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Antioxidant Activities of Fractions from *Sedum sarmentosum*

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Abstract

This study was conducted to evaluate the antioxidant activity of each fraction from *Sedum sarmentosum*. Antioxidant activity of each fraction was measured using the DPPH radical assay, the ferric thiocyanate (FTC) method, and thiobarbituric acid (TBA) method. The antioxidant activities were then compared with that of BHT (synthetic antioxidant). The ethyl acetate and butanol fractions were found to have significant DPPH radical scavenging activity, with scavenging potencies showing 90.61% and 87.02%, respectively. Total phenolic compound contents, determined according to the Folin-Denis method, were found to be in the order of ethyl acetate>butanol>ethanol>chloroform>aqueous fraction. From the results, we have been able to establish a positive correlation between the antioxidant activity and the total phenolic compound content of the sample. The antioxidant activity in a linoleic acid system was measured using the ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) method. The ethyl acetate fraction had the highest antioxidant activity among the tested fractions. On the basis of these results, the ethyl acetate fraction provided equivalent or higher antioxidant activity as compared to BHT. These results suggest that *Sedum sarmentosum* is a potentially useful antioxidant for foods, cosmetics, and medicine.

Key words: antioxidant activity, *Sedum sarmentosum*, fraction

INTRODUCTION

Antioxidants have a well defined role as preservatives because they neutralize free radicals by donating one of their own electrons, ending the electron-stealing reaction. These have been defined by the US Food and Drug Administration (FDA) as substances used to preserve food by retarding deterioration, rancidity or discoloration caused by oxidation (1). In the past synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), *tert*-butylhydroquinone (TBHQ), propyl gallate (PG) were widely used because of their strong antioxidant effects. In recent years, however, the use of some synthetic antioxidants have been restricted because of their possible toxic and carcinogenic effects (2-5). Thus, the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (6,7). Also, phytochemicals in fruits and vegetables have recently attracted a great deal of attention for their role in preventing diseases resulting from oxidative stress. Oxidative stress, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other auto-immune diseases besides ageing (8). Demonstrated antioxidant protection by phytochemicals contained in fruits and vegetables include prevention of cardiovascular diseases by sulfoxides and/or flavonoids from Alliums (9) and anticancer effects of glucosinolates and their derivatives (10) from Brassicas (11).

Antioxidant effects of fruits and vegetables can be mediated by phenolic compounds such as flavonoids, and phenolic acids or nitrogen compounds, such as alkaloids, chlorophyll derivatives, amino acids and amines. These flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (12-14). Antioxidant activity of a phenolic compound is correlated to structure-activity relationships, such as redox properties and the number and arrangement of the hydroxyl groups (15).

Recently, consumers are conscious of not only nutritional value of food, but also the safety of food ingredients. At the same time, there is a preference for natural food and food ingredients that are believed to be safer, healthier, and less subject to contamination than their artificial counterparts. Therefore, it is interesting and worthwhile to investigate and identify natural antioxidants from edible plants, even though they may not be comparable, in efficiency, to synthetic agents (16).

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It has been reported that *Sedum sarmentosum* has been used in the treatment of viral hepatitis in Asian countries (17). It also possesses an angiotension-converting enzyme inhibitory flavonoid (18) and antiproliferative activity on murine and human hepatoma cell lines (19). However, little research has been performed on antioxidative activity of *Sedum sarmentosum*.

The aim of the present study was to investigate the antioxidant properties of *Sedum sarmentosum* for further application in food industries as a natural antioxidant. Therefore, it is worthwhile to conduct a systematic fractionation and investigation antioxidant activities of each fraction.

MATERIALS AND METHODS

Material

Sedum sarmentosum was collected from a local market in Busan, Korea. The 1,1-diphenyl-2-picryhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, ammonium thiocyanate, and trichloroaceteic acid (TCA) were purchased from Sigma Chemical Co., Ltd. Ethanol, chloroform, ethyl acetate, 1-butanol, 2, 6-di-*tert*-butyl-4-methylphenol (BHT), and thiobarbituric acid (TBA) were purchased from Junsei Chemical Co. Sodium carbonate was from Tedia Company Co., ferrous chloride was from ICN Biomedicals, Inc., and linoleic acid was from Hayashi Pure Chemical Inc. All other chemicals used were of analytical grade.

Extraction and fraction procedures

Sedum sarmentosum was lyophilized (Bondiro freeze dryer, Ilshin Lab Co., Ltd.) for 2 days and then, ground into a fine powder for further use. The lyophilized *Sedum sarmentosum* was extracted three times with absolute ethanol at 59°C for 3 hrs and then, filtered. The resultant extract was combined and concentrated under reduced pressure to afford the residue. The ethanol extract was suspended in water and then, extracted successively with equal volumes of ethyl acetate, chloroform and *n*-butanol. Each fraction was evaporated in vacuo to obtain ethyl acetate, chloroform and *n*-butanol fractions, respectively. The extraction yields of *Sedum sarmentosum* with various solutions are presented in Table 1.

DPPH radical assay

The antioxidant activities of *Sedum sarmentosum* extracts and BHT were measured for their hydrogen donation and radical scavenging activities, using the stable radical DPPH. The DPPH-scavenging activity was performed as previously described (20) with some modifications. DPPH was dissolved in ethanol, and the experiments were performed on freshly prepared solutions.

Table 1. Yield of fractions from *Sedum sarmentosum*

Fraction	Dry weight, g	Yield, % (w/w)
Ethanol	30.28	62.11
Chloroform	2.83	5.81
Ethyl acetate	0.17	0.35
Butanol	7.38	15.19
Aqueous	6.19	12.70

$$\text{Yield } (\%) = \frac{\text{Dry weight of extracted fraction}}{\text{Dry weight of total fraction}} \times 100$$

In this assay, reaction mixture containing 0.1 mL sample was added to 2.9 mL of a DPPH solution and then, the mixture was shaken and left to stand for 10 min. Decolorisation of DPPH-donated protons was determined by measuring the absorbance at 525 nm with a spectrophotometer (Ultrospec 3000, Pharmacia Biotech).

The scavenging activity of DPPH radical was calculated using the following equation and shown as electron-donating activity (EDA):

$$\text{EDA } (\%) = \{1 - (\text{Absorbance at 525 nm in the presence of a sample/Absorbance at 525 nm in the absence of a sample})\} \times 100$$

Total phenolic compound index: Folin-Denis method

Total phenolic compounds were analyzed by the Folin-Denis method (21), using gallic acid as the standard. The assay conditions were as follows: 0.5 mL sample was added to 2.5 mL Folin-Ciocalteau reagent. After 5 min, 2 mL of 7.5% aqueous sodium carbonate solution was added to the mixture and then, incubated at 50°C for 5 min. Absorbance of the resulting mixture was measured at 760 nm. The content of total phenolic compounds was calculated from a standard curve with gallic acid the standard. Therefore, results are given as milligrams per mililiter of gallic acid equivalents (GAE).

Ferric thiocyanate (FTC) method

The FTC method was adapted from the method of Osawa and Namiki (22) with some modification. The 4 mg or 4 mL sample dissolved in 4 mL of 99.5% (w/v) ethanol was mixed with linoleic acid (2.51%, v/v) in 99.5% (w/v) ethanol (4.1 mL), 0.05 M phosphate buffer pH 7.0 (8 mL) and distilled water (3.9 mL) and kept in a screw-cap container in the dark at 40°C. Then, 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% ammonium thiocyanate were added to 0.1 mL of this solution. After precisely 3 min from the addition of 0.1 mL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance of the resulting red color was measured at 500 nm every 24 hr until the ab-

sorbance of the control reached maximum.

Thiobarbituric acid (TBA) method

The TBA test was conducted according to the method of Iwai et al. (23) with some modification. One milliliter sample prepared as described in FTC method was added to 35% trichloroacetic acid (0.5 mL) and 0.75 % thiobabituric acid solution (1.0 mL). This mixture was then placed in a boiling water bath at 100°C for 15 min and 70% trichloroacetic acid (1.0 mL) was added to the mixture. After cooling for 20 min, it was centrifuged at 2000×*g* for 15 min and the absorbance of the supernatant was then measured at 532 nm.

RESULTS AND DISCUSSION

DPPH radical-scavenging activity

DPPH radical-scavenging activity of each fraction from *Sedum sarmentosum* is shown in Table 2, showing activities in the order of ethyl acetate > butanol > ethanol > aqueous > chloroform fraction. Ethyl acetate fraction exhibited the highest antioxidant activity of 90.61%, which is a similar result as that of spinach (90.64%) (24). Xiao-Dong et al. (25) also reported that ethyl acetate fraction of *Chrysophyllum cainito* L. exhibited the highest antioxidant activity in DPPH assay.

Content of phenolic compounds

The contents of total phenolic compounds of *Sedum sarmentosum* are presented in Table 3. The obtained results showed that the ethyl acetate fraction had a total phenolic content, determined by Folin-Denis method and expressed as gallic acid equivalents, equal to 4921.56 μg/mL. Total phenolic compound content was in the following order: ethyl acetate > butanol > ethanol > chloroform > aqueous fraction. The results of DPPH radical assay and Folin-Denis test indicate strong association between antioxidant activity and phenolic compound, suggesting that phenolic compounds are probably responsible for the antioxidant activity of *Sedum sarmentosum*. Many of the previous studies conducted with vegetables or fruits have also found a positive correlation between total phenolic compound and the antioxidant activity, so high total phenols contents increase antioxidant activity (8,26,27).

Table 2. DPPH radical-scavenging effect of fractions from *Sedum sarmentosum* (Final concentration: 1 mg/mL)

Fraction	EDA[1)] (%)
Ethanol	68.30
Chloroform	1.57
Ethyl acetate	90.61
Butanol	87.02
Aqueous	6.78
BHT	81.79

[1)]Electron-donating ability.

Table 3. Total phenolic compound content of fractions from *Sedum sarmentosum* (GAE[1)], μg/mL)

Fraction	Total phenolic compound content
Ethanol	785.25
Chloroform	341.94
Ethyl acetate	4921.66
Butanol	900.46
Aqueous	268.20

[1)]Values are expressed as μg/mL gallic acid equivalents (GAE).

FTC method

To evaluate the antioxidant potential of each fraction from *Sedum sarmentosum*, their inhibitory activities against lipid peroxidation were compared with the selected standard antioxidant, BHT, by the ferric thiocyanate method. In the FTC method, the peroxides formed in emulsion during the initial stage of lipid oxidation were measured, and thus, high absorbance is an indication of a high concentration of the formed peroxides. Fig. 1 shows the antioxidant activity of each fraction, measured by the FTC method. The absorbance of linoleic acid emulsion without the addition of the added fractions or BHT increased rapidly. As can be seen in Fig. 1, the ethyl acetate, butanol, and aqueous fractions and BHT were able to reduce the formation of the peroxides in linoleic acid emulsion.

TBA method

During the oxidation process of linoleic acid emulsion, peroxides are gradually decomposed to lower molecular weight compounds. Such a compound is malonaldehyde which can be measured by the TBA method. Table 4

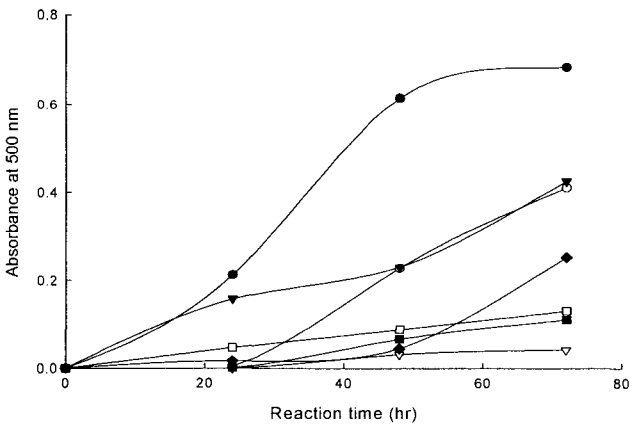

Fig. 1. Antioxidant activity of each fraction from *Sedum sarmentosum* and BHT as measured by the FTC method. The concentration of fractions used was 250 μg/mL.
(●), control; (○), ethanol fraction; (▼), chloroform fraction; (▽), ethyl acetate fraction; (■), butanol fraction; (□), aqueous fraction; (◆), BHT.

Table 4. Antioxidant activity of fractions from *Sedum sarmentosum* measured by TBA method

Fraction	Absorbance at 532 nm
Control	1.76
Ethanol	0.21
Chloroform	0.41
Ethyl acetate	0.19
Butanol	0.18
Aqueous	0.23
BHT	0.17

The concentration of the fractions used was 250 μg/mL.

shows the antioxidant activities of each fraction obtained from *Sedum sarmentosum*, measured by TBA. The results obtained were similar to that of the FTC method. The antioxidant activities of ethyl acetate and butanol fractions determined by FTC and TBA methods were not significantly different from that of BHT. Phenolic compounds are believed to be the major antioxidant phytochemicals in plants (12-14). Since ethyl acetate and butanol fractions contained higher phenolic compounds than others, as shown in Table 3, both fractions exhibited higher antioxidant activities. Therefore, *Sedum sarmentosum* could be utilized as a potential natural additive in foods, cosmetics and medical application. Further research is needed to identify the chemical structure responsible for the antioxidant activity in *Sedum sarmentosum.*

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