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Biochemical, Mineral Analysis and Anti-Oxidant Activity of Methanolic Extracts of *Ulva Flexuosa and Hypnea Valentiae*

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Abstract: ***Seaweeds have been used as a significant source of food because of their biochemical and nutrient composition. In the present study, biochemical composition and Macro and Micro nutrient content of selected seaweeds was examined. Quantitative analysis of protein content of Ulva flexuosa and Hypnea valentiae was ranged from 19.9 and 12.8%, High protein content was found in the green seaweed U. flexuosa and low protein content in the red seaweed H. valentiae. Carbohydrate content of seaweeds ranged from 35% and 50%. The maximum carbohydrate content was recorded in the red seaweed H. valentiae and the green seaweed U. flexuosa recorded the minimum value. The Lipid content of seaweeds varied from 4.6% to 5.4%. The maximum lipid content was recorded in red seaweed H. valentiae and the green seaweed U. flexuosa recorded the minimum content. The total fibre content was ranged from 2.6–2.1%. The maximum fibre content was recorded in the green seaweed U. flexuosa and red seaweed H. valentiae recorded the minimum content. The Macro and Micro nutrient contents of U. flexuosa and H. valentiae were investigated in order to gain more nutritional information. It was found that the two seaweed species contained high level Macro nutrient contents. of these, U. flexuosa was rich in Mg, Ca, Fe, Bo, Cu and Zn while H. valentiae was rich in K, Na, and Mn. This study suggested that both species could be potentially used as raw materials or ingredients to improve the nutritive value and quality of functional food and healthy products for human beings.***
Keywords: Biochemical properties, nutritional composition, U. flexuosa, H. valentiae, marine algae

I. INTRODUCTION

Marine macroalgae or seaweeds, are considered by their pigmentation, morphology, anatomy, and nutritional composition as red (Rhodophyta), brown (Phaeophyta) or green seaweeds (Chlorophyta) (Dawczynski et al., 2007). In India, seaweeds have been utilized since the ancient times as source of human food, animal feed, fertilizer, as well as herbicides (Fleurence, 1999; Sánchez Machado *et al*., 2004; Kumari, 2010). Seaweeds are valuable sources of Carbohydrate, protein, Fat, Fibre, and macro and trace elements, as well as important bioactive compounds (Ortiz *et al*., 2006). Thus, they have been recognized as being beneficial for human and animal health (Fleurence, 1999). However, the biochemical and nutrient compositions of seaweeds are depending on species, habitats, and environmental conditions (Ito and Hori, 1989).

Usually, green and red seaweeds contain higher protein contents (10–30% DW) than brown seaweeds (5–15% DW). Proteins are composed of several amino acids and their nutritional quality can be evaluated against the recommended amino acid pattern (Matanjun *et al*., 2009). The lipid content of marine seaweeds accounts for 1–6% DW and provides a low amount of energy (Kumari *et al*., 2010). The nutrient contents in seaweed are interpreted through their ash contents which range between 8–40% DW (Mabeau and Fleurence, 1993). Seaweeds are also good sources of dietary fibre (33– 50% DW), which can be classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) (Rupérez and Saura-Calixto, 2001). Red seaweed (*Hypnea* spp.) and green seaweed (*Ulva* spp.) have been abundant in the coastal area.

The *green seaweed, Ulva flexuosa, and red seaweed, Hypnea valentiae* are selected in this study. *U. flexuosa* is a species with hollow one layered thalloid green alga which grows rocks and shores in the shallow water. The cell wall of *Ulva* contains polysaccharides called ulvan, which comprises rhamnose, sulfate, xylose, iduronic acid, galactose, and glucose (Robic *et al*., 2009a). Ulvan can elicit responses and induced defence mechanism in cultivated organisms (Borsato *et al*., 2010). *H. valentiae species* has carrageenans polysaccharide which is found in red seaweeds (Sangha *et al*., 2010). It is commonly used as a thickening, suspending, gelling and stabilizing agents for food products as gelatin. It differs from agar mainly in higher sulphated fraction and higher ash content (Ramalingam *et al*., 2003). Therefore, the present study aimed to determine the Biochemical composition, and nutritional contents of *U. flexuosa* and *H. valentiae* collected from the Mandapam, Ramanathapuram, Tamil Nadu.

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II. MATERIALS AND METHODS

A. *Collection of algae*

The seaweeds, *Ulva flexuosa,* and *Hypnea valentiae* were collected from the intertidal shallow zone at depth of 0-1 m at Mandapam, Ramanathapuram, Tamil Nadu. The algae were obtained from the Mandapam coast, Gulf of Mannar region, Rameswaram (Latitude: 9°16'32.56" N and Longitude: 79°7'25.03" E) along the southern regions of Tamil Nadu. The harvested algae were washed with tap water to remove debris and epiphytes and packed in polythene bag tightly. The algae were further washed with distilled water to remove traces of salts and other contaminants, then shade dried at ambient temperature (28˚C) and the samples were grounded into fine powder using electric blender. The powdered samples were taken and stored at refrigerator at 4˚C for further use.

B. *Authentication of algae*

The collected algae were authenticated at Botanical Survey of India (BSI), Southern Regional centre, Coimbatore, Tamil Nadu. The algae were identified as *Ulva flexuosa, Hypnea valentiae* and voucher specimens No BSI/SRC/5/23/2018/Tech.1383).

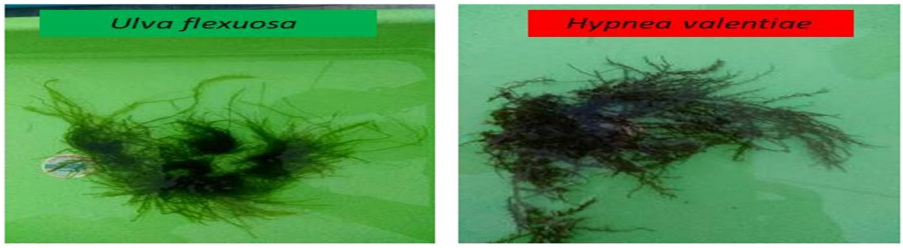

Fig. 1 Different types of Macro algae

C. *Bio-chemical analysis*

The total carbohydrate content of the powdered seaweed, *Ulva flexuosa, Hypnea valentiae* was estimated by phenol-sulphuric acid method. The Bradford assay was performed to measure the total protein concentration in the sample. Fat content (AOCS official method Ba 3-38), Moisture level (ISO6496), Crude fibre content, and Crude ash (ISO5984) was analysed using standard methods. Determination of Total phenolic and *flavonoid* content: TPC was expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE g^{-1}). FC was expressed as mg Rutin equivalents per gram of dried extract (mg RE g^{-1}).

D. *Determination of Elements by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)*

Sample Preparation: Weigh about 0.5 g of two types of seaweed sample in beaker separately and add 6 ml of Nitric acid and 2 ml of HCl. Keep it in hot plate till the sample is completely digested and then cool. Make up the solution to 50 ml with Mill-Q- Water. Standard Preparation: Prepare a Calibration mix of standards such as 10, 20, 30, 40, 50 µg/Kg using Mill-Q-Water containing 1 % Nitric acid. Preparation of the standard solution with 1 % Nitric acid using Mill-Q-Water and the concentration of 10, 20, 30, 40, 50 µg/Kg then add the Hydrochloric acid and Nitric acid prepare the calibration curve.

The analyses were performed by using ICP-MS system, (South India Textile Research Association, Coimbatore.) equipped with model Thermo Scientific -i-Cap-Q. The active components present in the seaweeds sample were separated using the inert gases Argon as the carrier gas; and the collision gas was Helium. The sample uptake time was 65 secs and probe rinse were 30 sec. The Retardation factor (Rf) power was up to 1600W. The plasma and carrier gas flowing rate was 20 ml/min. Axillary gas flowing rate was

0.78 ml/min. The Spray Chamber Temperature was 2.5ºC. All the samples were prepared in triplicate. The elements values were expressed in mg/kg. The ICP-MS sample quantification was done by Q- Tegra software by thermo ICP-MS (i-Cap-Q System).

E. *DPPH* free radical scavenging activity

DPPH radical scavenging activity was determined according to the method Zhang *et al*., (2007) with slight modifications. Each extract at 100 µl of various dilutions, were mixed with 100 µl of 0.16 mM DPPH solution. The mixture was vortexes for 30sec, kept for 30 mins. in dark place and then monitored at 517 nm in UV-VIS spec. Ascorbic acid was used as positive control. The antioxidant capacity was calculated using the following equation: % Inhibition = $(A_{cotrol} - (A_{sample} - A_{blank}) / A_{cotrol}) \times 100$

F. *Statistical analysis*

Data were expressed as mean, standard error and standard deviation of three replicate determinations. The correlation and regression analysis were performed between antioxidant activities using Microsoft Excel windows 16.

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III. RESULTS

A. *Bio-chemical analysis*

The determined for various bio-chemical constituents such as Moisture, carbohydrate, protein, total lipids, Fibre and ash content of *U. flexuosa*, and *H. valentiae* seaweeds were recorded and shown in the Table 1. The biochemical constituents, 7.6±0.2% of Moisture, 35±1% of carbohydrate, 15.9±.4% of protein, 4.6±0.2% of total fat, 2.6±0.2% of fibre, 14.6±0.1 of ash content were observed from the *U. flexuosa*. Whereas the biochemical constituents, 8.2±0.2% of Moisture, 50±2% of carbohydrate, 12.8±1% of protein, 5.4±0.3% of total fat, 2.1±0.2% of fibre, 15.4±0.2% of ash content was observed in the *H. valentiae*.

The amount of total phenolics in methanol extracts of *U. flexuosa*, and *H. valentiae* were determined spectrometric according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalent. Total phenolic (TPC) and flavonoid content (FC) of the algal extracts were also presented in Table 1. The content of phenolic compounds varied from 18.5± 0.5 (*U. flexuosa*) 25.2± 0.8 (*H. valentiae*) mg GAE g^{-1}. In general, the higher antioxidant capacity was resulted in higher total phenolic content. As shown in Table 1, the flavonoid content of algal extracts was varied 46±3 (*U. flexuosa*) and 36.7±1 (*H. valentiae*) mg RE g^{-1}.

Table 1 Bio-chemical contents of selected seaweeds.

S. No	Nutrient Analysis of Seaweed (N= 3)	*U. flexuosa* (%)	*H. valentiae* (%)
1	Moisture	7.6±0.2	8.2±0.2
2	Carbohydrate	35±1	50±2
3	Crude Protein	1.9±.4	12.8±1
4	Crude Fat	4.6±0.2	5.4±0.3
5	Crude Fibre	2.6±0.2	2.1±0.2
6	Total Ash	14.6±0.1	15.4±0.2
7	Total Phenol (mgGAEg-1)	18.5±0.5	25.2±0.8
8	Flavonoid (mg REg-1)	46±3	36.7±1

Mean ±SD

B. *ICP-MS Mineral Analysis of Seaweed*

The ICP-MS macro nutrient analyses were depicted in the Table 2**.** Among the 15 minerals analysed, Mg K, Ca, and Na, was macronutrients present in the selected seaweeds. Mg (16439.7±3.1) was higher in the green alga, *U. flexuosa*, K (2693.3±0.1) was higher in the red alga, *H. valentiae*, Ca (4823.4±0.1) level was higher in the green alga, *U. flexuosa* and Na(4600.2±0.03) was higher in the red alga, *H.valentiae*. The ICP-MS micro nutrient analyses were depicted in the Table 3. Fe, Mn, Bo, Cu, and Zn were micronutrients present in the seaweeds. Fe (2929.4±0.06) level was higher in green alga, *U. flexuosa*. Mn (37.7±0.03) level was higher in *H. valentiae*. Bo (405.7±0.03) was higher in the red alga, *H. valentiae*, Cu (33.7±0.04) was higher in the green alga, *U. flexuosa*, Zn (1.5±0.03) was higher in the green alga, *U. Flexuosa*.

Table 2 Macro nutrient analysis of seaweeds by ICP-MS

S. No	Macro nutrient Analysis (N= 3)	*U. flexuosa* (mg/kg)	*H. valentiae* (mg/kg)
1	Magnesium	16439.7±3.1	8914.1±0.3
2	Potassium	1155.3±0.3	2693.3±0.1
3	Calcium	4823.4±0.1	2373.5±0.01
4	Sodium	2763.3±0.3	4600.2±0.03

Mean ±SE

Table 3 Micro nutrient analysis of Macro algae by ICP-MS

S. No	Micro nutrient Analysis (N=3)	*U. flexuosa* (mg/kg)	*H. valentiae* (mg/kg)
1	Iron	2929.4±0.06	1699.9±0.2
2	Manganese	24.5±0.1	37.7±0.03
3	Boron	203.6±0.07	405.7±0.03
4	Copper	33.7±0.04	2.9±0.03
5	Zinc	1.5±0.03	1.15±0.08

Mean ±SE

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C. DPPH free radical scavenging activity of methanolic extract of seaweeds

DPPH radical scavenging method used to evaluate the antioxidant capacity of the seaweed extract. All seaweed extracts showed antioxidant activity in various degrees (Table 4, 5 and 6; Fig. 2). Lower IC_{50} value indicates higher antioxidant activity **(Table 7).** Comparison of IC_{50} value of ascorbic acid (21.8±2.0mgml^{-1}) as a standard antioxidant. *H. valentiae* exhibited relatively high antioxidant activity with a relatively low IC_{50} (14.2^{a}±0.3 mgml^{-1}) and compared with *U. flexuosa* (34.8^{c}±0.4 mgml^{-1}). The Pearson's correlation coefficients between the variables were presented in Table 8 and Graph 1, and 2. Strong positive significant correlations between DPPH radical scavenging and contents of phenolics and flavonoids. High positive correlations between IC_{50} and Phenolic content was r =0.1509, $p < 0.01$. and flavonoids r = 0.9990, $p < 0.01$.

Table 4 Free radical scavenging effects of Ascorbic acid

concentration	Absorbance of sample	control	Ctrl-sample/Ctrl	% inhibition
10	0.139	0.261	0.46743	46.7432
20	0.124	0.261	0.52490	52.4904
30	0.119	0.261	0.54406	54.4061
40	0.110	0.261	0.57854	57.8544
50	0.102	0.261	0.60919	60.9195
60	0.098	0.261	0.62452	62.4521
70	0.083	0.261	0.68199	68.1992
80	0.079	0.261	0.71264	71.2643
90	0.075	0.261	0.71264	71.2643
100	0.067	0.261	0.74329	74.3295

Table 5 DPPH free radical scavenging effects of methanolic extract of *Ulva flexuosa* seaweed.

concentration	Absorbance of sample	Control	Ctrl-sample/Ctrl	% inhibition
10	0.167	0.261	0.36015	36.0153
20	0.157	0.261	0.39846	39.8467
30	0.145	0.261	0.44444	44.4444
40	0.125	0.261	0.52107	52.1072
50	0.105	0.261	0.59770	59.7701
60	0.097	0.261	0.62835	62.8352
70	0.063	0.261	0.75862	75.8620
80	0.042	0.261	0.83908	83.9080
90	0.025	0.261	0.90421	90.4214
100	0.017	0.261	0.93486	93.4865

Table 6 DPPH Free radical Scavenging effects methanolic extract of *Hypnea valentiae*

concentration	Absorbance of sample	Control	Ctrl-sample/Ctrl	% inhibition
10	0.120	0.261	0.54023	54.0223
20	0.118	0.261	0.54789	54.7892
30	0.115	0.261	0.55939	55.9386
40	0.105	0.261	0.59770	59.7701
50	0.090	0.261	0.65517	65.5117
60	0.070	0.261	0.73180	73.1800
70	0.062	0.261	0.76245	76.2452
80	0.032	0.261	0.87739	87.7394
100	0.010	0.261	0.96168	96.1685

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Table 7 DPPH radical scavenging IC_{50} values of seaweeds extract and Ascorbic acid

S. No	Samples	IC50 value (µg/ml)
1	*U. flexuosa*	34.8±0.4
2	*H. valentiae*	14.2±0.3
3	Ascorbic acid	21.8±2.0

Mean ±SE

Table 8 Correlation between DPPH, Phenolic and flavonoid content of seaweeds

	DPPH radical Scavenging activity	Phenolic content	Flavonoid content
DPPH radical Scavenging activity	1	-	-
Phenolic content	0.1509	1	-
Flavonoid content	0.1946	0.999	1

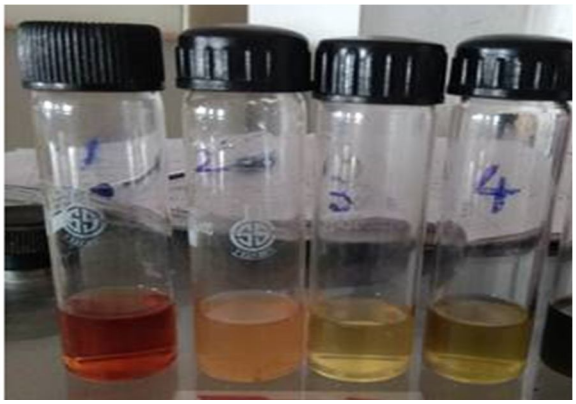
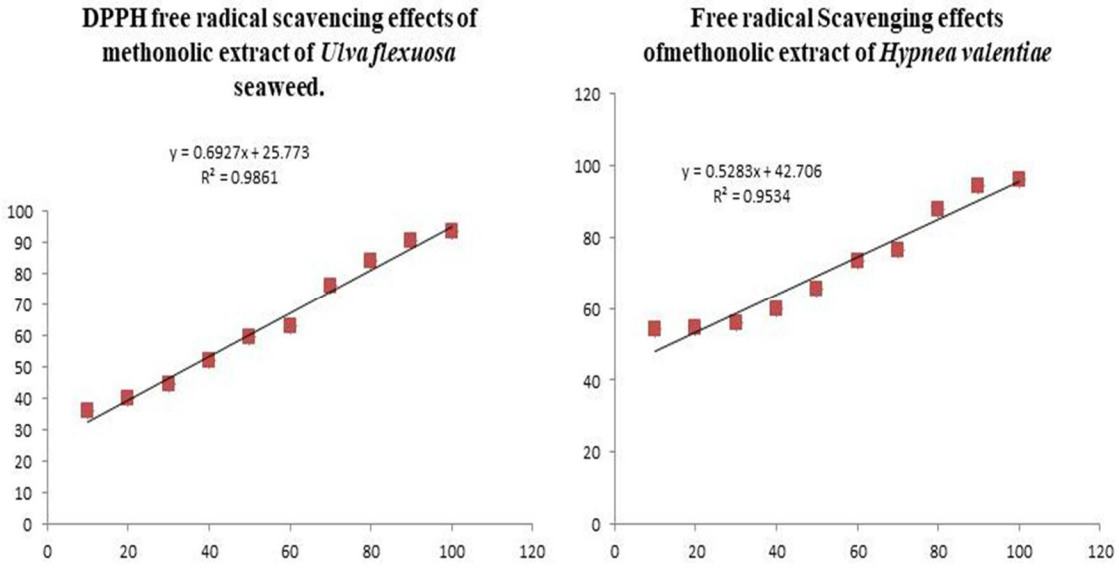


Fig. 2 Anti-oxidant and DPPH free radical scavenging activity of seaweeds



Graph 1 Regression analysis of Free radical Scavenging effects of *Ulva flexuosa* and *Hypnea valentiae*

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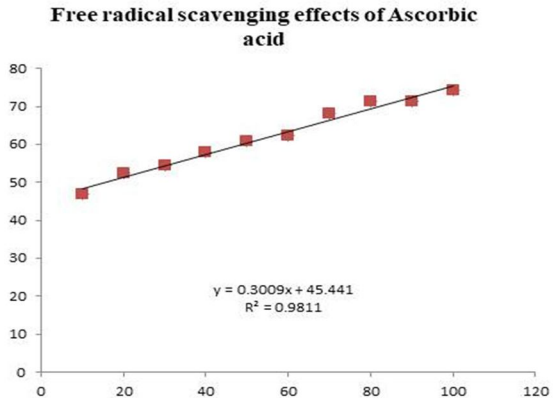

Graph 2 Regression analysis of Free radical Scavenging effects of Ascorbic acid

IV. DISCUSSION

The Bio-chemical composition of *U. flexuosa* and *H. valentiae* in the present study is shown in **Table 1**. Moisture content is an important measure in determining the self-life and quality of processed seaweed meals, as high moisture (16.9%) may rush the growth of microorganisms (Rohani-Ghadikolalel *et al*., 2012). Lower level of moisture content was observed in this study and *U. flexuosa* has lower moisture content (7.6%) whereas *H. valentiae* (8.2%) has quite higher but this level was acceptable and consistent with Marudhupandi and Inbakandan, (2015); Abirami and Kowsalya, (2011). Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other physiological processes (Gokulakrishnan *et al*., 2015). In the present study, the carbohydrate content was higher and followed by protein content. Among the selected seaweeds, the carbohydrate was varied from 35 to 50% of dry weight showed high carbohydrate content whereas *H. valentiae* has higher carbohydrate content than *U. flexuosa.* Omer *et al*., (2013) reported that proximate composition analysis of seaweeds, carbohydrates has the most abundant component constituting up to 90.83% of the dry matter of the seaweeds and this study report level was supported by Roy and Anantharaman, (2017).

Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation control. In the present study, the protein content of *U. flexuosa* (19.9±.4% DW) was significantly higher than that of *Hypnea valentiae* (12.8±1% DW). This result was consistent with the report of Fleurence (1999) who described that Ulva spp. had protein content within the range 10–26% (DW). (Manivannan *et al*., 2008) but lower than those of some red seaweed species.

The two most preferred and consumed algae, *Hypnea* and *Ulva*, has low protein contents. These results were supported by Angel *et al*., (2012). Selvi *et al*., (1999) reported that red alga *Hypnea valentiae* contains higher protein level to compare the other type of algae. Bouba *et al*., (2010) reported 22.22% of crude protein in *Ulva fasciata*. In general, the fat or lipid provides plentiful level of energy in oxidation process than other biological compounds. In the current study, the fat level of selected seaweeds was higher *H. valentiae* followed by *U. flexuosa*. Thus, this level was supported with Xiren and Abuduli, (2019).

Dietary fibres promote beneficial physiological effects including relaxation and blood cholesterol regulation. The fibre content of the selected seaweed was ranged from 2.1 to 2.6%. Fibre content was quite higher in *H. valentiae* and lower in *U. flexuosa*. Furthermore, the results were accordance with the reports of Tabarsa *et al*., (2012); Sakthivel and Pandima Devi, (2015). The consumption of dietary fibres and plant cell walls containing such fibre components protect humans against a number of chronic diseases reported by El-sayed and Hussein, (2017); Chai *et al*., (2012). From the previous literature reported that the soluble dietary fibre is regarded influential in absorption of nutrients, slower digestion, reduce levels of blood cholesterol and glucose. It also plays an important role in preventing constipation, colon cancer, cardiovascular disease and obesity (Ortiz, 2006). In contrast, insoluble dietary fibre is associated with faecal bulk increase and intestinal transit time decrease (Potty, 1996). Thus, the seaweed species appeared to be interesting source of raw material or ingredients for producing functional food or health promoting food known as abundant sources of high polysaccharide content which contain high level of soluble and insoluble dietary fibre (Lahaye, 1991).

The ash content in *the selected seaweed* was examined in this study ranged from 14.6 to 15.4%. The more ash content was found in red alga *H. valentiae* that indicated higher mineral content although suitable range of ash content was present in the *U. flexuosa* which level was agreed with Abirami and Kowsalya, (2011) was 10.5% and accordance with Khairy and El-Shafay, (2013) ranged between 17.56-24.49% and Abdel-Khaliq *et al.,* (2014) was 17.6%.

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Lower level was observed in Ortiz *et al*., (2006) was 11%, whereas higher content reported by Rohani-Ghadikolalel *et al.*, *(*2012) was 12.4% of ash level. Ash content in seaweeds is generally high which indicated that the essential minerals and trace elements were present in seaweeds.

In the present study reported the highest total phenol and flavonoid content was found in the red seaweed, *H. valentiae* and moderate level was found in *U. flexuosa.* Current report was supported by Wang *et al*., (2012). Phenolic compounds and flavonoid were affected growth and metabolism of bacteria, finding recommend that methanol extracts could be used as a best source of antimicrobial agent. Mary and Vimalabai, (2003) screened 4 brown seaweeds from Tuticorin coastal area for their phenol content; reported highest value in *Padina tetrastromatica*. The phenol content increased with the increasing age of the tissue and followed increasing salinity reported by Wang *et al*., (2012). The selected seaweeds have excellent nutrient source, containing high amounts of macro and micronutrients by inductively coupled plasma mass spectrometry (Table.2 and 3). Magnesium level was higher in *U. flexuosa*. Potassium level was more in *H. valentiae*. Calcium level was higher in *U. flexuosa*. Sodium level was more in *H. valentiae.* The Iron level was higher in *U. flexuosa.* Manganese level was higher in *H. valentiae*. The boron level was more in *H. valentiae.* Furthermore, the copper and Zinc level was higher in *U. flexuosa*. The macro and micro nutrient levels were supported by Chan and Matanjun, (2017); Matanjun *et al*., (2009) who found that Mg, K, Ca were the main mineral element. The results of the current study were agreed with the study of Tabarsa *et al*., (2012). In the current study, strong positive correlations were found between total phenol and flavonoid contents and antioxidant capacity. The antioxidant activity of selected seaweeds extract was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate), hydroxyl radical scavenging activity regression analysis was shown in the Graph 1, and 2; IC50 value was predicted in the Table 7. The antioxidant activities of selected seaweeds were in accordance with their amount of total phenolic and flavonoid contents (Table 1)**.** Lower IC50 value indicates higher antioxidant activity whereby *H. valentiae* exhibited relatively high antioxidant activity with relatively low IC50 was compared with *U. flexuosa*, and Ascorbic acid (21.8±2). Strong positive significant correlations between DPPH radical scavenging and contents of phenolics and flavonoids (Table 8). Moreover, High positive correlations between IC50 and Phenolic content was r =0.1509, ($p < 0.01$) and flavonoids was r = 0.9990, ($p < 0.01$) and these results were coincided with Chai and Wong, (2012). Luo *et al*., (2010) have demonstrated that phenolic compounds were one of the most effective antioxidants in marine algae (Zakaria *et al*., 2012). Flavonoids are more stable, less-reactive when they oxidized by radicals. A positive correlation has been documented between anti-oxidation capabilities and total polyphenol contents, but not with the contents of flavonoids (Liu *et al*., 2010; Chai and Wong, 2012)

V. CONCLUSION

India is endowed with 6000 km coastline and wet harvestable biomass of seaweeds belonging to 700 species. Of these, nearly 60 species to the tune of 30 % are economically important for their polysaccharides. Others amounting to 70 % of the biomass are underutilised. These underutilised or unutilised seaweed resources can be used as fodder or feed for animals either raw or as processed. Species of *Enteromorpha, Acanlhophora. Chnoospora, Chaetomorpha Caulerpa, Gracilaria, Hypnea, sargassum,* and *Ulva,* can be best tried as fodder. It was found that *U. flexuosa* and *H. valentiae* contained high levels of ash, appreciable protein and dietary fibre contents and relatively high levels of macro and micro elements, Thus, these two seaweeds can contribute to human and animal nutritional requirements. Their nutritional compositions together with their physicochemical properties suggest that *Ulva and Hypnea* species have a potential food to be functional ingredients in food industry. Moreover, its consumption has a positive effect on health because they can reduce blood lipid level, obesity and risk of coronary heart diseases. Further studies concerned vitamin, soluble and insoluble polysaccharide constituents, and toxic elements are necessary to provide more information for safer and more versatile utilization of these seaweeds.

VI. ACKNOWLEDGMENT

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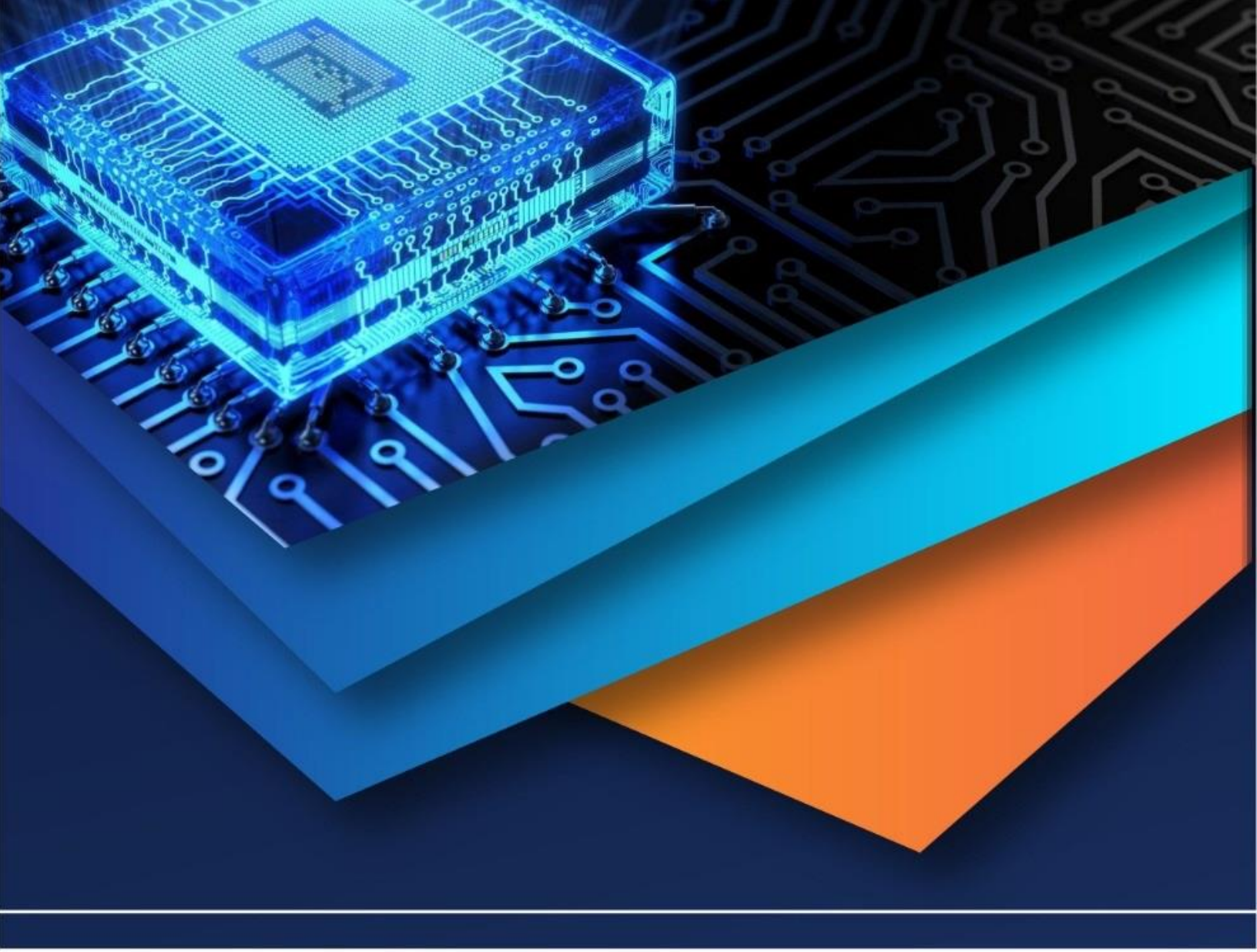

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