

Harnessing the Bioactive Potential of Indian Seaweeds through Multivariate Analysis

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Abstract

Seaweed is an important natural resource with wide-ranging applications in the food and health industries, due to its bioactive properties. In this study, 30 types of seaweed collected from the Indian coast were analyzed for their antioxidant potential, focusing on total phenolic content (TPC), flavonoid content (FC), and their ability to scavenge free radicals, such as DPPH and hydrogen peroxide. The results revealed considerable variation in these properties among the species. *Padina tetrastromatica* exhibited the highest TPC at 52.39 ± 0.51 mg GAE g⁻¹, while *Champia* sp. showed the lowest at 8.08 ± 1.85 mg GAE g⁻¹. For flavonoid content, *Spatoglossum asperum* had the highest value at 49.68 ± 1.4 mg QE g⁻¹, and *Padina gymnospora* the lowest at 14.84 ± 1.25 mg QE g⁻¹. In ferrous ion chelating ability, *Turbinaria* sp. exhibited the highest at $48.94 \pm 2.31\%$, while *Sciania fascicularis* recorded the lowest at $14.77 \pm 0.35\%$. *Caulerpa vervelansis* demonstrated the highest DPPH radical scavenging activity at $74.86 \pm 0.45\%$, and *Sciania hatei* showed the lowest at $24.98 \pm 2.33\%$. The highest hydrogen peroxide radical scavenging activity was found in *Ulva reticulata* at $34.55 \pm 0.56\%$, while *Sciania hatei* exhibited the lowest at $10.16 \pm 1.81\%$. The study also examined the mineral composition of these seaweeds, revealing a wide range of mineral content. Potassium content varied from 7.37% in *Turbinaria* sp. to 1.22% in *Hypnea musciformis*, sodium content ranged from 4.75% in *Gelidiella acerosa* to 2.85% in *Sargassum linearifolium*, and calcium content ranged from 8.41% in *Boodlea composita* to 1.06% in *Rhodymenia dissecta*. Magnesium levels were highest in *Gelidium micropertum* at 1.866% and lowest in *Portieria hornemannii* at 0.764%. Iron content peaked at 1.42% in *Gelidiella acerosa* and was lowest at 0.07% in *Grateloupia indica*. Cobalt content ranged from 0.06‰ in *Acrosiphonia orientalis* to 0.01‰ in *Sciania fascicularis*, while zinc content ranged from 1.796‰ in *Gelidium micropertum* to 1.087‰ in *Boodlea composita*. Manganese content varied from 0.053‰ in *Padina tetrastromatica* and *Sciania fascicularis* to 0.005‰ in species like *Spatoglossum asperum*. Principal Component Analysis (PCA) revealed strong positive correlations between TPC, FC, and antioxidant activities, emphasizing the bioactive potential of these seaweed species. These findings highlight the significant nutritional and antioxidant properties of seaweed, confirming its value as a promising resource for industrial applications in various fields.

Highlights

- Flavonoid, total phenolic and Antioxidant properties i.e. DPPH, HO scavenging, ferrous ion chelating ability of thirty seaweeds from Indian seacoasts reported.
- Certain brown and green algae have significantly higher antioxidant activities.
- PCA of antioxidant properties manifest chemotaxonomic relationship.

Introduction

Algae represent a diverse and specialized group of photosynthetic organisms, varying from microscopic blue-green algae to giant kelps that can grow several meters long. Among these, seaweeds or marine macroalgae are crucial components of the marine ecosystem and are classified into three main groups: Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae). These seaweeds are commercially valuable, serving as renewable marine resources. In many Asian countries, seaweeds have long been an integral part of the diet, owing to their exceptional nutritional and health benefits. Seaweeds are a rich source of carbohydrates, proteins, fatty acids, vitamins, and essential minerals, which make them vital in the diet of many coastal populations. Moreover, seaweeds are known for their high mineral content, which can vary depending on both exogenous factors (such as ocean salinity and temperature) and endogenous factors (like the specific species and growth stage) [1]. In addition to primary metabolites, seaweeds also contain a wide range of bioactive compounds, such as alkaloids, carotenoids, polysaccharides, polyunsaturated fatty acids, phycobilins, phlorotannins, phycocyanins, sterols, terpenes, tocopherols, and xanthophylls [2]. These bioactive compounds have attracted significant attention due to their potential health-promoting properties. Seaweeds are subjected to various environmental stressors, such as high salinity, fluctuating temperatures, and varying light conditions, which can induce oxidative stress. This oxidative stress can cause damage to cellular structures and metabolic pathways, but antioxidants found in seaweeds help to mitigate such damage by protecting the organisms against oxidation [3]. Antioxidants play a key role in reducing oxidative damage, and various seaweed species, including red, brown, and green algae, are known to possess potent antioxidant properties, which contribute to their therapeutic value [4][5]. In recent years, there has been growing interest in the potential of seaweeds as sources of novel pharmaceuticals and bioactive compounds with antioxidant and other health-promoting properties. The exploration of seaweeds for their medicinal benefits has led to the identification of a wide range of biologically active substances with potential applications in pharmaceuticals, nutraceuticals, and functional foods [6]. Given their wide array of bioactive compounds, seaweeds have become a focus of research aimed at discovering natural antioxidant compounds that can be utilized for human health benefits.

The present study was designed to evaluate the mineral content, total phenolic content (TPC), flavonoid content (FC), and antioxidant properties of various seaweed species collected from the Indian coastline. The seaweeds were subjected to multivariate analysis using Principal Component Analysis (PCA) to better understand the relationship between TPC, FC, and antioxidant properties. This study aims to contribute to the growing body of knowledge on the antioxidant potential of seaweeds and their bioactive properties, offering insights into their potential as valuable resources for industrial applications, particularly in the development of functional foods and natural therapeutics. The novelty of this work lies in its comprehensive analysis of a wide range of seaweed species from the Indian coast, employing PCA to elucidate the relationships among their bioactive properties, which has not been extensively explored in previous studies.

Material and methods

Collection of seaweeds

Seaweeds (30) were collected from various regions of Indian seacoast (Table 1), during low tide. Harvested seaweed was cleaned with seawater and shade dried on site of collection and transported to laboratory for further analyses.

Table 1

Antioxidant potential of various seaweeds. Values are mean $\pm$ Standard deviation (n = 3). For each individual experiment variable means with the same letter are not significantly different (p > 0.05). Collection Site* i.e. PO (Port Okha, Gujarat, India), M (Mandapam, Tamil Nadu, India), S (Shrivardhan, Maharashtra, India).

S. No.	Seaweed (Collection Site*)	Total Phenolic Content (mg GAE g ⁻¹)	Flavonoid Content (mg QE g ⁻¹)	Ferrous Ion Chelating Ability (%)	DPPH radical scavenging activity (%)	Hydrogen Peroxide Radical Scavenging activity (%)
1	<i>Ulva lactuca</i> (PO)	41.59 $\pm$ 1.75 ^{mn}	34 $\pm$ 1.02 ^g	46.45 $\pm$ 0.15 ^{hi}	56.09 $\pm$ 2.17 ^{jk}	23.47 $\pm$ 0.76 ^{ij}
2	<i>Ulva reticulata</i> (M)	45.59 $\pm$ 1.16 ^o	25.55 $\pm$ 0.72 ^e	48.59 $\pm$ 0.26 ^{ij}	61.04 $\pm$ 2.35 ^{lm}	34.55 $\pm$ 0.56 ^m
3	<i>Acrosiphonia orientalis</i> (PO)	37.04 $\pm$ 0.5 ^{kl}	43.66 $\pm$ 2.79 ⁱ	35.79 $\pm$ 0.22 ^{ef}	41.16 $\pm$ 2.55 ^e	15.39 $\pm$ 1.44 ^d
4	<i>Boodlea composite</i> (PO)	33.92 $\pm$ 1.6 ^j	15.12 $\pm$ 1.23 ^{abc}	24.59 $\pm$ 1.19 ^c	37.1 $\pm$ 4.25 ^d	15.49 $\pm$ 0.11 ^d
5	<i>Valonia utricularis</i> (PO)	12.88 $\pm$ 0.72 ^c	11.57 $\pm$ 1.08 ^a	18.69 $\pm$ 2.6 ^b	29.57 $\pm$ 2.06 ^c	12.23 $\pm$ 1.1 ^c
6	<i>Caulerpa vervelansis</i> (M)	48.92 $\pm$ 0.15 ^p	37.8 $\pm$ 2.47 ^{gh}	44.75 $\pm$ 0.31 ^{gh}	74.86 $\pm$ 0.45 ⁿ	27.54 $\pm$ 0.81 ^l
7	<i>Dictyopteris australis</i> (PO)	33.52 $\pm$ 0.75 ^j	37 $\pm$ 3.57 ^{gh}	23.75 $\pm$ 2.53 ^c	35.23 $\pm$ 0.25 ^d	15.32 $\pm$ 0.5 ^d
8	<i>Dictyota dicotoma</i> (PO)	38.08 $\pm$ 1.79 ^{kl}	34.78 $\pm$ 1.98 ^{gh}	38.02 $\pm$ 1.12 ^f	42.57 $\pm$ 2.38 ^{ef}	16.9 $\pm$ 0.66 ^e
9	<i>Lobophora variegata</i> (M)	35.94 $\pm$ 1.86 ^{jk}	37.77 $\pm$ 0.84 ^{gh}	37.62 $\pm$ 0.76 ^f	45.62 $\pm$ 0.55 ^{fg}	17.9 $\pm$ 0.68 ^e
10	<i>Padina gymnospora</i> (PO)	30.59 $\pm$ 0.75 ⁱ	14.84 $\pm$ 1.25 ^{abc}	38.42 $\pm$ 0.45 ^f	53.42 $\pm$ 2.7 ^{ij}	21.24 $\pm$ 0.31 ^{gh}
11	<i>Padina tetrastromatica</i> (M)	52.39 $\pm$ 0.51 ^q	14.81 $\pm$ 3.31 ^{abc}	43.45 $\pm$ 0.76 ^g	60.78 $\pm$ 3.78 ^{lm}	25.66 $\pm$ 1.25 ^k
12	<i>Spatoglossum asperum</i> (PO)	50.46 $\pm$ 0.36 ^{pq}	49.68 $\pm$ 1.4 ^j	49.38 $\pm$ 1.84 ^j	53.3 $\pm$ 2.75 ^{ij}	20.65 $\pm$ 0.42 ^g
13	<i>Stoechospermum marginatum</i> (PO)	41.65 $\pm$ 2.41 ^{mn}	16.29 $\pm$ 1.48 ^{bcd}	33.67 $\pm$ 1.21 ^e	53.9 $\pm$ 0.35 ^{ijk}	20.16 $\pm$ 1.34 ^{fg}
14	<i>Iyengaria stellata</i> (PO)	34.41 $\pm$ 1.92 ^j	27.34 $\pm$ 3.29 ^{ef}	38.33 $\pm$ 0.66 ^f	48.07 $\pm$ 1.89 ^{gh}	19.26 $\pm$ 0.05 ^f
15	<i>Sargassum linearifolium</i> (PO)	22.38 $\pm$ 0.14 ^g	29.53 $\pm$ 4.39 ^f	33.53 $\pm$ 1.12 ^e	57.65 $\pm$ 3.19 ^{kl}	22.37 $\pm$ 0.07 ^{hi}
16	<i>Turbinaria</i> sp. (M)	48.64 $\pm$ 0.45 ^p	18.45 $\pm$ 4.59 ^{cd}	48.94 $\pm$ 2.31 ^{ij}	62.95 $\pm$ 2.17 ^m	24.27 $\pm$ 0.12 ^j
17	<i>Porphyra</i> sp. (S)	33.7 $\pm$ 1.72 ^j	38.11 $\pm$ 1.72 ^h	47.32 $\pm$ 1.18 ^{hij}	63.02 $\pm$ 2.72 ^m	24.27 $\pm$ 0.08 ^j
18	<i>Sciania fascicularis</i> (PO)	15.84 $\pm$ 1.75 ^d	16.91 $\pm$ 0.3 ^{bcd}	14.77 $\pm$ 0.35 ^a	26.97 $\pm$ 2.06 ^{bc}	12.13 $\pm$ 0.09 ^c
19	<i>Sciania hatei</i> (PO)	17.63 $\pm$ 0.5 ^{de}	16.23 $\pm$ 1.13 ^{bcd}	18.09 $\pm$ 0.13 ^b	24.98 $\pm$ 2.33 ^b	10.16 $\pm$ 1.81 ^b
20	<i>Gelidium micropertum</i> (M)	28.45 $\pm$ 1.98 ^{hi}	28.29 $\pm$ 2.14 ^{ef}	28.9 $\pm$ 3.26 ^d	27.42 $\pm$ 1.93 ^{bc}	12.35 $\pm$ 0.01 ^c
21	<i>Gelidiella acerosa</i> (PO)	12.72 $\pm$ 0.39 ^c	16.72 $\pm$ 1.08 ^{bcd}	34.61 $\pm$ 1.19 ^e	62.52 $\pm$ 2.14 ^m	24.2 $\pm$ 0.21 ^j
22	<i>Grateloupia indica</i> (PO)	42.85 $\pm$ 1.91 ⁿ	30.55 $\pm$ 1.47 ^f	36.07 $\pm$ 2.23 ^{ef}	51 $\pm$ 1.85 ^{hi}	20.26 $\pm$ 0.09 ^f
23	<i>Halymenia venusta</i> (M)	27.57 $\pm$ 0.77 ^h	16.97 $\pm$ 1.39 ^{bcd}	23.06 $\pm$ 2.28 ^c	35.63 $\pm$ 1.15 ^d	15.17 $\pm$ 0.12 ^d
24	<i>Hypnea valentine</i> (M)	19.57 $\pm$ 1.97 ^{ef}	34.31 $\pm$ 1.5 ^{gh}	25.72 $\pm$ 0.4 ^c	29.37 $\pm$ 2.17 ^c	12.27 $\pm$ 0.96 ^c
25	<i>Hypnea musciformis</i> (PO)	21.85 $\pm$ 0.35 ^{fg}	36.29 $\pm$ 1.96 ^{gh}	28.43 $\pm$ 0.53 ^d	29.32 $\pm$ 0.35 ^c	12.33 $\pm$ 0.86 ^c
26	<i>Champia</i> sp. (M)	8.08 $\pm$ 1.85 ^a	11.97 $\pm$ 0.43 ^a	25.45 $\pm$ 2.26 ^c	33.56 $\pm$ 1.81 ^d	15.13 $\pm$ 0.98 ^d
27	<i>Botryocladia leptopoda</i> (PO)	37.11 $\pm$ 0.53 ^{kl}	18.29 $\pm$ 1.59 ^{cd}	23.98 $\pm$ 2.42 ^c	28.75 $\pm$ 0.35 ^{bc}	12.28 $\pm$ 0.68 ^c
28	<i>Rhodymenia dissecta</i> (PO)	39.51 $\pm$ 2.82 ^{lm}	13.6 $\pm$ 0.44 ^{ab}	28.77 $\pm$ 1.1 ^d	28.52 $\pm$ 2.25 ^{bc}	12.57 $\pm$ 0.19 ^c

S. No.	Seaweed (Collection Site*)	Total Phenolic Content (mg GAE g ⁻¹)	Flavonoid Content (mg QE g ⁻¹)	Ferrous Ion Chelating Ability (%)	DPPH radical scavenging activity (%)	Hydrogen Peroxide Radical Scavenging activity (%)
29	<i>Haloplegma duperreyi</i> (PO)	9.62 ± 0.39 ^{ab}	25.61 ± 1.26 ^e	16.17 ± 2.05 ^{ab}	15.68 ± 2.45 ^a	8.43 ± 0.01 ^a
30	<i>Portieria hornemannii</i> (M)	11.12 ± 2.36 ^{bc}	19.68 ± 2.52 ^d	23.12 ± 2.53 ^c	35.86 ± 2.3 ^d	15.17 ± 0.02 ^d

Preparation of seaweeds extract

Extract of 5g dried seaweed were prepared as per [7]. The extractions were done using methanol 10:1 (v/w), chloroform and methanol 1:1 (v/v) and finally with chloroform so that lower polar, polar and non-polar components of each sample, were represented. Three extracts (representing lower polar, polar, and non-polar components) from each sample were pooled and evaporated under decreased pressure using a rotary flash evaporator. The crude extract of each sample was weighed to calculate yield and then dissolved in 90% aqueous methanol. The yields (%) were calculated as followed;

Yield (%) = weight of extract (g) /weight of dry biomass (g) × 100

Total Phenolic Estimation

Phenolic contents of metabolically enriched extract were estimated by the method of Folin Ciocalteu reagent according to a procedure described by [8]. The absorbance was recorded at 650 nm and TPC was expressed as mg Gallic acid equivalents per gram (mg GAE g⁻¹).

Flavonoid estimation

Metabolically enriched extract seaweed sample was used for the determination of total flavonoid content using aluminium chloride colorimetric method described by [9]. Quercetin was used as standard used for construction of calibration curve and absorbance was recorded at 415 nm for standard curve. The concentration of total FC was given as mg Quercetin equivalents per gram of extract (mg QE g⁻¹).

Ferrous ion-chelating ability assay (Fe II-CA)

Fe II-CA of seaweed extracts (1 mg extract dissolved in 1 mL methanol) was determined according to [10]. Absorbance at 562 nm was recorded for standard curve of ascorbic acid. The percentage inhibition of the ferrous ion was calculated by comparing the results of test with that L-ascorbic acid of the control.

To calculate the Fe II-CA following formula is used:

Fe II-CA % = $[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})] / A_{\text{control}} \times 100$. Where

A_{control} = absorbance of control,

A_{sample} = absorbance of sample or standard and

A_{blank} = absorbance of blank.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-SA)

Scavenging activity of Metabolically enriched seaweed extract (2.0 ml) was done by the method of [11]. For activity 2.0 ml of 0.16 mM DPPH solution was added to the 2.0 ml aliquot of sample in test tube. After 30 min in the dark at Room temperature absorbance was measured at 517 nm. DPPH-SA (%) was determined by equation;

DPPH-SA (%) = $[1 - (A_{\text{sample}} - A_{\text{sample blank}}) / A_{\text{control}}] \times 100$

A_{control} = absorbance of control,

A_{sample} = absorbance of the sample, and

$A_{\text{sample blank}}$ = absorbance of the sample without DPPH solution

Hydrogen Peroxide Radical Scavenging Activity (H₂O₂ - SA)

Seaweed extracts were used to determine hydrogen peroxide radical scavenging activity (H_2O_2 - SA) according to the method by [12]. For scavenging activity test samples were added to the H_2O_2 solution and incubated for 10 min. absorbance was measured at 230 nm and phosphate buffer used as blank. The calculations were done by using formula;

$$H_2O_2 - SA \% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

A_{control} = absorbance of the control and

A_{sample} = absorbance in the presence of the seaweed extracts.

Estimation of Elements and Minerals

Mineral elements were analyzed by [13] method using 0.2 g oven dried seaweed samples and atomic absorption spectrophotometer (AAS). The calculations were done by following equation;

$$X (\mu\text{g/g}) = c(\text{ppm}) \times \text{Volume of solution} \times \text{D.F.} / \text{Weight of sample (g)}$$

X = Element,

C = Concentration and,

D.F. = Dilution Factor

Multivariate Analysis

Principal component analysis of data set was performed using XLSTAT. The score plot was used to display the distribution of samples of the data set and the loading plot to explain importance and interactions of the variables with possible grouping of samples.

Statistical Analysis

Data were expressed as $M \pm SD$ ($n = 3$) and were analyzed using ANOVA (one-way analysis of variance) by SPSS 16 and for significant difference Duncan multiple range post hoc test was used.

Results and Discussion

TPC and FC along with antioxidant activity (i.e. DPPH-SA, H_2O_2 - SA, Fe II-CA), of seaweed bioresource from different parts of Indian sea coast are shown in Table 1 to 3.

Table 2

Composition of microelements and macroelements of various seaweeds. Values are mean $\pm$ Standard deviation (n = 3). For each individual experiment variable means with the same letter are not significantly different ($p > 0.05$).

S. no	Seaweed	Potassium content (% dw)	Sodium content (% dw)	Calcium content (% dw)	Magnesium content (% dw)	Iron content (% dw)	Cobalt content (‰ dw)	Zinc content (‰ dw)	Manganese (‰ dw)
1	<i>Ulva lactuca</i>	3.46 $\pm$ 0.11f	3.48 $\pm$ 0.07bc	4.72 $\pm$ 0.14ef	1.841 $\pm$ 0.012fg	0.32 $\pm$ 0.02bcdefg	0.018 $\pm$ 0.000abcde	1.38 $\pm$ 0.038bcdefg	0.012 $\pm$ 0.001a
2	<i>Ulva reticulata</i>	2.46 $\pm$ 0.11d	3.65 $\pm$ 0.11bcde	4.52 $\pm$ 0.27e	0.921 $\pm$ 0.05bcd	0.15 $\pm$ 0.011ab	0.037 $\pm$ 0.003abdef	1.684 $\pm$ 0.029hi	0.007 $\pm$ 0.002a
3	<i>Acrosiphonia orientalis</i>	5.58 $\pm$ 0.01hi	4.35 $\pm$ 0.29fgh	6.69 $\pm$ 0.25h	1.848 $\pm$ 0.023fg	0.17 $\pm$ 0.01ab	0.06 $\pm$ 0.003f	1.532 $\pm$ 0.025fgh	0.016 $\pm$ 0.003a
4	<i>Boodlea composita</i>	4.47 $\pm$ 0.17	4.4 $\pm$ 0.33gh	8.41 $\pm$ 0.24j	0.898 $\pm$ 0.019abcd	0.28 $\pm$ 0.01abcdef	0.023 $\pm$ 0.002abcde	1.087 $\pm$ 0.015a	0.014 $\pm$ 0.003a
5	<i>Valonia utricularis</i>	2.69 $\pm$ 0.11de	3.47 $\pm$ 0.08b	6.53 $\pm$ 0.38h	0.851 $\pm$ 0.06abc	0.24 $\pm$ 0.03abcde	0.032 $\pm$ 0.002abcdef	1.624 $\pm$ 0.018gh	0.035 $\pm$ 0.002b
6	<i>Caulerpa vervelansis</i>	1.48 $\pm$ 0.03abc	3.59 $\pm$ 0.03bce	1.5 $\pm$ 0.23b	0.818 $\pm$ 0.058abc	0.26 $\pm$ 0.05abcef	0.032 $\pm$ 0.030abdef	1.186 $\pm$ 0.030abc	0.017 $\pm$ 0.003a
7	<i>Dictyopteris australis</i>	6.76 $\pm$ 0.15j	4.1 $\pm$ 0.08f	1.34 $\pm$ 0.28ab	1.811 $\pm$ 0.065fg	0.58 $\pm$ 0.1hi	0.013 $\pm$ 0.001abcd	1.15 $\pm$ 0.031abc	0.014 $\pm$ 0.002a
8	<i>Dictyota dicotoma</i>	2.46 $\pm$ 0.11d	3.79 $\pm$ 0.1de	2.4 $\pm$ 0.25c	0.767 $\pm$ 0.05a	0.42 $\pm$ 0.07cdefghi	0.029 $\pm$ 0.000abde	1.32 $\pm$ 0.08abcdef	0.006 $\pm$ 0.002a
9	<i>Lobophora variegata</i>	3.46 $\pm$ 0.18f	3.8 $\pm$ 0.03de	4.92 $\pm$ 0.08f	1.736 $\pm$ 0.082f	0.63 $\pm$ 0.031ijkl	0.016 $\pm$ 0.0013abcde	1.308 $\pm$ 0.0162abcdef	0.006 $\pm$ 0.001a
10	<i>Padina gymnospora</i>	1.41 $\pm$ 0.25ab	3.58 $\pm$ 0.11bcd	1.53 $\pm$ 0.12b	0.834 $\pm$ 0.009abc	0.36 $\pm$ 0.021bcdefgh	0.012 $\pm$ 0.001abcd	1.223 $\pm$ 0.091abcde	0.007 $\pm$ 0.002a
11	<i>Padina tetrastrumatica</i>	1.37 $\pm$ 0.25ab	3.58 $\pm$ 0.11bcd	1.53 $\pm$ 0.12b	0.771 $\pm$ 0.05a	0.14 $\pm$ 0.02ab	0.041 $\pm$ 0.005cdef	1.475 $\pm$ 0.025efgh	0.053 $\pm$ 0.005c
12	<i>Spatoglossum asperum</i>	2.69 $\pm$ 0.11de	4.34 $\pm$ 0.07fgh	5.54 $\pm$ 0.29g	0.922 $\pm$ 0.118bcd	0.17 $\pm$ 0.05ab	0.037 $\pm$ 0.0021abcdef	1.189 $\pm$ 0.009abcd	0.005 $\pm$ 0.002a
13	<i>Stoechospermum marginatum</i>	2.69 $\pm$ 0.11de	3.73 $\pm$ 0.14cde	7.65 $\pm$ 0.21i	0.948 $\pm$ 0.127cde	0.21 $\pm$ 0.06abcd	0.017 $\pm$ 0.002abcde	1.288 $\pm$ 0.084abcdef	0.005 $\pm$ 0.002a
14	<i>Iyengaria stellata</i>	2.79 $\pm$ 0e	4.31 $\pm$ 0.24fg	5.31 $\pm$ 0.21g	1.055 $\pm$ 0.212e	0.76 $\pm$ 0.08l	0.011 $\pm$ 0.001abc	1.226 $\pm$ 0.073abcde	0.008 $\pm$ 0.002a
15	<i>Sargassum linearifolium</i>	3.37 $\pm$ 0.16f	2.85 $\pm$ 0.04a	6.55 $\pm$ 0.35h	0.989 $\pm$ 0.162de	0.46 $\pm$ 0.03efghij	0.012 $\pm$ 0.000abcd	1.325 $\pm$ 0.041abcdef	0.009 $\pm$ 0.001a
16	<i>Turbinaria sp.</i>	7.37 $\pm$ 0.16k	3.85 $\pm$ 0.04e	1.22 $\pm$ 0.18ab	0.871 $\pm$ 0.055abcd	0.37 $\pm$ 0.15bcdefgh	0.042 $\pm$ 0.001cdef	1.34 $\pm$ 0.05abcdef	0.01 $\pm$ 0.001a
17	<i>Porphyra sp.</i>	1.71 $\pm$ 0.25c	3.82 $\pm$ 0.14de	1.47 $\pm$ 0.08b	0.852 $\pm$ 0.029abc	0.25 $\pm$ 0.02abcdef	0.023 $\pm$ 0.0019abcde	1.308 $\pm$ 0.018abcdef	0.006 $\pm$ 0.002a
18	<i>Sciania fascicularis</i>	5.79 $\pm$ 0.1i	4.27 $\pm$ 0.09fg	1.53 $\pm$ 0.12b	0.827 $\pm$ 0.052abc	0.52 $\pm$ 0.04ghi	0.01 $\pm$ 0.001ab	1.185 $\pm$ 0.018abc	0.053 $\pm$ 0.005c
19	<i>Sciania hatei</i>	2.69 $\pm$ 0.11de	4.31 $\pm$ 0.03fg	6.55 $\pm$ 0.37h	0.853 $\pm$ 0.003abc	0.49 $\pm$ 0.024fghij	0.019 $\pm$ 0.001abcde	1.134 $\pm$ 0.012ab	0.006 $\pm$ 0.002a
20	<i>Gelidium microperum</i>	2.7 $\pm$ 0.16de	3.58 $\pm$ 0.13bcd	2.55 $\pm$ 0.27c	1.866 $\pm$ 0.075g	0.45 $\pm$ 0.012efghij	0.033 $\pm$ 0.003abcdef	1.796 $\pm$ 0.092i	0.007 $\pm$ 0.003a
21	<i>Gelidiella acerosa</i>	2.69 $\pm$ 0.11de	4.75 $\pm$ 0.16i	1.53 $\pm$ 0.12b	0.821 $\pm$ 0.005abc	1.42 $\pm$ 0.032m	0.018 $\pm$ 0.001abcde	1.402 $\pm$ 0.236cdefg	0.037 $\pm$ 0.002b
22	<i>Grateloupia indica</i>	1.45 $\pm$ 0.28abc	3.72 $\pm$ 0.22cde	1.5 $\pm$ 0.32b	0.838 $\pm$ 0.002abc	0.07 $\pm$ 0.01a	0.011 $\pm$ 0.001abc	1.223 $\pm$ 0.091abcde	0.008 $\pm$ 0.001a
23	<i>Halymenia venusta</i>	1.35 $\pm$ 0.27ab	3.73 $\pm$ 0.05cde	3.74 $\pm$ 0.08d	0.832 $\pm$ 0.008abc	0.33 $\pm$ 0.0bcdefg	0.024 $\pm$ 0.002abcde	1.449 $\pm$ 0.043defgh	0.006 $\pm$ 0.002a
24	<i>Hypnea valentine</i>	1.37 $\pm$ 0.25ab	4.26 $\pm$ 0.12fg	1.56 $\pm$ 0.19b	0.819 $\pm$ 0.006abc	0.64 $\pm$ 0.016ijkl	0.016 $\pm$ 0.0013abcde	1.17 $\pm$ 0.012abc	0.048 $\pm$ 0.004bc
25	<i>Hypnea musciformis</i>	1.22 $\pm$ 0.01a	4.57 $\pm$ 0.2hi	6.45 $\pm$ 0.27h	0.803 $\pm$ 0.011ab	0.23 $\pm$ 0.1abcde	0.04 $\pm$ 0.0039bcdef	1.127 $\pm$ 0.003ab	0.01 $\pm$ 0.001a

S. no	Seaweed	Potassium content (% dw)	Sodium content (% dw)	Calcium content (% dw)	Magnesium content (% dw)	Iron content (% dw)	Cobalt content (‰ dw)	Zinc content (‰ dw)	Manganese (‰ dw)
26	<i>Champia sp.</i>	1.72 ± 0.06c	3.73 ± 0.05cde	1.07 ± 0.13a	0.832 ± 0.016abc	0.2 ± 0.03abc	0.026 ± 0.003abcde	1.344 ± 0.0102abcdef	0.007 ± 0.001a
27	<i>Botryocladia leptopoda</i>	1.31 ± 0.07ab	4.16 ± 0.13fg	1.53 ± 0.12b	0.815 ± 0.003abc	0.68 ± 0.06jkl	0.045 ± 0.0019ef	1.493 ± 0.0403fgh	0.006 ± 0.001a
28	<i>Rhodymenia dissecta</i>	1.54 ± 0.35bc	4.24 ± 0.16fg	1.06 ± 0.09a	0.844 ± 0.001abc	0.47 ± 0.08efghij	0.017 ± 0.001abcde	1.302 ± 0.0144abcdef	0.006 ± 0.001a
29	<i>Haloplegma duperreyi</i>	5.38 ± 0.06h	4.22 ± 0.09fg	3.67 ± 0.1d	0.818 ± 0.026abc	0.37 ± 0.07bcdefgh	0.043 ± 0.002def	1.233 ± 0.099abcde	0.043 ± 0.004bc
30	<i>Portieria hornemannii</i>	3.46 ± 0.18f	3.46 ± 0.04b	1.53 ± 0.12b	0.764 ± 0.029a	0.73 ± 0.02kl	0.023 ± 0.0019abcde	1.116 ± 0.005a	0.007 ± 0.002a

Table 3
Pearson's correlation analysis.

	TPC	TF	DPPH-RSA	H2O2-SA	Fe II – CA
TPC	1				
TF	0.343498	1			
DPPH-RSA	0.605645	0.226987	1		
H2O2-SA	0.592906	0.162333	0.93943	1	
Fe II – CA	0.760204	0.441801	0.866752	0.850268	1
Values in bold are different from 0 with a significance level alpha = 0.05					

Extraction yield of metabolically enriched extract

The yields of the extracts from thirty different seaweeds were found to have significant ($p < 0.05$) difference (Fig. 1). Extraction yield of extract (on dry weight basis) ranged 19.54 ± 0.43 to $1.07 \pm 0.03\%$. The highest extraction yield was recorded in *D. dicotoma* from Phaeophyceae and minimum was found in *H. duperreyi* from Rhodophyceae. Maximum yield of extract was recorded in Phaeophyceae (i.e. $19.54 \pm 0.43\%$ to $4.21 \pm 0.14\%$) which was followed by Rhodophyceae (i.e. $16.59 \pm 0.91\%$ to $1.07 \pm 0.03\%$) and Chlorophyceae (i.e. $12.69 \pm 0.21\%$ to $7.57 \pm 0.66\%$). Extract was used in present study as it had shown highest antioxidant activity with TPC previously in many seaweed species [14].

Total Phenolic and flavonoid contents

Seaweeds are rich in naturally occurring phenolic compounds with hydroxyl groups bond directly to an aromatic hydrocarbon group. Phenolic compounds require two OH groups (o-diphenol) to chelate metal ions [14, 15]. Their ability to react with radicals correlates with the number of phenolic rings and catecholic structures [16]. Earlier reports showed bioactivities in seaweed extracts, mainly due to polyphenols which have antioxidant activity [17]. In the present study TPC and FC metabolically enriched extracts of thirty different marine seaweeds showed significant ($p < 0.05$) differences and are given in Table 1. TPC in seaweeds varied from 52.39 ± 0.51 mg GAE g^{-1} (*P. tetrastromatica*) to 8.08 ± 1.85 mg GAE g^{-1} (*Champia* sp.). Maximum amount of TPC was recorded in Phaeophyceae (i.e. 52.39 ± 0.51 – 22.38 ± 0.14 mg GAE g^{-1}) followed by Chlorophyceae (48.92 ± 0.15 to 12.88 ± 0.72 mg GAE g^{-1}) and Rhodophyceae (i.e. 39.51 ± 2.82 to 8.08 ± 1.85 mg GAE g^{-1}). Earlier reports also show high TPC contents in brown seaweeds. Brown seaweeds were reported to have high polyphenolic components, phlorotannins [18, 19]. Amongst phenolic compounds, flavonoids are the largest category, reported to possess antioxidant as well as free radical scavenging properties [20]. Significant ($p < 0.05$) differences in TF (total flavonoid content) were also observed in different seaweeds extracts, where it ranged from 49.68 ± 1.4 – 11.57 ± 1.08 mg QE g^{-1} (Table 1).

Maximum amount of TF was observed in *Spatoglossum asperum* belonging to Phaeophyceae while minimum was observed in *Valonia utricularis* from Chlorophyceae. TF in seaweed extracts was maximum among Phaeophyceae (49.68 ± 1.4 to 14.81 ± 3.31 mg QE g^{-1}) followed by Chlorophyceae (43.66 ± 2.79 to 11.57 ± 1.08 mg QE g^{-1}) and Rhodophyceae (38.11 ± 1.72 to 11.97 ± 0.43 mg QE g^{-1}). In previous study of flavonoids distribution in seaweeds from Indian sea coast also reported high amount of TF in brown seaweeds in compare to green and red seaweeds [21]. Brown seaweeds generally have high polyphenol secondary metabolites compared to red and green algae [18]. These polyphenolic compounds exhibit the chemical characteristics of tannins and are likely to be bound to proteins and carbohydrates [22]. Phlorotannins are a group of highly bioactive marine polyphenols found exclusively in brown algae (Phaeophyceae). These phlorotannins are efficient antioxidants and have potential as multifunctional natural antioxidants. Previous works have observed relationship linking TPC of herbs, vegetables, fruits, and antioxidant activity [23]. These phenolic components also possess a wide range of health-promoting properties such as antimicrobial, antiviral, antioxidant and

antitumor activities. In particular, seaweed polyphenols have strong antioxidant capacity, which endow them good anticancer effect [24]. Several polyphenolic compounds also isolated from brown seaweeds and explore their role in preventing degenerative diseases such as [25]. Seaweeds contain a wide range of bioactive components like sulfated polysaccharides, peptides, amino acids, and polyphenols, all exhibiting multiple antioxidant properties [26]. For exploring these antioxidant properties of seaweeds, utilization of solitary method is inadequate so for identification of all antioxidant activities different procedures were involved. Thus, different *in vitro* antioxidant assays, Fe II-CA, DPPH-SA, and H₂O₂-SA were selected to evaluate the antioxidant activities of seaweed species.

Ferrous Ion Chelating Ability (Fe II - CA)

Amongst, the mechanisms of antioxidant activity, metal ion chelating capability are reported as most important assay. Ferrous ions are the utmost significant pro-oxidants to transition metals within food structure [27]. Metal ion chelating capability of seaweed extracts were analyzed using ferrous ions and showed in Table 1. A Significant ($p < 0.05$) difference was seen in metal ion chelating capability of seaweed extracts. Highest chelating ability was observed in *Spatoglossum asperum* ($49.38 \pm 1.84\%$ of methanolic extract) which belongs to Phaeophyceae and minimum was observed in *Sciania fascicularis* ($14.77 \pm 0.35\%$) of Rhodophyceae. Metal chelating activity in seaweed extracts in Phaeophyceae ranged from $49.38 \pm 1.84\%$ to $23.75 \pm 2.53\%$ which was followed by Chlorophyceae, $48.59 \pm 0.26\%$ to $18.69 \pm 2.6\%$ and Rhodophyceae range of metal chelating activity was $47.32 \pm 1.18\%$ to $14.77 \pm 0.35\%$. According to [28], Phenolic acids as important antioxidant agents have demonstrated DPPH scavenging activity and have exhibited Fe²⁺ chelating potential as well as reducing agents and inhibitors of lipid peroxidation. These seaweeds with high radical scavenging activity have found utilization as natural resource for antioxidants in cosmetic industries, food industries as well as in medicine.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-SA)

2,2-Diphenyl-1-picrylhydrazyl had largely been used for detection of free radical scavenging activities of compounds[29]. DPPH-SA is a quick, simple, stable and replicable procedure which has been utilized to preview new antioxidants and to determine radical scavenging activity from organic or natural bioresource [30]. Methanol extract from *Caulerpa vervelans* showed high, significant ($p < 0.05$) radical scavenging activity (i.e. i.e. $74.86 \pm 0.45\%$) followed by *Porphyra* sp. ($63.02 \pm 2.72\%$), *Turbinaria* sp. ($62.95 \pm 2.17\%$) and *Gelidiella acerosa* ($62.52 \pm 2.14\%$) (Table 1). Among the thirty seaweeds DPPH radical scavenging activity (DPPH-SA) of Chlorophyceae ranged from $74.86 \pm 0.45\%$ to $29.57 \pm 2.06\%$, while Phaeophyceae ranged from $62.95 \pm 2.17\%$ to $35.23 \pm 0.25\%$. Among Rhodophyceae DPPH radical scavenging activity of seaweed samples ranged from $63.02 \pm 2.72\%$ to $15.68 \pm 2.45\%$ activity. The DPPH-SA radical scavenging activities (%) were consistently higher in Chlorophyceae and Phaeophyceae (Table 1). The present study showed correlation that seaweeds having high TPC in addition showed high DPPH-SA as well. Hence, indicating that antiradical activity among seaweeds is primarily due to the presence of algal polyphenols. Other antioxidant components like fucoxanthin which simultaneously extracted in some amount may have contribution to the overall activities. Similar tendency of DPPH-SA in seaweeds extract was observed by [18]. Phenolic compound Phloroglucinol found in brown seaweeds has significant total antioxidant potential as well as DPPH and superoxide scavenging activities and metal chelating properties [31].

Hydrogen peroxide radical scavenging activity (H₂O₂ - SA)

H₂O₂ -SA of extracts from thirty seaweeds, exhibits significant ($p < 0.05$) difference (Table 1). H₂O₂ - SA ranged from 34.55 ± 0.56 – $8.43 \pm 0.01\%$, found in *Ulva reticulata* and *Haloplegma duperreyi* respectively. H₂O₂ - radical scavenging activity was exhibited among Chlorophyceae (i.e. $34.55 \pm 0.56\%$ to $12.23 \pm 1.1\%$) followed by Phaeophyceae (i.e. $25.66 \pm 1.25\%$ to $15.32 \pm 0.5\%$). Among Rhodophyceae, it ranged from (i.e. $24.27 \pm 0.08\%$ to $8.43 \pm 0.01\%$).

All species of red, brown and green seaweeds showed varied range of significant antioxidant activities. Carotenoids, phlorotannins, and sulfated polysaccharides (fucoidans) are amongst the most important and significant antioxidants which can be derived from algae. Amongst these three, bioactive marine polyphenol known as phlorotannins are an important antioxidant with potential as a natural antioxidant and contributing up to 25% of the DW in brown algal species [32]. Another antioxidant from brown algae is Fucoidans, which consist of sulfated groups attached to the fucose residues found in the cell wall. Phlorotannins and Fucoidans possess *in vitro* antioxidant activity such as hydroxyl radical scavenging activity, iron-chelating ability, including superoxide and reducing power [33, 34]. On the other hand, in the red, green, and brown algae carotenoids, provide protection against photooxidative processes by scavenging peroxy radicals and singlet oxygen[35]. Prior studies correlated the carotenoid contents and antioxidant activity of different algae [4]. Fucoxanthin, a xanthophyll is most abundant and efficient quenchers of singlet oxygen. *Fucus vesiculosus* a brown alga contained 1 µg/mg DW fucoxanthin and 0.2 µg/mg dry weight (DW) β-carotene [36]. Tocopherol is another antioxidant of carotenoid family and is widely used in the food industry because of its widely efficient radical scavenging activity in the extracts [37]. Antioxidant activities in seaweeds from coastal region are due to fluctuating environmental conditions in the intertidal zone [38], acclimation strategies to salinity and desiccation stress in seaweeds [39] air exposure during low spring tides, intense UV radiation, and other factors that may contribute to oxidative stress. antioxidant potential may be related to the presence of phenolic compounds that absorb UV radiation and increase antioxidants mainly in brown seaweeds [40].

Mineral Composition

Seaweeds are good source of minerals, compared to meat, spinach [41] and most land plants [42]. As per Dietary Reference Intake (DRI), the recommended daily intake of (~ 25g) can be fulfilled by a minute amount of seaweeds [43]. Significant amount of minerals in seaweeds provides benefit to human health like, treatment of thyroid goiter as well as also benefits vegetarians and vegans [44]. In the present study, total potassium,

sodium, calcium, magnesium and iron content of macro elements ranged from 7.37% – 1.31% dw, 4.57% – 2.85% dw, 8.41–1.06% dw, 1.866% – 0.764% dw and 1.42% – 0.07% dw respectively (Table 2). Phaeophyceae had highest potassium content on the other hand Chlorophyceae had highest calcium content. Sodium content, magnesium content and iron content along with microelements like cobalt content, Zinc content and manganese were highest in Rhodophyceae.

Seaweeds possess elevated amounts of mineral elements such as calcium and magnesium, which are known for their nutritional value such as *Ulva* sp. which can have up to 3.25 g of calcium per kg of dry weight [45]. Calcium content of seaweeds has better prospects of being utilized as nutraceuticals compared to calcium content of cow's milk which can be attributed to high content and easy ingestion in calcium carbonate form from seaweed, rather than calcium phosphate form of cow's milk [44]. Like calcium, magnesium participates in cementing of bones and teeth whereas iron is an important constituent of hemoglobin specific to 15 mg/day for female and 10–12 mg/day for male [43]. Not only macronutrient but also micronutrients (cobalt, manganese, and zinc) play various important roles in metabolism. Variations of element accumulation in seaweeds are mainly affected by physicochemical conditions of water, topology, stage of plant development, seasonality, and nearby anthropological activities.

Principal component analysis (PCA)

PCA of TPC, FC and Antioxidant activity

Relatively little work about the systematic antioxidant properties of seaweeds were done. Although there are many publications on the antioxidant activity of numerous seaweeds from Indian seacoast, there are very few reports on systematic analysis of antioxidant properties. Principal component analysis (PCA) was performed for TPC, FC, and antioxidant properties of thirty seaweeds of Phaeophyceae, Rhodophyceae and Chlorophyceae. The results of PCA for TPC, FC and different antioxidant activity were depicted on plot graphs (Fig. 2).

Principal components first (PC1) and second (PC2) explained 88.16% of total variance (PC1 accounts 69.22% and PC2 accounts 18.93%) present in data set. In the present study PCA help in understanding and visualization of complex and large data set, and antioxidant activity among thirty seaweeds. Analysis shows that TPC, FC and all antioxidant activity assays i.e. DPPH – SA, H_2O_2 – SA and Fe II – CA were closely loaded and grouped together on PC1, which suggests, a positive correlation among grouped variables. PC1 showed high correlation with TPC, FC, DPPH – SA, H_2O_2 – SA and Fe II – CA. seaweeds belongs to Phaeophyceae except *Dictyopteris australis* and Chlorophyceae except *Boodlea composite*, *Valonia utricularis* are grouped at right side along PC1. Accordingly, Phaeophyceae and Chlorophyceae seaweed member's shows highest correlation with TPC, FC, and these three antioxidant properties were positioned at right side on PC1. Seaweed species *Gelidiella acerosa*, *Grateloupia indica* and *Porphyra* spp. belongs to Rhodophyceae also loaded on positive side along PC1 and suggest that these species also have high antioxidant properties. Seaweeds belongs to Rhodophyceae except *Gelidiella acerosa*, *Grateloupia indica* and *Porphyra* sp. are loaded in negative side along PC1 indicating these seaweeds have comparatively less TPC, FC, and antioxidant properties. PC2 mainly differentiate the seaweeds have high TPC, FC and Fe II – CA. PCA of antioxidant components for chemotaxonomy and to investigate interrelation between biochemical components were also carried by some researchers [18, 46]. Pearson's correlation analysis also supports the results, Table 3 revealed a positive correlation in between the TPC, FC and all antioxidant activity assays i.e. DPPH – SA, H_2O_2 – SA and Fe II – CA also show a positive correlation (Table 3). Previous studies revealed that phenolic compounds are the main contributors to the antioxidant activity of various seaweeds. A positive correlation has been documented between TPC and antioxidant activity of different seaweed extracts by many researchers [18, 47]. Similar results of PCA were also presented by [46] in tropical seaweeds from Saurashtra coast of India. In present study, PCA biplot of antioxidant properties of seaweeds also shows chemotaxonomic relationship.

Conclusion

The present screening study indicated that seaweeds from different parts of Indian sea coast was found to contain varied range of significant antioxidant activities with TPC and FC. PCA indicates that seaweeds have positive and highly significant relationships between TPC and antioxidant properties i.e. DPPH – SA, H_2O_2 – SA and Fe II – CA. Accordingly on the basis of antioxidant properties, PCA discriminates three classes of seaweeds in three closely loading groups according to their taxonomic positions which manifest chemotaxonomic relationship antioxidant properties and other nutraceutical components present in these seaweeds increase their potential health benefits if used as alternative food source. Seaweeds may be utilized as a source of natural antioxidant components in food, pharmaceuticals and various other enterprises. Today seaweeds have become economically as well as ecologically significant bioresource of the world. Therefore, constant exploration is the need of hour to facilitate the useful and valuable information from this diverse and fascinating group of organisms.

Abbreviations

DPPH, 2,2-Diphenyl-1-picrylhydrazyl; DPPH-SA, 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; Dw, Dry weight; FC, Flavonoid contents; Fe II-CA, Ferrous ion-chelating ability assay; GAE g^{-1} , Gallic acid equivalents per gram; H_2O_2 –SA, Hydrogen peroxide radical scavenging activity; IA, Index of Atherogenicity; PC1, Principal component first; PC2, Principal component second; PCA, Principal Component Analysis; PUFA, Polyunsaturated fatty acids; QE g^{-1} , Quercetin equivalents per gram of extract; TPC, Total phenolic content; UI, Unsaturation Index

Declarations

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Declaration of authors' contributions

PV, DK, GM and DS have role in design of the study, performed experiments, analysis and interpretation of data and all authors contributed to manuscript writing and approved the final manuscript.

Declarations of conflict of interest

No conflicts, informed consent, human or animal rights applicable.

Ethics, Consent to Participate, and Consent to Publish declarations: Not applicable.

Financial conflicts of interest

No financial conflicts of Interest or any other interest.

Clinical trial number: Not applicable.

Data availability: The data that support the findings of this study are available upon request.

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Figures

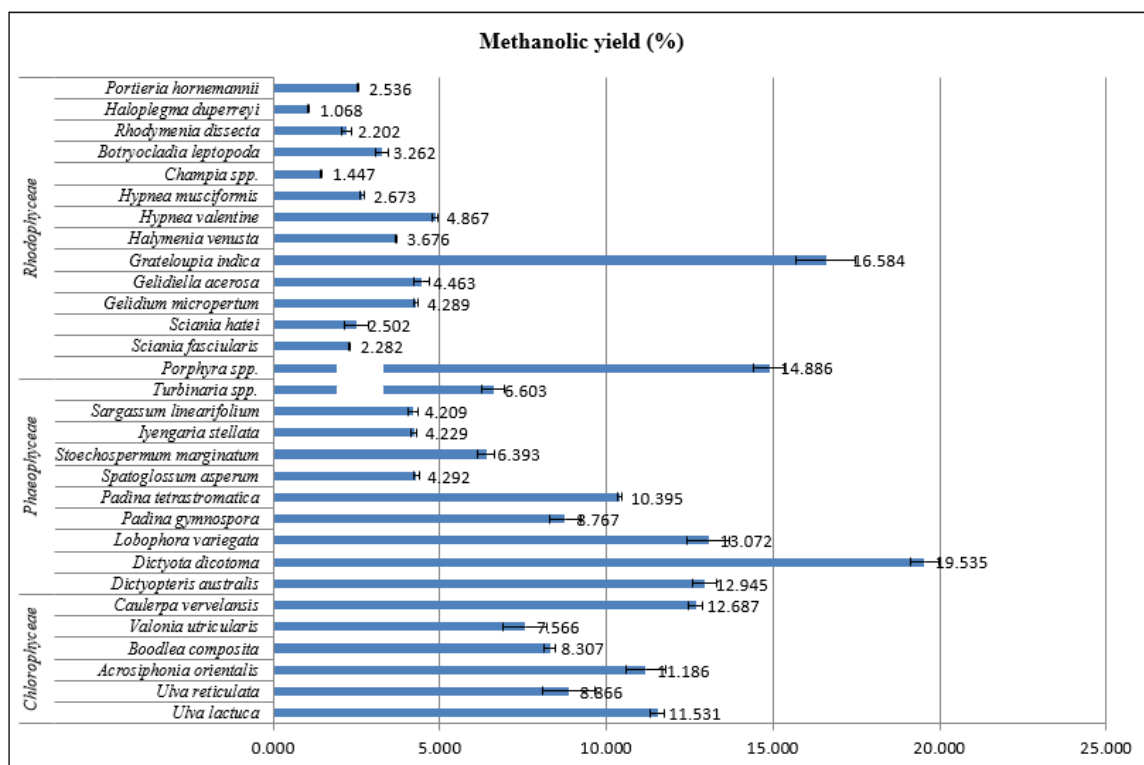


Figure 1

Methanolic yield of seaweeds. Values are mean $\pm$ Standard deviation (n = 3).

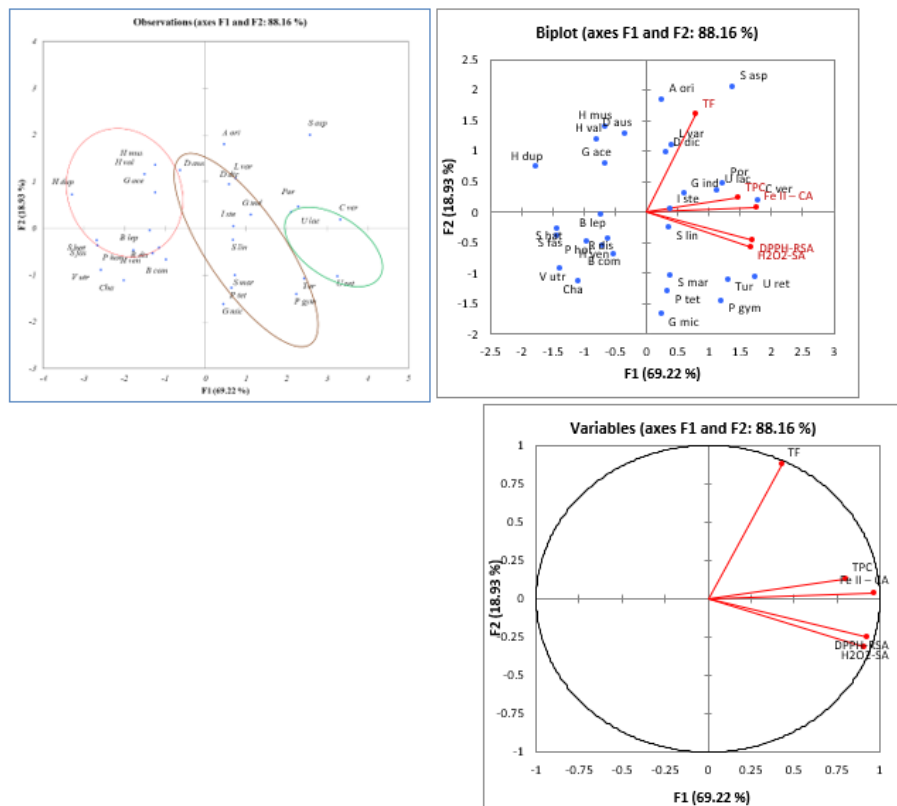


Figure 2

Principal component analysis (PCA): Bi plot shows Principal component analysis (PC1 and PC2) of Total phenolic content (TPC), flavonoid content (FC) and Antioxidant properties i.e. DPPH radical scavenging activity (DPPH-SA), Hydrogen peroxide radical scavenging activity (H₂O₂ – SA) and Ferrous Ion Chelating Ability (Fe II - CA) of 30 different seaweeds from Indian Sea coast.