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Research Paper

Verification of the antioxidant activity of a subterranean part of *Suaeda japonica* Makino

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ABSTRACT

We report the biological activity of the ethanolic extractions of *Suaeda japonica* Makino. The layer fraction contains hexane, chloroform, ethyl acetate, butyl alcohol, and H_2O and dried the samples. The antioxidant and reducing potential activity as well as the polyphenolic, tannin and flavonoid compounds extracted from *Suaeda japonica* Makino were evaluated by different assays. The subterranean parts was used for these standardized assays. The maximum antioxidant activity was observed in ethyl acetate. The total phenolic and total flavonoid content were highest with ethyl acetate and the butyl alcohol fraction. High reducing potential was observed in the ethyl acetate fraction. A positive correlation was observed between the antioxidant activity and polyphenolic compounds (total phenolic content and total flavonoid content). Similarly, there was significant correlation between antioxidant activities and reducing potential indicating that the reducers present in the ethyl acetate fraction are major contributors to the antioxidant potential. Thus, the ethyl acetate fraction of this plant could be used for pharmaceutical and functional materials applications.

1. Introduction

Oxygen is needed for energy conversion and metabolism; it can also remove antioxidants. Active oxygen is present in the form of a free radical including superoxide radical (O_2^-) and the hydroxy radical (HO·). This active oxygen is unstable; its reactivity is stronger than intracellular proteins or lipids. It can induce a variety of diseases that inflict serious damage to cells and DNA (Chang et al., 1997).

Antioxidants are found in plants and are bioactive substances. They are widely used in cosmetics, medicine, and food. Medicinal plants can have greater meaning in terms of the development of functional foods (Kim et al., 1995; Choi et al., 1992).

Antioxidant substances are widely distributed in the animal and plant kingdom. Fruits and vegetables contain many phenolic compounds, flavone derivatives, tocopherol acids, selenium, vitamin C, carotenoids natural non-enzymatic antioxidant and amino acids such as glutathione. Some antioxidant substances form metal complexes. The main feature is primarily represented by the antioxidative activity to delay or prevent lipid oxidation as well as the prevention and delay of cancer, cardiovascular diseases. They also have anti-aging properties (Bors and Saran, 1987).

These antioxidants are used by free radical reactions and do not remove oxygen or minimize the loss of the oxygen absorption. Vitamins and essential amino acid can protect the body (Lee et al., 2005). Foods with vitamins, minerals, and polyphenols have antioxidant activity. (Kang et al., 1995; Yoon et al., 1990; Lee and Lee, 1990).

However, the antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have a high antioxidant capacity and low price. They can cause serious illness with excessive intake (Seog et al., 2002). This causes toxicity to the human body. On the other hand, α-tocopherol is a natural antioxidant. Ascorbic acid is an affordable antioxidant. It is safer and more effective (Sa et al., 2004).

Suaeda japonica Makino is a member of the family Chenopodiaceae and is an annual succulent herb and one of the most salt-tolerant plant species seen abundantly on muddy seashores along the western coast of Korea, Europe, Japan and Iran (Han et al., 2003). It is grown in arid areas and lowland flooded areas. It is a flame retardant in these habitats, and it can be grown in flooded conditions for long periods of time. It has been used in traditional medicines, and most current research is on growth characteristics and environmental factors. Recently, *S. japonica* has been used as a functional food and a medicinal plant due to

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its beneficial effects including α-glusidase inhibition and antioxidant/anti-diabetic activities (Huang et al., 2011; Kunkel, 1984). However, additional verification of these observations is needed.

2. Material and methods

2.1. Chemicals required

We used the following agents: ascorbic acid, 1,1-diphenyl, 2-picryl hydrazyl (DPPH), gallic acid, vanillin, (+)-catechin, sulfuric acid, thiobarbituric acid (TBA), rutin trihydrate, sodium dodecyl sulphate (SDS), trichloroacetic acid (TCA), 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH), sulfuric acid (H_2SO_4), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, fluorescein, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), potassium ferricyanide ($K_3[Fe(CN)_6]$), ferrous sulphate ($FeSO_4$) and ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), copper(II) chloride ($CuCl_2$), iron(II) chloride ($FeCl_2$), ethanolic neocuproine, Folin–Ciocalteuphenol reagent, 2,2′-bipyridyl, ethylene-diamine-tetra-acetic acid (EDTA), ammonium acetate, and dimethyl- sulfoxide. All chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA. We also purchased these items: potassium persulfate (Junsei, Japan), HPLC grade methanol, and ethanol (J.T Baker, U.S.A).

2.2. Material collection and preparation of extracts

The subterranean part of *Suaeda japonica* Makino (SJSP) were collected from the neighboring Masan-ri 809-22, Byeollyang-myeon, Suncheon-si, Jeollanam-do, Korea, from June to October. The subterranean parts were washed under running tap water to remove dust and mud and extracted (3 kg dry weight in 3.0 L of H_2O). This was successively partitioned with *n*-hexane (1.5 L, 3 times), chloroform ($CHCl_3$, 1.5 L, 3 times), ethyl acetate (EtOAc, 1.5 L, 3 times), and water-saturated *n*-butanol (BuOH, 1.5 L, 3 times). Each layer was centrifuged, filtered, and concentrated with freeze-drying. Samples were stored in the refrigerator before use. Samples were diluted to various concentrations for further experiments (Fig. 1).

2.3. Antioxidant activity

2.3.1. Condensed tannin content

Catechins and pro-anthocyanidins reactive to vanillin were analyzed by the vanillin method of Richard and William (1978) with slight modification. Each extract and fraction (1 mL) was placed in a test tube. This was followed with 2 mL of vanillin (1% in 7 M H_2SO_4) in an ice bath and incubated at 25 °C for 15 min. The absorbance of the solution was read at 500 nm. Concentrations were calculated as g catechin equivalents (CE)/kg dry mass from a calibration curve. The tannin concentration was expressed as mg CE/g.

2.3.2. Total flavonoid content

The total flavonoid content method of Thomas et al. (2012) was used with slight modifications. Distilled water (100 μL) was added to each of the 96 wells followed by 10 μL of 50 g/L $NaNO_2$ and 25 μL of standard or sample solution. After 5 min, 15 μL of 100 g/L $AlCl_3$ was added to the mixture; 6 min later, 50 μL of 1 M NaOH and 50 μL of distilled water were added. The plate was shaken for 30 s in the plate reader prior to absorbance measurements at 510 nm. Quercetin was used as a standard at 1–500 μg/mL to generate a calibration curve. The total flavonoid concentration was expressed as mg QE/g.

2.3.3. Total phenolic content

The total phenolic content method of Thomas et al. (2012) and Zhang et al. (2006) was used with slight modifications. To each of the 96 wells, 75 μL of distilled water was added followed by 25 μL of either sample or standard and 25 μL of F–C reagent (diluted 1:1 (v/v) with distilled water). All reagents other than samples and standard were delivered through a repeating pipette. After solutions were mixed and incubated for 6 min. Then, 100 μL of 75 g/L Na_2CO_3 was added to each well. The solutions were mixed again, and the plates were covered and left in the dark for 90 min after which the absorbance at 765 nm was read. Each standard and sample solution were analyzed in triplicate and assayed against a sample control (i.e. sample solution without F–C reagent and Na_2CO_3). Gallic acid was used as a standard at 1–500 μg/mL to produce a calibration curve. The total phenolic concentration was expressed as mg GAE/g.

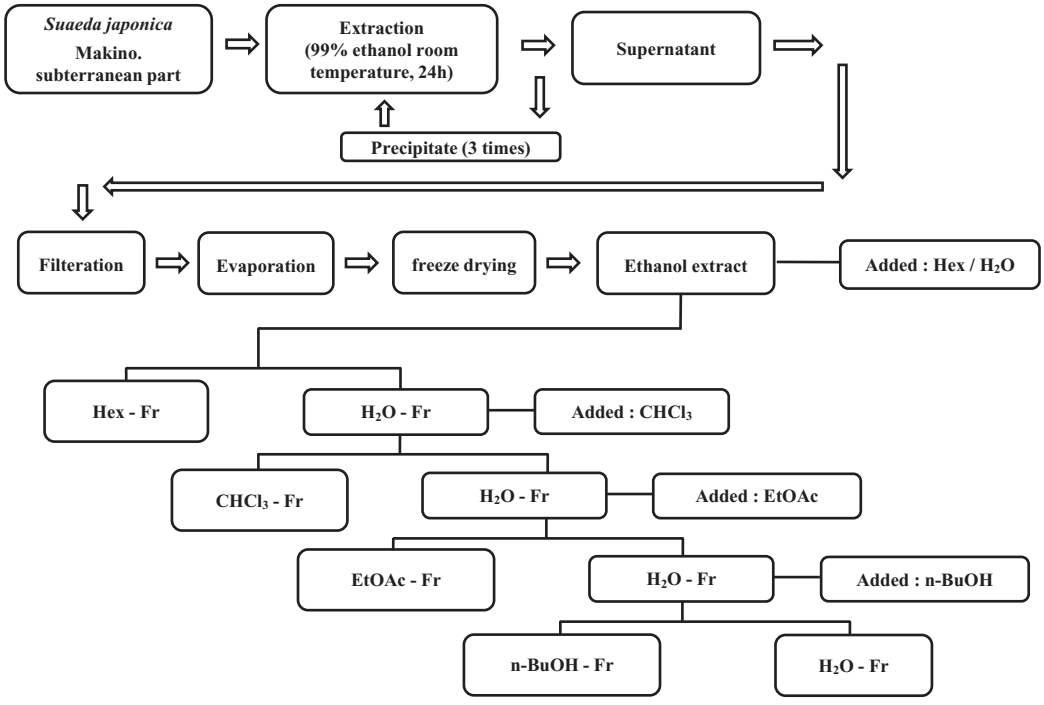

Fig. 1. The procedure of ethanol extract and fractions from *Suaeda japonica* subterranean part (SJSP).