Antioxidant Activities of *Ulva lactuca* Extracts with Different Solvents

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The fractions of *Ulva lactuca* were studied to verify the antioxidant activities. The fractions from the ethanol extract of *U. lactuca* were prepared by the systematic extraction procedure with the solvents such as hexane, ethyl ether, ethyl acetate, butanol and H_2O. Furthermore, ethyl ether, ethyl acetate and aqueous fractions of *U. lactuca* were purified using HPLC. The antioxidant activities of purified samples from ethyl ether, ethyl acetate, and aqueous fractions were investigated using 1,1-diphenyl-2-picryl-hydrazil (DPPH). L-ascorbic acid, a positive control showed the highest DPPH radical scavenging activity. In addition, purified sample from aqueous fraction also showed relatively high activity. Purified sample from ethyl acetate fraction showed moderate activity, but purified sample from ethyl ether fraction showed the lowest activity. Dose dependent patterns were observed on all three samples tested. The lipid peroxidation inhibition activities of these three purified samples were also investigated. Purified sample from ethyl ether fraction of *U. lactuca* showed the highest activity and as strong activity as that of α-tocopherol, a positive control. These results suggest that *U. lactuca* may be a useful candidate for a natural antioxidant agent.

Key words – *Ulva lactuca*, HPLC, antioxidant, DPPH, lipid peroxidation

Introduction

In the Far East and Asian Pacific, people have a long tradition of consuming seaweeds as part of their diet. The nutritional properties of seaweeds are not completely known yet. Compared to land plants, the chemical composition of seaweeds has been poorly investigated. The chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions[9]. Nevertheless, in general, seaweeds are rich in non-starch polysaccharides, minerals and vitamins[2,12].

Ulva lactuca, a green alga, is the type species of the *Ulva* genus; also known as sea lettuce. It is a small green alga (up to 30 cm across) with a broad, crumpled frond that is tough, translucent and membranous. It is attached to rock via a small hold-fast and represents green to dark green color. This Chlorophyta is a sheet forming alga composed of two layers or cells, as seen in cross section here. *Ulva*, among other green algae, is very prolific in areas were there are lots of nutrients available.

Oxidative damage in the human body plays an important causative role in disease initiation and progression [10]. Damage from free radicals and reactive oxygen species (ROS) has been linked to some neuro-degenerative disorders and cancers[4,13]. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers[3]. Recently, interest has considerably increased in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity[14]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods[11]. Hence, the studies on natural antioxidant have gained increasingly greater importance.

The aim of the present study was to evaluate the profitable properties of *U. lactuca* for human food or additives. *U. lactuca* was extracted with different organic solvents and components of each fraction was determined by HPLC method. We investigated the antioxidant activities of each fraction by DPPH radical scavenging assay and lipid peroxidation inhibition assay.

Materials and Methods

Plants and chemicals

U. lactuca was obtained from the sea in Busan (Korea),

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desalted and dried. Except for acetonitrile (HPLC-grade), all of the other chemicals used were of analytical grade. All solvents and sample solutions were filtered through 0.2-μm nylon membrane filters before HPLC analysis.

Preparation of sample extracts

One hundred gram of dried sample was dipped in 2 L of 95% ethanol. The ethanol extract solution was collected after 8 h at 60℃ and evaporated to eliminate the ethanol. Then this solution was fractionated by hexane, diethyl ether, ethyl acetate, butanol and water, sequentially (Fig. 1). Each fraction was collected and evaporated to dry with CentriVap concentrator (LABCONCO Corporation, Kansas, USA).

HPLC analysis

All fractions were dissolved with ethanol and identified by analytical HPLC with comparison of their retention times. HPLC analysis was carried out by HPLC system of Young Lin Instrument Co. Ltd. (Anyang, Korea) equipped with a vacuum degasser and mixer (Model SDV50A), a solvent delivery pump (Model SP930D), a column oven system (Model CTS30) and a UV-Vis. absorbance detector (Model UV730D). Analysis was performed on a TSK-gel ODS-120T column (4.6 mm × 150 mm, 5 μm) (TOSOH, Japan). The binary gradient elution system consisted of 20% acetonitrile in water (A) and 80% acetonitrile (B). The gradient started with 100% A to 100% B linearly for 30 min. The flow-rate was 1 ml/min and injection volume was 20 μl (1 mg/ml) The UV detection wavelength was set at 201 nm.

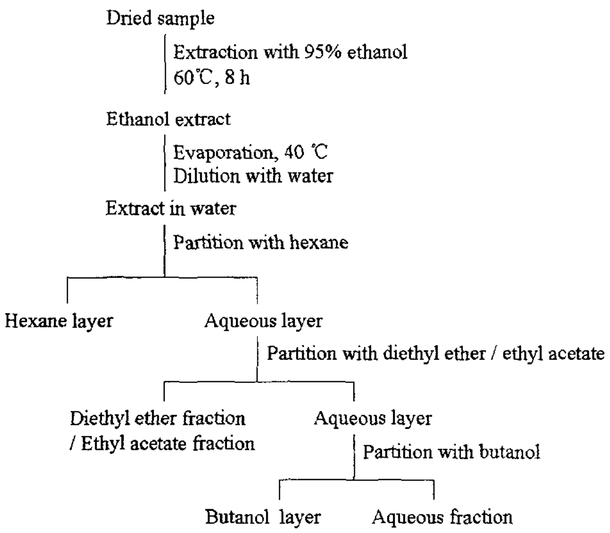

Fig. 1. Fractionation procedure of *Ulva lactuca*.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) method proposed by Brand-Williams et al.[1] with slight modification. Briefly, 1 ml of DPPH solution (0.1 mM, in ethanol anhydrous) was added to 0.5 ml of sample solution. The mixture was shaken vigorously and left to stand (25℃) in the dark for 20 minutes. Then the absorbance was measured at 525 nm in a spectrophotometer. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH• scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance of the sample. L-ascorbic acid, was used as a positive control.

Total antioxidant activity in linoleic acid emulsion

The antioxidant activity of the extracts on inhibition of linoleic acid peroxidation was assayed using the thiocyanate method[7] with some modification. Each sample solution (2.5 ml, 0.01 mg/ml) was added to 2.5 ml linoleic acid emulsion, respectively. The mixed solution (5 ml) was incubated at 37℃ in the dark. The degree of oxidation was measured by sequentially adding ethanol (5 ml, 75% v/v), ammonium thiocyanate (0.5 ml, 30% w/v), and ferrous chloride (0.5 ml, 0.02 M v/v) to sample solution (0.5 ml) which was taken after five days' incubation. Finally, the absorbance was read at 500 nm and the data used were the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 was absorbance of the control reaction and A_1 was the absorbance in the presence of the sample. Alpha-tocopherol was used as a positive control.

Statistical analysis

The experimental data were expressed as mean±S.D. of triplicate measurements. The results were processed by Microsoft Office Excel 2003 software.

Results

HPLC analysis

HPLC chromatogram of each fraction was shown Fig. 2, 3 and 4. All of the three fractions showed one main peak at the chromatography condition used in this study and their retention time was 18.6167 min (Fig. 2), 17.9833 min (Fig. 3) and 15.4667 min (Fig. 4), respectively, for diethyl ether, ethyl acetate and aqueous fraction.

DPPH radical scavenging activity

The percentage scavenging activity of each extract against DPPH was shown in Fig. 5. All of the three fractions possessed the strong scavenging ability on DPPH radical and their abilities were concentration-dependant. Aqueous fraction showed best scavenging activity among the three fractions, however, they were lower than L-ascorbic acid. At the concentration of 0.1 mg/ml, the sequence of scavenging activity was L-ascorbic acid (85.0%), aqueous fraction (70.5%), ethyl acetate fraction (56.1%), and diethyl ether fraction (38.3%).

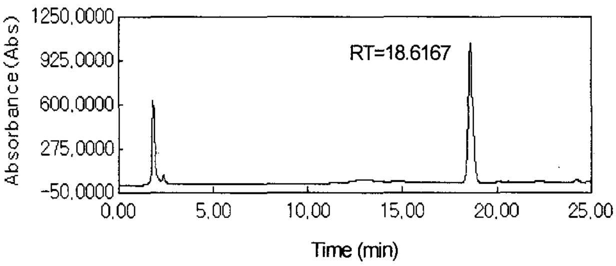

Fig. 2. HPLC chromatogram of diethyl ether fraction. RT: retention time.

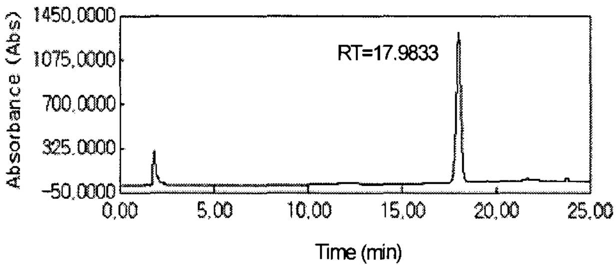

Fig. 3. HPLC chromatogram of ethyl acetate fraction. RT: retention time.

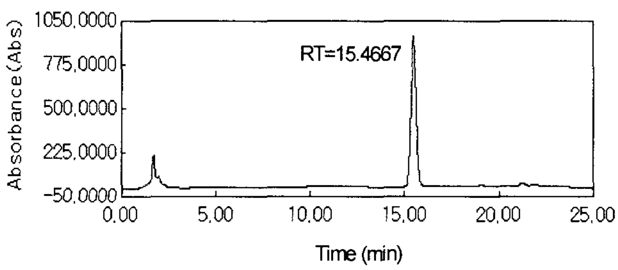

Fig. 4. HPLC chromatogram of aqueous fraction. RT: retention time.

Total antioxidant activity in linoleic acid emulsion

The results for linoleic acid peroxidation after addition of α-tocopherol, diethyl ether, ethyl acetate and aqueous fraction determined by measuring the absorbance at 500 nm were showed in Fig. 6. All of the fractions showed effective antioxidant activity at the concentration of 0.1 mg/ml, which were in the order of diethyl ether, ethyl acetate, and aqueous fraction. Ehtyl-ether fraction exhibited similar strong activity with α-tocopherol and much higher than the other two fractions.

Discussion

In the present study, we focused on natural antioxidants

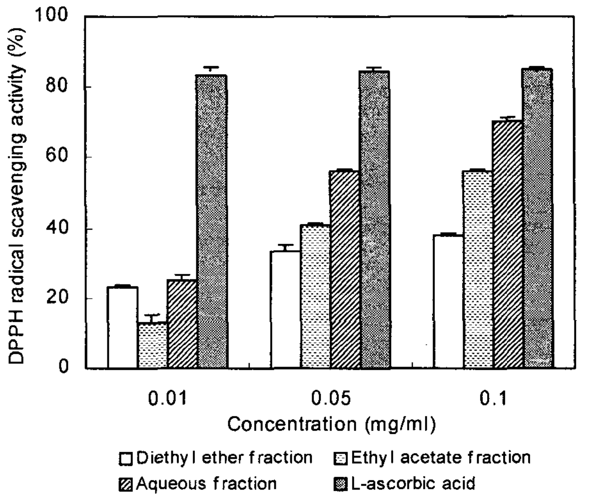

Fig. 5. DPPH radical scavenging activity of *Ulva lactuca* fractions at different concentrations. Results were mean± S.D. of three parallel measurements.

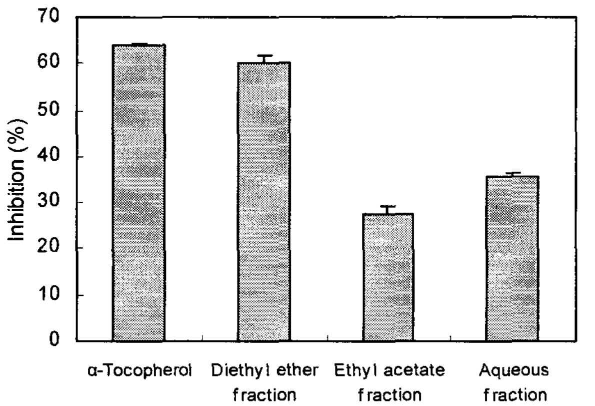

Fig. 6. Lipid peroxidation inhibition activity of the *Ulva lactuca* fractions at the concentration of 0.1 mg/ml. Results were mean±S.D. of three parallel measurements.

from *U. lactuca*, which prepared by fractionation using different organic solvents, and their antioxidant effects were evaluated in two different assays including free radical (DPPH) scavenging assay and lipid peroxidation inhibition assay.

According to the results showed in Fig. 2- Fig. 4, each fraction showed a main peak at different retention time, which were 18.6167 min, 17.9833 min and 15.4667 min, respectively, for diethyl ether, ethyl acetate and aqueous fraction. It meant that each fraction of *U. lactuca* contained different main components. The peak in each chromatogram might stand for one single component. But they also probably consisted of several components which characteristics were quite similar. So, we are trying NMR analysis with these peaks for further identification.

DPPH is a free radical donor, which has been widely used to evaluate the free radical scavenging effect of natural antioxidants[5]. All of the three fractions showed good DPPH radical scavenging activity (Fig. 5). Their activities were concentration-dependent. Among the three fractions, aqueous fraction showed highest scavenging activity which was 70.5%, however, it was lower than L-ascorbic acid at the same concentration. Another antioxidant activity measured in this study showed quite different result. During the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{2+} to Fe^{3+}. Then, Fe^{3+} form a complex with SCN^- and this complex has a maximum absorbance at 500 nm[7]. diethyl ether fraction demonstrated much higher activity than the other two fractions, which was 60%. It was similar with α-tocopherol at the concentration of 0.1 mg/ml. Such results indicated that different fraction of *U. lactuca* has different kinds of antioxidant activities.

In conclusion, *U. lactuca* fractionated with different organic solvents and their HPLC chromatogram and antioxidant activities were different which need to identify in further study. All of the three fractions showed good antioxidant activities. Aqueous fraction showed highest DPPH radical scavenging activity and diethyl ether fraction showed highest lipid peroxidation inhibition activity. These results should assist the development of a new natural antioxidant from *U. lactuca*.

Acknowledgement

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References

1. Brand-Williams, W., M. E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. u.-Technol.* **28**, 25-30.
2. Darcy-Vrillon, B. 1993. Nutritional aspects of the developing use of marine macroalgae for the human food industry. *Int. J. Food Sci. Nutr.* **44**, 23-35.
3. Duh, P. D. 1998. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free radical and active oxygen. *J. Am. Oil Chem. Soc.* **75**, 455-465.
4. Frankel, E. N., J. Kanner, J. B. German, E. Parks and J. E. Kinsella. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **341**, 454-457.
5. Goodwin, J. S. and M. Brodwick. 1995. Diet, aging and cancer. *Clin. Geriatr. Med.* **11**, 577 - 589.
6. Gulcin I., B. Dali and G. Akcahan. 2005. Antiradical and antioxidant activity of total anthocyanins from *Perilla pankinensis* decne. *J. Ethnopharmacol.* **101**, 287-293.
7. Gulcin, I., M. Oktay, E. Kirecci and O. I. Kufrevioglu. 2003. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum L.*) seed extracts. *Food Chem.* **83**, 371 - 382.
8. Heo, S. J., E. J. Park, K. W. Lee and Y. J. Jeon. 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technol.* **96**, 1613-1623
9. Ito, K. and K. Hori. 1989. Seaweed: chemical composition and potential food uses. *Food Rev. Int.* **5**, 101-144.
10. Jacob, R. A. and B. J. Burri. 1996. Oxidative damage and defense. *Am. J. Clin. Nutr.* **63**, 985-990.
11. Lai, L. S., S. T. Chou and W. W. Chao. 2001. Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. *J. Agr. Food Chem.* **49**, 963-968.
12. Mabeau, S. and J. Fleurence. 1993. Seaweed in food products: bio-chemical and nutritional aspects. *Trends Food Sci. Tech.* **4**, 103-107.
13. Youdim, K. and J. A. Joseph. 2001. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radical Bio. Med.* **30**, 583-594.
14. Zheng, W. and S. Y. Wang. 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agr. Food Chem.* **49**, 5165-5170.

초록 : 갈파래(*Ulva lactuca*) 용매별 분획의 항산화활성

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갈파래(*Ulva lactuca*)의 용매별 분획의 항산화활성 측정을 위해 갈파래를 95% ethanol로 추출하고 diethyl ether, ethyl acetate, H_2O 등의 용매로 분획하였다. 각 분획을 HPLC로 분석한 결과 각 분획에서 단일 peak가 검출되었으며 용출시간이 서로 달랐다. 각 분획의 항산화 활성을 DPPH 라디칼 소거능, 지질과산화억제활성 방법으로 검증하였다. 각 분획의 DPPH 라디칼 소거활성은 농도의존성을 나타내었고 활성은 수층, ethyl acetate 층, diethyl ether층 분획의 순서였다. 지질과산화 억제활성은 diethyl ether 분획에서 가장 높게 나타났으며 같은 농도의 α-tocopherol과 비슷하였다. 본 연구 결과는 갈파래를 이용한 천연 항산화제 생산에 응용이 가능할 것으로 생각된다.