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Multivariate analysis of fatty acid and biochemical constitutes of seaweeds to characterize their potential as bioresource for biofuel and fine chemicals

Priyanka Verma [a], Manoj Kumar [a], Girish Mishra [a], Dinabandhu Sahoo [a,b,c,*]

[a] Department of Botany, University of Delhi, Delhi, India
[b] Institute of Bioresource and Sustainable Development, Imphal, Manipur, India
[c] Institute of Life Science, Bhubaneshwar, Odisha, India

HIGHLIGHTS

- Bio prospecting of seaweeds from tropical environment on the basis of composition.
- C20:5n-3 eicosapentaenoic acid was detected in ten seaweeds.
- Manifestation of chemotaxonomic relationship among thirty seaweeds.

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ABSTRACT

In the present study bio prospecting of thirty seaweeds from Indian coasts was analyzed for their biochemical components including pigments, fatty acid and ash content. Multivariate analysis of biochemical components and fatty acids was done using Principal Component Analysis (PCA) and Agglomerative hierarchical clustering (AHC) to manifest chemotaxonomic relationship among various seaweeds. The overall analysis suggests that these seaweeds have multi-functional properties and can be utilized as promising bioresource for proteins, lipids, pigments and carbohydrates for the food/feed and biofuel industry.

1. Introduction

Algae are photosynthetic organisms of great diversity. Algae, which constitute ninety percent of the marine plants, act as primary producers in the food chain and contribute to nearly 50% of global photosynthesis. Among these, seaweeds, the marine macroalgae play a vital role of being the key component of the coastal ecosystems. Consumption of seaweeds has been reported for over three thousand years, predominantly from Asia, where seaweeds are prized for their nutritional and health benefits (Kumar et al., 2015). Several seaweeds such as *Ulva*, *Fucus*, *Undaria*, *Porphyra*, *Palmaria*, *Gracilaria* and *Chondrus* etc. have been utilized in salads, soups and as low-calorie foods (Jiménez-Escrig and Sánchez-Muniz, 2000). Seaweeds are also known for many important compounds such as antioxidants, proteins, polyunsaturated fatty acids (PUFAs), pigments, minerals, fibers and vitamins (Fernández-Martín et al., 2009) making them useful as functional foods.

The biochemical content of seaweed is mostly investigated to explore its possible use as sources of protein, carbohydrate, lipid, fatty acid and pigments as well as for the understanding the behavior of seaweed species towards environmental conditions. There is a fair amount of information regarding carbohydrate and lipid of seaweeds and their applications but little information is available on the protein content (Fleurence, 1999) and nutritional value of seaweeds. However, despite low lipid content, seaweeds comprises of high proportion of fatty acids. Fatty acids from seaweeds can be used as an alternate of fish oils (Pereira et al., 2012) and play a vital role in growth, development and reproduction of fishes and marine invertebrates (Kumar et al., 2011). Polyunsaturated fatty acids

* Corresponding author at: Department of Botany, University of Delhi, Delhi, India.
E-mail address: dbsahoo@hotmail.com (D. Sahoo).

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(PUFAs) are rich in C18 and C20 and being explored for cosmetic, food, feed, and pharmaceutical application (Chandini et al., 2008). A high intake of long-chain n–3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic (DHA, C 22:6 n–3) and eicosapentaenoic (EPA, C 20:5 n–3) acid consumption has proved to have favorable effects in humans (Colombo et al., 2006) and are being explored for nutraceutical applications. Seaweeds are also gaining popularity as an alternative renewable source of biomass for production of biofuels (Singh et al., 2011). Seaweeds with low lignin or non-lignin content with substantial amount of sugars (carbohydrate) could be used during fermentation for bioethanol production (Shukla et al., 2016). Diversification of seaweed bioresources for biofuel and food (or for nutraceutical components) has become an important issue in recent time due to food versus fuel debate. So seaweeds can be classified into biomass as a feedstock for biofuel and food/feed for consumption. Seaweeds with high protein and essential fatty acid could be promising source for food/feed while seaweed species with high cellulosic or carbohydrate components which are usually considered as waste can be used as biomass or bioethanol. On the other hand, seaweeds with high lipid content can be used as biomass for biodiesel production.

Seaweeds are usually classified into three broad groups based on pigmentation: brown, red and green referred as Phaeophyceae, Rhodophyceae and Chlorophyceae, respectively. Chlorophyll '*a*' are natural pigments found in all seaweeds along with carotenoids. Carotenoids are one of the naturally occurring pigments responsible for distinct colures in higher plants as well as in micro and macroalgae. Among carotenoids, Fucoxanthin forms an important component of brown seaweeds. It belongs to the group Xanthophyll, among carotenoids and displays potent antioxidant activity making it a replacement for synthetic antioxidants (Sudhakar et al., 2013). Rhodophyceae contain a particular protein called Phycobiliproteins (R-phycoerythrin, R-phycocyanin, and R-allophycocyanin). These, R-phycoerythrin (R-PE) are already being used in alcoholic and non-alcoholic beverages, candies, jellies, chewing gum, ice sherberts, popsicles, soft drinks, dairy products, cosmetics like lipstick, eyeliners as well as widely used in clinical and immunological research laboratories (Spolaore et al., 2006; Sekar and Chandramohan, 2008; Sudhakar et al., 2013).

Considering the indispensable importance of seaweeds, an attempt has been made to evaluate the possible bioresource utilization of seaweed species from the Indian sea coast. In the present study economically important biochemical constituents of seaweed were analyzed to assess their potential use as a therapeutic natural food/feed resource or biochemical products and potentials of seaweed biomass as a feedstock for biofuel production.

2. Material and methods

2.1. Sample collection

A total of thirty seaweeds were collected during low tide from various parts of Indian coast i.e. Port Okha, (Gujarat), Mandapam (Tamil Nadu) and Shrivardhan (Maharashtra). Among these thirty species nineteen were collected from Port Okha (Gujarat) namely *Ulva lactuca*, *Acrosiphonia orientalis*, *Boodlea composita* and *Valonia utricularis,* from Chlorophyceae; *Dictyopteris australis*, *Dictyota dicotoma*, *Padina gymnospora*, *Spatoglossum asperum*, *Stoechospermum marginatum*, *Iyengaria stellata* and *Sargassum linearifolium* from Phaeophyceae; while *Sciania fasciularis*, *Sciania hatei*, *Gelidiella acerosa*, *Grateloupia indica*, *Halymenia venusta*, *Botryocladia leptopoda*, *Rhodymenia dissecta* and *Haloplegma duperreyi* from Rhodophyceae. Ten species were collected from Mandapam (Tamil Nadu) *Ulva reticulata* and *Caulerpa vervelansis* were from Chlorophyceae while *Lobophora variegata*, *Padina tetrastromatica* and *Turbinaria* sp. were from Phaeophyceae; *Gelidium micropertum*, *Hypnea valentiae*, *Hypnea musciformis*, *Champia* sp. and *Portieria hornemannii* belonged to Rhodophyceae. Only a single species *Porphyra* sp. belonging to class Rhodophyceae was collected from Shrivardhan (Maharashtra). The samples were manually harvested and cleaned thoroughly of epiphytes as well as undesirable material. The seaweed samples were shade dried and brought to laboratory for further analyses.

2.2. Estimation of total protein

1 g of dried seaweed samples was dissolved in 100 ml of 10% NaCl, stirred for 15 min and then filtered through Whatman filter paper No. 1. Filtrates were used as a crude extract of protein and estimated by Bradford's method (1976).

2.3. Total soluble carbohydrates estimation

For estimation of total soluble carbohydrate, 0.1 g of dried seaweed samples was treated with 10 ml of 70% ethanol at 80 °C in a water bath for 2 h. After cooling it down to room temperature the slurry was retreated with 70% ethanol at 80 °C in water bath for complete extraction of soluble sugars. Filtrates from both batches were mixed thoroughly and volume was made up to 100 ml with distilled water. This solution was used for the estimation of total soluble carbohydrate using Phenol–Sulphuric acid method (Dubois et al., 1956).

2.4. Estimation of total lipid

Total lipid was estimated as per Bligh and Dyer method (1959). For the determination of total lipid content, 1 g of seaweed was homogenized in chloroform: methanol (10:20 v/v) mixture.

2.5. Estimation of ash content

For the determination of total ash content 1 g of seaweed content was analyzed as per Kumar et al. (2015).

2.6. GC–MS FAMES

The fatty acids were converted to their fatty acid methyl esters (FAMES) by trans methylation of samples with, adding 1 ml of 2% methanolic HCl and heating for 1 h at 80 °C then adding 1 ml of 0.9% NaCl in H_2O. It was followed by addition of 2 ml of hexane, vortex mixing for 30 s and centrifugation at 2000 rpm for 5 min. The upper phase (hexane) was moved into fresh tube and dried under N_2 flow. 50 µl of hexane was added to dried FAMES and 1 µl was injected to GC–MS for analysis. The GC–MS analysis of FAMES was carried out on a gas chromatography–mass spectrometer (GC-7890B coupled with GC–MS5977A MSD) equipped with an autosampler (G4513A) from Agilent Technologies (USA) using a DB-Wax fused silica capillary column, 30 m × 0.25 µm × 0.25 µm (Agilent Technologies). Helium (99.9% purity) was used as the carrier gas with the column flow rate of 1.8 ml/min and the pre-column pressure of 20.90 psi. The column temperature regime was 50 °C for 1 min, followed by a 25 °C/min ramp up to 200 °C followed by 18 min at 230 °C. The mass spectrometer was operated in electron compact mode with electron energy of 70 eV. Both the ion source temperature and the interface temperature were set at 230 °C. FAMES peaks were identified via NIST (National Institute of Standards and Technology) library. Post run analysis and quantified by area normalization.