et al.* (2022). Previous studies indicated higher flavonoid contents in other species, revealing that extraction efficacy are influenced by solvent type used.

The mean phenol content of 0.35% has been reported in *G. bursa-pastoris* (Yildiz *et al.*, 2011) while Murugan and Iyer (2012) reported phenol in MeOH extract of *Gracilaria salicornia* higher than *G. edulis*. Moreover, the aqueous extract minimum level in *G. salicornia* in contrast result showed in *Gracilariopsis longissima* (Elalla and Shalaby, 2009). Maximum amount of phenol was observed in *J. rubens* (29.55 ± 11.73 mg g^{-1}) (Ismail *et al.*, 2017). *G. salicornia* showed the high quantity of phenol content of 23.37 mg (Nursid *et al.*, 2020). *A. spicifera, L. papillosa, and G. corticata* had a good quantity of polyphenol contained at ranged from 4.20 to 10.74 mg-^{1} extract (Dixit *et al.*, 2018).

Table 2: Phycobiliprotein content (mg g^{-1}) in selected red seaweeds (fresh weight basis)

Red seaweeds	Phycocyanin	Allophycocyanin	Phycoerythrin
Gracilaria salicornia	1.245 ± 0.11	1.032 ± 0.14	2.455 ± 0.11
Jania rubens	1.558 ± 0.31	3.014 ± 0.35	2.923 ± 0.21
Hypnea musciformis	1.145 ± 0.15	1.043 ± 0.18	4.564 ± 0.91
Gracilaria folifera	1.220 ± 0.30	1.798 ± 0.25	3.567 ± 0.10
Gracilaria crassa	2.324 ± 0.60	2.600 ± 0.70	4.154 ± 0.19

Among the selected seaweeds, phycobiliprotein content was maximum in *G. crassa* and *J. rubens* [Table 2]. The allophycocyanin and phycocyanin contents were maximum in *J. rubens* and *G. crassa*, respectively, and minimum in *H. musciformis* and *G. salicornia*. The phycoerythrin content was maximum in *H. musciformis*, minimum in *G. folifera*. Earlier studies have also reported abundant phycocyanin and phycoerythrin contents in *G. crassa* (Baghel *et al.*, 2014). Conversely higher levels of phycobiliproteins has been recorded in *G. gracilis* (Pereira *et al.*, 2020) and very low amount of phycobiliprotein pigments in *G. salicornia* by Senthilkumar *et al.* (2013).

Conclusion: This study revealed the richness of test red seaweeds with respect to the carbohydrates, protein, phycobiliprotein and ash content; least in lipids, chl. *a* and chl. d, phenol and flavonoids. The present study concluded that these marine red seaweeds had some beneficial biochemical and secondary metabolites. Future studies needs to explore the characterization of compounds that play a role in the biological activity and effectiveness of biological systems.

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