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The Antioxidant Activity of *Ecklonia stolonifera*

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The antioxidant activity of *Ecklonia stolonifera* was determined by measuring lipid peroxide produced when a mouse liver homogenate was exposed to the air at 37°C, using 2-thiobarbituric acid (TBA) and the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The methanol extract of *Ecklonia stolonifera* showed strong antioxidant activity. And the methanol extract was fractionated with several solvents. With regard their fractions, the antioxidative activity were in the order of ethyl acetate>dichloromethane insoluble intermediated phase>dichloromethane>*n*-butanol>water fraction. The ethyl acetate soluble fraction exhibiting the strongest antioxidant activity was further purified by repeated silica gel and Sephadex LH-20 column chromatography. Antioxidant phloroglucinol was isolated and identified by ^{1}H-NMR and ^{13}C-NMR. Its antioxidant activity was similar to that of L-ascorbic acid.

Key words : *Ecklonia stolonifera*, phloroglucinol, antioxidant activity, marine algae

INTRODUCTION

Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defence of living cells against oxidative damage. The toxic and otherwise unfavorable effects of synthesized food antioxidants have been widely noted. Nevertheless, phenolic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are still used extensively as food antioxidants because of their excellent results and low cost. When slightly larger doses (50 mg/kg/day) of these phenolic antioxidants are administered to rodents and monkeys, however, certain pathological, enzyme and lipid alterations as well as carcinogenic effects have been observed (Branen, 1975). The development of alternative natural antioxidants has, therefore, assumed an increased importance. Many investigators have found different types of antioxidants in various kinds of plants (Larson, 1988). We have been interested in different kinds of seaweed as a new source of natural antioxidants, not only because many marine algae such as green and brown algae are commonly used as foods but also because these algae are abundant in Korea. Although the antioxidant activity of seaweed extracts and their substituted phenols and polyphenols has been known for some time (Kurata and Amiya, 1975; Fujimoto *et al.*, 1985; Fujimoto and Kaneda, 1980), the antioxidant activity of *Ecklonia stolonifera* has not yet been investigated.

We previously tested out the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical using methanol extracts of 19 kinds of seaweeds to discover some effective new natural antioxidants and reported that 4 extracts exhibited a strong antioxidative effect (Choi *et al.*, 1993). Of these 4 types of seaweeds, *Ecklonia stolonifera* had the strongest effect and was therefore used in the present study.

In this paper the antioxidant activity of *Ecklonia stolonifera*, a brown algae, was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-thiobarbituric acid (TBA).

MATERIALS AND METHODS

Algae material

All the seaweed of *Ecklonia stolonifera* used was collected at Tae Jong Dae, Pusan in July, 1990. The algae were identified by the botanist Prof. H. G. Kim, and a voucher specimen is now deposited in the author's laboratory (J. S. Choi). All the seaweed was washed with fresh water and air-dried in the shade.

Extraction and fractionation

The dried material (2.9 kg) was extracted three

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times with methanol, and the solvent was removed under reduced pressure to a dark blue semisolid (500 g). Succesive partitioning yielded dichloromethane (80.3 g), dichloromethane insoluble intermediated phase (7.5 g), ethyl acetate (17 g), *n*-butanol (13.8 g) and water soluble (275.5 g) fractions, respectively.

Isolation of phloroglucinol

The ethyl acetate-soluble fraction was chromatographed over silica gel using a $CHCl_3$-MeOH mixture and further separated by Sephadex LH-20 (solvent: MeOH) to yield the phloroglucinol in the form of hygroscopic powder, m.p. 218°C, which was identified by direct comparison with an authentic sample (m.m.p., ^{1}H- and ^{13}C-NMR).

DPPH radical scavenging effect

The DPPH radical scavenging effect was carried out according to the method first employed by M. S. Blois (Blos, 1958). Four milliliters of MeOH solution of varying sample concentrations was added to 1.0 ml DPPH methanol solution (1.5×10^{-4} M). After standing at room temperature for 30 min., the absorbance of this solution was determined at 520 nm using a spectrophotometer and the remaining DPPH was calculated. The results were calculated by taking the mean of all triplicate values.

TBA assay

The antioxidant activity was determined by measuring lipid peroxides using 2-thiobarbituric acid (TBA) (Hiroshi *et al.*, 1979). The mouse liver homogenate (0.3 ml) was mixed with 0.3 ml of aqueous 8.1% sodium dodecyl sulfate (SDS) and 0.1 ml of sample/or distilled water (D.W.) in a test tube. The mixtures were incubated at 37°C for 6 hr in a water bath. 1.5 ml of 20% acetic acid and 1 ml of 1.2% TBA solution were added. The test solutions were boiled at 100°C for 30 min and then cooled at room temperature. The solutions were centrifuged at 2,500 rpm for 15 min, and the absorbance of the upper layer was measured at 532 nm. One TBA unit corresponded to 0.1 optical density at 532 nm and calculated to TBA value per g liver weight (Han *et al.*, 1994). The results were calculated by taking the mean of all triplicate values.

RESULTS AND DISCUSSION

The radical scavenging effect of the methanol extract and their fractions of *Ecklonia stolonifera* on DPPH radical

The DPPH stable radical loses its characteristics purple color when supplied with electrons or hydrogen ions. The capacity of the tested substances to donate electrons can be estimated from the degree of their loss of color (Park *et al.*, 1991). The DPPH radical scavenging effect for the methanol extract and their fractions are shown in Fig. 1. The radical scavenging effect for the methanol extract and all fractions obtained from the methanol extract was observed in all cases apart from the water fraction; the effect of this was dependent on their concentration. The radical scavenging effect of the ethyl acetate and dichloromethane insoluble intermediated phase fraction were stronger than the others. Their IC_{50} was 18.4 μg/4 ml and 22.6 μg/4 ml, respectively. The results suggest that the methanol extract and the ethyl acetate and the dichloromethane insoluble intermediated phase fraction of *Ecklonia stolonifera* are effective radical scavengers.

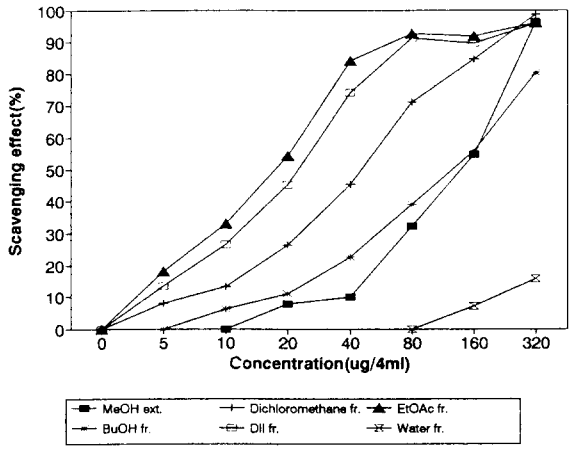

Fig. 1. The radical scavenging effect of the methanol extract and their fractions of *Ecklonia stolonifera* on DPPH radical. DII fr.; dichloromethane insoluble intermediated fraction.

The radical scavenging effect of phloroglucinol on DPPH radical

The effect of phloroglucinol and L-ascorbic acid on the DPPH radical scavenge is shown in Fig. 2. The addition of phloroglucinol at a concentration of 1.25 to 160 μg/4 ml scavenged the DPPH radical by 7 to 73%. The radical scavenging effect of these compounds increased according to their respective concentrations. Their IC_{50} was 55.7 μg/4 ml and 6.4 μg/4 ml, respectively. Although the effects of phloroglucinol was slightly weaker than that of L-ascorbic acid, the results suggest that phloroglucinol is also an effective radical scavenger.

The effects of the methanol extract of *Ecklonia stolonifera* on lipid peroxidation of a mouse liver as a function of the incubation time

A mouse liver homogenate was incubated at 37°C,

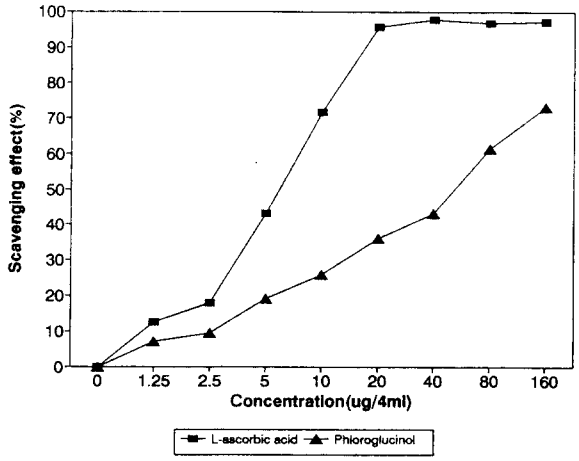

Fig. 2. The radical scavenging effect of phloroglucinol and L-ascorbic acid on DPPH radical.

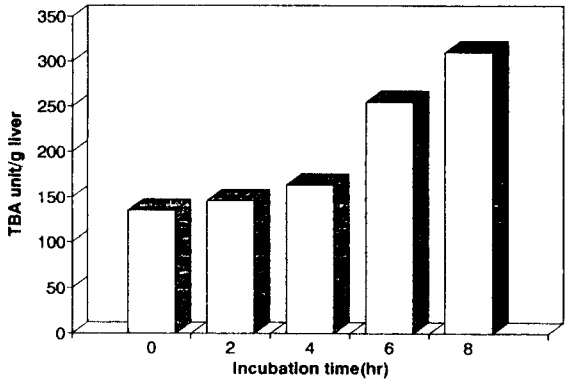

Fig. 3. The auto-oxidation of the lipid in a mouse liver homogenate as a function of the incubation time.

and the auto-oxidation of the lipid as a function of the incubation time is shown in Fig. 3. There was an increase in the TBA value as a function of the incubation time, and a rapid increase in the TBA value after 6hr. Because of this rapid increase, the antioxidant activity of the methanol extract was measured for values under 6hr of the incubation time.

The effects of the methanol extract of *Ecklonia stolonifera* on the lipid peroxidation of the mouse liver is shown in Fig. 4. The lipid peroxidation of the control group without a sample was increased as a function of incubation time. However, the addition of the methanol extract of *Ecklonia stolonifera* inhibited the lipid peroxidation of the mouse liver homogenate by about 46% after 6 hr. The results suggest that the methanol extract of *Ecklonia stolonifera* has an antioxidant effect which counters the lipid peroxidation.

The TBA value of the methanol extract of *Ecklonia stolonifera* was higher than that for the control group at incubation 0 hr because of the presence of certain compounds such as a chlorophyll influenced TBA pigment in the methanol extract of *Ecklonia stolonifera.*

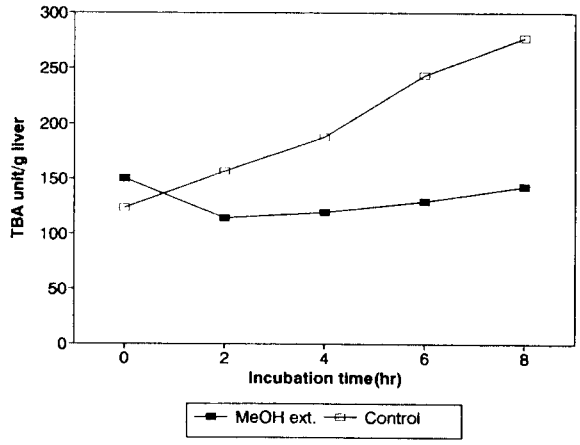

Fig. 4. The effect of the methanol extract of *Ecklonia stolonifera* on lipid peroxidation of a mouse liver as a function of the incubation time.

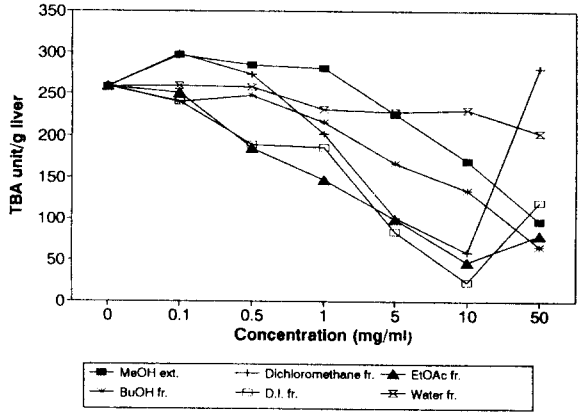

Fig. 5. The effect of various fractions obtained from the methanol extract of *Ecklonia stolonifera* on lipid peroxidation of liver homogenate. TBA values were measured after incubation for 6 hr. DII fr.; dichloromethane insoluble intermediated fraction.

The effects of the methanol extract of *Ecklonia stolonifera* and their various solvent fractions on lipid peroxidation of a mouse liver homogenate

The effects of various solvent fractions on lipid peroxidation is shown in Fig. 5. With the previously noted exception of the water fraction, the addition of each fraction inhibited the lipid peroxidation significantly. The dichloromethane insoluble intermediated phase, ethyl acetate, and dichloromethane fractions showed greater activity than the others in terms of antioxidant activity and inhibited the lipid peroxidation at a concentration of 10 mg/ml by 90.9, 81.8, 77.2% respectively. For dichloromethane, dichloromethane insoluble intermediated and ethyl acetate fraction at a concentration of 50 mg/ml, the TBA value was slightly increased again due to their own pigments.

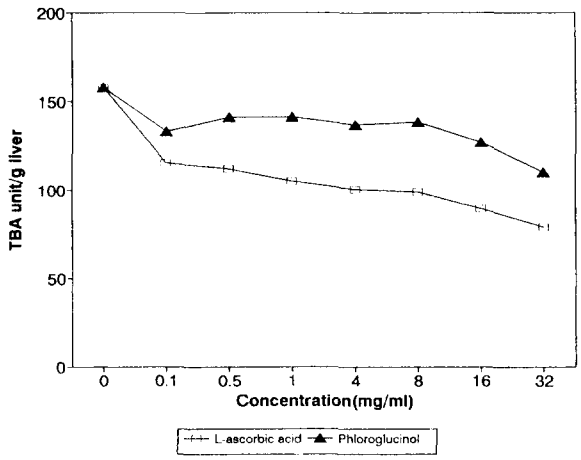

Fig. 6. The effect of phloroglucinol and L-ascorbic acid on lipid peroxidation of liver homogenate. TBA values were measured after incubation for 6 hr

The effects of phloroglucinol and L-asorbic acid on lipid peroxidation

The effects of phloroglucinol and L-ascorbic acid on lipid peroxidation is shown in Fig. 6. The addition of phloroglucinol isolated and L-ascorbic acid inhibited the lipid peroxidation; at a concentration of 10 μg/ml this inhibition was 35.3% and 46.5% respectively. The results suggest that phloroglucinol, as well as L-ascorbic acid, demonstrates antioxidant activity.

In the present study, antioxidant activity was found in the methanol extract of the *Ecklonia stolonifera*. We also separated this methanol extract into dichloromethane, dichloromethane insoluble intermediated phase, ethyl acetate, n-butanol and water fractions, and examined their effects. The strong antioxidant activity was also found in the ethyl acetate fraction and phloroglucinol was isolated as one of the active components. The antioxidant effect of phloroglucinol was more marked than in the case of the methanol extract.

Generally, algae has been used as a form of folk medicine in the curing of curare, helmintics, gout, eczema and gallstones in Korea. In a sense of successive screening tests for antioxidant principles in marine algae, Fujimoto *et al.* (1980) reported that more than half of them showed this effect. In particular, the chloroform-soluble fractions extracted from several species of brown algae, *Eisenia bicyclis* and *Undaria pinnatifida*, showed excellent antioxidant activities. And they also found that bromophenols which were isolated from a red algae, *Polyshiponia ulceolate*, showed a marked antioxidant activity (Fujimoto *et al.*, 1985). Park *et al.* (1991) demonstrated the presence of two effective natural antioxidant compounds in three edible algae *Laminaria sinclairii, Undaria pinnatifida* and *Enteromorpha linza*; these were confirmed to be benzene-derivative substances.

Phloroglucinol has been isolated from many plants, for example, *Eucalyptus kino* and *Acacia arabica* (Dictionary of Natural Products, 1994), including marine algae, such as *Ecklonia stolonifera, Ecklonia cava, Cystophyllum hakodatense, Sargassum ringgoldianum*, and *Fucus vesiculosus* (Scheuer, 1981; Taniguchi *et al.*, 1994); the antispasmodic effect and nitrite scavenging effect of phloroglucinol have also been reported (Dctionary of Natural Products, 1994; Choi *et al.*, 1989).

However, no report on the antioxidant activity of the methanol extract of *Ecklonia stolonifera* and its component, phloroglucinol, has yet appeared.

The findings of the present work would tend to indicate that the methanol extract of *Ecklonia stolonifera* and its component, phloroglucinol, may be useful for the treatment of oxidative damage.

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REFERENCES CITED

Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature*, 26, 1199-1200, (1958).

Branen, A. L. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *JAOCS*, 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, 4, 299-303 (1993).

Choi, J. S., Park, S. H. and Choi, J. H.: Nitrite scavenging effect by flavonoids and its structure-ef fect relationship. *Arch. Pharm. Res.*, 12, 26-33 (1989).

Dictionary of Natural Products, Chapman & Hall, London, p.627, 1994.

Fujimoto, K. and Kaneda, T. Screening test for antioxygenic compounds from marine algae and fractionation from *Eisenia bicyclis* and *Undaria pinnatifida. Bull. Jap. Soc. Sci. Fish.*, 16, 1125-1130 (1980).

Fujimoto, K., Ohmura, H. and Kaneda, T. Screening for antioxygenic compounds in marine algae and bromophenols as effective principles in a red alga

Polysiphonia ulceolate. Bull. Jap. Soc. Sci. Fish., 51, 1139-1143 (1985).

Han, Y. N., Oh, H. K., Hwang, G. H. and Lee, M. S. Antioxidant components of *Gardenia* fruit. *Kor. J. Pharmacogn.*, 25, 226-232 (1994).

Hiroshi, O., Nobuko, O. and Kunio, Y. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95, 351-358 (1979).

Kurata, K. and Amiya, T. Chemical studies on constituents of marine algae-II Constituents of the red alga, *Rhodomela subfusa. Bull. Jap. Soc. Sci. Fish.*, 41, 657-659 (1975).

Larson, R. A. The antioxidants of higher plants. *Phytochemisrty*, 27, 4, 969-978 (1988).

Park, J. H., Kang, K. C., Baek, S. B., Lee, Y. H. and Lee, K. S. : Separation of antioxidant compounds from edible marine algae. *Korean J. Food Sci. Technol.*, 23, 256-261 (1991).

Scheuer, P. J. Marine Natural Products, Vol. IV, Academic Press, 1981, pp.119.

Taniguchi, K., Kurata, K. and Suzuki, M. Feeding-deterrent effect of phlorotannins from the brown alga Ecklonia stolonifera against the abalone *Haliotis discus hannai. Bull. Jap. Soc. Sci. Fish.*, 57, 2065-2071 (1991).

Yamasaki, K., Hashimoto, A., Kokusenya, Y., Miyamoto, T. and Sato, T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. *Chem. Pharm. Bull.*, 42, 1663-1665 (1994).