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Biofunctional Activities of *Sanguisorbae officinalis* L. Leaves Ethanol Extract

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ABSTRACT: This study was conducted to investigate the antioxidant activity and the bioactive compounds found in 70% ethanol extracts taken from *Sanguisorbae officinalis* L. leaves(SO) cultivated in Korea. The extracts were tested for their total phenolic contents (TPC), total flavonoid contents (TFC), phenolic compounds, and antioxidative activities using various *in vitro* assay such as DPPH, ABTS radical scavenging activity, FRAP activity, and reducing power. The TPC and TFC were found to be 119.3±1.54 mg gallic acid and 59.6±1.43 mg rutin at mg of 70% ethanol extracts, respectively. Catechin was the major material among the phenolic compounds in SO extracts. The DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP activity, and reducing power of SO extracts were increased in a dose-dependent manner. These results suggest that SO extracts could be considered as a good source of natural antioxidants and functional food ingredient.

Keywords: *Sanguisorbae officinalis* L. leaves, antioxidant activities, radical scavenging activity, total phenolic content

INTRODUCTION

Reactive oxygen species (ROS), including superoxide anion($\cdot O^{2-}$), hydroxyl radical ($\cdot$ HO), hydrogen peroxide (H_2O_2), peroxyl radical (ROO·), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$), can be generated from unbalance pro-oxidant and antioxidant enzyme response systems (Buesal E and Gülçin İ 2011). Furthermore, different environmental stress factors such as pollution, drought, temperature, excessive light intensities and nutritional limitation increase the production of ROS (Ehling-Schulz M and Scherer S 1999). To inhibit the physiological damages caused by excess ROS, a wide array of enzymatic and non-enzymatic endogenous antioxidant defense system have been evolved to compensate the generation of ROS (Fridovich I 1997).

Typical natural antioxidants include tocopherols, carotenoids, falvonoids, and polyphenolic compounds (Amro B et al 2002) l that can potentially provide protection against the development of certain oxidation-linked chronic diseases (Škerget M et al 2005). Regular consumption of bioactive compounds from plant and fruit may associated with protecting oxidative damage and lowering the risk factor of chronic diseases such as cancer, heart disease, and cerebrovascular disease (Block G et al 1992). Moreover, several recently reported that the phytochemicals including phenolic compounds have gastroprotective effects due to their antioxidant properties (Martin MJ et al 1998).

Sanguisorbae officinalis L. is a perennial herb whi-

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ch belong to Rosaceae and that is usually found in mountain and fields over the Korea. Generally, the rhizomes of *Sanguisorbae officinalis* L. grow lengthy sideways and its stem grows straight, some of which reach 1.5 meters tall. The dark red flowers bloom on a long stalk like spikelets one by one between July and September. The length of the flower is about 2.5 cm, and there are four sepals and four stamens. The quadrangle fruits are winged achene that ripen in October. It is called different names from region to region. If you cut its leaves, it smells like a refreshing cucumber, so we call it "O I Pul" (O'Reilly M et al 1999). Sanguisorbae radix, the dried root of *Sanguisorbae officinalis* L., it roots have hemostatic, analgesic, and astringent properties, and have been used in traditional Chinese medicine for the treatment of burns, scalds, inflammation and internal haemorrhage (East J 1955). They control bloody pus, treat various boils, supplement damaged tissue, relieve alcohol poisoning, quench one's thirst, and brighten one's eyes (Park JH et al 1997). So far, saponin components such as triterpense and their glycoside have been reported as major active compounds principles that possess these in vitro and in vivo pharmacological effects (Park KH et al 2004). Although numerous activities of this plant have been reported and published so far, and molecular immunopharmacological mechanisms of extracts are studied by Yu T et al(2011). Many reports explicitly explain the molecular immunopharmacological mechanisms that underlie the anti-inflammatory properties of *Sanguisorbae officinalis*, there are no reports documenting radical radical scavenging-linked antioxidant activity of *Sanguisorbae officinalis* cultivated in Korea.

The objective of this study was to investigate whether the total phenolic, total flavonoids, DPPH (2,2-diphenyl-1-pic-rylhydrazy) radical scavenging activity, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay, ferric reducing ability of plasma (FRAP) assay, reducing power assay, major phenolic compounds of *Sanguisorbae officinalis* L. cultivated in Korea were evaluated by various *in vitro* assay and by HPLC with photodiode array detector. Additionally, we examined the correlation between antioxidant activity and phenolic compounds.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu reagent, gallic acid, rutin, catechin, vitamin C were obtained from Sigma-Aldrich (St. Louis, MO, USA). The chemicals used for antioxidant activities such DPPH (2,2-diphenyl-1-pic-rylhydrazy), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), TPTZ (2,4,6-Tripyridyl-s-triazine), trichloroacetic acid, ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of reagent grade.

Sample Preparation

The *Sanguisorbae officinalis* L. leaves(SO) used in this study was purchased from Daekwangherb (Chuncheon, Korea). The dried leaf sample was ground to 20～30 mesh using a grinder (IKA M 20, IKA, Staufen, Germany). The sample power (3 kg) was refluxed with 10 volumes (v/w) of 70% ethanol at 70℃ for 3 hr, and extraction was repeated three times. The extracts were filtered through Whatman filter paper (No. 2), concentrated with a vacuum evaporator, and completely dried with a freeze drier. The freeze-dried powder was dissolved in 50% DMSO (dimethyl sulfoxide) and filtered through a membrane filter (0.45 μm) and used for antioxidant activity.

Determination of Total Phenol Contents

The total phenol contents of extracts from SO was determined by modified of Gutfinger (1981). The sample solution (1 mL) was placed in a test tube with Folin-Ciocalteu reagent (1 mL) and sodium carbonate solution (1 mL). After incubation for 1 hr at 25℃, the absorbance was measured at 750 nm and total phenol content was calculated as gallic acid equivalents (mg GAE/ g).

Determination of Total Flavonoids Contents

The total flavonoids contents of extracts from SO were determined according to the method of Moreno MIN et al (2000). The sample solution (0.5 mL) were mixed with 1.5 mL ethanol (95%), followed by 0.1 mL of aluminum chloride (10%), 0.1 mL of potassium acetate (1 M) and distilled water. After in-

cubation at room temperature for 30 min, the absorbance was 415 nm and total flavonoids content was calculated as rutin equivalents (mg RE/ g).

Analysis of the Vitamin C Contents

For the analysis of the vitamin C content, the HPLC equipment was Agilent 1260 with photodiode array detector at 270 nm. HPLC analysis was carried out using a C_{18} column (Agilent Technologies, Palo Alto, CA, USA) (4.6×150 mm, 3.5 um). A binary gradient from mobile phase A consisting of 1% (v/v) acetic acid in distilled water (milipore) to mobile phase B consisting of 60% (v/v) methanol in distilled water (milipore) was used with the following elution program: a linear gradient from 10% to 30% (A) for 5 min and from 30% to 100% (B) for 30 min. Identification and quantification of vitamin C in sample was performed compared to chromatographic retention times and areas of external standards.

The Antioxidant Activity of These Extracts DPPH Radical

The antioxidant activity of these extracts was measured on basis of their electron donating ability (EDA) of the stable DPPH as previously described (Chu YH et al 2000) with slight modification. Different concentrations (10～500 μg/mL) of SO was prepared. Then, 1 mL of ethanolic DPPH solution (4×10^{-4} M) was added to the samples. These samples were vortexed and incubated in the dark for 10 min at room temperature. DPPH radical scavenging activities were measured by spectrophotometer at 490 nm and were calculated and expressed as a percentage using the following formula:

DPPH radical scavenging activity (%)
$=[1-(A/B)] \times 100$
A: absorbance value of testing solution
B: absorbance value of control solution

ABTS Radical Scavenging Activity

The antioxidant activity using ABTS free radical as previously described Re R et al (1999). Before analysis, the stock solution was prepared by stirring ABTS (7 mM) and potassium persulfate (2.45 mM) in water at room temperature for 16 hr. The ABTS solution was diluted with ethanol to achieve an absorbance of 0.75±0.025 at 750 nm. Then, 1 mL ABTS solution was added to 10 μL of different concentrations (10～500 μg/mL) of SO extracts. These samples were vortexed and incubated in the dark for 6 min. ABTS radical scavenging activities were measured by spectrophotometer at 750 nm, were calculated and expressed as a percentage using the following formula:

ABTS radical scavenging activity (%)
$=[1-(A/B)] \times 100$
A: absorbance value of testing solution
B: absorbance value of control solution

FRAP Assay

The ferric reducing ability of SO extract was by using the method of Benzine IFF and Strain J (1996) with modification. FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM iron chloride in proportion of 10:1:1(v/v), respectively. Different concentrations (10～500 μg/mL) of sample solution (50 μL) was mixed with distilled water (150 μL) and FRAP reagent (1.5 mL) was added. The absorbance of the reaction mixture was then measured at 595 nm after 4 min.

Reducing Power

The reducing power SO extract was evaluated by the method of Jayaprakasha G et al (2001). Different concentrations (10～500 μg/mL) of SO extract in distilled water (0.5 mL) were mixed with 2.5 mL of sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50℃ for 20 min. 2.5 mL of trichloroacetic acid (10%) was then added and mixture was centrifuge 1,790 ×g for 10 min. Then, 2.5 mL of the supernatant was taken out and mixed with 2.5 mL of distilled water. After adding 0.5 mL of iron (III) chloride (0.1%), the absorbance was spectrophotometrically measured at 750 nm.

Separation and Quantification of Phenolic Acids

The amount of free phenolic acid (gallic acid, catechin, chlorogenic acid,caffeic acid, *p*-coumaric acid, and ferulic acid) were analyzed using a high-performance liquid chromatography (HPLC, Waters M-600E; Milfold, MA, USA) system equipped with a UV

absorbance detector (280 nm 0.05 AUFS). The separations of free phenolic acids standards were achieved in the reverse phase mode using Waters Spherisorb ODS_2 column (250 mm×4.6 mm I.D., 5 μm). The mobile phases were solvent A, 1 mM trifluoroacetic acid (TFA) in 10% (v/v) acetonitrile; solvent B, 1 mM TFA in 40% (v/v) methanol and 40% (v/v) acetonitrile. The gradient profile was used as follows (Waldron KW et al 1996): A 90%, B 10% (initial); A 90%, B 10% (0～10 min); A 60%, B 40% (10～15 min); A 60%, B 40% (15～24 min); A 0%, B 5%, C 100%, (24～40 min); A 90%, B 10%, C 100%, (40～45 min); A 90%, B 10%, C 100%, (45～50 min) at a flow rate of 1 mL/min. For standard curves, pure phenolic acids were dissolved in acetonitrile and diluted to 10, 50, 100 mg/mL. Peaks were identified by retention times corrected with library spectra established from standard solution. The amounts of free phenolic acid were quantified used the standard curves.

Statistical Analysis

All measurements were repeated three times. The results were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $p<0.05$ (SAS Institute. 1998). Correlation analyses were performed using the Pearson's correlation coefficient (R).

RESULTS AND DISCUSSION

Total Phenol, Flavonoids Vitamin C Contents of Extracts from Sanguisorbae officinalis L. Leaves

The recent focus of interest on phenolic compounds stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). The extraction yields of SO was 19.34%. In addition, the total phenol content of SO determined using regression equation of calibration curve (y=19.069×—0.0024, R^2: 0.9997) and expressed in gallic acid equivalents was found to be 119±1.54 mg GAE/g (Table 1). The flavonoids are the most common group of polyphenolic compounds in human diet and are found commonly in fruits and vegetables. They can prevent coronary heart disease and have antioxidant properties (Pietta PG 2000). The total flavonoids contents of SO extract were determined using regression equation of calibration curve (y=3.884×+0.0305, R^2: 0.9997) and expressed in rutin equivalents was found to be 59.6±1.43 mg RE/g (Table 1). Phenols are very important plant constituents because of their scavenging ability, which is attributed to their hydroxyl (—OH) groups (Hatano T et al 1989) and the methyloxy (—OCH_3) substituent in the molecules (Cai YZ et al 2006). It has been reported that the levels of plasma antioxidant vitamins and minerals such as vitamin C, E, folic acid, and zinc declined and oxidative damage increased in stressed animals (Sahin K et al 2002). The content of vitamin C in the SO was 17.2±0.94 mg /100 g (Table 1).

Compounds of Phenolic Acids

Amounts of phenolic acids in the SO extracts are

Table 1. Extraction yields, total phenol, flavonoids, vitamin C, and phenolic acid contents of 70% ethanol extracts from *Sanguisorbae officinalis* L. leaves

	Sanguisorbae officinalis L. leaves
Extraction yields (%)	19.34*
Total phenol content (mg GAE[e]/g)	119.3±1.54*
Total flavonoids content (mg RE[f]/g)	59.6±1.43*
Vitamin C content (mg /100 g)	17.2±0.94*
Gallic acid (mg /100 g)	141.53±0.12[c]
Catechin (mg /100 g)	762.88±0.17[a]
Chlorogenic acid (mg /100 g)	180.94±0.31[d]
Caffeic acid (mg /100 g)	286.17±0.53[b]

Significantly different at $p<0.05$ by student t-test. The values are mean±standard deviation from three replications.

[a～d)] mean±S.D. was significantly different within the same column ($p<0.05$).

[e)] GAE: Gallic acid equilibrium.

[f)] RE: Rutin equilibrium.

shown in Table 1. In SO extract, the catechin (762.88 mg/100 g) was major compound, followed by caffeic acid (286.17 mg /100 g), chlorogenic acid (180.94 mg /100 g), and gallic acid (141.53 mg /100 g).

DPPH Radical Scavenging Activity

In DPPH assay, the natural and synthetic antioxidants were able to reduce the stable radical DPPH to the yellow-colored DPPH. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. The use of DPPH provides an easy and way to evaluate antioxidant activity (Oyaizu M 1986). The DPPH radical scavenging activities of SO extract, ascorbic acid, and BHT were evaluated in the following order: ascorbic acid > SO extract > BHT in presence of same concentration of test samples (1,000 g/mL) (Figure 1). The DPPH radical scavenging percentages of various concentrations (200, 400, 600, and 800 g/mL) of SO extract was 61.6, 82.0, 82.8, and 83.11%, respectively. Our results show that the DPPH radical scavenging activity of wild herb extracts was increased in a dose-dependent manner. Benavente-Garcia O et al (1996) reported that the radical scavenging activity of plant extracts depends on the amount of polyphenolic compounds in the extract.

ABTS Radical Cation Scavenging Activity

Another effective method to measured radical scavenging activity is the ABTS radical cation de-

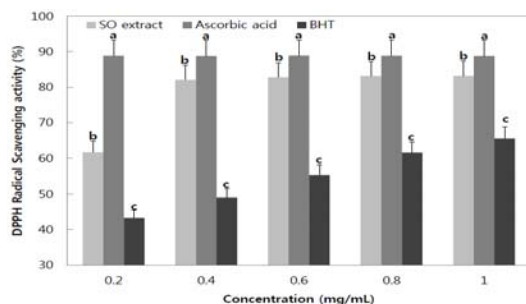

Figure 1. DPPH radical scavenging ability of *Sanguisorbae officinalis* L. leaves. All values are presented as the means±SD. [a~c] Means in the same column not sharing a common letter are significantly different (p<0.05) by Duncan's test.

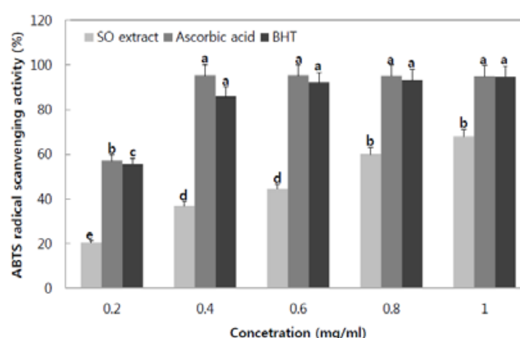

Figure 2. ABTS radical cation scavenging ability of *Sanguisorbae officinalis* L. leaves. All values are presented as the means±SD. [a~e] Means in the same column not sharing a common letter are significantly different (p<0.05) by Duncan's test.

colorization assay, which showed similar results to those obtained in the DPPH reaction. The ABTS radical scavenging activities of SO, ascorbic acid, and BHT was showed in the following order: ascorbic acid > BHT > SO extract in presence at a concentration of 1,000 μg/mL (Figure 2) The ABTS radical scavenging percentages of various concentrations (200 ~800 μg/mL) of SO extract were 20.5, 37.0, 44.6, and 60.1%, respectively. *Gardenia jasminoides* extracts appeared that SRA-B extracts have the efficacy of 20% per 62.5 μg/mL in ABTS radical scavenging activity (Yang HJ et al 2011). With this study, SO extracts has the lower efficacy at an equal concentration.

Determination of FRAP Assay in SO

The FRAP assay is based on the ability of the antioxidant to reduced Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorbance maximum at 593 nm, which is pH-dependent. The absorbance decrease is proportional to the antioxidant activity (Benzie IFF and Srain J 1996). In the present study, the trend for ferric ion reducing activities of SO extract, ascorbic acid, and BHT are shown in Figure 3. For SO extract, the absorbance was increased due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration (Figure 3). The SO extract at a concentration of 1,000 μg/mL had similar FRAP as 200 μg/mL of ascorbic acid.

Determination of Reducing Power in SO

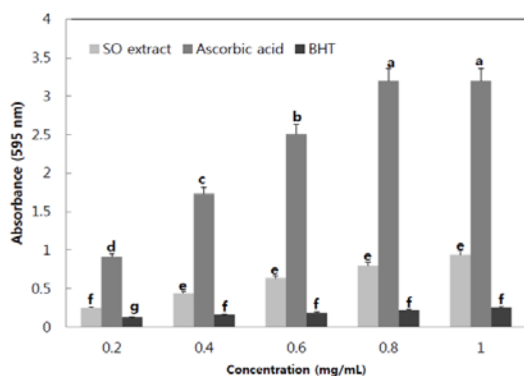

Figure 3. FRAP (ferric reducing ability of plasma) assay of *Sanguisorbae officinalis* L. leaves. All values are presented as the means±SD. [a~g] Means in the same column not sharing a common letter are significantly different (p<0.05) by Duncan's test.

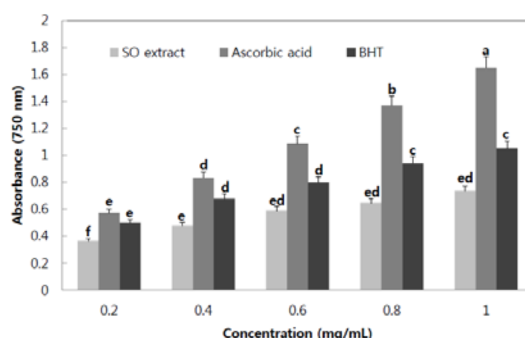

Figure 4. Reducing power activity of *Sanguisorbae officinalis* L. leaves. All values are presented as the means±SD. [a~f] Means in the same column not sharing a common letter are significantly different (p< 0.05) by Duncan's test.

In this reducing power assay, the presence of reducers (i.e., SO extract) converted the Fe^{3+}/ferricyanide complex present in the assay system to the ferrous form. By measuring the absorbance at 750 nm to determine the Fe^{2+} concentration, it is possible to estimate the reducing power of the reducers. The reducing power of SO extracts was increased along with the treatment concentrations (Figure 4). The SO extract at a concentration of 1,000 μg/mL had similar reducing power (about 0.83 at 750 nm) as a concentration of 400 μg/mL of ascorbic acid.

Correlation among the Antioxidant Characteristics

Total phenolic and flavonoid contents have been reported to be responsible for the antioxidant activities of botanical extracts. DPPH, hydroxyl radical scavenging activity, and superoxide anion radical scavenging activity have been used to measure anti-

Table 2. Correlation (r^a) between different antioxidant capacity parameters (by DPPH, ABTS, FRAP, reducing power activity) and total phenolic contents, total flavonoid content of *Sanguisorbae officinalis* L. leaves

	TPC[b]	TFC[c]	DPPH[d]	ABTS[e]	FRAP[f]	RPA[g]
TPC	-	0.151	−0.746	−0.361	−0.143	−0.587
TFC	-	-	−0.771	0.867***	0.975***	0.711**
DPPH	-	-	-	−0.351	−0.552	0.101
ABTS	-	-	-	-	0.975***	0.967***
FRAP	-	-	-	-	-	0.885**
RPA	-	-	-	-	-	-

[a)] r, correlation coefficient.
[b)] TPC, Total phenolic content.
[c)] TFC, Total flavonoid content.
[d)] DPPH radical scavenging activity.
[e)] ABTS radical scavenging activity.
[f)] FRAP, ferric reducing ability of plasma.
[g)] RPA, reducing power activity.
** Correlation in significant at p<0.05; *** Correlation in significant at p<0.001.

oxidant activity and these results should correlate with those of total phenolic and flavonoids content. A recent report (Zheng W and Wang S 2011; Do JR et al 2004) demonstrated that some bioactive compounds present in medicinal plants possessed high total antioxidant activity, which was due to the presence of phenolic, carotenoids and flavonoids. A regression analysis was used to correlate the results of the six assays (Table 2). High correlation coefficients were found between the total flavonoid content and FRAP (r=0.975, p<0.05), between the ABTS radical scavenging activity and FRAP radical scavenging activity (r=0.957, p<0.001), and between the ABTS radical scavenging activity and reducing power activity (r=0.967, p<0.001). However, a non-significant correlation coefficient was found between flavonoid content and total phenolic content, between DPPH radical scavenging activity and reducing power activity (p>0.05). As the aluminum chloride method is specific only for flavones and flavonols, total flavonoid content could be underestimated by the method (Meda et al 2005), which probably accounts for a lower correlation observed between antioxidant activity and flavonoid count.

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오이풀잎 에탄올 추출물에 대한 기능성 연구

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국문초록

본 연구의 목적은 한국에서 재배된 70% 오이풀 에탄올 추출물을 이용하여 총페놀, 총플라보노이드, 페놀릭 화합물과 항산화성(DPPH, ABTS, FRAP, 환원력)에 대해 실험결과를 제공하는 것이다. 연구결과 총페놀과 총플라보노이드의 함량은 각각 119.3±1.54 mg gallic acid and 59.6±1.43 mg rutin를 나타내었다. 또한, 추출물에 함유된 페놀릭 화합물의 주된 물질은 Catechin으로 나타났으며, 항산화성을 나타내는 DPPH, ABTS, FRAP, 환원력은 농도의존적으로 증가하는 경향을 나타내었다. 이상의 결과로 오이풀 추출물은 천연 항산화성 및 기능성 식품 첨가물로서 좋은 재료가 될 것으로 판단된다.

주제어: 오이풀잎, 항산화 활성, 총페놀, 총프라보노이드