ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Antioxidant Potential of Seaweeds from Kunakeshwar along the West Coast Maharashtra

Mole Megha N*. Sabale Anjali B.
Department of Botany, Shivaji University, Kolhapur

Received:
19th July 2013
Received in revised form:
25th July 2013
Accepted:
30th July 2013
Available online:
10th Aug 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

Antioxidant activity of edible species of seaweeds consisting of green seaweeds (*Chaetomorpha media* and *Enteromorpha intestinalis*), brown seaweeds (*Padina tetrastromatica* and *Dictyota dichotoma*) and red seaweeds (*Gracilaria corticata* and *Gelidiella acerosa*) was determined using methanol and ethanol as extraction media. FRAP, ferrous ion chelating activity, DPPH assay, reducing power and total antioxidant capacity along with the total phenolic content were determined in ethanol and methanol extracted seaweeds. The methanolic extract of *Enteromorpha intestinalis* and ethanolic extract of *Dictyota dichotoma* had an appreciable radical scavenging activity, total antioxidant activity and reducing power than the other seaweeds. There was a significant correlation between the DPPH activity scored from the methanolic seaweed extracts, total antioxidant capacity analyzed from ethanolic extracts and the total phenol content (R^2 = 0.769 and 0.782 resp.).

Keywords: Seaweeds, Antioxidant activity, FRAP, DPPH, Phenols, TAC, Ferrous ion chelating activity.

1. INTRODUCTION:

Marine algal compounds are reported to possess antioxidant properties which have multiple functions in biological systems, such as defense against oxidative damage and participation in the signaling pathways of cells [1]. One of the major actions of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species [2]. Oxidative stress and reactive oxygen species have been associated with the onset of a variety of chronic diseases in humans [3]. Free radicals are responsible for aging and their presence in excess constitutes the cause of various human diseases.

Seaweeds are known to be a rich source of antioxidant compounds [4, 5, 6]. Marine algae are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents [7, 8] but absence of the oxidative damage in the structural components (Polyunsaturated fatty acids) of seaweeds [9] and their stability to oxidation during storage, [10] suggest that their cells have protective antioxidative defense systems [11]. Phenolic compound such as flavonoids, phenolic acids and tannins are considered to be the major contributor to the antioxidant capacity of plants. In edible brown, green and red seaweeds antioxidant properties have been correlated to their phenolic content [6, 12, 13].

*Corresponding author: Mole Megha N | Department of Botany, Shivaji University, Kolhapur.
e-mail: m_pranju@rediffmail.com | Telephone: +91 9975513667

In the present study antioxidant potential of six species of seaweeds is investigated and discussed by measuring the ferric reducing antioxidant power, reducing power, DPPH assay, total antioxidant capacity and ferrous ion chelating activity. It is worthwhile to carry out more than one type of antioxidant activity measurement as various mechanisms for combating oxidative damage are in progress at a time in cells [14, 15,] and a single assay may not accurately reflect all of the radical sources or all antioxidants in a mixed or complex system [16]. Total content of phenols is also estimated in these seaweeds using the classical Folin-Ciocalteu reagent and the relationship between the antioxidant capacities and phenolic content is also predicted.

2. MATERIALS AND METHODS

2.1 Collection and preparation of algal extracts

Fresh, mature thalli of *Chaetomorpha media* (C. Agardh) Kutz*, Enteromorpha intestinalis* (L.) Nees, *Padina tetrastromatica* Hauck, *Dictyota dichotoma* (Hudson) Lamouroux*, Gracilaria corticata* (J. Agardh) and *Gelidiella acerosa* (Forsskal) J.Feldmann & G. Hamel were collected during the low tide from submerged marine rocks at Kunakeshwar (164'0.120'N latitude and 7328'0.120'E longitude) in Sindhudurg district along the west coast of Maharashtra (India). Collected samples were washed thoroughly with fresh water to remove salt, sand particles and epiphytes. It was then dried in shade and ground to obtain a fine powder. One gram of algal powder was extracted in 10 ml of solvent (methanol or ethanol) for 24h on a rotary shaker. The extract was filtered through Whatman no.1 filter paper and stored in glass vials in refrigerator at 0-4^{0}C [17].

2.2 DPPH radical scavenging activity

DPPH (2, 2- diphenyl -1- picrylhydrasyl) radical scavenging activity was determined according to the method of Wang et al. [18]. 0.5ml of extract was mixed with 3ml of 25mM DPPH solution prepared in methanol and incubated for 30 min. in the darkness at room temperature. Then absorbance was measured at 517 nm against a blank of methanol without DPPH. Methanol with DPPH solution was used as the control. Results are expressed as percent inhibition of the DPPH radical and calculated using the following formula.

$$\text{DPPH activity (\%)} = \frac{\text{Abs. (Control)} - \text{Abs. (Sample)}}{\text{Abs. (Control)}} \times 100$$

2.3 Ferric reducing antioxidant property (FRAP)

FRAP assay was performed according to the method given by Benzie & Strain [19]. Antioxidant activity of the standard was estimated by measuring increased absorbance caused by generated ferrous ions. The working FRAP reagent contained 0.3M acetate buffer (pH 3.6), 10mM TPTZ (2,4,6-tri(2-pyridyl)-S-triazine), 40mM HCl and 20mM $Fecl_3.6H_2O$ in the ratio of 10:1:1 (freshly prepared and warmed to 37^{0}C.) 2.7 ml of this working solution was mixed with 100 µl of algal extract to initiate the reaction. Absorbance was recorded after 10 min. at 593 nm. Antioxidant capacity is expressed as ascorbic acid equivalent.

2.4 Ferrous ion chelating ability

Ferrous ion chelating ability was determined according to the method of Decker & Welch [20]. 0.5 ml of individual algal extract was mixed with 0.1 ml of 2mM $Fecl_2$ 0.2 ml of 5mM ferrozine solution and reaction mixture was incubated for 10 min. at room temperature. Absorbance was measured at 562 nm. Percentage of chelating ability was calculated using the following equation.

$$\text{Ferrous ion chelating ability (\%)} = 1 - \frac{\text{A (Sample)}}{\text{A (Control)}} \times 100$$

2.5 Reducing power:

Reducing power of the extract was evaluated according to the method of Yen & Chen [21]. This assay measures the total antioxidant capacity of the sample evaluating the redox potentials of the compounds. Extract was mixed with one ml phosphate buffer (0.2M, pH 6.6) and one ml 1% potassium ferricyanide and incubated at 50^{0}C for 20 min. After cooling it was mixed with 1ml of trichloroacetic acid (10%). 1.5 ml of this mixture was transferred to other test tube to which 1.5 ml distilled water and 0.5 ml $Fecl_3.6H_2O$ (0.1%) were added. The mixture was centrifuged and kept at room temperature for 10 min. before recording the absorbance at 700 nm. The results are expressed as ascorbic acid equivalent per gram of sample.

2.6 Total antioxidant capacity

Total antioxidant capacity (TAC) was determined according to Prieto et al [22]. The extract (100 mg/ml) was mixed with 3 ml reagents containing 0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate and incubated at 95^{0}C for 90 min. in water bath. The absorbance was recorded at 695 nm. Results are expressed as ascorbic acid equivalent per gram of sample.

2.7 Determination of phenolic content:

Total content of phenolic compounds of algal extracts was determined spectrophotometrically using Folin Ciocalteu reagent as per the method described by Sadasivam & Manickam [23]. Extract (0.5ml) was diluted to 3ml with distilled water and mixed with 0.5ml of folin ciocalteu reagent. After three min. 2ml of 20% sodium carbonate were added and the contents were thoroughly mixed. Then it was placed in a boiling water bath for exactly one min., cooled and then absorbance was recorded at 650 nm against a reagent blank. Total amount of phenols was calculated based on a standard curve of catechol and expressed in mg/100gm of dry weight.

3. RESULTS

3.1 DPPH assay:

This assay is used to test the ability of the antioxidant compounds present in the algal extracts to function as proton radical scavengers or hydrogen donors [24]. DPPH activity exceeded 60% in all the seaweeds in the present study when ethanol extract was tested. In the methanol extracted samples DPPH activity was less but more than 50% except in *C. media.* Similar observations were reported by Senthilkumar & Sudha [25] for methanol extracted *Chaetomorpha linum* which contained 45.32 % DPPH activity. The ethanolic extract of green and red seaweeds exhibited a high scavenging activity (73– 88 % & 68– 80 % resp.) in the present study. It was interesting to note that ethanolic extract of brown seaweeds exhibited slightly higher DPPH activity (59.39% & 63.54 %) than the methanol extract (58.74% & 64.71%). The DPPH scavenging activity of a commercial antioxidant ascorbic acid was 94.75% when used at a concentration of 10mg/ml. A higher DPPH scavenging capacity in brown and green seaweeds compared to red seaweeds has been reported in several studies [26, 27, 28]. In the present study DPPH activity was consistently higher in methanolic extracts of Phaeophyta and Rhodophyta members. A High DPPH scavenging activity has also been reported by Ganesan et al.[29] in green, brown and red seaweeds.

3.2 FRAP assay:

FRAP method is based on the comparison of the total amount of antioxidant with the reducing capacity of the sample. The FRAP assay revealed a maximum antioxidant activity in ethanolic extract of both the brown algae (1.16 mg/g) and methanolic extract of red alga *G. corticata* (1.076 mg/g). In green algal extracts the activity was more or less similar in both the solvents. Methanolic extract of *P. tetrastromatica* also exhibited a high FRAP value (0.96mg/g). High FRAP values for brown seaweeds *Sargassum polystum* and *Padina* sp. have been shown by Matanjun [3]

3.3 Ferrous ion chelating activity:

The ferrous ion chelating ability of methanolic and ethanolic extract is shown in Tables 1&2. Metal chelating ability of seaweed extract was tested at a concentration of 100mg/ml. The ethanol extracted samples showed a higher ferrous ion chelating ability than the methanolic samples. The activity was 70-80% in most of the seaweeds studied. For *Chaetomorpha* both the extracts exhibited a lower value of FIC (34%). Budhiyanti et al [30] have reported a higher ferrous ion chelating ability in *Sargassum hystrix.* An extract with higher binding ability would prevent or inhibit reaction such as fenton type reaction which generates reactive hydroxyl radicals [31].

3.4 Reducing power:

The reducing power of the methanolic extract of all species was high and ranged from 0.624 – 1.088 mg/g. In the ethanolic samples it was remarkably less. Methanolic extract of *D. dichotoma* (1.088mg/g) and *E. intestinalis* (1.019mg/g) had the maximum reducing power than all other seaweeds under investigation. The reducing property indicated that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process. So that they can act as primary and secondary antioxidants [32].

3.5 Total antioxidant capacity:

The total antioxidant activity was remarkable in methanolic extract of all the six species of seaweeds and it was greater than 100mg/ml. Maximum value was scored in *C. media* and *P. tetrastromatica* (211.20 mg/g & 144.33 mg/g resp.). In ethanolic extract of *D. dichotoma* the highest antioxidant activity (112.54 mg/g) was recorded. Ganesan et al [29] have also noticed a higher total antioxidant capacity in the methanolic extract of several brown and green seaweeds.

3.6 Total phenolic content:

In the present study phenolic content was found higher in methanolic extracts than in ethanolic (Tables 1& 2). In both the green algal ethanolic extracts phenolic content was less as compared to other species. Maximum phenols were present in the methanolic extract of *E. intestinalis* (3.72 mg/g) and *G. acerosa* (3.50 mg/g). Ethanolic extract of *D. dichotoma* (3.24 mg/g) presented a higher value of phenolic content. In members of Chlorophyta phenolic content was very less than in the members of Phaeophyta [32].

Correlation between the total phenolic content and the antioxidant capacity was used to compare the sensitivity of the relevant tests as shown in fig. 1. From the graph it is clear that the correlation between the total phenolic content and antioxidant capacity scored from the DPPH assay in methanol extract is the highest (R^2= 0.769) and with FRAP assay is the lowest (R^2= 0.018). In ethanolic extract the correlation between phenolic content and total antioxidant capacity is maximum (R^2= 0.782) and with FRAP assay is minimum (R^2= 0.401). The correlation between total antioxidant capacity and phenolic content was positive and very significant in ethanol extracted samples whereas it was negative in the methanol extracted seaweeds.

Recently many studies have shown that phenolic compounds such as flavonoids, isoflavone, anthocyanins and lignins are known to be important inhibitors of oxidative molecules. These phenolic compounds have many hydroxyl groups in their structure and have ability to catch free radicals [33]. The lower correlation between FRAP values and the phenolic contents in both the seaweed extracts indicated that only the phenolic compounds are not involved in the antioxidant activity through this pathway but there might be some effects involving other active compounds.

This study showed that *C. media, E. intestinalis, P. tetrastromatica, D. dichotoma, G. corticata* and *G. acerosa* possessed varying degrees of antioxidative activities in methanol and ethanol extracts. The methanolic and ethanolic extracts of green seaweeds *E. intestinalis* and the brown seaweed *D. dichotoma* showed a better ferrous ion chelating activity, total antioxidant capacity and reducing power ability and a higher phenolic content to correlate with.

Sr.no	Seaweeds	DPPH (% inhibition)	FRAP (mg AA/g)	Reducing Power (mg AA/g)	Ferrous ion chelating capacity (%)	Total antioxidant capacity (mg AA/g)	Phenolic content (mg CE/g)
1	*Chaetomorpha media*	34.46 ± 0.10	0.571± 0.002	0.624± 0.001	27.60± 0.10	211.20 ± 0.005	2.77 ± 0.005
2	*Enteromorpha intestinalis*	75.56 ± 0.63	0.631± 0.002	1.088 ± 0.002	73.20 ± 0.10	159.91 ± 0.38	3.72 ± 0.02
3	*Padina tetrastromatica*	64.71 ± 0.10	0.964± 0.006	1.019 ± 0.002	72.00 ± 0.10	144.33 ± 0.21	3.40 ± 0.005
4	*Dictyota dichotoma*	58.74 ± 0.10	0.691± 0.001	0.847 ± 0.003	71.70 ± 0.10	134.54 ± 0.38	3.38 ± 0.005
5	*Gracilaria corticata*	50.47 ± 0.15	1.076±0.001	0.682 ± 0.001	47.20 ± 0.10	112.54 ± 0.52	2.87 ± 0.005
6	*Gelidiella acerosa*	53.80 ± 0.10	0.853± 0.004	0.746 ± 0.005	48.40 ± 0.10	86.88 ± 0.032	3.50 ± 0.032

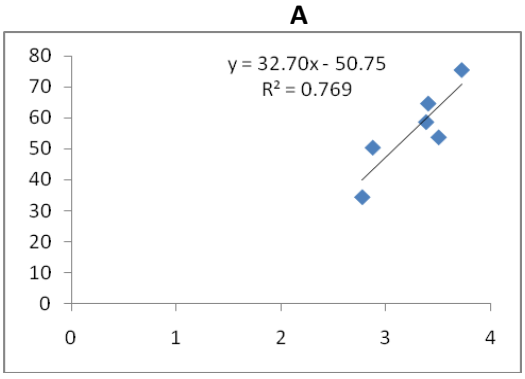
Table 1: Antioxidant properties of seaweeds in methanolic extract

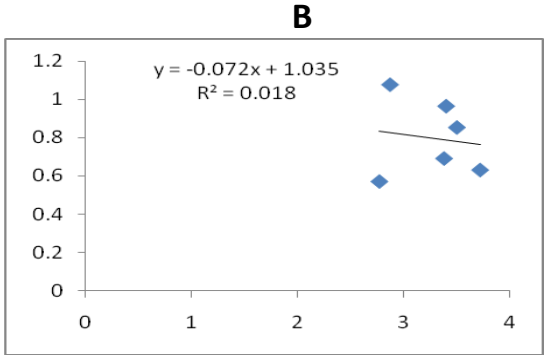
Values represent mean of three observations with standard deviation (SD) ±

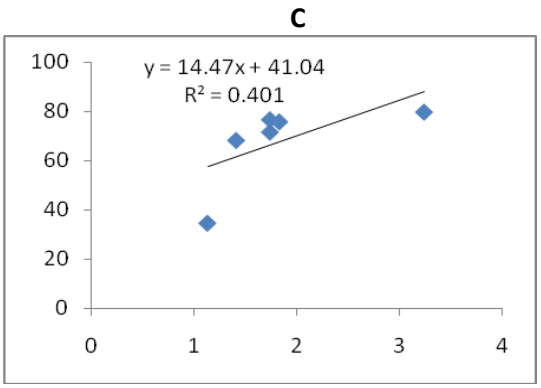
Sr.no	Seaweeds	DPPH (% inhibition)	FRAP (mg AA/g)	Reducing Power (mg AA/g)	Ferrous ion chelating capacity (%)	Total antioxidant capacity (mg AA/g)	Phenolic content (mg CE/g)
1	*Chaetomorpha media*	88.06 ± 0.10	0.554 ± 0.001	0.167 ± 0.0005	34.76 ± 0.30	37.35± 0.28	1.13 ± 0.005
2	*Enteromorpha intestinalis*	73.07 ± 0.15	0.688 ± 0.002	0.120 ± 0.0005	68.26 ± 0.30	55.08 ± 0.51	1.41 ± 0.005
3	*Padina tetrastromatica*	63.54 ± 0.15	1.168 ± 0.003	0.208 ± 0.0005	71.60 ± 0.36	62.97 ± 0.28	1.74 ± 0.00
4	*Dictyota dichotoma*	59.39 ± 0.15	1.165 ± 0.0032	0.331 ± 0.0005	79.73 ± 0.58	109.27± 0.86	3.24 ± 0.005
5	*Gracilaria corticata*	80.07 ± 0.15	0.763 ± 0.001	0.256 ± 0.001	76.63 ± 0.35	90.13 ± 0.40	1.74 ± 0.00
6	*Gelidiella acerosa*	68.31 ± 0.10	0.797 ± 0.0015	0.147 ± 0.001	75.80 ± 0.51	76.02 ± 0.37	1.83 ± 0.005

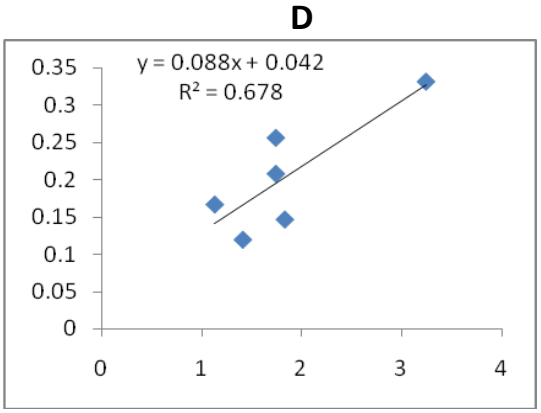
Table 2: Antioxidant properties of seaweeds in ethanolic extract

Values represent mean of three observations with standard deviation (SD) ±









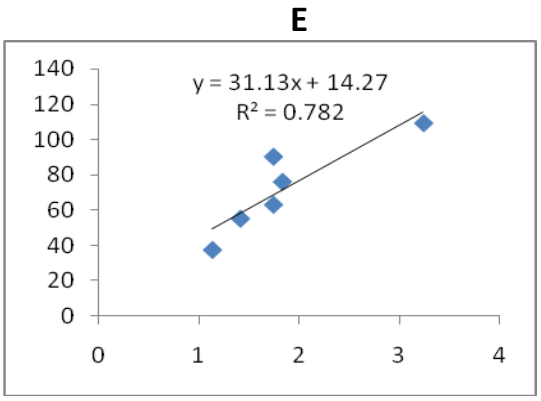


Fig 1: Correlation between the content of total phenols in methanolic seaweed extract and their antioxidant capacity as determined by DPPH method (A), FRAP assay (B), Ferrous ion chelating ability (C), Reducing power (D) and Total antioxidant capacity(E).

4. REFERENCES

1. Cox S, Abu-Ghannam N and Gupta S An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweed. Inter J Food Res 2010; 17: 205 – 220.
2. Kumar K. S, Ganesan K, Subba Rao P. V.Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty- An edible seaweeds. Food Chem 2008; 107:289–295.
3. Matanjun P, Mohamed S, Mustapha N. M, Muhammad K, Ming C. H. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J Appl Phycol 2008; 20: 367– 373.
4. Jimenez-Escrig A, Goni C. L. Nutritional evaluation and physiological effects of edible seaweeds, Arch Latinoam Nutr 1999; 49(2):114-120
5. Risso S, Escudero C, Estevao S, De Portela M. L, Fajardo M. A. Chemical composition and seasonal fluctuations of the edible green seaweed *Monostroma undulatum* Wittrock from the southern Argentia coast. Arch Latinoam Nutr 2003; 53(3): 306–311.
6. Cardozo K. H. M, Guaratini T, Barros M. P, Falcao V. R, Tonon A. P, Lopes N. P, Campos S, Torres M. A, Souza A. O, Colepicolo P, Pinto E. Metabolites from algae with economical impact. Comp Biochem Physiol, Part C: Parmacol, Toxicol Endocrinol 2007; 146: 60
7. Dykens J. A, Shick J. M, Benoit C, Buettner G. R Winston G. W. Oxygen radical prduction in the sea anemone *Anthopleura elegantissima* and endosymbiotic algae. J Exp Biol 1992; 168: 219–241.
8. Namiki M. Antioxidant/ antimutagens in food. Crit Rev Food Sci Nutr 1990; 29:273–300.
9. Matsukawa R, Dubinsky Z, kishimoto E, Masakki K, Masuda Y, Takeuchi T. C A comparison of screening methods for antioxidant activity in seaweeds. J Appl Phycol 1997; 9: 29 – 35.
10. Ramarathnam N, Osawa T, Ochi H, Kawakishi S. The contribution of plant food antioxidants to human health. Trends Food Sci Technol 1995; 6: 75–82.
11. Jime´nez-Escrig, A, Jime´nez-Jime´nez I, Pulido R, Saura-Calixto F. Antioxidant activity of fresh and processed edible seaweeds. J Sci Food Agric 2001; 81: 530–534.
12. Burrit D, Larkindale J, Hurd C. L. Antioxidant metabolism in the intetidal red seaweed *Stictosiphonia arbuscula* following dessication. Planta 2002; 215(5): 829 –838.
13. Othman A, Ismail A, Ghani N. A, Adenan I. Antioxidant capacity and phenolic content of Cocoa beans.Food Chem 2007; 100: 1523–1530.
14. Frankel E. N, Meyer A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agric 2000; 80: 1925–1941.
15. Prior R. L, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Rad Biol Med 1999; 27: 1173–1181
16. Prior R. L, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005; 53: 4290–4302.
17. Zubia M, Robledo D. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J Appl Phycol 2007; 19:449–458.
18. Wang Li., Rangarajan M., Shao Y., Lavoie E Huang T. and Ho C. Antioxidative phenolic compounds from sage (*salvia officinalis*) J Agric Food Chem 1998; 46: 4869-487
19. Benzie I. F. F, Strain J. J. Ferric reducing (antioxidant) power as ameasure of antioxidant capacity: the FRAP assay. In: Packer L(ed) Oxidants and Antioxidants Volume 299 of Methods in Enzymology. Academic Press, Orlando, 1999; 15–27
20. Decker E. A, Welch B. Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem 1990; 38: 674-677.
21. Yen G. C, Chen H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995; 43: 27-32.
22. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application of vitamin e. Anal Biochem 1999; 269: 337-341.
23. Sadasivam, S, A Manickam. Biochemical methods for agricultural science, Wiley Eastern Ltd, Madras. 2008
24. Singh N, Rajini P. S. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 2004; 85: 611-616.
25. Senthilkumar P, Sellappa Sudha. Antioxidant and antibacterial properties of methanolic extract of green seaweed *Chaetomorpha linium* from gulf of mannar: southeast coast of India. Jundishapur J Microbiol 2011; 5(2): 411–415.
26. Devi K. P, Suganthy N, Kesika P, Pandian S. K. Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complement Altern Med 2008 8: 38–49.

27. Wang T, Jonsdottir R, Olafsdottir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem 2009; 116: 240–248.
28. Zubia M, Fabre M. S, Kerjean V, Lann K. L, Stigerpoureau V, Fauchon M, Deslandes E. Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. Food Chem 2009; 116: 693–701.
29. Ganesan P, Chandini S. K, Bhaskarn. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresour Technol 2008; 99(8): 2717– 2723.
30. Budhiyanti S. A , Raharjo S, Marseno D.W, Iwan Y.B. Lelana. Antioxidant activity of brown algae *Sargassum* species extract from the coastline of Java Island. American J Agric Biol Sci 2012; 7(2): 337–346.
31. Chew Y. L, Lima Y. Y, Omar M, Khoo K.S. Antioxidant activity of three edible seaweeds from two areas in south east asia. LWT Food Sci Technol 2008; 41: 1067–1072.
32. Heo S. J, Park E. J, Lee K. W, Jeon Y. J.Antioxidant activites of enzymatic extract from brown seaweeds. Bioresour Technol 2005; 96: 1613–1623.
33. Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E. Effect of interaction of tannins with coexisting substances. VI. Effect of tannins and related polyphenols om superoxide anion radical and on DPPH radical. Chem Pharm Bull 1989; 37: 2016–2021.

Conflict of Interest: None Declared

Cite this article as:
MOLE MEGHA N. AND SABALE ANJALI B. Antioxidant Potential of Seaweeds from Kunakeshwar along the West Coast Maharashtra. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (22), 45-50.

