Arch Pharm Res Vol 27, No 2, 194-198, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

# Inhibitory Phlorotannins from the Edible Brown Alga *Ecklonia stolonifera* on Total Reactive Oxygen Species (ROS) Generation

**Hye Sook Kang, Hae Young Chung[1], Ji Young Kim[1], Byeng Wha Son[2], Hyun Ah Jung[3], and Jae Sue Choi**

*Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea, [1]College of Pharmacy, Pusan National University, Busan 609-735, Korea, [2]Department of Chemistry, Pukyong National University, Busan 608-737, Korea, and [3]Korea Maritime University, Busan 606-791, Korea*

*(Received November 21, 2003)*

Reactive oxygen species (ROS) play an important role in the pathogenesis of many human degenerative diseases such as cancer, aging, arteriosclerosis, and rheumatism. Much attention has been focused on the development of safe and effective antioxidants. To discover sources of antioxidative activity in marine algae, extracts from 17 kinds of seaweed were screened for their inhibitory effect on total ROS generation in kidney homogenate using 2',7'-dichlorofluorescein diacetate (DCFH-DA). ROS inhibition was seen in three species: *Ulva pertusa, Symphyocladia latiuscula,* and *Ecklonia stolonifera*. At a final concentration of 25 μg/mL, *U. pertusa* inhibited 85.65±20.28% of total ROS generation, *S. latiscula* caused 50.63±0.09% inhibitory, and the *Ecklonia* species was 44.30±7.33% inhibition. *E. stolonifera* OKAMURA (Laminariaceae), which belongs to the brown algae, has been further investigated because it is commonly used as a foodstuff in Korea. Five compounds, phloroglucinol (**1**), eckstolonol (**2**), eckol (**3**), phlorofucofuroeckol A (**4**), and dieckol (**5**), isolated from the ethyl acetate soluble fraction of the methanolic extract of *E. stolonifera* inhibited total ROS generation.

**Key words:** *Ecklonia stolonifera,* Phlorotannins, Total ROS, Antioxidant

## INTRODUCTION

It is well known that reactive oxygen species (ROS) such as $\cdot O_2^-$ (superoxide anion), $H_2O_2$ (hydrogen peroxide), and ·OH (hydroxyl radical) are closely involved in various human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis, and atherosclerosis (Singh, 1989; Freeman, 1984; Squadrito and Pryor, 1998). For several years, many researchers have been searching for powerful but non-toxic antioxidants from natural sources, especially edible or medicinal plants. Such natural antioxidants could prevent the formation of the above reactive species-related disorders in human beings without the use of synthetic compounds, which may be carcinogenic and harmful to the lungs and liver (Branen, 1975). From this viewpoint, marine algae have attracted special interest as a natural antioxidant source.

Correspondence to: Jae Sue Choi, Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea
Tel: 82-51-620-6335, Fax: 82-51-620-6330
E-mail: choijs@pknu.ac.kr

In the present study, the extracts of various algae were screened for their inhibitory activity on total ROS. Various fractions and isolated components from the active alga, *Ecklonia stolonifera*, were further tested for inhibitory activity on total ROS.

*E. stolonifera* OKAMURA IS a perennial edible brown alga belonging to the Laminariaceae family. It grows at a water depth of 2-10 m around countries such as Korea and Japan, where it is commonly used as a foodstuff along with *Laminaria japonica* and *Undaria pinnatifida*. Phloroglucinol, phlorotannins (Taniguchi *et al.*, 1991), and ecklonialactones (Kurata *et al.*, 1989; 1993) have been isolated from *E. stolonifera.* Antioxidant activity (Choi *et al.*, 1993; Lee *et al.*, 1996a;), antimutagenic activity (Lee *et al.*, 1996b; 1998; Han *et al.*, 2000), feeding-deterrent effect (Taniguchi *et al.*, 1991), and tyrosinase inhibitory activity (Park *et al.*, 2000) have also been reported in this species.

## MATERIALS AND METHODS

### Plant material

Leafy thalli of *E. stolonifera* and other seaweed species

used in the present study were collected at Gi-jang-gun in Busan, Korea in February 2000 and identified by Prof. H. G. Kim of the Faculty of Marine Bioscience and Technology, Kangnung National University. A voucher specimen (no. 20000228) has been deposited in the author's laboratory (J. S. Choi).

### General

Column chromatography was done on silica gel 60 (230-400 mesh, Merck, Germany), RP-18 Lichroprep (Merck, Germany), and Sephadex LH-20 columns (Sigma, St. Louis, MO, USA). TLC was carried out on precoated Merck Kieselgel 60 $F_{254}$ plates (0.25 mm) and spots were detected under UV light using 50% $H_2SO_4$ reagent. 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Molecular Probes (Eugene, Oregon, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Solvents for column chromatography were of reagent grade.

### Isolation of phlorotannins

Lyophilized powders (3 kg) of *E. stolonifera* were refluxed with MeOH (3×9 L) for 3 h. The extract (700 g) was suspended in water and extracted successively with *n*-hexane (27.9 g), $CH_2Cl_2$ (25.6 g), EtOAc (25.0 g), and *n*-BuOH (99.6 g). The EtOAc fraction (25.0 g) was applied on a silica gel (Merck, 70~230 mesh, 800 g) column (4× 80 cm). The column was eluted using mixtures of EtOAc/MeOH under gradient conditions (50:1-5:1) to yield 10 fractions (F1~F10). The first fraction, F1 (3.44 g, $IC_{50}$ = 20 μg/mL) was further separated on a 3×70 cm silica gel column (70~230 mesh, 250 g) with a solvent of *n*-hexane and EtOAc (1:1) to give 11 fractions (F1-1~F1-11). Compound **1** (98 mg) was obtained by RP-18 column chromatography (20% MeOH ~100% MeOH, gradient) of F1-4 (257 mg). Compounds **2** (60 mg) and **3** (135 mg) were yielded by RP-18 column chromatography (20% MeOH ~100% MeOH, gradient) of F1-5 (1.01 g). Compounds **4** (57 mg) and **5** (87 mg) were isolated from F1-6 (945 mg) by RP-18 column chromatography (20% MeOH ~100% MeOH, gradient) followed by purification with Sephadex LH-20 (MeOH).

### Measurement of inhibition on total ROS generation (DCF Method)

The generation of ROS was assessed using the ROS-sensitive fluorescence indicator DCFH-DA (LeBel and Bondy, 1990). Rat kidney homogenates were prepared from the kidneys of freshly killed male Wistar rats weighing between 150-200 g. Ten μL of each test sample (final concentration, 0.5 mg/mL) was added to 190 μL of kidney postmitochondrial fraction in a 50 mM potassium phosphate buffer. Then the mixture was loaded with 50 μL of DCFH-DA (12.5 mM) in a potassium phosphate buffer and shaken for 5 min. Fluorescence of 2′,7′-dichlorofluorescein (DCF), the oxidation product from DCFH-DA was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm for 30 min on a microplate fluorescence spectrophotometer FL 500 (Bio-Tek instruments, USA). Trolox was used as a positive control.

### Statistical analysis

All results were expressed as mean values ± standard error of triplicate experiments.

## RESULTS AND DISCUSSION

Reactive oxygen/nitrogen species may act as potent oxidizing and nitrating agents to damage several cellular components such as nucleic acids, proteins, and lipids (Beckman *et al.*, 1990; Pincemail, 1995). Compounds in this group include superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($\cdot$OH), singlet oxygen ($^1O_2$), nitric oxide (NO$\cdot$) and peroxynitrite ($ONOO^-$). Free radicals of lipids such as alkoxyl radical (RO$\cdot$) and peroxyl radical (ROO$\cdot$) can also cause cellular damage. Accumulated ROS/RNS cause further aggravation of inflammation, leading to the eventual loss of cellular homeostasis, i.e. aging (Chung *et al.*, 2000). Free or non-free radicals including reactive oxygen species cause a variety of diseases such as inflammation, cardiovascular diseases, cancer, Alzheimer's disease, rheumatoid arthritis, and atherosclerosis (Beckman and Koppenol, 1996; Podrez *et al.*, 1999). These diseases have been reported to be ameliorated by radical scavengers (Aruoma, 1999; Hermann *et al.*, 1999).

In this study, we investigated the inhibitory activity of 17 kinds of seaweed extracts on total ROS generation in kidney homogenates using the ROS-sensitive indicator 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is a nonfluorescent probe that diffuses into cells. Mitochondrial esterase hydrolyzes DCFH-DA to 2′,7′-dichlorodihydrofluorescein (DCFH), a non-fluorescent compound that remains in the cell. ROS oxidize DCFH to the highly fluorescent dye, 2′,7′-dichlorofluorescein (DCF).

As shown in Table I, three of these species, *Ulva pertusa* (Chlorophyta), *Symphyocladia latiuscula* (Rhodophyta), and *Ecklonia stolonifera* (Phaeophyta), inhibited ROS production. At a final concentration of 25 μg/mL, *U. pertusa* caused 85.65 ± 20.28% inhibition, *S. latiuscula* was 50.63 ±0.09% inhibitory, and 44.30±7.33% of total ROS generation was inhibited by *E. stolonifera*. The brown algae *E. stolonifera* OKAMURA (Laminariaceae), has been further investigated, because it is commonly used as a foodstuff in Korea, along with *Laminaria japonica* and *Undaria*

**Table I.** Inhibitory effects of seaweed on total ROS generation

| | Seaweed species | Inhibition (%)[a] |
|---|---|---|
| Chlorophyta | *Enteromorpha crinita* | -37.55 ± 3.66[b] |
| | *Ulva pertusa* | 85.65 ± 20.28 |
| Phaeophyta | *Ecklonia stolonifera* | 44.30 ± 7.33 |
| | *Sargassum cinereum* | -125.32 ± 10.09[b] |
| | *Sargassum miyabei* | -15.61 ± 6.17[b] |
| | *Sargassum thunbergii* | -124.89 ± 3.91[b] |
| Rhodophyta | *Acrosorium flabellatum* | -54.02 ± 19.83[b] |
| | *Chondrus crispus* | -49.37 ± 12.91[b] |
| | *Chondrus ocellatus* | -88.61 ± 21.23[b] |
| | *Galasaura fastigiata* | -113.50 ± 18.79[b] |
| | *Gelidium amansii* | -54.85 ± 11.98[b] |
| | *Gigartina tenella* | -110.97 ± 16.55[b] |
| | *Grateloupia elliptica* | -97.47 ± 19.21[b] |
| | *Gymnogongrus flabelliformis* | -78.90 ± 17.25[b] |
| | *Lomentaria catenata* | -34.60 ± 7.39[b] |
| | *Rhodymenia intricate* | -69.62 ± 10.17[b] |
| | *Symphyocladia latiuscula* | 50.63 ± 0.09 |

[a]Inhibitory activity was expressed as the mean±S.E. of inhibitory percentage of triplicate determines obtained at the final concentration of 25 μg/mL.
[b]These indicates total ROS generation under experimental conditions

*pinnatifida*.

In our ongoing study to identify the active principles, we also evaluated the fractions soluble in the following solvents: *n*-hexane, $CH_2Cl_2$, EtOAc, and *n*-BuOH. The $H_2O$ layer derived from *E. stolonifera* was also studied. As summarized in Fig. 1, $CH_2Cl_2$, EtOAc, and *n*-BuOH fractions exhibited an inhibitory effect on total ROS. The EtOAc fraction exhibited a strong scavenging activity on total ROS of 40.81±0.34% at 25 μg/mL. $CH_2Cl_2$ fraction showed an activity of 16.02±5.07% and *n*-BuOH fraction showed 25.42±1.47% activity. In contrast, *n*-hexane (-345.13±9.91%) and $H_2O$ (-3.82±1.77%) fractions generated ROS. Trolox, a positive control, showed an inhibitory activity of 40.36±2.82% on total ROS generation at the same concentration.

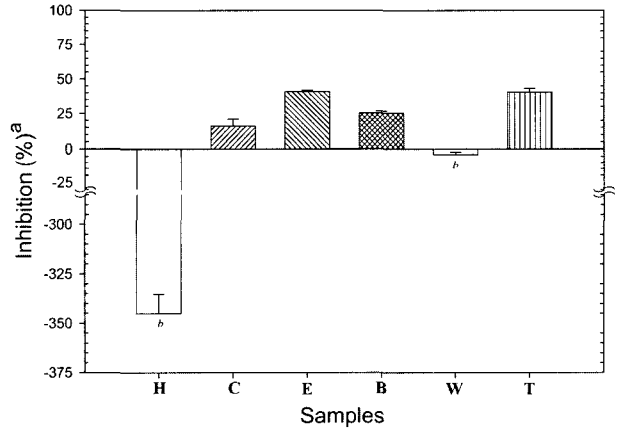


**Fig. 1.** Inhibitory effects of various fractions obtained from the methanolic extract of *E. stolonifera* on total ROS. Inhibitory activity was expressed as the mean±S.E. of inhibitory percentage of triplicate determines obtained with a final concentration of 25 μg/mL. [b]These indicates total ROS generation under experimental conditions. H: *n*-Hexane fr.; C: $CH_2Cl_2$ fr.; E: EtOAc fr.; B: *n*-BuOH fr.; W: $H_2O$ fr.; T: Trolox.

Five compounds (**1~5**, Fig. 2), isolated from EtOAc fraction, were identified as phloroglucinol (**1**), eckstolonol (**2**), eckol (**3**), phlorofucofuroeckol A (**4**), and dieckol (**5**) on the basis of chemical and physicochemical evidence, and compared with those of reported values in the literatures (Nakamura *et al.*, 1996; Fukuyama *et al.*, 1985; 1989a; 1990; Kang *et al.*, 2003). Compounds **1~5** inhibited total ROS generation with $IC_{50}$ values of 30.82±2.53, 12.17±0.73, 4.04±0.04, 3.80±0.09, and 12.68±0.34 μM, respectively (Table II). The activity of compounds **3** and **4** were comparable to that of Trolox ($IC_{50}$, 5.70±0.62 μM), a well-known ROS scavenger.

Phlorotannins are known to have various biological activities: anti-plasmin inhibitory activity (Fukuyama *et al.*, 1985, 1989a, 1989b, 1990; Nakayama *et al.*, 1989), algicidal effects (Nagayama *et al.*, 2003), bactericidal effects (Nagayama *et al*, 2002), feeding deterrent effects on marine herbivores (Hay, 1988; Altena and Steinberg, 1992; Boettcher and Targett, 1993; Targett *et al.*, 1995), UV protection (Swanson and Druehl, 2002), and chemopreventive effects against vascular risk factors (Kang *et al.*, 2003). Nakamura *et al.*(1996) reported that the phlorotannins

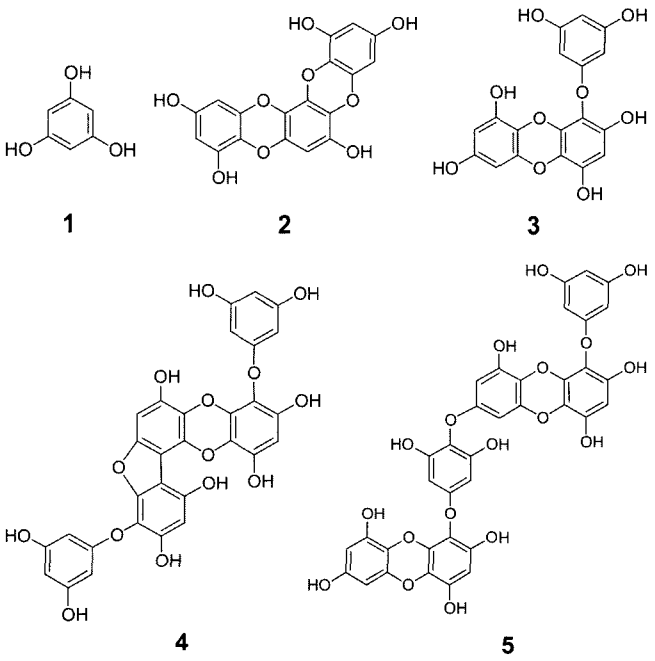


**Fig. 2.** Structures of the phlorotannins from *E. stolonifera*

**Table II.** Inhibitory effect of isolated compounds **1~5** from the EtOAc fraction of the MeOH extract of *E. stolonifera* on total ROS

| Compounds | $IC_{50}$ (μM)[a] |
|---|---|
| Phloroglucinol (**1**) | 30.82 ± 2.53 |
| Eckstolonol (**2**) | 12.17 ± 0.73 |
| Eckol (**3**) | 4.04 ± 0.04 |
| Phlorofucofuroeckol A (**4**) | 3.80 ± 0.09 |
| Dieckol (**5**) | 12.68 ± 0.34 |
| Trolox | 5.70 ± 0.62 |

[a]Inhibitory activities were expressed as the mean±S.E. of 50% inhibitory concentrations of triplicate determines obtained by interpolation of concentration-inhibition curve.

extended the induction time of autoxidation of methyl α-linolenate. However, no reports have appeared yet on inhibitory activity of phlorotannins on total ROS.

This work showed that the inhibitory abilities of phlorotannins, natural compounds found in edible brown algae on total ROS can be useful in the prevention and treatment of free radical-related disease. Investigations into further isolation of inhibitory principles on total ROS are now in progress.

## ACKNOWLEDGEMENT

This study was supported by a grant from the "2002 Korea Sea Grant Program (2002 KSGP)", Ministry of Maritime Affairs and Fisheries (2002).

## REFERENCES

Altena, A. and Steinberg, P. D., Are differences in the responses between North American and Australasian marine herbivores to phlorotannins due to differences in phlorotannin structure? *Biochem. Syst. Ecol.*, 20, 493-499 (1992).

Aruoma, O. I., Antioxidant actions of plant foods: use of oxidative DNA damage as a tool for studying antioxidant efficacy. *Free Radical Res.*, 30, 419-427 (1999).

Beckman, J. S., Beckman, T. W., Chen, J., Marshell, P. A., and Freeman, B. A., Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA*, 87, 1620-1624 (1990).

Beckman, J. S. and Koppenol, W. H., Nitric oxide, superoxide and peroxynitrite: the good, the bad and ugly. *Am. J. Physiol.*, 271, C1424-C1437 (1996).

Boettcher, A. A. and Targett, N. M., Role of polyphenolic molecular size in reduction of assimilation efficiency in *Xiphister mucosus*. *Ecology*, 74, 891-903 (1993).

Branen, A. L., Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Am. Oil Chem. Soc.*, 52, 59-63 (1975).

Choi, J. S., Lee, J. H., Park, H. J., Kim, H. G., Young, H. S., and Mun, S. I., Screening for antioxidant activity of plant and marine algae and its active principles from *Prunus davidiana*. *Kor. J. Pharmacogn.*, 24, 299-303 (1993).

Chung, H. Y., Kim, H. J., Jung, K. J., Yoon, J. S., Yoo, M. A., Kim, K. W., and Yu, B. P., The inflammatory process in aging. *Rev. Clin. Gerontol.*, 10, 207-222 (2000).

Freeman, B. A., Biological sites and mechanism of free radical production, In: Armstrong D, Sohal R, Culter RG, Slater T. (eds), *Free radicals in molecular biology, aging, and disease*, Raven Press, New York, pp. 43-52 (1984).

Fukuyama, Y., Miura, I., Kinzyo, Z., Mori, H., Kido, M., Nakayama, Y., Takahashi, M., and Ochi, M., Eckols, novel phlorotannins with a dibenzo-*p*-dioxin skeleton possessing inhibitory effects on $\alpha_2$-macroglobulin from the brown alga *Ecklonia kurome* OKAMURA. *Chem. Lett.*, 739-742 (1985).

Fukuyama, Y., Kodama, M., Miura, I., Kinzyo, Z., Kido, M., Mori, H., Nakayama, Y., and Takahashi, M., Structure of an anti-plasmin inhibitor, eckol, isolated from the brown alga *Ecklonia kurome* OKAMURA and inhibitory activities of its derivatives on plasma plasmin inhibitors. *Chem. Pharm. Bull.*, 37, 349-353 (1989a).

Fukuyama, Y., Kodama, M., Miura, I., Kinzyo, Z., Mori, H., Nakayama, Y., and Takahashi, M., Anti-plasmin inhibitor. V. Structures of novel dimeric eckols isolated from the brown alga *Ecklonia kurome* OKAMURA. *Chem. Pharm. Bull.*, 37, 2438-2440 (1989b).

Fukuyama, Y., Kodama, M., Miura, I., Kinzyo, Z., Mori, H., Nakayama, Y., and Takahashi, M., Anti-plasmin inhibitor. VI. Structure of phlorofucofuroeckol A, a novel phlorotannin with both dibenzo-1,4-dioxin and dibenzofuran elements, from *Ecklonia kurome* OKAMURA. *Chem. Pharm. Bull.*, 38, 133-135 (1990).

Han, E. S., Kim, J. W., Eom, M. O., Kang, I. H., Kang, H. J., Choi, J. S., Ha, K. W., and Oh, H. Y., Inhibitory effects of *Ecklonia stolonifera* on gene mutation on mouse lymphoma $tk^{+/-}$ locus in L5178Y-3.7.2C cell and bone marrow micronuclei formation in ddY mice. *Environ. Mutagen. Carcinogen.*, 20, 104-111 (2000).

Hay, M. E., Marine plant-herbivore interactions: the ecology of chemical defense. *Ann. Rev. Ecol. Syst.*, 19, 111-145 (1988).

Hermann, M., Kapiotis, S., Hofbauer, R., Exner, M., Seelos, C., Held, I., and Gmeiner, B., Salicylate inhibits LDL oxidation initiated by superoxide/nitric oxide radicals. *FEBS Lett.*, 445, 212-214 (1999).

Kang, H. S., Chung, H. Y., Jung, J. H., Son, B. W., and Choi, J. S., A new phlorotannin from the brown alga *Ecklonia stolonifera*. *Chem. Pharm. Bull.*, 51, 1012-1014 (2003)

Kang, K., Park, Y., Hwang, H. J., Kim, S. H., Lee, J. G., and Shin, H. C., Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agent against vascular risk factors. *Arch. Pharm. Res.*, 26, 286-293 (2003).

Kurata, K., Taniguchi, K., Shiraishi, K., Hayama, N., Tanaka, I., and Suzuki, M., Ecklonialactone-A and -B, two unusual metabolites from the brown algae *Ecklonia stolonifera* Okamura. *Chem. Lett.*, 267-270 (1989).

Kurata, K., Taniguchi, K., Shiraishi, K., and Suzuki, M., Ecklonialactones C-F from the brown alga *Ecklonia stolonifera. Phytochemistry,* 33, 155-159 (1993).

LeBel, C. P. and Bondy, S. C., Sensitive and rapid quantitation of oxygen reactive species formation in rat synaptosomes. *Neurochem. Int.*, 17, 435-440 (1990).

Lee, J. H., Park, J. C., and Choi, J. S., The antioxidant activity of *Ecklonia stolonifera. Arch. Pharm. Res.*, 19, 223-227 (1996a).

Lee, J. H., Oh, H. Y., and Choi, J. S., Preventive effect of *Ecklonia stolonifera* on the frequency of benzo(a)pyrene-induced chromosomal aberrations. *J. Food Sci. Nutr.*, 1, 64-68 (1996b).

Lee, J. H., Kim, N. D., Choi, J. S., Kim, Y. J., Moon, Y. H., Lim, S. Y., and Park, K. Y., Inhibitory effects of the methanolic extract of an edible brown alga, *Ecklonia stolonifera* and its component, phloroglucinol on aflatoxin $B_1$ mutagenicity *in vitro* (Ames test) and on benzo(a)pyrene or *N*-methyl *N*-nitrosourea clastogenicity *in vivo* (mouse micronucleus test). *Nat. Prod. Sci.*, 4, 105-114 (1998).

Nagayama, K., Iwamura, Y., Shibata, T., Hirayama, I., and Nakamura, T., Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome. J. Antimicro. Chemother.,* 50, 889-893 (2002).

Nagayama, K., Shibata, T., Fujimoto, K., Honjo, T., and Nakamura, T., Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae. *Aquaculture,* 218, 601-611 (2003).

Nakamura, T., Nagayama, K., Uchida, K., and Tanaka, R., Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicyclis. Fish. Sci.*, 62, 923-926 (1996).

Nakayama, Y., Takahashi, M., Fukuyama, Y., and Kinzyo, Z., An anti-plasmin inhibitor, eckol, isolated from the brown alga *Ecklonia kurome* OKAMURA. *Agric. Biol. Chem.*, 63, 3025-3030 (1989).

Park, D. C., Ji, C. I., Kim, S. H., Jung, K. J., Lee, T. G., Kim, I. S., Park, Y. H., and Kim, S. B., Characteristics of tyrosinase inhibitory extract from *Ecklonia stolonifera. J. Fish. Sci. Tech.*, 3, 195-199 (2000).

Pincemail, J. J., Free radicals and antioxidants in human diseases. In Favier AE, Cadet J, Kalyanaraman B, Fontecave M, Pierre JL. (eds) *Analysis of Free radicals in Biological Systems.* Birkhauser Verlag, Berlin, pp.83-98 (1995).

Podrez, E. A., Schmitt, D., Hoff, H. F., and Hazen, S. L., Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form *in vitro. J. Clin. Invest.*, 103, 1547-1560 (1999).

Singh, A., Physicochemical and physiological aspects, In: Miquel J, Quintanilha AT, Weber H. (eds), *CRC handbook of free radicals and antioxidants in biomedicine* Vol. I, CRC press Inc., Boca Raton, Florida, pp.17-25 (1989).

Squadrito, G. L. and Pryor, W. A., Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic. Biol. Med.*, 25, 392-403 (1998).

Swanson, A. K. and Druehl, L. D., Induction, exudation and the UV protective role of kelp phlorotannins. *Aquatic Botany,* 73, 241-253 (2002).

Taniguchi, K., Kurata, K., and Suzuki, M., Feeding-deterrent effect of phlorotannins from the brown alga *Ecklonia stolonifera* against the abalone *Haliotis discus* Hannai,. *Nippon Suisan Gakkaishi*, 57, 2065-2071 (1991).

Targett, N. M., Boettcher, A. A., Targett, T. E., and Vrolijk, N. H., Tropical marine herbivore assimilation of phenolic-rich plants. *Oecologia,* 103, 170-179 (1995).