Asian Journal of Chemistry Vol. 20, No. 7 (2008), 5347-5352

Antioxidants and Free Radical Scavenging Activity of Brown Algae of Visakhapatnam Coast

S.V. RAJAGOPAL*, B.V. RAMAN, I. BHASKAR REDDY†, M. RAMA RAO† and K. SIVAKUMAR
Department of Biotechnology, GITAM Institute of Science GITAM University, Rushikonda, Visakhapatnam-530 045, India
Fax: (91)(891)2790032; E-mail: rajsaladi@yahoo.com

Antioxidant, free radical scavenging activity, total phenolics, total carotenoids, vitamin C and vitamin E content of *Padina tetrastomatica, Sargassum illicifolium* and *Sargassum vulgare* were carried out in order to expand their utilization in pharmaceutical and food industry. Fractions rich in phenolics were extracted from three brown algal species using methanol as solvent. Free radical scavenging activity was studied using DPPH photometric assay. *Padina tetrastomatica* exhibited higher levels of radical scavenging activity, total carotenoids, total phenolics and high vitamin C contents. Whereas, *Sargassum* species contain high content of vitamin E and low content of phenolics, carotenoids and vitamin C.

Key Words: Brown 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 the effect of seaweeds on human health[1]. Early in the 1950's, the medicinal properties of seaweeds are restricted to traditional and folk medicines[2]. Compounds with biological activities and pharmacological properties were isolated from marine algae[3]. It has been asserted that seaweeds may have curative properties for tuberculosis, arthritis, colds and influenza, worm infestations and even tumors[4-6]. 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[7].

†Departmentof Biochemistry, College of Science, GITAM University, Visakhapatnam-530 045, India.

Vitamin C, vitamin E and carotenoids which are ubiquitous in marine algae acts as natural antioxidants and have proven their importance in the food industry and human health[8]. Phenolic compounds are also a major determinant of antioxidant potentials of food[9] 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[10]. These phenolic compounds exhibit a wide range of physiological properties such as antiallergic, antiartherogenic, antiinflammatory, antimicrobial, antioxidant, antithrombotis, cardio protective and vasodilatory effects[11-15]. This paper aims to examine the non-enzymatic antioxidant potential of three brown algal species of Visakhapatnam coast in terms of their vitamin C, vitamin E, carotenoids and phenolics contents.

The rocky coast line of Visakhapatnam support the growth of about 98 different species of seaweeds[16]. These species show seasonal variation in their growth pattern. Most of the green seaweeds attain maximum growth in the month of November. December and January; where as red and brown seaweeds grow luxuriantly from February to April. However, few species of green, brown and red seaweeds grow through out the year.

EXPERIMENTAL

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), while Folin-Ciocalteau reagent was purchased from (Merck).

Collection of algae samples: Three algae species namely *Padina tetrastomatica, Sargassum ilicifolium* and *Sargassum vulgare* were collected during low tide along the Visakhapatnam coast line (Lat. 17°41' N. Long. 83°17' E.) from November 2006 to January 2007.

Sample preparation: The collected algae samples are immediately carried to the lab and washed with tap water. They are allowed to shade dried and powdered. 25 g of powdered algae samples were thoroughly mixed with 50 mL of methanol in 250 mL Erlynmeyer flask for 5 h and centrifuged at 10,000 g for 15 min. 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.*[17]. To 0.2 mL of each extract 2 mL of Folin's reagent and 2 mL 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 0.2 mL of water, 2 mL of Folin's reagent

and 2 mL 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)/g of dry weight.

Estimation of vitamin C and vitamin E contents: The vitamin contents were estimated according to method of Varley *et al.*[18]. In the estimation of vitamin C, 1 mL of glacial acetic acid was added to 5 mL of algae extract and titrated against the 2,6-dichlorophenol indophenol until the colour changes to pale pink. Standard was prepared using ascorbic acid (0.04 mg/mL). The amount of vitamin C present in the sample was determined using standard value and expressed as mg/g of dry weight.

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

Estimation of total chlorophyll and total carotenoids: Total chlorophyll and total carotenoids were estimated according to the method of Lichtenthaler *et al.*[19]. 1 g of dry algae powder was dissolved in 5 mL of methanol:acetone mixture (4:1) The components were then centrifuged at 5000 g and the supernatant was used for measuring the absorbance at 775 nm to determine the total chlorophyll content and at 510 and 456 nm to determine the total carotenoid content of algae extracts by using the following formula:

Total chlorophyll = O.D at 775 × 2.19
Total carotenoids = A (456)-(A (775) × 0.1) + A (510) – (A (775) × 0.05)
The chlorophyll and carotenoid levels were expressed as mg/g of dry weight.

Antioxidant activity determination by radical scavenging activity: Radical scavenging activity was carried out according to the method of Mensor *et al.*[20]. It involves a stoichiometric reaction, based on a change in colour from purple to yellow as the free radicals are scavenged. To 1 mL of 0.3 mM DPPH solution, 2.5 mL of sample solution was added and allowed to react at room temperature for 0.5 h. 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.

The results were expressed in terms of % of antioxidant activity using the following equation:

$$\% \text{ of scavenging activity} = 100 - \frac{\text{OD of sample} - \text{OD of blank}}{\text{OD of negative control}} \times 100$$

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 extract of algae species were studied by their ability to scavenge the free radicals in terms of phenolic, vitamin C, vitamin E, chlorophyll and carotenoid contents. It is evident from the Figs. 1and 2, *Padina tetrastomatica* contain high content of phenolics, vitamin C and free radical scavenging activity. Whereas, *Sargassum* species contain relatively high levels of vitamin E, but low in phenolic content and free radical scavenging activity.

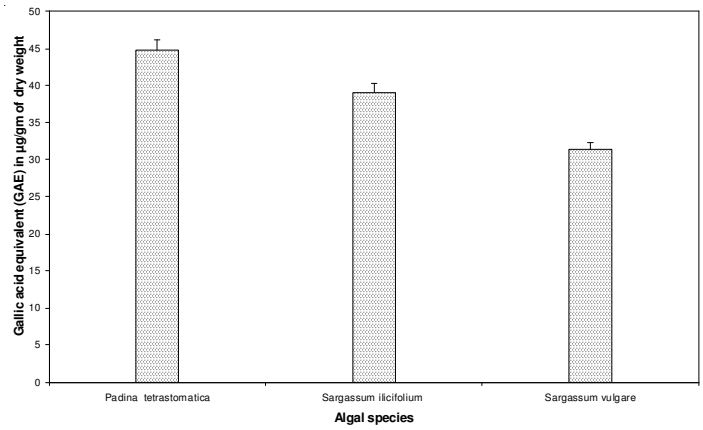

Fig. 1. Total phenolic content of algae extracts

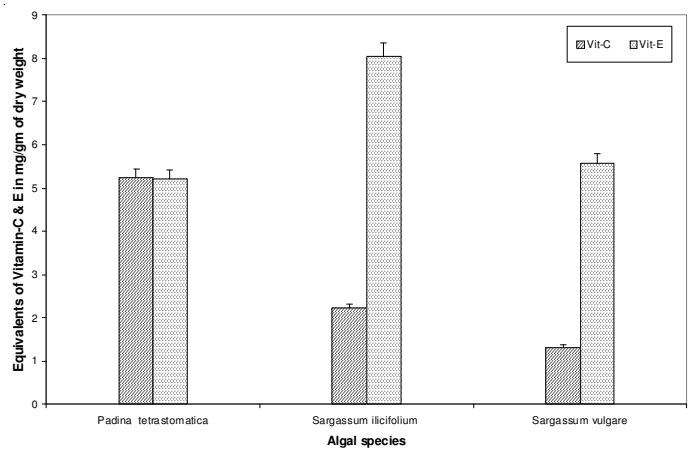

Fig. 2. Vitamin C and vitamin E levels of algae extracts

As shown in Fig. 3 the total chlorophyll and carotenoids contents were high in *Padina tetrastomatica* and low in *Sargassum* species.

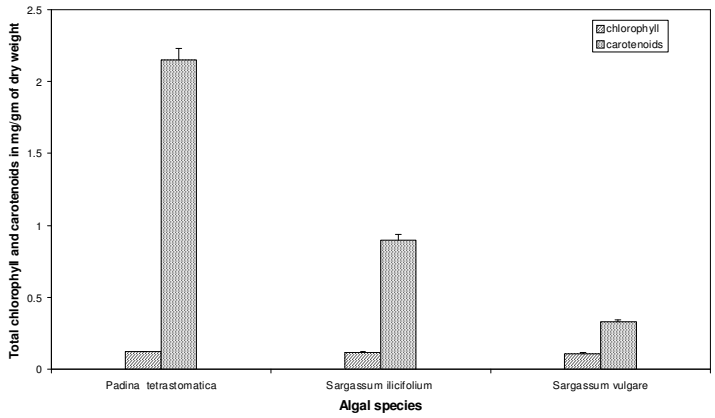

Fig. 3. Total chlorophylls and carotenoid levels of algae extracts

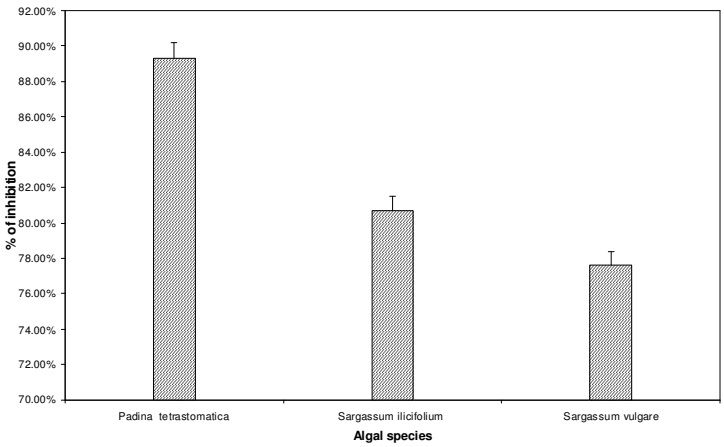

Fig. 4. Radical scavenging activity of algae extracts

In the DPPH test, the reduction of DPPH suggest the presence of electron or hydrogen donors in algae extracts. The high picryl radical scavenging activity of *Padina tetrastomatica* may be due to the presence of high content of phenols, vitamin C and carotenoids which acts as a natural antioxidants. Whereas, in *Sargassum* species the scavenging activity may be due to the high vitamin E content.

It is evident from the literature vitamin C, tocopherols, carotenoids and phenolics are capable of acting as electron or hydrogen donors[21]. In the present study, the high radical scavenging effect of *Padina tetrastomatica*

and the low scavenging effect *Sargassum* vulgare correlates with their phenolics, vitamin C and vitamin E and carotenoid contents, respectively. Because of the presence of high levels of vitamins and carotenoids seaweeds are used as human food and animal fodder[22,23].

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

ACKNOWLEDGEMENTS

The authors thank the GlTAM management for providing the necessary facilities to carry out this work. Thanks are also due to Dr. M. Umamaheswar Rao for identification of Algae species.

REFERENCES

1. G.M. Smith, Marine Algae of the Monterey Peninsula, Stanford University, California, edn. 2 (1994).
2. R.A. Lincoln, K. Strupinski and J.M. Walker, *Life Chem. Rep.*, **8**, 97 (1991).
3. A.M.S. Mayers and V.K.B. Lehmann, *Pharmacologist*, **42**, 62 (2000).
4. D. Landsborough, A Popular History of British Seaweeds (1857).
5. F. Visioli, L. Borsani and C. Galli, *Cardiovascular Res.*, **47**, 419 (2000).
6. P. Greenwald, C.K. Clifford and J.A. Milner, *Eur. J. Cancer*, **37**, 948 (2001).
7. T. Nakamura, K. Nagayama, K. Uchida and R. Tanaka, *Fisheries Sci.*, **62**, 923 (1996).
8. F. Shahidi, in ed.: F. Shahidi, Natural Antioxidants: An Overview, Natural Antioxidants, Chemistry, Health Effects and Applications, Champaign, IL, USA, AOCS Press, pp. 1-10 (1997).
9. A.J. Parr and G.P. Bolwell, *J. Sci. Food Agric.*, **80**, 985 (2000).
10. P.K.J.P.D. Wanasundara and F. Shahidi, *J. Food Sci.*, **61**, 604 (1996).
11. O. Benavente-Garcia, J. Castillo, F.R. Marin, A. Ortuno and J.A. Del Rio, *J. Agric. Food Chem.*, **45**, 4505 (1997).
12. C. Manach, A. Mazur and A. Scalbert, *Curr. Opin. Lipidol.*, **46**, 77 (2005).
13. E. Middleton, C. Kandaswami and T.C. Theoharides, *Pharmocol. Rev.*, **52**, 673 (2000).
14. R. Puupponen-pina, L. Nohynek, C. Meier, M. Kahko nen, M. Heinonen and A. Hopia, *J. Appl. Microbiol.*, **90**, 494 (2001).
15. S. Samman, P.M. Lyons Wall and N.C. Cook, in eds.: C.A. Rice-Evans and L. Packer, Flavanoids and Coronary Heart Disease: Dietary Perspectives, Flavanoids in Health and Disease, New York, Marcel Dekker, pp. 469-482 (1998).
16. M. Uma maheswaerao and T. Sreeramulu, *Bot. J. Linn. Soc.*, **63**, 23 (1970).
17. J. Javanmardi, C. Stushnoff, E. Locke and J.M. Vivanco, *Food Chem.*, **83**, 547 (2003).
18. H. Varley, A.H. Gowen lock and M. Bell, in eds.: H. Varley and A.H. Gowen lock, Vitamins In: Practical Clinical Biochemistry, William Heinemann Medical Books Ltd., London Vol. 2, pp. 215-217 (1984).
19. H.K. Lichtenthaler, in eds.: L. Packer and R. Douce, Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, Methods in Enzymology, Washington, Academic Press, Vol. 148, pp. 350-382 (1987).
20. L.L. Mensor, F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. Dossantos, C.S. Coube and S.G. Leitao, *Phytother. Res.*, **15**, 127 (2001).
21. R. Matsukawa, Z. Dubinsky, E. Kishimoto, K. Masaki, Y. Masuda, T. Takeudhi, M. Chiharal, Y. Yamamoto, E. Niki and Karube, *J. Appl. Phycol.*, **9**, 29 (1997).
22. K. Ito and K. Hori, *Food Rev. Int.*, **5**, 101 (1989).
23. J. Fluerence, E. Chenard and M. Lucon, *J. Appl. Phycol.*, **11**, 231 (1999).

(*Received*: 11 August 2007; *Accepted*: 26 April 2008) AJC-6544