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 H2O. 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 Chlorophyllb 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 carcinogenicity[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°C 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.

Dried sample
   Extraction with 95% ethanol
   60°C, 8 h
Ethanol extract
   Evaporation, 40°C
   Dilution with water
Extract in water
   Partition with hexane

Hexane layer
   Aqueous layer
   Partition with diethyl ether / ethyl acetate
Diethyl ether fraction / Ethyl acetate fraction
   Aqueous layer
   Partition with butanol
Butanol layer
   Aqueous fraction

Fig. 1. Fractionation procedure of *Ulex 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°C) 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:

\[ \text{DPPH} \bullet \text{ scavenging effect (\%)} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100 \]

where \( A_0 \) was the absorbance of the control reaction and \( A_t \) 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°C 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:

\[ \text{Inhibition (\%)} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100 \]

where \( A_0 \) was absorbance of the control reaction and \( A_t \) 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-dependent. 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%).

**Total antioxidant activity in linoleic acid emulsion**

The results for linoleic acid peroxidation after addition of $\alpha$-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. Ethyl-ether fraction exhibited similar strong activity with $\alpha$-tocopherol and much higher than the other two fractions.

**Discussion**

In the present study, we focused on natural antioxidants...
from *U. lactua*, 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. lactua* 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 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. lactua* has different kinds of antioxidant activities.

In conclusion, *U. lactua* 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. lactua*.

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References

초록: 갈과래(Ulva lactuca) 응답별 분획의 항산화활성

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갈과래(Ulva lactuca)의 응답별 분획의 항산화활성 측정을 위해 갈과래를 95% ethanol로 추출하고 diethyl ether, ethyl acetate, H2O 등의 용매로 분획하였다. 각 분획을 HPLC로 분석한 결과 각 분획에서 단일 peak가 검출되었으며 용용시간이 서로 달랐다. 각 분획의 항산화 활성을 DPPH 라디칼 소거능, 지질과산화억제활성 방법으로 측정하였다. 각 분획의 DPPH 라디칼 소거능은 농도의존성을 나타내었고 활성은 수증, ethyl acetate 중, diethyl ether중 분획의 순서였다. 지질과산화 억제활성은 diethyl ether 분획에서 가장 높게 나타났으며 같은 농도의 α-tocopherol과 비슷하였다. 본 연구 결과는 갈과래를 이용한 선언 항산화제 생산에 융용이 가능할 것으로 생각된다.