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The variation in distribution of total phenols and antioxidant activity in five species of marine macro algae

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ABSTRACT

The total phenol content of five species of marine macro algae belonging to Chlorophyceae and Rhodophyceae were analysed for one year. Of the five species that were analysed, three species belong to Chlorophyceae and two species to Rhodophyceae. The annual mean of phenols was high in C. sertularioides followed by G. corticata, U. fasciata, E. compressa and H. musciformis. The phenols ranged from 110 to 520 µg/g among the five species. The seasonal variations in distribution was also observed in these five species. The phenols were observed high in winter season for E. compressa, C . sertularioides and G. corticata and during summer season for U. fasciata and H. musciformis. The environmental variables such as pH, dissolved oxygen, light and silicate were correlated positively at $P \geq 0.05$ level with the phenols of these macro algal species. The ANOVA showed a significant variation between U. fasciata, E. compressa; U. fasciata and C. sertularioides; E. compressa and C. sertularioides at $P < 0.05$. The variation is also significant between G. corticata and H. musciformis at $P < 0.05$ level. The variations in the distribution of total phenols may be due to variation in species, sediment and surrounding water quality. All the five macro algae tested showed antioxidant activities and these algae have significantly possessed 31 to 56% antioxidant activity indicating that these species may exert potential natural antioxidant properties for various applications in industry.

Key words: antioxidant activity, distribution, environmental variables, marine macroalgae, polyphenols, seasonal variation

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INTRODUCTION

Phenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plant poly phenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases. Phenols are compounds possessing one or more aromatic rings with one or more hydroxyl groups. With more than 8,000 phenol structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenols are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenols are widespread constituents of plant foods (fruits, vegetables, cereals, olives, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partially responsible for the overall organoleptic properties of plant foods. For example, phenols contribute to the bitterness and astringency of fruit and fruit juices, because of the interaction between phenolics, mainly procyanidin, and the glycoprotein in saliva. Anthocyanins, one of the six subgroups of a large group of plant polyphenol constituents known as flavonoids, are responsible for the orange, red, blue and purple colours of many fruits and vegetables such as apples, berries, beets

and onions. It is known that phenols are the most important compounds affecting flavor and colour difference among white, pink and red wines; they react with oxygen and are critical to the preservation, maturation and aging of the wine.

Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall [8]. The phenolic compounds have diverse biological activities ranging from toxicity to hormonal mimicry and act as cell wall material, colourfull attractants for birds and insects belong to seed dispersal and pollination. Phenols are important in human food since they function as antioxidant, anti – carcinogenic, anti – inflammatory, anti – allergenic, anti – antherogenic, anti - microbial, anti – thrombolic, cardioprotective and vasodilatory [5, 21]. The Poly phenols have high radical free scavenging activity which help to reduce the risk of chronic age related neurological degenerative diseases [12].

The antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantity of reactive oxygen and/ or nitrogen species ROS/RNS. Oxidative stress is important in development of chronic degenerative disease including coronary heart disease, cancer and aging [2].The analysis of phenol contents and antioxidant activity in seaweeds is reported earlier from many geographical areas [6, 13,16, 19, 20 ,24, 26, 28, 30]. Yet, the seasonal variation on phenols in red and green algae was not reported so far. So, with this in view five species belonging to red and green algae were analysed for their seasonal variations in phenols. The antioxidant activity of these five species of macro algae was also studied for their percentage of inhibition.

MATERIALS AND METHODS

Macroalgae material collection and processing

Five marine macroalgae belonging three to Chlorophyceae viz., *U. fasciata, E. compressa*, *C. sertularioides,* and two species to Rhodophyceae *viz., G. corticata* and *H. musciformis* were collected from Visakhapatnam coast, east coast of India, at Tenneti Park area. These algae were collected during February 2012 to January 2013 from the intertidal zone of the coast at low tide periods. The algae were collected carefully and were washed with fresh water and were cleaned for all the extraneous matter. They were shade dried, powdered, labeled and preserved for further analysis.

Measurement of environmental parameters

The environmental variables were also collected simultaneously at the macroalgae collecting site for one year. The environmental parameters such as temperature, pH, salinity and light were measured using standard meters. The dissolved oxygen, nitrite, phosphate and silicate were measured according to the standard methods for sea water analysis.

Determination of total phenols

One gram powdered material of macro algae was extracted with 5 ml of 80% ehanol and the extracts were filtered with Whatman no 1 filter paper and the residues were repeatedly extracted with the same solvent until they were colourless. The ethanol in the extracts was removed and concentrated. Total phenol contents were determined with Folin – ciocalteu reagent [22] using Gallic acid as a standard phenol compound. The concentration of total phenol contents was measured as milligram of Gallic acid equivalent (GAE in mg/g of the sample). All the determinations were carried out in triplicates.

Determination of antioxidant scavenging activity

3 g of each alga sample collected in December 2012 was extracted with 30 ml of ethanol. The scavenging activity of DPPH free radical was measured using these crude extracts. An aliquot of 2 ml of 0.004% DPPH solution in ethanol and plant extract at various concentrations were mixed. The mixtures were made to reach a steady state at room temperature for 30 minutes. Decolourisation of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 2 ml of respective vehicle in place of plant extract/ ascorbic acid.

$$\text{Percentage inhibition} = \frac{\text{Average control OD} - \text{Test sample OD}}{\text{Average control}} \times 100$$

Statistical analysis

The mean and standard deviation were calculated**.** The correlation coefficients were anlysed for 12 months for physical, chemical variables and phenols of seaweeds. The ANOVA is tested for the level of variance between the species. The statistical analysis was done using standard statistical packages.

RESULTS AND DISCUSSION

The range and the mean values of environmental variables during the sampling period are presented in Table 1. The temperature of the sea water ranged from 27 to 30 ◦C with an annual mean of 29.5 ◦C. The pH ranged from 8.2 to 9.2 with an annual mean of 8.5. The salinity ranged from 20 to 32 ppt (parts per thousand) with a mean of 29.5 ppt. The atmospheric light ranged from 26.8 to 68.6 K lux with a mean of 50.5 K lux. The dissolved oxygen ranged from 3.1 to 10.4 mg/l with a mean of 5.6 mg/l. The nitrite ranged from 0.10 to 4.05 µmoles with a mean of 1.23 µmoles. The phosphate ranged from 0.3 to 1.71 µmoles with a mean of 0.80 µmoles. The silicate ranged from 0.13 to 12.6 µmoles with a mean of 7.75 µmoles. The seasonal variations in environmental variables are presented in table 2. The temperature was high in summer and rainy period with 30 ◦C and 30.5 ◦C respectively. It was low with 28 ◦C in the winter period. The pH was high in winter with 9.0 and low with 8.25 in rainy period. The salinity was high with 31 ppt in summer and low with 28 ppt in winter period. The light was high in winter with 60.87 K lux and it was low with 44.35 K lux in rainy period. The dissolved oxygen was high with 7.04 mg/l in summer and it was low with 4.18 mg/l in winter. The nitrite was high with 1.97 µmoles in rainy period and it was low with 0.67 µmoles in winter. The phosphate was high with 1.21 µmoles in rainy period and low with 0.47 µmoles in winter. The silicate was high with 8.85 µmoles in summer and low with 5.86 µmoles in winter. The present results agreed with the earlier findings [3, 23] on the coastal waters of Bay of Bengal. The high salinity value was observed during summer which could be due to high temperature and shallow nature of the coastal area. High dissolved oxygen during north east monsoon could be attributed to low temperature and high wave action dissolving much atmospheric air. High concentration of phosphate during winter due to desorption of phosphate taking place from the sediment with increasing salinity during this period [25].

The total phenol content of five species of marine macroalgae was analysed for a period of one year. Variation in distribution of phenols was found among the five species studied. The phenols ranged from 110 to 520 µg/g among the five species. Among the Chlorophyceae the mean varied from 217.3 to 391.8 µg/g and between Rhodophyceae it was from 210.0 to 306.0 µg/g. The variation in annual mean distribution of phenols was shown in Fig.1. The range and mean values were given in table 3. *In C. sertularioides* it was high with an annual mean of 391.8 µg/g followed by *G. corticata* with 306.0 µg/g, *U. fasciata* 226.6 µg/g, *E. compressa* with 217.3 µg/g and *H. musciformis* with 210.4 µg/g . The highest range was also observed in *C. sertularioides* varying from 110 to 520 µg/g. In *G. corticata* it varied from 240 to 410 µg/g. In *H. musciformis* it was varied from 180 to 280 µg/g. In *U. fasciata* it was 160 to 280 µg/g and the lowest range was 110 to 280 µg/g occurred in *E. compressa*. The earlier reports on total phenol content of U. fasciata was 30, E. *compressa* was 25 µg/g dry weight [16. It was much lesser than the present values. The phenolic content 3.24 mg/g in *G. corticata* [18] and 0.61 mg/g in *H. musciformis* (Balamurugan *et al*., 2013) which is comparable to the present values. In brown algae the phenols ranged from 0.11 to 0.84 mg/g, as reported in our earlier study [24]. This may be due to brown algae which contain more phenols than the other two classes of macro algae [10, 18]. The present values on these sea plants are comparable to the green leafy vegetables such as *Coriandrum sativum* 2.2 mg/g and *Brassica oleraceae* var. *capitata* with 3.0 mg/g [9].

The monthly variation in distribution of phenol varied among the five species. In U. fasciata the peak values for phenols was found in March to April 2012 with 280 µg/g. It was also high during February 2012 with 260 µg/g and also in December 2012 with 250 µg/g. It was varied from 210 to 240 µg/g during June, August, October, and November and also in January 2013. It was 190 µg/g in May and July 2012 and the lowest value was 160 µg/g occurred in September 2012. In *E. compressa* the peak value was 280 µg/g for phenol occurred in November, December 2012 and January 2013. Higher content was also found in April, June 2012 with 270 µg/g. In February it was 240 µg/g, in October 2012 it was 221 µg/g and in September it was 210 ug/g. In March and May 2012, it was 180 µg/g and 170 µg/g respectively. The lowest value was 160 µg/g found in July 2012. In *C. sertularioides* the peak value for phenol was 520 µg/g found in February 2012. It was followed by 480 µg/g in Aug 2012 and 460

µg/g in October 2012. It was 430 µg/g in January 2013, 420 µg/g in July 2012. It was 390 µg/g in November 2012, 330 µg/g from April to June 2012. The lowest value of 310 µg/g was found in September and December 2012. In *G. corticata* the peak value for phenol was 410 µg/g occurred in January 2013. It was followed by 350 µg/g in June and October 2012. It was 325 µg/g in November and December 2012, 280 µg/g in February and August 2013 and 250 µg/g in April and September 2012. The lowest value 240 µg/g was occurred in March 2012. In *H. musciformis* the phenol content is relatively lower than the other four species mentioned above. The higher content was 280 µg/g was found in March 2012. It was followed by 250 µg/g in February and October 2012 and 210 µg/g in July 2012 and 200 µg/g in April and November 2012. It was 190 and 180 µg/g respectively in December and May 2012. The lowest values of 140 and 135 µg/g respectively occurred in August and September 2012.

Table 1. The range and mean values of environmental variables

Variable	Range	Mean	Standard deviation
Temperature ◦C	27.0 – 33.0	29.5	± 1.2
pH	8.2 – 9.2	8.5	± 0.40
Salinity (ppt)	20.0 – 32.0	29.5	± 3.4
Light (K lux)	26.8 -68.6	50.5	± 15.4
Dissolved oxygen (mg/l)	3.10 – 10.4	5.6	± 0.48
Nitrite (µ moles)	0.10 – 4.05	1.23	± 0.16
Phosphate (µmoles)	0.30 – 1.71	0.80	± 0.43
Silicate (µ moles)	0.13 – 6.02	7.75	± 3.8

Table 2. The seasonal variations in environmental variables

Variable	Summer*	Rainy**	Winter***
Temperature ◦C	30.0	30.5	20.0
pH	8.7	8.25	9.0
Salinity (ppt)	31.0	30.5	28.0
Light (K lux)	46.52	44.35	60.87
Dissolved oxygen (mg/l)	7.04	5.41	4.18
Nitrite (µ moles)	0.78	1.97	0.67
Phosphate (µmoles)	0.71	1.21	0.47
Silicate (µ moles)	8.85	8.59	5.86

** summer – February to May: ** Rainy – June to September*
**** Winter - October to January*

Table 3. The mean and range values of phenols (µg/g dry weight) in macroalgae

Name of the algae	Range	Mean	SD
Ulva fasciata	160-280	226.6	± 38.6
Enteromorpha compressa	110 -280	217.3	± 56.9
Caulerpa sertularioides	310 -520	391.8	± 74.8
Gracilaria corticata	240 - 410	306.0	± 52.3
Hypnea musciformis	180 -280	210.4	± 46.5

Table 4. The seasonal variations in phenols of marine macroalgae

Name of the algae	Summer	Rainy	Winter
Ulva fasciata	252.5	197.5	130.0
Enteromorpha compressa	215.0	187.5	260.33
Caulerpa sertularioides	393.33	385.0	410.0
Gracilaria corticata	256.6	293.3	352.5
Hypnea musciformis	227.5	183.75	220.0

** summer – February to May: ** Rainy – June to Septembe*
**** Winter - October to January*

Table 5. DPPH free radical scavenging activity of ethanolic extracts of macroalgae species.

Name of the algae	Concentration mg/l	% inhibition
Ulva fasciata	100	52.4
Enteromorpha compressa	100	31.2
Caulerpa sertularioides	50	55.04
Gracilaria corticata	75	56.1
Hypnea musciformis	100	50.8

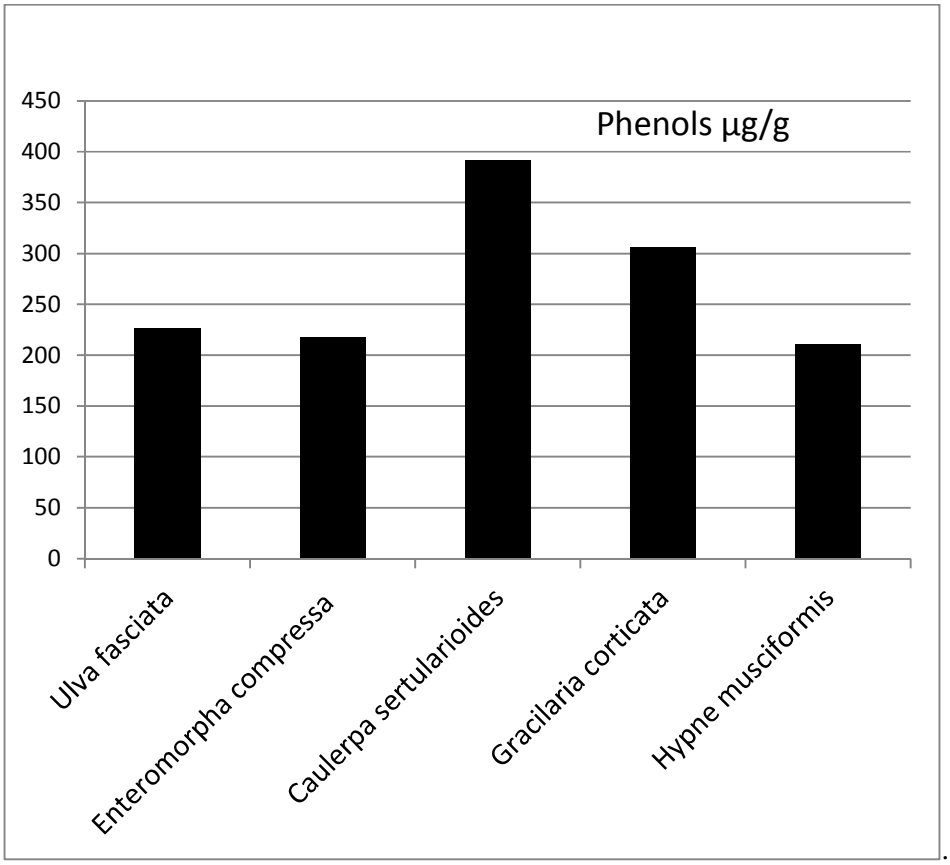
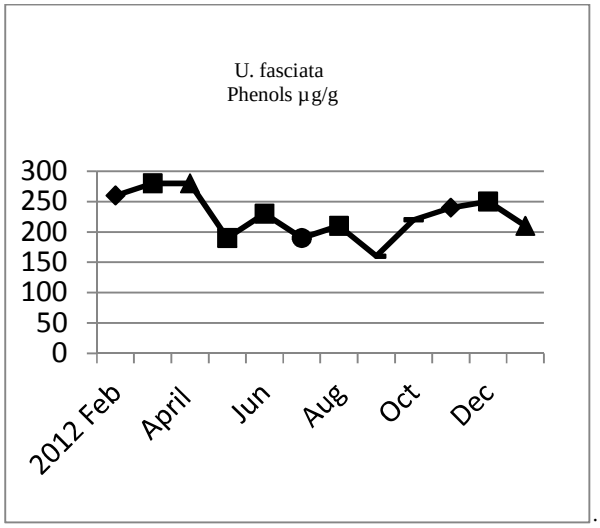
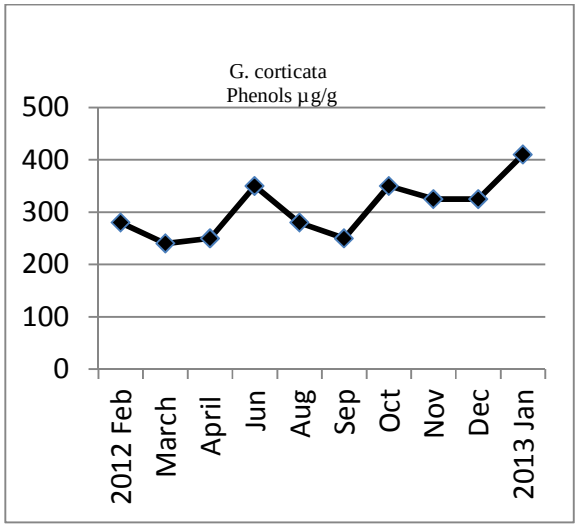


Fig. 1. The annual mean variation in distribution of phenols in macroalgae





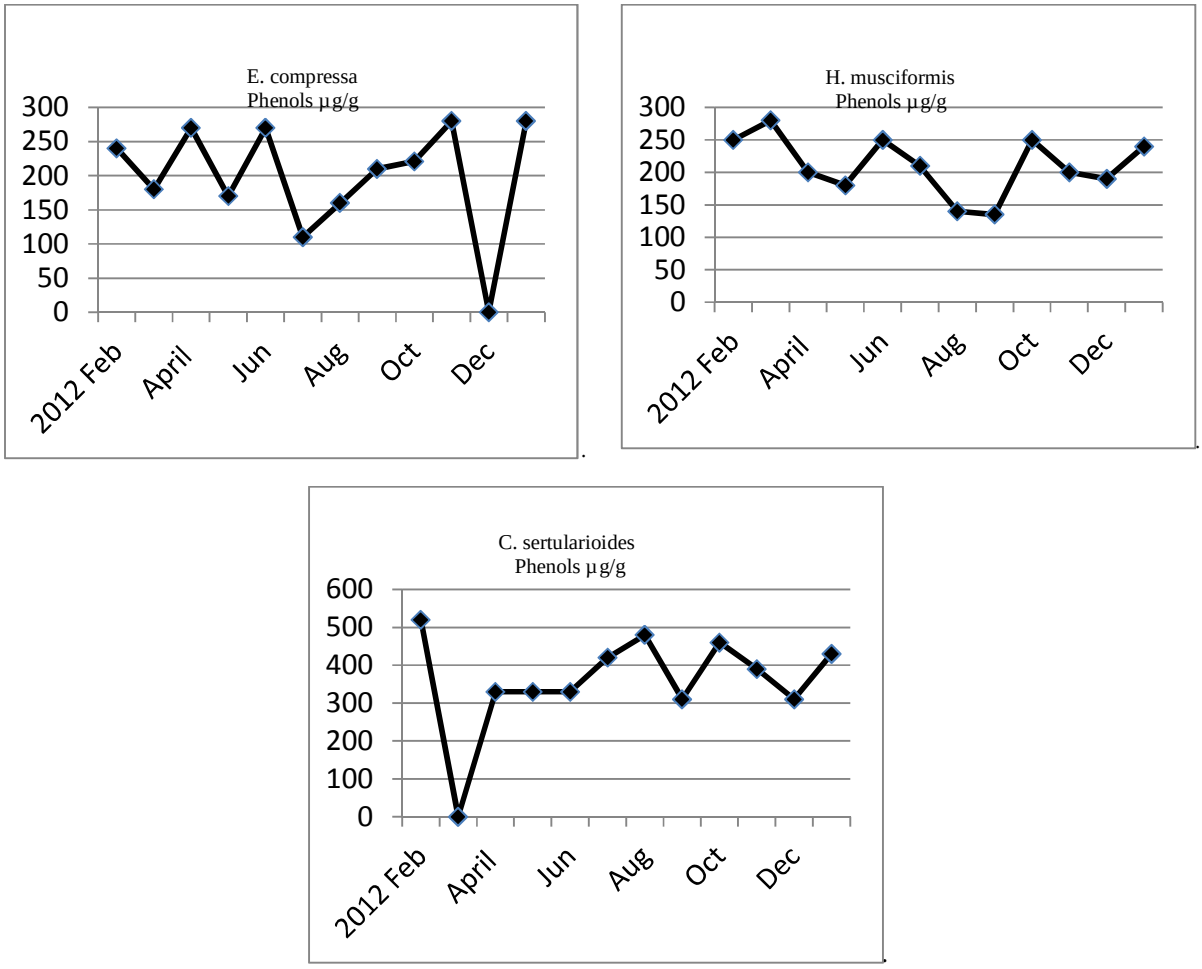


Fig.2. The monthly variation in distribution of phenols in five marine macro algae

The seasonal variation in phenol was given in table 4. In *U. fasciata* the maximum content 252.5 µg/g was found in summer. It was 197.5 µg/g in rainy period and 230.0 µg/g in winter period. In *E. compressa* the highest phenol 260.33 µg/g was found in winter. It was 215.0 in summer and 187.5 µg/g in rainy period. In *C. sertularioides* the highest content 410 µg/g was found in winter, 393.33 µg/g in summer and 385.0 µg/g in rainy period. In *G. corticata* the highest phenol was 352.5 µg/ occurred in winter, 293.3 µg/g was in rainy period and 256.6 µg/g was in summer. In *H. musciformis* the highest content 227.5 µg/g was found in summer, 220.0 µg/g in winter and 183.75 µg/g in rainy period. The seasonal variation in phenolic content of macroalgae varied with the species. It was highest during winter for *E. compressa, C. sertularioides, G. corticata* and during summer for *U. fasciata* and *H. Musciformis*. Sarojini *et al.,* (2013) reported that the high content of phenol in *P. tetrastromatica* and *S. vulgare* during winter. Similar findings were also reported by Kumar *et al*., 2015 in *S. wightii*. The phenol content varied with the ontogenic stage of seaweeds, field site and season [27]. It has been reported that the level of phenol compounds of algae usually increases with excessive exposure to UV radiations [11]. In the present study the moderate levels of environmental variables instead of higher or lower levels influenced the hike of total phenols during winter and summer seasons.

Correlation coefficients were calculated between environmental variables and phenols. In *U. fasciata* the pH correlated with phenol at $P \geq 0.05$, where r is 0.41, and df is 10. The Dissolved oxygen correlated with phenol at $P \geq 0.05$ where r is 0.44 and df is 10. In *E. compressa* the pH correlated with phenol at $P \geq 0.05$ where r is 0.70 and df is 10. The light also correlated with phenol at $P \geq 0.05$ where r is 0.419 and df is 10. In *C. sertularioides* the silicate correlated with phenol at $P \geq 0.05$ where the r is 0.64 and df is 10. In *G. corticata* the light correlated with phenol at $P \geq 0.05$ where the r is 0.61 and the df is 8. In *H. musciformis* the silicate correlated with phenol at $P \geq$

0.05 where r is 0.510 and df is 10. The higher levels of polyphenols were reported under increasing salinity [1, 14]. Single factor ANOVA was conducted between the phenol contents of these species. In *U. fasciata* and *E. compressa* the variation between species is significant at P > 0.05 level where the F critical is 4.35. Between *U. fasciata* and *C. sertularioides* the variation is significant at P > 0.05 where F critical is 4.35 and F table is 50.13. Between *E. compressa* and *C. sertularioides* the variation is significant at P > 0.05 where F critical is 4.41 and F table is 30.26. Between *G. corticata* and *H. musciformis* the variation is significant at P > 0.05 where F critical is 4.413 and F table value is 15.71.

All the five species that were analyzed showed antioxidant activities. The DPPH radical scavenging activity of the above mentioned five species of macro algae was presented in table 5. In U. fasciata the inhibition was 52.4% at 100 mg/ml. In *E. compressa* it was 31.2%, at 100 mg/ml, which was the lowest inhibition of all species. In *C. sertularioides*, it was 55.04% for 50 mg/ml, which was the highest inhibition observed. In *G. corticata* it was 56.105 in 75 mg/ml and in *H. musciformis* it was 50.8% for 100 mg/ml. The DPPH activity reported from other places [4, 15] of east coast of India, on these species are in agreement with the present study. The production of antioxidant compounds, like phenolics is influenced by several factors: extrinsic factors such as pressure exerted by the herbivores, irradiance, depth, salinity, nutrients and the intrinsic factors like type, age and reproductive stage of the species [7]. Therefore antioxidant activity of seaweeds could be subjected to great interspecific variations, even at very small scales [7].

CONCLUSION

The phenol content of five macroalgae that was analysed varied among the species. These values were low to the earlier reports on Phaeophyceae species such as *Padina tetrastromatica* and *Sargassum vulgare [24]* since Phaeophyceae members, which are good sources of phenol than the Chlorophceae and Rhodophyceae members. All the five species showed antioxidant property, sugesting that these tropical macroalgae develop an effective antioxidant defense system. The antioxidant activity of Phaeophyceae species as reported earlier [24] was also higher than the Chlorophyceae and Rhodophyceae species of the present study. These results will lead to their evaluation in medicine, food products and cosmetic industry. Based on our current scientific understanding polyphenols offer great hope for the prevention of chronic human degenerative diseases.

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