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Antioxidant effect of the marine algae *Chlorella vulgaris* against naphthalene-induced oxidative stress in the albino rats

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Abstract Alcoholic extract of the marine algae *Chlorella vulgaris* was examined for its free radical scavenging effect with reference to naphthalene-induced lipid peroxidation in serum, liver, and kidney of rats. Initially, upon naphthalene intoxication (435 mg/kg body weight, intraperitoneally), the lipid peroxidation activity increased significantly ($P < 0.001$), and in contrast, the enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymic antioxidants (glutathione, ascorbic acid, and α-tocopherol) levels decreased remarkably. When the naphthalene stressed rats were treated with *Chlorella vulgaris* extract (70 mg/kg body weight, orally), the lipid peroxidation activity reduced significantly ($P < 0.001$) and the activities of both the enzymic and non-enzymic antioxidants increased reaching near control values. The minimum concentration (70 mg/l) of the extract that exhibited maximum (85%) free radical scavenging activity was chosen for the experimental study. The present results suggest that *Chlorella vulgaris* extract exerts its chemopreventive effect by modulating the antioxidants status and lipid peroxidation during naphthalene intoxication.

Keywords *Chlorella vulgaris* · Free radical scavengers · Naphthalene · Lipid peroxidation

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Introduction

Naphthalene is a bicyclic aromatic hydrocarbon, widely used commercially in moth repellents, lavatory scent discs, soil fumigants, raw material for manufacturing naphthylamines, anthranilic and phthalic acids, and synthetic resins. It is one of the predominant compounds found in crude oil and refined petroleum products [1]. Naphthalene is of considerable toxicological interest because of its widespread human exposure and the potential to generate reactive oxygen species (ROS) as it undergoes extensive microsomal metabolism leading to the generation of quinone, hydroquinone and semiquinone free radical intermediates [2]. The free radicals generated by microsomal metabolism may augment an oxidative stress by the formation of hydrogen peroxide and superoxide anions [3]. The free radicals cause cellular injury, the consequences of which are often exhibited as a measure of lipid peroxidation (LPO). However, cells possess antioxidant system viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), ascorbic acid (Vitamin C), α-tocopherol (Vitamin E) etc. to protect them against the oxidative stress and consecutive LPO. Findings by previous researchers indicate that antioxidant system play a crucial role in the prevention of naphthalene toxicity by counteracting the oxidative stress thereby modulating their levels [4, 5].

ROS are fundamental to any biochemical processes. Under normal conditions, equilibrium exists between the amounts of free radicals being generated and antioxidants available to quench or scavenge them thus protecting the organism against deleterious effects of oxidative stress. The status of LPO and antioxidants in an organism reflects the dynamic balance between the antioxidants defense and pro-oxidant conditions in animals, which is

an useful index for assessing the risk of oxidative damage [1, 6].

Marine algae have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals. The unicellular algae *Chlorella vulgaris* contains many bioactive substances with medical properties. Experimental studies have demonstrated the medicinal properties of *Chlorella* viz. antitumor effect [7, 8], hepato protective properties, antioxidant properties [9], and antibacterial effects [10], etc. Chemical analysis performed by Hasegawa et al. [11] revealed that *Chlorella* extract contained 44.3 g protein, 39.5 g carbohydrates and 15.4 g nucleic acids in 100 g (dry weight) whole material. Biochemically, *Chlorella* contains many dietary antioxidants such as lutein, α-carotene, *β*-carotene, ascorbic acid and α-tocopherol [12–14]. These bioactive compounds have the capacity to scavenge the free radicals.

Major avenues of research remain to be explored to establish the scope of dietary, nutritional and pharmacological prospective of antioxidant regimes in the preventive and palliative therapy of a number of oxidative stress-related disorders. Hence, the present research utilized rats as an animal model to investigate biochemical changes elicited by naphthalene intoxication and attempt to screen the free radical savaging action of *Chlorella vulgaris*.

Materials and methods

Chemicals

Naphthalene was procured from Sigma Chemical Co., Ltd, St. Louis, MO, USA. The readymade formulation of *Chlorella vulgaris* strains (tablets) was obtained from Taiwan Chlorella Manufacturing Co., Ltd (Taiwan). Other chemicals used for biochemical assays were of analytical grade and obtained from local suppliers.

Chlorella vulgaris culture

Chlorella vulgaris was cultured in a glass tank (20 l) of seawater. The seawater (30 ppt) collected from pollution free zone was filtered (0.5 μm pore-size Millipore filters), sterilized at 120°C for 30 min, and enriched with Erd-Schreiber medium. The cultures were then maintained at 28 ± 1°C with sufficient aeration and constant illumination.

Extract preparation

The algae were harvested by continuous-flow centrifugation (10 l/h) at 2,000*g* for 40 min at 4°C. The resulting whole-cell pellet was weighed and air-dried to remove moisture. The dried algal powder was packed in a permeable cellulose thimble and subjected to continuous Soxhlet extraction with ethanol. The resulting extract was concentrated to dry residue using a rotary evaporator and refrigerated until use.

Free radical scavenging activity of *Chlorella vulgaris*

The radical scavenging activity of *Chlorella vulgaris* extract was assayed by the DPPH (1,1-Diphenyl-2-picrylhydrazyl) method as described by Abe et al. [15]. A 0.5 ml aliquot of 10, 20, 30, 40, 50, 60, 70, 80, 90 and -100 mg l^{-1}ethanol dissolved *Chlorella vulgaris* extract was mixed with 0.25 ml of ethanolic, 0.5 mM DPPH solution and 0.5 ml of 100 mM acetate buffer (pH 5.5). The decrease in absorbance of DPPH radicals at 517 nm was measured by a UV-vis spectrophotometer (Shimadzu UV-1201). The inhibition (%) of DPPH* was calculated based on the formula $(A_{\text{control}} - A_{\text{test}})/A_{\text{control}} \times 100$, where A_{control} is the absorbance of the control (DPPH solution without the test sample), and A_{test} is the absorbance of the test sample. The free radical scavenging activity of the test samples was expressed as the effective concentration of the extract (mg l^{-1}) required for inhibiting the free radicals. All tests were performed in triplicate.

Experimental animals

Male Wistar strain albino rats, weighing about 120 ± 150 g were obtained from the International Institute of Biotechnology and Toxicology (IIBAT), Padappai, Chennai, India. The animals were maintained in a 12-h dark and light cycle at 22 ± 3°C, fed with commercial pelleted diet (M/s. Hindustan Foods Ltd., Bangalore, India) and had free access to water.

Experimental design

The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines. The rats were randomized into three groups, each group comprising six animals. Group I rats served as normal control which received corn oil in addition to food and water. Group II rats were administered with naphthalene dissolved in corn oil (435 mg/kg body weight, intraperitoneally), and group III rats were treated with *Chlorella vulgaris* extract in 0.9% NaCl solution (70 mg/kg body weight, orally) and naphthalene in corn oil intraperitoneally (435 mg/kg body weight, intraperitoneally) daily for a period of 15 days. At the end of experiment, the rats were anesthetized with sodium pentobarbitone (35 mg/kg body weight, intraperitoneally), sacrificed by

cervical decapitation, and blood was collected to obtain serum. The liver and kidney were quickly dissected and placed in ice cold 0.9% NaCl solution. Portions (100 mg) of liver and kidney tissues were homogenized in 5 ml ice-cold 0.125 M Tris-HCl (pH 6.8) and then centrifuged at 5000 g for 15 min. The clear supernatants were used for biochemical assays.

Biochemical assays

Protein concentration was estimated according to the method of Lowry et al. [16] using bovine serum albumin as a standard. LPO was determined by the method of Okawa et al. [17] following a reaction of thiobarbituric acid (TBA) with malonyldialdehyde (MDA) formed by the peroxidation of lipids. SOD activity was measured at absorbance 420 nm as the degree of inhibition of autoxidation of pyrogallol in an alkaline pH by the method of Marklund and Marklund [18]. The amount of hydrogen peroxide consumed per minute per milligram of protein was assayed as the CAT activity. The color developed was read at 570 nm in a spectrophotometer by the method of Sinha [19]. GPx activity was estimated using the procedure of Rotruck et al. [20] in which available GPx catalyses the oxidation of reduced GSH by hydrogen peroxide. In the presence of GSH reductase and NADPH, oxidized GSH is converted to its reduced form with concomitant oxidation of NADPH to $NADP^+$. Oxidation of NADPH was measured by the decrease in the absorbance at 340 nm in a spectrophotometer. GSH was estimated by the method of Moron et al. [21] by reading the optical density (412 nm) of the yellow substance formed when 5′5′-dithio-2-nitro benzoic acid (DTNB) is reduced by GSH. Ascorbic acid is oxidized by copper to form dehydroascorbate, which reacts with 2,4, dinitrophenyl hydrazine to form a colored substance with an absorption maximum at 520 nm. This was measured to estimate the ascorbic acid (Vitamin C) content using ascorbic acid as standard by following the method of Omaye et al. [22]. Vitamin E (α-tocopherol) levels were estimated by the method of Desai [23], where the ferric ions are reduced to ferrous ions in the presence of tocopherol and bathophenanthroline to form a pink colored substance, which was read at 520 nm using a spectrophotometer. α-tocopherol was used as the standard.

Analysis of data

Values were expressed as mean ± S.D. for six rats in each group and significance differences between the mean values were determined by one-way analysis of variance (ANOVA). The significance was given as $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

The DPPH scavenging activity of *Chlorella vulgaris* extract is presented in Fig. 1. The minimum concentration (70 mg/l) of the extract that exhibited maximum (85%) free radical scavenging activity was chosen for the animal experimental study. The levels of LPO and antioxidants system in serum, liver, and kidney of control and experimental groups are presented in Table 1. There was a marked increase in the LPO activity in serum, liver, and kidney of rats administered with naphthalene in corn oil ($P < 0.001$). In contrast, the antioxidants level was found to decrease in tested samples of naphthalene-intoxicated rats when compared to control rats. Treatments of naphthalene-intoxicated rats with *Chlorella vulgaris* extract significantly ($P < 0.001$) reduced the LPO activity and increased the antioxidants levels thus bringing back their activity to near normal.

Discussion

Naphthalene toxicity is considered primarily due to prooxidant and antioxidant imbalance that is associated with increased production of free radicals during its metabolism and perturbation of intracellular redox equilibrium resulting in the development of oxidative stress [3]. In the present investigation, there is a significant increase ($P < 0.001$) in the levels of TBARS in the serum, liver, and kidney of naphthalene-administered rats when compared to control. The observed oxidative stress may be due to the generation of the intermediates like quinone, hydroquinone and semiquinone which further stimulates the production of various radicals like superoxide anion, hydrogen peroxide and hydroxyl radical during the metabolism of naphthalene in the cell [24]. The results presented in this study reveal that *Chlorella vulgaris* extract reduced the lipid membrane peroxidation by inhibiting the production

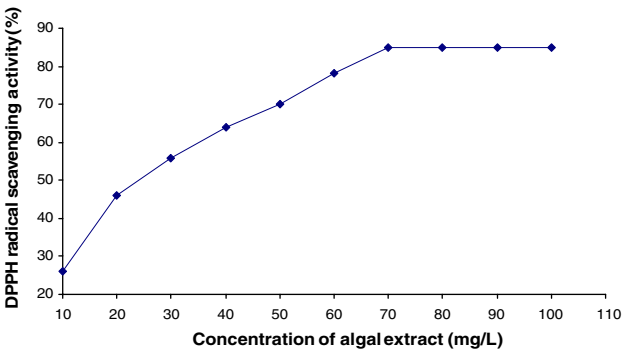

Fig. 1 Free radical scavenging capacity of *Chlorella vulgaris* extract on DPPH

Table 1 Effect of *Chlorella vulgaris* extract on lipid peroxidation and antioxidants status in serum, liver, and kidney of naphthalene-intoxicated rats

Parameters	Serum			Liver			Kidney		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
LPO	173.41 ± 1.16	295.27*** ± 1.22	175.15*** ± 1.05	194.22 ± 1.26	311.75*** ± 1.21	188.26*** ± 1.58	181.69 ± 1.63	266.83*** ± 1.85	177.46*** ± 1.66
SOD	43.52 ± 0.11	26.36** ± 0.19	41.86** ± 0.24	51.13 ± 0.17	23.68*** ± 0.19	49.47** ± 0.24	48.31 ± 0.15	32.46** ± 0.21	42.65*** ± 0.36
CAT	134.81 ± 1.10	97.42** ± 0.24	129.62* ± 1.36	146.84 ± 1.22	91.38*** ± 0.33	133.29*** ± 1.51	141.74 ± 1.69	87.53*** ± 1.24	124.86** ± 1.08
GPx	6.45 ± 0.03	3.55** ± 0.01	5.91* ± 0.02	7.42 ± 0.09	3.17** ± 0.06	6.88*** ± 0.08	6.52 ± 0.17	4.11** ± 0.35	5.82*** ± 0.24
GSH	4.63 ± 0.012	2.51** ± 0.07	4.13** ± 0.17	5.47 ± 0.13	3.44** ± 0.09	4.95** ± 0.04	5.16 ± 0.03	2.72** ± 0.01	4.22* ± 0.02
Vitamin C	3.15 ± 0.01	1.87*** ± 0.08	2.84** ± 0.02	3.92 ± 0.13	1.62** ± 0.06	3.16*** ± 0.019	3.74 ± 0.02	2.07* ± 0.05	2.84* ± 0.01
Vitamin E	2.62 ± 0.09	1.36* ± 0.06	2.17* ± 0.03	2.83 ± 0.04	1.02** ± 0.07	2.30* ± 0.05	2.30 ± 0.08	1.28** ± 0.07	2.14** ± 0.05

Values are expressed as mean ± S.D. for six rats in each group

LPO, nanomoles of malondialdehyde released/mg protein; SOD, units/(min mg protein); CAT, micromoles of H_2O_2 consumed/(min mg protein); GPx, micromoles of GSH oxidized/(min mg protein); GSH, μg/mg protein; Ascorbic acid, μg/mg protein; α-tocopherol, μg/mg protein

Group I, control; Group II, Naphthalene; Group III, Naphthalene + *Chlorella vulgaris*

Group II compared with Group I and Group III compared with Group II

* Values are statistically significant at $P < 0.05$

** Values are statistically significant at $P < 0.01$

*** Values are statistically significant at $P < 0.001$

of ROS with their free radical scavenging effect or by improving the antioxidant capacity of the cells.

Antioxidant enzymes are considered to be the primary defense that prevents biological macromolecules from oxidative damage. Among the antioxidant system, GSH (L-γ-glutamyl-cysteinyl-glycine) has an important role as it is a ubiquitous non-protein thiol present in the cell in its reduced form acts as an intracellular reductant. SOD catalyzes the transformation of superoxide radicals to H_2O_2 and O_2, CAT is responsible for the reduction of hydrogen peroxide, and GPx catalyzes the reduction of both hydrogen and lipid peroxides. Thus by using GSH as a cofactor, the antioxidant system scavenges the oxygen radicals and protects the biological molecules from damage [3, 5, 24, 25].

The reduction in the level of SOD activity in naphthalene-intoxicated rats might be due to the increase of superoxide anion radical during its metabolism. The observed reduction in the CAT activity may be explained by the insufficient supply of NADPH, which is required for the activation of CAT for its regeneration from its inactive form. Therefore, it is clear that deficiency of NADPH production during naphthalene stress could alter the CAT activity. Decline in the CAT activity in the naphthalene administered rats may be due to the increased accumulation of hydrogen peroxide thereby leading to augmented LPO. Decrease in the activity of GPx point outs the reduction in the levels of GSH and amplification in the levels of peroxides.

Maintenance of normal cell functions in the presence of oxygen largely depends on the efficiency of the defense mechanisms against free radical mediated oxidative stress, where non-enzymic antioxidants play a crucial role [1]. A significant decline in the levels of GSH, ascorbic acid and α-tocopherol was observed in naphthalene-exposed rats. GSH is responsible for the regulation of intracellular levels of LPO and also acts as a reactant in conjugation with electrophillic substances. Therefore, changes in GSH level may be a very important marker of the detoxification ability of an organism. Lowered levels of GSH in serum, liver, and kidney of naphthalene stressed rats represent an excess free radical production, which may be due to the binding of naphthalene with various sulfhydryls that exist in the cell [25]. The dehydroascorbic acid formed in the reaction is reduced to ascorbic acid by non-enzymatic reaction with GSH, which is eventually converted into oxidized GSH [26]. Since GSH reduces the dehydroascorbate to ascorbate, the decreased level of ascorbic acid observed in naphthalene-exposed rats might be due to the diminishing availability of GSH.

The rats administered with naphthalene along with *Chlorella vulgaris* extract exhibited reduction in LPO activity and an increase in the antioxidants activity of

serum, liver, and kidney. The chief mechanism involved in the reduction of LPO activity occurs primarily through the rearrangement of cellular redox status. This is evidenced by the fact that serum, liver, and kidney antioxidants reserve show substantial increase in their activity after *Chlorella vulgaris* extract treatment when compared to naphthalene-stressed rats. The increase in the activities of these antioxidants may be due to the ability of α-carotene and β-carotene in *Chlorella vulgaris* extract to react with various ROS as well as to interfere with oxidation processes in the lipid and in the cellular compartment [13].

The supplementation of *Chlorella vulgaris* extract to naphthalene-intoxicated rat showed an increase in the level of reduced-GSH, which may be due to the regeneration of reduced GSH. The antioxidants GSH, ascorbic acid and α-tocopherol are interrelated by a recycling process. Moreover, ascorbic acid present in *Chlorella vulgaris* might influence the increase in the affinity for GSH reductase, which has a key role in GSH recycling and maintenance of cellular GSH levels [27]. The algal extract is also responsible for reducing the oxidative stress either by removing the toxic metabolites from the target organ and/or by directly scavenging ROS by its sulfhydryl groups.

Carotenes are ubiquitously distributed in algae exhibiting a wide pharmacological spectrum of effects. The inhibition of LPO may be due to the free radical scavenging property of carotenes which can scavenge singlet oxygen and terminate peroxides by their low redox potential due to the presence of a hydroxyl group [9]. Previous phytochemical investigations on *Chlorella* and other marine algae demonstrated that carotenes exhibit free radical scavenging action [27–29] and their presence in *Chlorella* extract could explain the antioxidant property reported here. The free radical scavenging activity of *Chlorella vulgaris* could also be related to their interaction with enzymic and non-enzymic antioxidants system attenuating and resisting the oxidative stress and further LPO consequences. The ethanolic extract of *Chlorella vulgaris* expressed significant antioxidant activity against naphthalene-induced oxidative stress in the studied rat model. Considering the free radical scavenging property of *Chlorella vulgaris*, this marine alga can be used as a supplement in traditional medicine.

References

1. Vijayavel K, Gomathi RD, Durgabhavani K, Balasubramanian MP (2004) Sublethal effect of naphthalene on lipid peroxidation and antioxidants status of the marine edible crab *Scylla serrata*. Mar Pollut Bull 48:429–433
2. Wilson AS, Davis CD, Williams DP, Buckpitt AR, Pirmohamed M, Park BK (1996) Characterisation of the toxic metabolite(s) of naphthalene. Toxicology 114:233–242
3. Vuchetich PJ, Bagchi D, Bagchi M, Hassoun EA, Tang L, Stohs SJ (1996) Naphthalene-induced oxidative stress in rats and the protective effects of vitamin E succinate. Free Radic Biol Med 21(5):577–590
4. Greene JF, Zheng J, Grant DF, Hammock BD (2000) Cytotoxicity of 1,2-epoxynaphthalene is correlated with protein binding and in situ glutathione depletion in cytochrome P4501A1 expressing Sf-21 cells. Toxicol Sci 53:352–360
5. Stohs SJ, Ohia S, Bagchi D (2002) Naphthalene toxicity and antioxidant nutrients. Toxicology 180:97–105
6. Vijayavel K, Anbuselvam C, Balasubramanian MP (2005) Naphthalene-induced hematological disturbances and oxidative stress in an estuarine edible crab, *Scylla serrata*. Environ Toxicol 20:464–466
7. Konishi F, Tanaka K, Himeno K, Taniguchi K, Nomoto K (1985) Antitumor effect induced by a hot water extract of *Chlorella vulgaris* (CE): resistance to Meth-A tumor growth mediated by CE-induced polymorphonuclear leukocytes. Cancer Immunol Immunother 19:73–78
8. Morimoto T, Nagatsu A, Murakami N, Sakakibara J, Tokuda H, Nishino H (1995) Anti-tumor-promoting glyceroglycolipids from the green alga, *Chlorella vulgaris*. Phytochemistry 40:1433–1437
9. Singh A, Singh SP, Bamezai R (1998) Perinatal influence of *Chlorella vulgaris* (E-25) on hepatic drug metabolizing enzymes and lipid peroxidation. Anticancer Res 18(3A):1509–1514
10. Tanaka K, Koga T, Konishi F, Nakamura M, Mitsuyama K, Nomoto K (1986) Augmentation of host defense by a unicellular green alga, *Chlorella vulgaris*, to *Escherichia coli* infection. Infect Immun 53:267–271
11. Hasegawa T, Okuda M, Makino M, Hiromatsu K, Nomoto K, Yoshikai Y (1995) Hot water extracts of *Chlorella vulgaris* reduce opportunistic infection with *Listeria monocytogenes* in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. Int J Immunopharmacol 17:505–512
12. Shibata S, Oda K, Onodera-Masuoka N, Matsubara S, Kikuchi-Hayakawa H, Ishikawa F, Iwabuchi A, Sansawa H (2001) Hypocholesterolemic effects of indigestible fraction of *Chlorella regularis* in cholesterol-fed rats. J Nutr Sci Vitaminol 47:373–377
13. Shibata S, Natori Y, Nishhara T, Tomisaka K, Matsumoto K, Sansawa H, Nguyen VC (2003) Antioxidant and anti-cataract effects of *Chlorella* on rats with streptozocin-induced diabetes. J Nutri Sci Vitaminol 49:334–339
14. Hashimoto S, Setoyama T, Yokokura T, Mutai M (1982) Effects of soybean phosphilid, chlorella phospholipid and clofibrate on collagen and elastin synthesis in the aorta and on the serum and liver lipid contents in rats. Exp Mol Pathol 36:99–106
15. Abe N, Murata T, Hirota A (1998) Novel 1,1-diphenyl-2-picrylhydrazyl-radical scavengers, bisorbicillin and demethyltrichodimerol, from a fungus. Biosci Biotechnol Biochem 62:661–662
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin-ciocalteau reagent. J Biol Chem 193:265–273
17. Okawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
18. Marklund S, Marklund G (1974) Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem 47:469–474
19. Sinha AK (1972) Coliometric assay of catalse. Anal Biochem 47:389–395
20. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588–590
21. Moron M, Depierre JW, Mannervik BT (1979) Levels of glutathione, glutathione reductase and glutathione *S*-transferase activities in rat lung and liver. Biochem Biophys Acta 582:67–78

22. Omaye ST, Turnbull JB, Sauberlich HE (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol 62:1–11
23. Desai ID (1984) Vitamin E analysis method for animal tissues. Methods Enzymol 105:138–143
24. Pandya U, Saini MK, Jin GF, Awasthi S, Godley BF, Awasthi YC (2000) Dietary curcumin prevents ocular toxicity of naphthalene in rats. Toxicol Lett 115(3):195–204
25. Vijayavel K, Anbuselvam C, Balasubramanian MP (2006) Free radical scavenging activity of the marine mangrove *Rhizophora apiculata* bark extract with reference to naphthalene induced mitochondrial dysfunction. Chem Biol Inter 163(1–2):170–175
26. Ramanathan K, Balakumar BS, Panneerselvam C (2002) Effects of ascorbic acid and α-tocopherol on arsenic-induced oxidative stress. Hum Exp Toxicol 21:675–680
27. Miranda MS, Sato S, Mancini-Filho J (2001) Antioxidant activity of the microalga *Chlorella vulgaris* cultured on special conditions. Boll Chim Farm 140:165–168
28. Kudaa T, Tsunekawaa M, Gotoa H, Arakib Y (2005) Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J Food Comp Anal 18:625–633
29. Wu LC, Ho JAA, Shieh MC, Lu IW (2005) Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. J Agric Food Chem 53:4207–4212