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Antioxidants and free radical scavenging activity of red algae of Visakhapatnam coast

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ABSTRACT

In the present study antioxidant, free radical scavenging activity, total phenolics, total carotenoids, vitamin-C and vitamin-E contents of four algal species were carried out to expand their utilization in pharmaceutical and food industry. Fractions rich in phenolics were extracted from four Red algal species using methanol as solvent and Free radical scavenging activity was studied using DPPH photometric assay. *Gracilaria corticata* exhibited higher levels of radical scavenging activity, total phenolics, and high Vitamin C and vitamin E contents. Whereas, carotenoids are rich in *Gracilaria corticata.*

Key words: Red algae, antioxidants, radical scavenging activity, phenolics, carotenoids.

INTRODUCTION

Seaweeds are extensively used as food particularly in East Asian countries like China, Japan, Korea, and Taiwan. In the biomedicine and pharmaceutical industries a number of research studies have been conducted to investigate claims of seaweeds effects on human health (Smith, 1944). Early in the 1950's the medicinal properties of seaweeds are restricted to traditional and folk medicines. (Lincolnn *et al.,* 1991).Compounds with biological activities and pharmacological properties were discovered in marine algae during last one decade (Mayers *et al.,* 2000). It has been asserted that seaweeds may have curative properties for tuberculosis, arthritis, colds and influenza, worm infestations and even tumors (Landsborough, 1857; Visioli *et al.,* 2000; Greenwald *et al.,* 2001). Seaweeds are of rich nutritive value as they contain high levels of vitamins and carotenoids. Marine algae are rich in polyphenols and they constitute an extremely heterogenous group of molecules providing a wide range of potential biological activity (Nakamura *et al.,* 1996).

Vitamin C, vitamin E and various carotenoids are ubiquitous in marine algae acts as natural antioxidants that have proven their importance in the food industry and human health (Shahidi, 1997). phenolic compounds could be a major determinant of antioxidant potentials of food (Parr and Bolwell, 2000) and could therefore be a natural source of antioxidants. Among natural antioxidants phenolic compounds are reported to quench oxygen derived free radicals by donating a hydrogen atom or an electron to the free radical (wanasundara *et al.,* 1996). These phenolic compounds exhibit a wide range of physiological properties such as antiallergic, antiartherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotis, cardio protective and vasodilatory effects. (Banavente - garcia *et al.,* 1997; Mallach *et al.,* 2005; Middleton *et al.,* 2000; puupponen-pimia *et al.,* 2001; Samman *et al.,* 1998). This paper aims to examine the non enzymatic antioxidant potential of four Red algal species in terms of their vitamin C, vitamin E, carotenoids and phenolic contents.

The rocky coast line of visakhapatnam support the luxuriant growth of about 98 different

species of seaweeds of these 25 belong to chlorophyceae, 18 phaeophyceae and 55 are Rhodophyceae (Umamaheswar Rao and Sreeramulu, 1970). These species show seasonal variation in their growth pattern. Most of the green seaweeds attain maximum growth from November to January; whereas, red and brown seaweeds grow luxuriantly from February to April. However few species of green, brown and red seaweeds grow through out the year.

MATERIAL AND METHODS

Chemicals

2.2-diphenyl-l-picrylhydrazyl(DPPH) and rutin were purchased from Sigma Chemicals Co. (St. Louis, Mo, USA). Ascorbic acid, 2, 6,-dichlorophenolindophenol, bathophenanthroline, vitamin-E, ferric chloride sodium carbonate, monobasic sodium phosphate, disodium hydrogen phosphate were purchased from (Hi-media, Mumbai, India), Folin-ciocalteau reagent was purchased from (Merck).

Collection of algal samples

Four algal species namely *Gracilaria corticata, Gelidiopsis variabilis, Hypnea musciformis, Amphiroa fragilissima* were collected during low tide along the Visakhapatnam coast line (LAT. 17° 41' N. LONG. 83° 17' E.) from November 2006 to January 2007.

Methods

Sample preparation

The collected algal samples are immediately carried to the lab and washed with tap water. They are allowed to shade dried and powdered. 25 gms of powdered algal samples were thoroughly mixed with 50 ml of methanol in 250 ml Erlynmeyer flask for 5hrs and centrifuged at 10,000g for 15min. The supernatant was collected in separate vials and preserved at -20°C for further studies.

Estimation of total phenolic content

The total phenolic content was estimated according to the method of Javanmardi *et al.,* (2003). To 0.2ml of each extract 2ml of Folin's reagent and 2ml of 7.5 %(w/v) sodium carbonate was added followed by incubation for 15 min at 45°C. A blank was simultaneously set up using 0.2ml of water, 2 ml of Folin's reagent and 2ml of 7.5 %(w/v) sodium carbonate. The absorbance values were measured at 765 nm against blank.

Gallic acid was used to construct the standard calibration curve and the results were expressed as µg of Gallic acid equivalent (GAE) per gm of dry weight.

Estimation of vitamin C and vitamin E

The vitamin content was estimated according to method of varley *et al.,* (practical clinical biochemistry) In the estimation of vitamin-C, 1 ml of glacial acetic acid was added to 5ml of algal extract and titrated against the 2, 6-dichlorophenol indophenol until the color changes to pale pink. Standard was prepared using ascorbic acid (0.04mg/ml).the amount of vitamin present in the sample was determined using standard value and expressed as mg/gm of dry tissue.

In the estimation of vitamin-E, to 1 ml of methanolic extract 0.2mlof bathophenanthroline (0.2%in ethanol) and 0.2ml of ferric chloride (5mM in alcohol) was added ,followed by the addition of 0.2ml of 1mM phosphoric acid reagent. The absorbance was measured at 534 nm against the alcohol. A standard caliburation curve was constructed using DL -tocopherol, and the amount of vitamin-E present in the algal extract was expressed as mg equivalents of DL- tocopherol per gm of dry weight.

Estimation of total chlorophyll and total carotenoids

Total chlorophyll and total carotenoids were estimated according to the method of Lichtenthaler, (1987). 1gm of dry algal powder was dissolved in 5ml of methanol: acetone mixture (4:1). The components were then centrifuged at 5000g and the supernatant was used for measuring the absorbance at 775nm to determine the total chlorophyll content and at 510nm and 456nm to determine the total carotenoids content of algal extracts by using the following formula.

Total chlorophyll = O.D at 775 x 2.19

Total carotenoids = A (456) - (A (775) x 0.1) +
A (510) - (A (775) xO.05)

The chlorophyll and carotenoid levels were expressed as mg/gm of dry weight.

Antioxidant activity determination by radical scavenging activity

Radical scavenging activity was carried out according to the method of Mensor *et al.,* (2001). It involves a stoichiometric reaction, based on a change in color from purple to yellow as the free radicals are scavenged. To 1ml of 0.3mM DPPH in ethanol solution 2.5ml of sample solution was added and was allowed to react at room temperature for 30min and the absorbance was measured in a Hitachi UV-VISIBLE model U -200 Spectrophotometer at 518 nm. Rutin was used as the positive control. DPPH solution plus methanol is used as negative control and methanol is used as blank. The results were expressed in terms of % of antioxidant activity using the following equation:

% of scavenging activity =
100 - [(O.D of sample – O.D of blank) x 100/
O.D of negative control].

Statistical analysis

Results were expressed as the mean values with standard deviation (mean±SD) of n determinations (n=5). Statistical analysis was performed by means of student's *t*-test and the difference was considered at the level of $p < 0.05$

RESULTS AND DISCUSSION

The antioxidant activity of methanolic extracts of algal species and their ability to scavenge the free radicals in terms of phenolics, vitamin C, vitamin E, chlorophyll and carotenoid contents.

It is evident from the Fig. 1 and 2, *Gracilaria corticata* contains high content of phenolics, vitamin C and vitamin E followed by *Hypnea musciformis.* Whereas, *Amphiroa fragilissima* and *Gelidiopsis variabilis* contain relatively low content of phenolics and vitamins.

The total chlorophyll and carotenoids contents were presented in Fig. 3. *Gracilaria corticata* contain high amount of chlorophyll and carotenoids. Whereas, chlorophyll content was lowest in *Amphiroa frsgilissima* and carotenoids in *Gelidiopsis variabilis*.

In the DPPH test the reduction of DPPH suggests the presence of electron or hydrogen

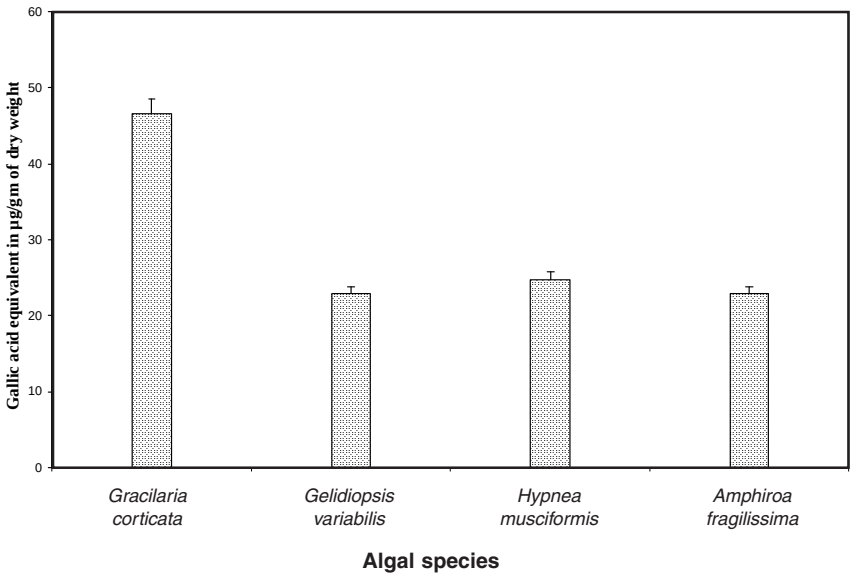

Fig. 1: Total phenolic content of algal extract

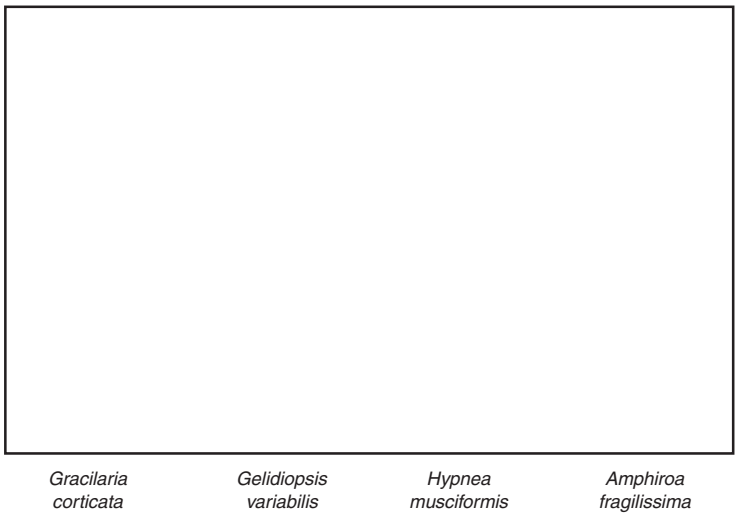

Fig. 2: Vitamin-C and vitamin E contents of Algal extracts

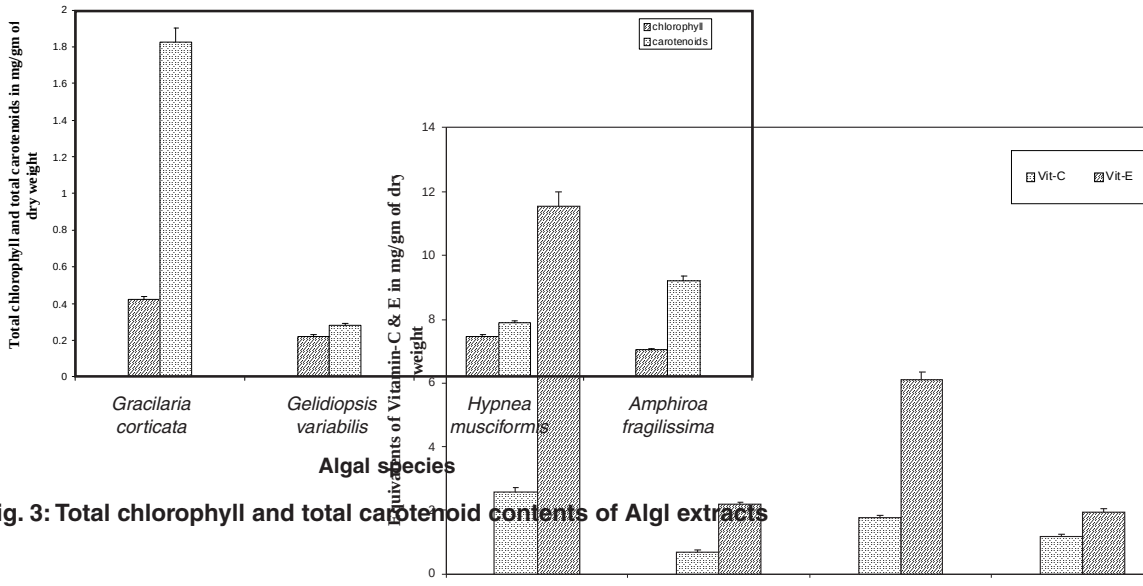

Fig. 3: Total chlorophyll and total carotenoid contents of Algl extracts

donors in algal extracts. In the present study the high picryl radical scavenging activity (Fig. 4) of *Gracilaria corticata* may be due to the presence of high content of phenolics, carotenoids, vitamin C and vitamin E, which acts as natural antioxidants. Whereas, in *Hypnea musciformis* the scavenging activity may be due to the presence of high content of vitamin C and Vitamin E.

It is evident from the literature vitamin C, vitamin E, carotenoids and phenolics acts as a electron donors (Matsukawa *et al*., 1997) Because of the presence of high levels vitamins and carotenoid the seaweeds are used as human food and animal fodder (ltu and Hori, 1989; Joel fleurence, Estelle Chenard and Michel Lucon.,1999).

Thus the seaweeds have great potential as a source of drugs and tools for use in biochemical, pharmaceutical and medical research.

Figure 2: Vitamin-C and vitamin E contents of Algal extracts

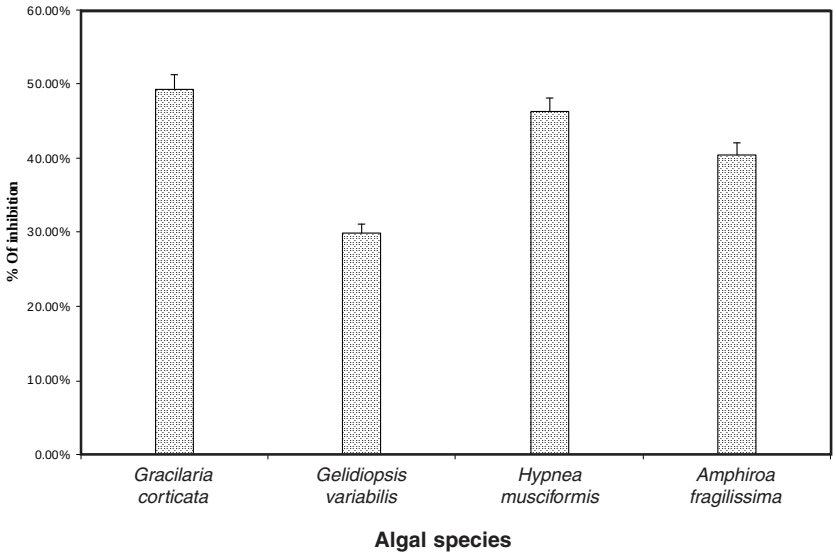

Fig. 4: Radical scavenging activity of Algal extracts

Conclusion

In conclusion the results clearly indicated that the 4 species of seaweeds tested in this investigation possess antioxidant activity to various degrees. The present findings appear useful in leading to the development of therapeutic products to protect against certain diseases. At the present time no direct linkage could be established with certainty between the estimated presence of phenolic compounds in the extract, fractions, and their activities. Yet the results presented in this report provide useful data that make it possible to classify the marine algal extracts in respect to their antioxidant potential. This should lead to a better understanding of the antioxidant activity and the active principal and furthermore, may allow for rational recommendations regarding their use in folk medicinal systems.

The above study thus show's that common seaweeds contain materials with antioxidants. In order to use these seaweeds as antioxidants for food or other purposes, further studies are now required to assess their effectiveness and possible toxicity.

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REFERENCES

1. Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuno, A., and Del Rio, J.A.,:Uses and properties of citrus flavonoids. *Journal of Agricultural and Food chemistry*. **45**: 4505-4515 (1997).
2. Greenwald, P., Clifford, C.K., Milner, J.A.,:Diet and cancer prevention. *Eur. J. Cancer*. **37**: 948-965 (2001).
3. Ito, K., Hori, K., Seaweed: Chemical composition and potential food uses. *Food Rev.Int.* **5:** 101-144 (1989).
4. Javanmardi, J., Stushnoff, C., Locke, E., and Vivanco, J.M., Antioxidant activity and total phenolic content of Iranian *Ocimum*

accessions. *Food chemistry.* **83**: 547-550 (2003).

5. Joel fluerence, Estelle chenard and Michel Lucon.,:Determination of nutritional value of proteins obtained from *Ulva armoricana. Journal of Applied Phycology.* **11**: 231-239. (1999).
6. Landsborough, D., A Popular History of British seaweeds. (1857).
7. Lichtenthaler, H. K., Chlorophylls and carotenoids :pigments of photosynthetic biomembranes. In: PACKER, L. and DOUCE, R.eds. *Methods in Enzymology.* Washing ton, academic press, Vol. **148**: 350-382 (1987).
8. Lincoln, R.A., Strupinski, K., Walker, J.M., Bioactive compounds from Algae. *Life Chem. Rep.* **8**: 97-183 (1991).
9. Manach, C., Mazur, A., and Scalbert, A., Polyphenols and preservation of cardiovascular diseases. *Current opinions in Lipidology*. **46**: 77-84 (2005).
10. Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masaki, K., Masuda, Y., Takeudhi, T., Chiharal, M., Yamamoto, Y., Niki E., and Karube: A Comparison of Screening Methods for Antioxidant activity in Seaweeds. *Journal of Applied Phycology.* **9**: 29-35 (1997).
11. Mayers, AMS., Lehmann VKB., Marine pharmacology in 1998:Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antihelminthic, antiplatelet, antiprotozoal and antiviral activities; with actions on the cardiovascular, endocrine, immune and nervous systems and other miscellaneous mechanisms of action. *Pharmacologist* **42**: 62-69 (2000).
12. Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., Dossantos, T.C., Coube, C.S., Leitao, S.G., : Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytother. Res*. **15**: 127-130 (2001).
13. Middleton, E., Kandaswami, C., and Theoharides, T.C., :The effect of plant flavonoids on mammalian cells: implications for inflammations, heart disease,and cancer. *Pharmocological reviews,* **52**: 673-751 (2000).
14. Nakamura, T., Nagayama, K., Uchida, K., Tanaka, R., Antioxidant activity of phlorotannins isolated from brown Algae *Eisenia bicyclis. Fisheries Science* **62**(6): 923-926 (1996).
15. Parr, A.J., and Bolwell, G.P., Phenols in the plant and in man. The potential for possible nutrient enhancement of the diet by modifying the phenols content or profile. *Journal of the science of Food and Agriculture.* **80**: 985-1012 (2000).
16. Puupponen-pina, R., Nohynek, L., Meier, C., Kahko nen, M., Heinonen, M., Hopia, A., Antimicrobial properties of phenolic compounds from berries. *Journal of Applied microbiology*. **90**: 494-507 (2001).
17. Samman, S., LyonsWall, P.M., and Cook N.C., Flavanoids and coronary heart disease: Dietary perspectives. In C.A. Rice-Evans and L. Packer 9Eds, Flavanoids in health and disease.. New York; Marcel Dekker. 469-482 (1998).
18. Shahidi, F., Natural antioxidants: An overview. In F. Shahidi 9Ed, Natural Antioxidants, chemistry, health effects and applications. Champaign, IL, USA; AOCS Press. 1-10 (1997).
19. Smith, G.M.,: Marine Algae of the Monterey Peninsula, California. Stanford Univ., 2nd Edition (1994).
20. Uma maheswaerao, M., and Sreeramulu, T., An annotated list of the Marine Algae of Visakhapatnam (India). *Bot. J. Linn. Soc.* **63**: 23-45 (1970).
21. Varley, H., Gowen lock, A.H., and Bell, M., Vitamins In: practical clinical biochemistry, Eds, Varley, H., and Gowen lock, A.H., William Heinemann medical books Ltd., London. **2**: 215-217 (1984).
22. Visioli, F., Borsani, L., Galli, C., Diet and prevention of coronary heart disease: The potential role of phytochemicals. *Cardio-vascular Research*. **47**(3): 419-425 (2000).
23. Wanasundara, P. K. J. P. D., and Shahidi, F., Optimization of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology. *Journal of Food Science*. **61**: 604-607 (1996).