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Physiological and Functional Properties of *Salicornia herbacea* (Tungtungmadi) Leaf Extracts

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Abstract

The physiologically relevant functional properties of various solvent extracts from *Salicornia herbacea* leaves were investigated by measuring lipid peroxidation, DPPH radical scavenging, nitrite scavenging, and xanthine oxidase inhibition. Ethyl ether, chloroform, ethyl acetate and n-butanol fractions obtained from the 80% aqueous ethanol extracts of *Salicornia herbacea* leaves showed strong antioxidative activities in linoleic acid methyl esters. Peroxide values (POV) were not significantly different among the samples treated with the different fractions; the incubation time required to reach a peroxide value of 80 meq/kg was about 40 hrs. However, control linoleic acid methyl esters had POV of more than 480 meq/kg after 40 hrs. The DPPH radical scavenging activity of the ethyl acetate fraction was much more effective than diethyl ether, n-butanol, chloroform and water fractions, with an IC_{50} of 279 μg/mL, but less effective than ascorbic acid (IC_{50} = 67 μg/mL). The nitrite scavenging activities of all fractions increased as pH decreased. Among the fractions, nitrite scavenging activities of diethyl ether and ethyl acetate fractions at pH 1.2 were highest at 59.0 and 56.2%, respectively. The diethyl ether fraction obtained from the 80% aqueous ethanol extract of *Salicornia herbacea* leaves was the most effective inhibitor of xanthine oxidase of all the solvent extracts at 84% inhibition for a 1 mg/mL concentration. These results suggest that *Salicornia herbacea* leaf extracts may be effective antioxidants, not only in food stability, but also in human health.

Key words: *Salicornia herbacea*, antioxidative activity

INTRODUCTION

Recently, *Salicornia herbacea* (Tungtungmadi, a member of the glassworts) has been used as food and medicine in Korea. It is one of the most salt tolerant plant species along the western coast of Korea, and is an annual succulent shrub growing in the coastal wetlands. *Salicornia herbacea* has adapted to salt environments and/or is resistant to salinity stress, and exhibits maximum growth between 100 and 300 mM NaCl (1). Felix et al. (2) reported that H^+-ATPases in *Salicornia bigelovii* are important in salt tolerance and provide a biochemical framework for understanding mechanisms of salt tolerance in plants. Joshi (3) reported that there are seasonal changes in free amino acids and mineral constituents in halophytes. Studies on the nutritional values of *Salicornia herbacea* have focused its contents of protein, salt, amino acids and minerals (4). However, no studies have investigated the functional properties of *Salicornia herbacea*.

In the present study, we investigated the lipid peroxidation, DPPH radical scavenging, nitrite scavenging, and xanthine oxidase inhibition of organic solvents extracts obtained from the 80% aqueous ethanol extract of *Salicornia herbacea* leaves.

MATERIALS AND METHODS

Materials

Salicornia herbacea leaves were purchased from the Ham-Cho market in Haenam, Jeollanam-Do. The sun-dried leaves (120 g) were ground, passed through a 10 mesh screen, and soaked in cooled water for 20 min to remove salt. The precipitates were collected by centrifuge (1,000 × g, 20 min) oven dried at 60°C (82 g), and extracted with 80% aqueous ethanol.

Sample preparation

Extracts were prepared by placing 82 g of dried leaves in 1 L of 80% aqueous ethanol at room temperature for 48 hrs; the extract was further fractionated as schematically outlined in Fig. 1.

Measurement of peroxide value

Inhibition of lipid peroxidation by each fraction was measured by the method of Ando et al. (5), using linoleic

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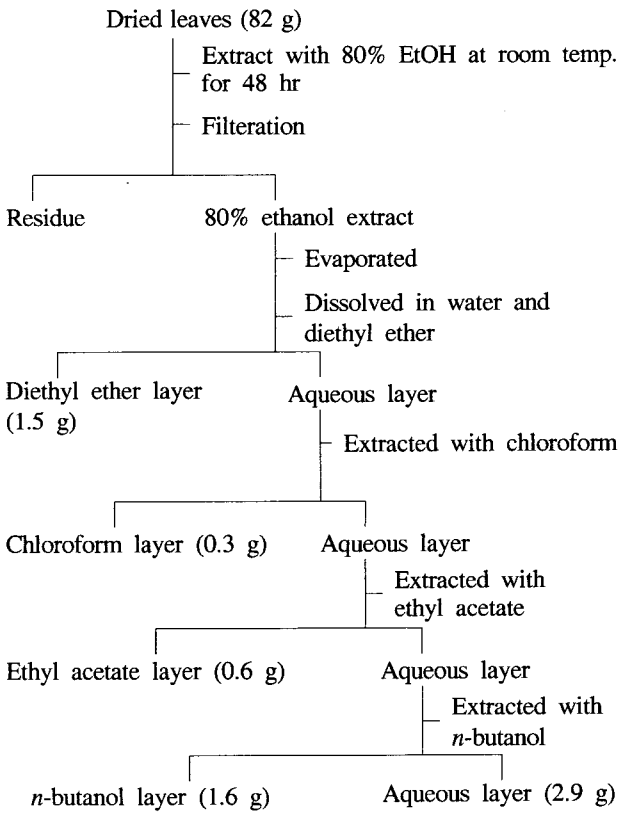

Fig. 1. Flow diagram for the extraction and fractionation of *Salicornia herbacea* leaves.

acid methyl ester as the substrate. The reaction mixtures were composed of 100 μL of linoleic acid methyl ester and 50 μL of sample. The mixtures were incubated at 50°C in capped tubes, and then 35 mL of chloroform-acetic acid (2 : 3, v/v) and saturated potassium iodide solution (1 mL) were added to each tube. 75 mL of distilled water and 1 mL of a 1% starch solution were added to all of the tubes and POV determined, and then 0.01 N disodiumsulfoxide solution was added to each tube.

Measurement of DPPH radical scavenging activity

The DPPH radical scavenging activity of each fraction was measured according to the α, α'-diphenyl-β-pycryl-hydrazyl (DPPH) method (6). Various concentrations of each fraction and L-ascorbic acid (0.2 mL) were added to the 4×10^{-4} M DPPH solutions (0.8 mL), and mixed thoroughly for 5 sec. After 10 min of incubation at room temperature, absorbance was measured at 525 nm. The antioxidant activity of each fraction was expressed in terms of the IC_{50} (concentration in micrograms per milliliter required to inhibit DPPH radical formation by 50%) calculated from the log-dose inhibition curve.

Measurement of nitrite scavenging activity

According to the method described by Kato et al. (7) using Griess reagent, 10% of the fraction was used to determine the nitrite scavenging activity under different conditions (pH 1.2, 4.2 and 6.0) by measuring the absorbance at 520 nm. The nitrite scavenging activity (%) was calculated using the following equation:

$$\text{Nitrite scavenging activity (\%)} = (1 - \frac{\text{Abs}}{\text{Abc}}) \times 100$$

Measurement of xathine oxidase inhibition activity

Xanthine oxidase inhibitory activity of each fraction was tested spectrophotometrically with xanthine as the substrate by the method of Noro et al. (8) and was calculated by the following equation; where A and B are the activities of the enzyme with and without the test material, respectively.

$$\text{Xanthine oxidase inhibitory activity (\%)} = (1 - \frac{B}{A}) \times 100$$

RESULTS AND DISCUSSION

Lipid peroxidation

Inhibition activities against lipid peroxide are shown in Fig. 2. Lipid peroxidation causes oxidative damage not only in food systems, but also in the human body. Since some synthetic antioxidants, such as butylated hydroxyanisole (BHA), have been found to be toxic to experimental animals (9), natural plant products have received much attention as sources of safe antioxidants (10). Recently, an intensive search for novel natural antioxidants from numerous plant materials has been undertaken including those used as foods (11,12). Therefore, in the present study, natural plant extracts were tested for antioxidative activity using a lipid peroxidation system.

All the fractions obtained from the 80% aqueous ethanol extract of *Salicornia herbacea* had strong antioxidative activity in linoleic acid methyl esters, and there no significant

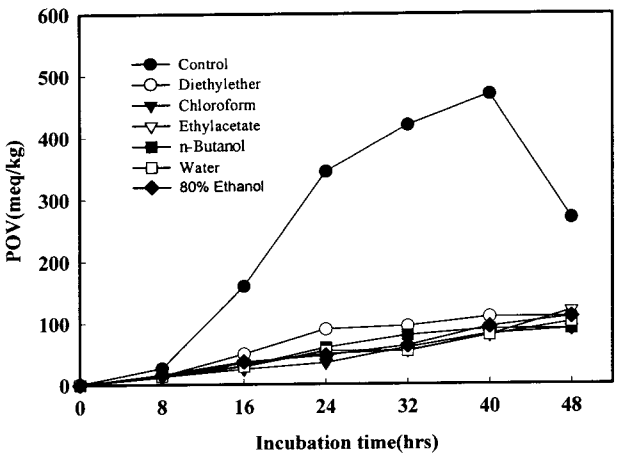

Fig. 2. Antioxidative effects of each fraction extracted from *Salicornia herbacea* leaves by peroxide values.

differences in peroxide values (POV) among fractions. The incubation time to reach a peroxide value of 80 meq/kg in each treated fraction was about 40 hrs, however, the controls had a high value of more than 480 meq/kg after 40 hrs. These results demonstrate that solvent fractions obtained from *Salicornia herbasea* leaves can be highly efficacious antioxidants.

DPPH radical scavenging activity

In the DPPH free radical method, antioxidant efficiency is measured at ambient temperature because it is stable in ethanolic solution for more than 60 min and can be used to evaluate antioxidative activities in a relatively short time. Antioxidants react with the DPPH free radicals directly and restore them by the transfer of electrons or hydrogen (13). Therefore, we used this system for assessing the radical scavenging ability of *Salicornia herbacea* leaf extracts.

Free radical scavenging activities of diethyl ether, chloroform, ethyl acetate and n-butanol fractions on 4×10^{-4} M DPPH were measured and compared with L-ascorbic acid (Table 1).

The ethyl acetate fraction was much more effective than the diethyl ether, n-butanol, chloroform and water fractions, at an IC_{50} of 279 μg/mL, but less effective than ascorbic acid (IC_{50} = 67 μg/mL).

Nitrite-scavenging activity

Nitrite is an important food additives, from both an economical as well as technical standpoint. It protects foods against the risk from outgrowth and toxin production by *Clostridium botulinum* (14). Addition of nitrite also stabilizes desirable flavors and colors of meats and fish (15, 16). However, the use of nitrite in food results in the occurrence of very low levels of nitrosamine, which at higher levels have been shown to be carcinogenic in laboratory animals (17). The formation of nitrosoamine results from interaction of components in foods, and can also be formed in the human stomach through the reaction of amines with nitrite (18). Therefore, we investigated the nitrite-scavenging potential of *Salicornia herbacea* leaf extracts.

As shown in Table 2, the nitrite scavenging activities of diethy ether, chlorofrm, ethyl acetate and n-butanol fractions increased as pH decreased. Among the fractions, nitrite scavenging activities of diethyl ether and ethyl acetate fractions at pH 1.2 were most effective at 59.0 and 56.2%, respectively. These results were similar to those for *Glechoma hederacea* (19), Bamboo leaves (20), and various other Korean medicinal plants (21).

These results demonstrate that the diethyl ether and ethyl acetate fractions of *Salicornia herbacea* extracts have potent nitrite scavenging activities and are potentially useful antioxidants in processed foods, and may have similar activity nitrosation inhibition in the stomach.

Xanthine oxidase inhibitory activity

Xanthine oxidase catalyzes the oxidation of hypoxanthine or xanthine to form uric acid and superoxide anion in the presence of molecular oxygen, and is an important mediator of purine metabolism. The increase of urate in blood can cause gout and kidney stones (22). Hayashi et al. (23) reported that different plant flavonoids have different inhibitory activities on xanthine oxidase because of their different positions of hydroxyl groups.

Fig. 3 shows inhibitory activities on xanthine oxidase of diethy ether, chloroform, ethyl acetate and n-butanol fractions obtained from *Salicornia herbacea* leaf extract. The diethyl ether fraction was the most effective inhibitor of xanthine oxidase with 84% inhibition at 1 mg/mL con-

Table 1. DPPH (4×10^{-4} M) radical scavenging concentration of each fraction obtained from *Salicornia herbacea* leaves

Fractions	IC_{50} (μg/mL)
80% Ethanol	649±54
Diethyl ether	501±42
Chloroform	804±34
Ethyl acetate	279±57
n-Buthanol	559±42
Water	1,798±67
Ascorbic acid	67±18

Table 2. Nitrite scavenging activity of each fraction obtained from *Salicornia herbacea* leaves under different pH conditions (%)

Fractions	pH		
	1.2	4.2	6.0
80% Ethanol	39.7±5.4	19.2±3.7	12.8±4.0
Diethyl ether	59.0±5.2	21.6±2.7	7.7±3.1
Chloroform	38.5±3.7	18.4±3.5	7.0±3.4
Ethyl acetate	56.2±5.0	22.2±3.5	6.2±2.6
n-Butanol	43.5±6.1	17.7±4.1	10.3±3.4
Water	36.4±6.9	10.3±3.0	8.6±2.5

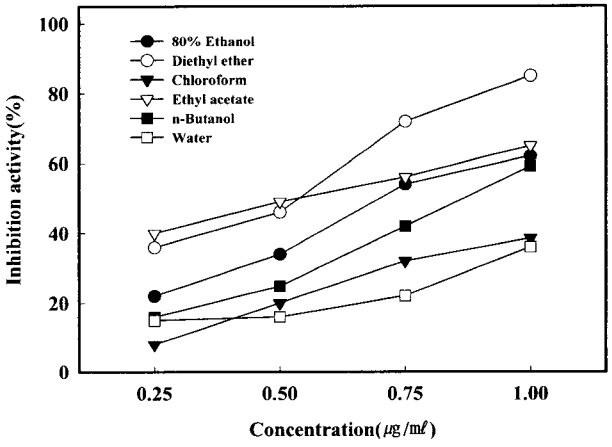

Fig. 3. Changes of xanthine oxidase inhibition activity of each fraction extracted from *Salicornia herbacea* leaves.

centration, whereas ethyl acetate and n-butanol fractions had 62 and 59% inhibitions, respectively. The inhibitory activity of the diethyl ether fraction was similar to that of the diethyl ether fraction from a methanol extract of *E. cava* (24). We conclude, therefore, that the diethyl ether fraction of *Salicornia herbacea* leaves is an effective inhibitor of xanthine oxidase.

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