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Exploration of the Antioxidant Chemical Constituents and Antioxidant Performance of Various Solvent Extracts of Eighteen Plants

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ABSTRACT: This study examined the antioxidant chemical constituents (total phenolic and total flavonoid contents) and antioxidant activities [1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging, and ferric reducing antioxidant power (FRAP)] of different solvent extracts of eighteen functional plants. The active components of the target plants were extracted using four different solvents (methanol, ethanol, chloroform, and water). *Mentha piperascens* leaf exhibited a higher total phenolic content (chloroform and water extracts), total flavonoid content (all solvent extracts), DPPH radical scavenging activity (methanol, ethanol, and water extracts), ABTS radical scavenging activity (water extract), and FRAP radical scavenging activity (water extract) (in all solvent extracts). Similarly, *Petasites japonicas* root was another excellent and competent extract with a high total flavonoid content (in all four solvent extracts), DPPH and ABTS radical scavenging activity (methanol, ethanol, and water extracts), and FRAP activity (methanol, ethanol, and water extracts) (in all solvent extracts). *Rubus coreanus* fruit had the highest total phenolic content (methanol, ethanol, and water extracts), DPPH and ABTS radical scavenging activate (in all solvent extracts), and FRAP in its water extract were the two most effective functional plants. Based on the abundance of antioxidant chemical constituents and the most potent antioxidant activity demonstrated in this study, extracts from *M. piperascens* leaf, *P. japonicas* root, and *R. coreanus* fruit appear to be promising candidates to meet the current demand for natural preservatives in food and pharmaceutical industries.

Keywords: antioxidant, natural preservatives, radical scavenging, solvent

INTRODUCTION

Food quality degradation during storage is a critical issue for the food industry (Nychas and Panagou, 2011). Food spoilage occurs when a food product undergoes microbiological, chemical, or physical changes that render it unfit for consumption (Petruzzi et al., 2017). Oxidation is an important driver of foodstuff deterioration (Nychas and Panagou, 2011). The oxidation process alters numerous interactions between food ingredients, resulting in unpleasant products (Ahmed et al., 2016). Moreover, food lipids are particularly susceptible to oxidation, and their degradation reactions are a frequent cause of food degradation during processing, storage, distribution, and final preparation (Ahmed et al., 2016). Rancidity can manifest itself in various forms, including off-flavors, color loss, altered nutrient value, and the production of toxic substances (Keller et al., 2015; Li et al., 2015). Moreover, mutagenic, carcinogenic, and cytotoxic lipid oxidation products are considered health risk factors (Keller et al., 2015; Li et al., 2015).

Certain preservatives are used by food manufacturers to extend the shelf life and maintain the quality of food products (Sharma, 2015; Kumari et al., 2019). Preservatives are natural or synthetic substances added to foods to keep them from spoiling due to microbial growth or undesirable chemical changes (Kumari et al., 2019). Presently, most preservatives are synthetic rather than natural (Anand and Sati, 2013). Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate are added to foods to protect them from oxidation (Kumari et al., 2019).

In recent years, artificial/synthetic preservatives have raised various health concerns (Saeed et al., 2019). Many of these preservatives are thought to be toxic (Kumari et al., 2019), while others can be fatal (Anand and Sati,

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2013). One of the most pernicious effects of synthetic preservatives on food is their ability to mutate into carcinogens. Certain food items contain preservatives that contain carcinogenic chemicals (Mirza et al., 2017). Additionally, synthetic preservatives have been linked to serious health problems such as hypersensitivity, allergy, asthma, hyperactivity, and neurological damage (Kumari et al., 2019). They can also affect mental acuity, behavior, and immune response (Pandey and Upadhyay, 2012). Therefore, consumers' criteria for evaluating food safety have gradually evolved in recent years to include both the potential for immediate physical harm and the long-term effects of artificial preservative consumption (Erickson and Doyle, 2017). Additionally, consumer demand for foods free of artificial preservatives is steadily increasing, necessitating significant research into new natural additives. The growing demand for chemical-free food paved the way for the use of natural preservatives in food industries and inspired scientists to find an alternative to natural agents (Saeed et al., 2019).

Natural preservatives are not harmful to health when used to preserve food 'as is' because they are not mixed with synthetic items, and the chemical composition is not altered. Numerous studies have established that various naturally occurring and beneficial alternative preservatives derived from plants, animals, microbes, and minerals possess antioxidant, antimicrobial, and anti-enzymatic properties (Anand and Sati, 2013). However, they are rarely used as additives because they are more expensive than synthetic antioxidants (Li et al., 2009). This study was conducted to assess the antioxidant chemical constituents (total phenolic and total flavonoid contents) and antioxidant potential [1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging, and ferric reducing antioxidant power (FRAP)] of different solvent extracts of 18 functional plants. The plant parts used in this study are not identical for all target plants; seed, leaf, needle, steam, or flower were chosen according to the trend in previous traditional applications and the yield potential, abundant availability, and commercial application.

MATERIALS AND METHODS

Collection of functional plants

Approximately 120 different functional plants regarded as potential natural preservatives were collected from the National Institute of Biological Resources for use in the current study (Incheon, Korea). For 7 days at 50°C, the plant parts were oven-dried (10~15% moisture content). Samples were collected in polyethylene plastic bags at the experimental site: Daegu University's Life and Natural Science College. A preliminary screening of the antioxidant constituents and activities of 120 different functional plants was conducted before the study. Ethanol and water are food-grade solvents that are nontoxic (required no additional purification steps) and can be used directly as food preservatives. Therefore, ethanolic extracts of functional plant parts were chosen for screening, and their total phenolic content [mg catechin (CAT) equivalents/g] and DPPH radical scavenging activity (mg BHA equivalents/g) were determined. Subsequently, 18 functional plants with a higher total phenolic content (>0.15 mg CAT equivalents/g) and DPPH radical scavenging activity (>125 mg BHA equivalents/g) were chosen for the subsequent and actual study. These screening cut points were chosen considering the extract's performance variability. Those plants not selected for the actual study were below the cutoff value (the cut points), indicating no noticeable variation.

Extract preparation

The study samples were ground to a fine powder using a Multi Grinder (HMF1265, Hanil Electric, Bucheon, Korea), separated using a 1-mm mesh sieve, packed in polythene bags, and stored in a desiccator (Auto C-3W, Sanplatec Corp., Osaka, Japan) until extraction. Extractions from powdered samples of the target plants were carried out using four different solvents: 100% chloroform, 100% methanol, 70% ethanol, and hot water. Four different lots of fifteen grams powdered samples were weighed and poured into four separate 500 mL conical flasks (flask A~D). 150 mL chloroform, methanol, ethanol, and distilled water were added to the sample containing flasks A, B, C, and D, respectively, for different solvent extractions. The sample was boiled for 30 min at 80°C with the addition of distilled water in a water bath (VS-8480, Vision Science Co., Ltd., Gyeongsan, Korea). The four mixtures were shaken every 6 h for two days. The filter paper was used to filter the extracts (Whatman no. 1, GE Healthcare, Buckinghamshire, UK). The extracts (filtrate) were concentrated at 45°C under reduced pressure using an evaporator (EYELA CCA-1110, Tokyo Rikakicai Co., Tokyo, Japan). The extracts were then lyophilized using a freeze dryer and stored in a desiccator until further use (Biotron Inc., Bucheon, Korea).

Measurement of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965), with some modifications. Briefly, samples were prepared from various solvent extracts of the functional plants used in this study at various concentrations (50, 100, 200, 300, 400, and 500 μg/mL). Next, 20 μL of the extracted sample was added to 100 μL of distilled water and incubated for 5 min with 20 μL of Folin-Cio-

calteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA). After 30 min of incubation at room temperature, 10 μL of 20% sodium carbonate (Na_2CO_3) was added to the reaction mixture. The absorbance at 760 nm was measured spectrophotometrically. Catechin was used as a reference standard, and the sample readings were expressed as mg CAT equivalents/g dry weight.

Measurement of total flavonoid content

The total flavonoid content was determined using a modified version of the Meda et al. (2005) method. In brief, 0.25 mL of sample (1 mg/mL) was added to 1 mL of double-distilled water in a tube. Next, 0.075 mL of 5% $NaNO_2$, 0.075 mL of 10% $AlCl_3$, and 0.5 mL of 1 M NaOH were added sequentially at 0, 5, and 6 min. Finally, the reacting solution was adjusted to a volume of 2.5 mL using double-distilled water. A spectrophotometer was used to determine the solution's absorbance at 410 nm (Ultrospec 2100 pro, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Rutin was used as a standard to determine the total flavonoid content. The results are given as mg rutin equivalents (RE)/g dry weight.

DPPH radical scavenging activity

The samples' DPPH free radical scavenging activity was determined using a method adapted from Brand-Williams et al. (1995). For the current study, samples from various solvent extracts of the functional plants were prepared into various concentrations [(0, control), 50, 100, 200, 300, 400, and 500 μg/mL] using 70% ethanol. The study used 0.1 mM DPPH in ethanol. Then, each sample was mixed in a 1:1 ratio with the DPPH solution. The mixture was vigorously shaken and left for 30 min, and the absorbance was measured spectrophotometrically at 517 nm. Reduced absorbance of the reaction mixture indicates increased free radical scavenging efficiency. The following equation was used to determine the DPPH free radical scavenging capacity. BHA was used as the standard. The results were expressed as BHA equivalent antioxidant capacity based on the mean of three readings.

$$\text{Scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

ABTS radical scavenging assay

The ABTS radical scavenging activity of extracts was determined using a modified version of the method described by Re et al. (1999). To prepare ABTS-potassium persulfate solution ($ABTS^{\bullet+}$), 7 mM ABTS was mixed with 2.45 mM potassium persulfate (2:1, v/v^{+}). For approximately 16 h, the ABTS radical cations were incubated at 20°C. By diluting the solution with 95% (v/v) ethanol, the absorbance of the solution was adjusted to 0.70±0.02 at 734 nm. Samples were prepared from various solvent extracts of functional plants at various concentrations [(0, control), 50, 100, 200, 300, 400, and 500 μg/mL]. The sample and $ABTS^{\bullet+}$ solution were then mixed (1:1, v/v), and the mixture's absorbance at 734 nm was monitored after 6 min. In place of the sample, 95% (v/v) ethanol was used as the blank, each sample was measured in triplicate, and BHT was used as a reference compound. The percentage of inhibition was calculated in the formula indicated below. The results were expressed as BHT equivalent antioxidant capacity based on the mean of three readings:

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

FRAP assay

The antioxidant activity of extracts against ferric reduction was determined using the FRAP assay developed by Benzie and Strain (1996). The FRAP method determines the difference in absorbance that occurs when the complex 2,4,6-tripyridyl-*S*-triazine (TPTZ)-Fe^{3+} is reduced to TPTZ-Fe^{2+} (colored form) in the presence of antioxidants (Halvorsen et al., 2002). 25 mL of 300 mmol/L acetate buffer, 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl, and 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ were combined to produce the solution for this assay (solutions were mixed together 10:1:1 ratio). We prepared samples from various solvent extracts of selected functional plants at concentrations ranging from 50 to 500 μg/mL. Subsequently, the extracted sample, distilled water, and FRAP reagent were mixed in a 1:3:30 ratio and absorbance measurements at 593 nm were taken during the 5-min reaction. The calibration curve was plotted for various concentrations of Fe(II) (501,000 μM), with quercetin serving as the standard. The average of three readings was calculated and expressed as mg of quercetin equivalents (QE)/g ferric reducing activity.

Statistical analysis

The current study compared different solvent extracts of the same plant part and the same solvent extracts of different target plants. The experimental data were statistically analyzed using one-way ANOVA with SAS software version 9.4 (SAS Institute, Cary, NC, USA). A significance level of $P<0.05$ was used for all evaluations; Duncan's multiple range tests were used to detect differences between means.

RESULTS AND DISCUSSION

The present study examined the antioxidant properties

Table 1. Total phenolic content of different solvent extracts of parts of selected plants (unit: mg CAT equivalents/g)

Explored plant	Various solvent extract				SEM
	Methanol	Ethanol	Chloroform	Water	
Brassica juncea seed	0.28^{Bgh}	0.51^{ABef}	0.33^{Bde}	0.74^{Ae}	0.13
Caragana sinica leaf	0.75^{Bf}	0.99^{Acde}	0.34^{Cd}	0.78^{Be}	0.08
Cornus officinalis fruit	0.28^{Agh}	0.30^{Af}	0.18^{Bgh}	ND	0.04
Cudrania tricuspidata fruit	1.99^{Ac}	0.39^{Bf}	0.29^{Bdef}	0.15^{Ch}	0.07
Lepidium sativum seed	1.30^{Ae}	1.42^{Ac}	0.14^{Ch}	0.97^{Bd}	0.13
Liriope platyphylla stem	0.13^{Bhi}	0.19^{Af}	0.05^{Ci}	ND	0.01
Lonicera japonica leaf	0.61^{Bf}	0.61^{Bef}	0.67^{Ab}	0.47^{Cfg}	0.03
Mentha piperascens leaf	0.61^{BCf}	0.64^{Bef}	0.46^{Cc}	0.97^{Ad}	0.08
Moringa stenopetala leaf	0.65^{Cf}	1.16^{Bcd}	0.44^{Dc}	1.27^{Ac}	0.05
Morus bombycis fruit	1.95^{Ac}	1.22^{Bcd}	1.12^{Ba}	1.08^{Bd}	0.09
Paeonia lactiflora root	2.69^{Ab}	1.24^{Bcd}	0.17^{Cgh}	ND	0.09
Petasites japonicus root	0.10^{Ci}	0.62^{Aef}	0.31^{Bde}	0.14^{Ch}	0.07
Pinus deniflora needle	3.49^{Aa}	2.71^{Bb}	1.11^{Ca}	0.34^{Dg}	0.22
Poncirus trifoliata fruit	0.61^{Af}	0.49^{ABef}	0.32^{Cde}	0.42^{Cg}	0.07
Rubus coreanus fruit	3.58^{Ba}	5.39^{Aa}	0.29^{Cdef}	4.78^{Aa}	0.56
Scutellaria baicalensis root	1.63^{Ad}	1.38^{Bc}	0.51^{Cc}	1.81^{Ab}	0.13
Taraxacum coreanum flower	0.73^{Af}	0.72^{Adef}	0.24^{Befg}	0.62^{Aef}	0.10
Zanthoxylum piperitum seed	0.32^{Ag}	0.22^{Bf}	0.21^{Bfgh}	0.33^{Ag}	0.02
SEM	0.10	0.29	0.05	0.12	

Means in a row (different solvent extracts of the same target plant) followed by uppercase letters (A–D) are significantly different ($P<0.05$).
Means in a column (the same solvent extracts of different target plants) followed by lowercase letters (a–i) are significantly different ($P<0.05$).
CAT, catechin equivalents; SEM, standard mean error (n=3); ND, not detected.

of 18 diverse functional plants. Prior to this study, a preliminary screening of 120 different plant parts for their antioxidant potential was conducted, and 18 of the best-performing plant parts were selected for further investigation. Extracts of the selected target plant part were analyzed in four different solvents (methanol, ethanol, chloroform, and water) for five different antioxidant quality attributes (antioxidant constituents and activities), including total phenolic content, total flavonoid content, DPPH radical scavenging, ABTS radical scavenging, and FRAP. As a result, a single target plant was subjected to 20 observations (4 types of solvent extracts ×5 attributes).

Total phenolic content of explored plants

There has been a surge in interest in phenolic compounds in recent years due to their potential therapeutic benefits. The anticarcinogenic, antimutagenic, and cardioprotective properties of phenolic compounds have been attributed to their antioxidant properties, which neutralize free radicals and inhibit lipid peroxidation (Potter, 2005). According to Soobrattee et al. (2005), phenolic compounds are important plant components with redox properties that contribute to antioxidant activity. The hydroxyl groups found in plant extracts aid in free radical scavenging. The total phenolic content (mg CAT equivalents/g) of various solvent extracts of the plants studied is shown in Table 1. In all eighteen considered target plants, the total phenolic content varied significantly ($P<0.05$) between different solvent extracts of a plant part. Different solvent systems have varying degrees of polarity, resulting in significant differences in the extraction of active compounds. Compared to their counter solvent extracts, methanolic extracts of *Cornus officinalis* fruit, *Cudrania tricuspidata* fruit, *Lepidium sativum* seed, *Morus bombycis* fruit, *Paeonia lactiflora* root, *Pinus deniflora* needle, and *Scutellaria baicalensis* root, ethanolic extracts of *Caragana sinica* leaf, *Liriope platyphylla* stem, *Petasites japonicus* root, and *Rubus coreanus* fruit, chloroform extract of *Lonicera japonica* leaf, and water of *Mentha piperascens* leaf and *Moringa stenopetala* leaf, both methanolic and ethanolic *Poncirus trifoliata* fruit and *Taraxacum coreanum* flower, both methanolic and water extracts of *Zanthoxylum piperitum* seed, and *Brassica juncea* seed of both ethanolic and water extracts exhibited a significantly highest total phenolic content in mg CAT equivalents/g. Altogether, phenolic compounds extraction potential of solvents, methanol solvent> ethanolic> water> chloroform had 10, 7, 4, and 1 active extracts, respectively, in 18 target plants considered. There is a significant ($P<0.05$) difference in the total phenolic content of four solvent extracts from different plants used in the current study (Table 1).

The total phenolic content of methanolic extracts ranged between 0.10 and 3.58 mg CAT equivalents/g, a variation of approximately thirty-six-fold. Compared to the other 18 plants analyzed in this study, the following seven

plants had significantly ($P<0.05$) higher total phenolic compounds in methanolic extracts, in the following order: *R. coreanus* fruit, *P. deniflora* needle> *P. lactiflora* root> *C. tricuspidata* fruit, *M. bombycis* fruit> *S. baicalensis* root> *L. sativum* seed having the content of 3.58, 3.49, 2.69, 1.99, 1.95, 1.63, and 1.30 mg CAT equivalents/g, respectively. The lowest content of <0.20 mg CAT equivalents/g is exhibited in the *L. platyphylla* stem and *P. japonicus* root extracts. From ethanolic extracts, substantially ($P<0.05$) highest levels of total phenolic compounds were exhibited in the following seven plants in decreasing order: *R. coreanus* fruit> *P. deniflora* needle> *L. sativum* seed and *S. baicalensis* root> *P. lactiflora* root, *M. bombycis* fruit, and *M. stenopetala* leaf with 5.39, 2.71, 1.42, 1.38, 1.24, 1.22, and 1.16 mg CAT equivalents/g, respectively. Furthermore, the lowest contents <0.25 mg CAT equivalents/g were exhibited in *Z. piperitum* seed and *L. platyphylla* stem. The total phenolic content range was 0.19 to 5.39 mg CAT equivalents/g which is about 28-fold in variation, whereas the total phenolic compounds from chloroform solvent were markedly ($P<0.05$) higher in the following six plants: *M. bombycis* fruit and *P. deniflora* needle> *L. japonica* leaf> *S. baicalensis* root, *M. piperascens* leaf, and *M. stenopetala* leaf with the content of 1.12, 1.11, 0.67, 0.51, 0.46, and 0.44 mg CAT equivalents/g, respectively. The lowest content of <0.20 mg CAT equivalents /g was exhibited in *C. officinalis* fruit, *P. lactiflora* root, *L. sativum* seed, and *L. platyphylla* stem extracts. The content ranged from 1.12 mg CAT equivalents/g in *M. bombycis* fruit to 0.05 mg CAT equivalents/g in *L. platyphylla* stem, which resulted in a 22-fold variation. Furthermore, from the water extracts, the higher levels of total phenolic compounds were exhibited as follows: *R. coreanus* fruit> *S. baicalensis* root> *M. stenopetala* leaf> *M. bombycis* fruit, *L. sativum* seed, and *M. piperascens* leaf corresponding with 4.78, 1.81, 1.27, 1.08, 0.97, and 0.97 mg CAT equivalents /g, respectively. *P. lactiflora* root, *L. platyphylla* stem, and *C. officinalis* fruit water extracts did not possess total phenolic compounds in mg CAT equivalents/g.

Total flavonoid content of explored plants

The phenolic compounds found in plants are classified into numerous categories. The most important are flavonoids, which exhibit significant antioxidant properties (Nunes et al., 2012). Flavonoids are found naturally in plants and are beneficial to human health. Flavonoid derivatives have been shown to have antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic properties (Di Carlo et al., 1999; Montoro et al., 2005). Flavonoids are extremely effective at scavenging the majority of oxidizing chemicals, including singlet oxygen and other free radicals implicated in various diseases (Bravo, 1998). Table 2 summarizes the total flavonoid content of various solvent extracts of functional plants used in this study. Among the 18 plants analyzed in this study, a significant ($P<0.05$) difference in total flavonoid (mg RE/g) con-

Table 2. Total flavonoid content of various plant solvent extracts (unit: mg RE/g)

Explored plant	Various solvent extract				SEM
	Methanol	Ethanol	Chloroform	Water	
Brassica juncea seed	ND	ND	ND	ND	0.00
Caragana sinica leaf	0.11^{Be}	0.15^{Ad}	ND	ND	0.01
Cornus officinalis fruit	ND	ND	0.08^{e}	ND	0.06
Cudrania tricuspidata fruit	ND	ND	ND	ND	0.00
Lepidium sativum seed	5.90^{Aa}	5.17^{Ba}	2.46^{Cb}	ND	0.10
Liriope platyphylla stem	ND	ND	ND	ND	0.00
Lonicera japonica leaf	ND	ND	ND	ND	0.00
Mentha piperascens leaf	0.73^{Dc}	1.55^{Bc}	6.01^{Aa}	1.32^{Ca}	0.11
Moringa stenopetala leaf	ND	ND	ND	ND	0.00
Morus bombycis fruit	ND	ND	ND	ND	0.00
Paeonia lactiflora root	ND	ND	ND	ND	0.00
Petasites japonicus root	0.35^{Cd}	2.08^{Ab}	0.60^{Cd}	1.13^{Bb}	0.16
Pinus deniflora needle	ND	ND	ND	ND	0.00
Poncirus trifoliata fruit	ND	ND	ND	ND	0.00
Rubus coreanus fruit	ND	ND	ND	ND	0.00
Scutellaria baicalensis root	ND	5.31^{a}	ND	ND	0.55
Taraxacum coreanum flower	ND	ND	ND	ND	0.00
Zanthoxylum piperitum seed	2.34^{Ab}	0.04^{Dd}	1.46^{Bc}	0.28^{Cc}	0.08
SEM	0.06	0.27	0.05	0.02	

Means in a row (different solvent extracts of the same target plant) followed by uppercase letters (A–D) are significantly different ($P<0.05$).
Means in a column (the same solvent extracts of different target plants) followed by lowercase letters (a–e) are significantly different ($P<0.05$).
RE, rutin equivalents; SEM, standard mean error (n=3); ND, not detected.

tent was observed between their various solvent extracts in *C. sinica* leaf, *C. officinalis* fruit, *L. sativum* seed, *M. piperascens* leaf, *P. japonicus* root, *S. baicalensis* root, and *Z. piperitum* seed. In contrast, the rest of the plants did not own total flavonoid in mg RE/g for any solvents used in the extraction. Moreover, unlike their counter solvents extracts methanolic extracts of *L. sativum* seed and *Z. piperitum* seed, ethanolic extracts of *L. sativum* seed and *S. baicalensis* root, and chloroform extracts of *M. piperascens* leaf and *L. sativum* seed exhibited a substantially ($P<0.05$) higher total flavonoid (mg RE/g) content. When the extraction potential of the solvents was considered in seven of the target plants with high total flavonoid content, ethanol was found to be superior to methanol. Chloroform was found to be superior to water (ethanol> methanol and chloroform> water), with 3, 2, and no active extracts, respectively.

Of the eighteen plants examined in the present study, the total flavonoid content of methanolic solvent extracts was exhibited in the following five plants and listed as *L. sativum* seed> *Z. piperitum* seed> *M. piperascens* leaf> *P. japonicus* root> *C. sinica* leaf having 5.90, 2.34, 0.73, 0.35, and 0.11 mg RE/g, respectively, and other extracts from methanolic solvent did not yield total flavonoid in mg RE/g. Similarly, a significantly ($P<0.05$) higher level of total flavonoid from ethanolic extracts was found in the following plants in decreasing order: *S. baicalensis* root and *L. sativum* seed> *P. japonicus* root> *M. piperascens* leaf having a content of 5.31, 5.17, 2.08, and 1.55 mg RE/g, respectively. Extracts from *C. sinica* leaf and *Z. piperitum* seed exhibited a noticeably ($P<0.05$) lower content<1 mg RE/g compared to the total flavonoid content of the other extracts in mg RE/g. The *M. piperascens* leaf> *L. sativum* seed> *Z. piperitum* seed extracts in total flavonoid content from chloroform extracts followed by *P. japonicus* root and *C. officinalis* fruit with contents of 6.01, 2.46, 1.46, 0.60, and 0.08 mg RE/g, respectively. Furthermore, we observed no total flavonoid content in mg RE/g in the other chloroform extracts. Water extracts of other plants examined, except for *M. piperascens* leaf, *P. japonicus* root, and *Z. piperitum* seed, did not produce a total flavonoid value in mg RE/g. The contents in the three indicated extracts were also limited to 1.32, 1.13, and 0.28 mg RE/g, respectively.

DPPH radical scavenging activity of explored plants

The free radical scavenging activity of DPPH has been widely used to determine the antioxidant activity of natural products derived from plants and microbes (Shyur et al., 2005). The DPPH assay has several advantages over other methods, including increased stability, credible sensitivity, ease of use, and feasibility (Ozcelik et al., 2003). Furthermore, the assay determines an extract's ability to donate hydrogen to the DPPH radical, thereby bleaching the DPPH solution; the greater the bleaching action, the higher the antioxidant activity (Prabhune et al., 2013).

Table 3. DPPH radical scavenging activity of various plant solvent extracts (unit: mg BHA equivalents/g)

Explored plant	Various solvent extract				SEM
	Methanol	Ethanol	Chloroform	Water	
Brassica juncea seed	358.18^{Ae}	281.05^{Bg}	101.58^{Dg}	239.70^{Cdef}	9.12
Caragana sinica leaf	231.45^{Bh}	324.48^{Af}	80.00^{Ch}	222.70^{Befg}	9.80
Cornus officinalis fruit	415.87^{Ad}	380.06^{Bd}	37.84^{Ci}	ND	14.34
Cudrania tricuspidata fruit	353.55^{Ae}	209.55^{Bj}	116.84^{Cf}	192.48^{Bh}	9.15
Lepidium sativum seed	275.23^{Cg}	477.33^{Aab}	119.61^{Df}	322.59^{Bc}	16.84
Liriope platyphylla stem	97.19^{Ci}	130.33^{Bk}	165.78^{Ad}	ND	4.36
Lonicera japonica leaf	397.98^{Bd}	419.81^{Ac}	310.95^{Cb}	258.82^{Dd}	8.68
Mentha piperascens leaf	498.67^{Ab}	413.32^{Bc}	119.15^{Cf}	479.15^{Aa}	18.45
Moringa stenopetala leaf	351.45^{Ae}	321.64^{Bf}	96.92^{Cg}	344.51^{Ac}	7.34
Morus bombycis fruit	304.99^{Bf}	249.78^{Chi}	378.46^{Aa}	214.16^{Dfgh}	13.76
Paeonia lactiflora root	537.41^{Aa}	492.41^{Ba}	177.33^{Cd}	ND	14.55
Petasites japonicus root	487.56^{Ab}	490.06^{Aab}	127.19^{Bf}	501.01^{Aa}	16.55
Pinus deniflora needle	508.27^{Aab}	357.88^{Bde}	289.98^{Cc}	149.13^{Di}	6.13
Poncirus trifoliata fruit	239.14^{Ah}	240.09^{Ai}	148.25^{Be}	209.01^{Agh}	17.85
Rubus coreanus fruit	514.45^{Aab}	465.70^{Bb}	171.30^{Cd}	502.65^{Aa}	11.95
Scutellaria baicalensis root	451.51^{Ac}	344.42^{Bef}	146.55^{Ce}	487.14^{Aa}	23.23
Taraxacum coreanum flower	303.80^{Bf}	323.89^{Bf}	118.77^{Cf}	395.83^{Ab}	21.00
Zanthoxylum piperitum seed	220.10^{Ch}	269.22^{Agh}	ND	245.30^{Bde}	11.40
SEM	16.83	14.37	7.80	15.26	

Means in a row (different solvent extracts of the same target plant) followed by uppercase letters (A–D) are significantly different ($P<0.05$).
Means in a column (the same solvent extracts of different target plants) followed by lowercase letters (a–k) are significantly different ($P<0.05$).
DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHA, butylated hydroxyanisole; SEM, standard mean error (n=3); ND, not detected.

Table 3 summarizes the DPPH radical scavenging antioxidant activity of various solvent extracts of explored plants. The type of solvent used in the extraction had a significant ($P<0.05$) effect on the DPPH radical scavenging activity in all eighteen plants examined. The stability of different extracts from the same plant material is determined by the solvent used to extract the active components, and antioxidant concentrations and activity of extracts from the same plant material can vary significantly (Akowuah et al., 2005). Compared to their counter solvents, a noticeably ($P<0.05$) strong DPPH radical scavenging activity was observed in methanolic extracts of *B. juncea* seed, *C. officinalis* fruit, *C. tricuspidata* fruit, *P. lactiflora* root, and *P. deniflora* needle, ethanolic extracts of *C. sinica* leaf, *L. sativum* seed, *L. japonica* leaf, and *Z. piperitum* seed, chloroform extracts of *L. platyphylla* stem and *M. bombycis* fruit, and water extract of the *T. coreanum* flower. Similarly, we observed markedly potent DPPH radical scavenging activity in both methanolic and water extracts of *M. piperascens* leaf, *M. stenopetala* leaf, *R. coreanus* fruit, and *S. baicalensis* root. Methanolic, ethanolic, and chloroform extracts of *P. japonicus* root and *P. trifoliata* fruit had a similar highest ($P<0.05$) DPPH radical scavenging activity than their water extracts. Based on the scavenging potential of solvents we observed an increasing trend: methanol> water> ethanolic> chloroform with 11, 7, 6, and 2 potent extracts, respectively.

Among 18 target plants considered in this study, the following five plants exhibited the most effective ($P<0.05$) DPPH radical scavenging activity from methanolic extracts and ranked as *P. lactiflora* root> *R. coreanus* fruit and *P. deniflora* needle> *M. piperascens* leaf and *P. japonicus* root having the scavenging values of 537.41, 514.45, 508.27, 498.67, and 487.56 mg BHA equivalents/g, respectively. All other extracts exhibited similar DPPH scavenging values above 220.00 mg BHA equivalents/g except for *L. platyphylla* stem, which had the least score of 97.19 mg BHA equivalents/g. Similarly, the *L. platyphylla* stem ethanolic extracts had the least scavenging activity for DPPH radical with 130.33 mg BHA equivalents/g. Meanwhile, all other ethanolic extracts presented scavenging activity above 200.00, and substantially ($P<0.05$) higher DPPH radical scavenging activity was observed in the following six plants in decreasing order: *P. lactiflora* root> *P. japonicus* root and *L. sativum* seed> *R. coreanus* fruit> *L. japonica* leaf and *M. piperascens* leaf with corresponding values of 492.41, 490.06, 477.33, 465.70, 419.81, and 413.32 mg BHA equivalents/g, respectively. *M. bombycis* fruit, *L. japonica* leaf, *P. deniflora* needle, *P. lactiflora* root, *R. coreanus* fruit, and *L. platyphylla* stem exhibited noticeably ($P<0.05$) effective DPPH radical scavenging from chloroform extracts with 378.46, 310.95, 289.98, 177.33, 171.30, and 165.78 mg BHA equivalents/g, respectively. *C. officinalis* fruit extracts exhibited the least scavenging activity of 37.84 mg BHA equivalents/g, and *Z. piperitum* seed exhibited significant DPPH radical scavenging activity. Moreover, from water extracts, high DPPH radical scavenging activity ($P<0.05$) (>395.00 mg BHA equivalents/g) was observed in the following five listed extracts in decreasing order: *R. coreanus* fruit, *P. japonicus* root, *S. baicalensis* root, and *M. piperascens* leaf, *T. coreanum* flower, and their scavenging values were 502.65, 501.01, 487.14, 479.15, and 395.83 mg BHA equivalents/g, respectively. However, the water extracts of three plants, namely *P. lactiflora* root, *L. platyphylla* stem, and *C. officinalis* fruit, did not show scavenging activity for DPPH radical in mg BHA equivalents/g.

ABTS radical scavenging activity of explored plants

The ABTS radical cation scavenging assay is widely used to determine the radical scavenging ability of samples (Brand-Williams et al., 1995; Ilyasov et al., 2020). Due to the ease with which this procedure can be applied, it is frequently used to rapidly determine the antioxidant activity of various plant extracts (Prior et al., 2005). Single-electron transfer deactivates radicals in the ABTS assay. When ABTS reacts with potassium persulfate, the $ABTS^{\bullet+}$ chromophore is formed, converting ABTS to its radical cation. This radical cation is blue in color and absorbs light at a wavelength of 734 nm (Re et al., 1999). The antioxidant activity of different solvent extracts of functional plants considered in this study was summarized in Table 4. In all 18 plants examined in this study, there is a significant ($P<0.05$) difference in ABTS radical scavenging activity between their various solvent extracts. In comparison to their counter solvent extracts, methanolic extracts exhibited significantly ($P<0.05$) increased ABTS radical scavenging activity of *C. tricuspidata* fruit, *L. sativum* seed, *M. bombycis* fruit, *P. lactiflora* root, and *P. deniflora* needle, and ethanolic extracts of *C. sinica* leaf, *C. officinalis* fruit, *L. platyphylla* stem, *L. japonica* leaf, and *S. baicalensis* root, and water extracts of *M. piperascens* leaf, *P. japonicus* root, and *T. coreanum* flower. Moreover, the methanolic and ethanolic extracts of *P. trifoliata* fruit, methanolic and water extracts of *Z. piperitum* seed, and chloroform and water extracts of *B. juncea* seed exhibited a noticeably ($P<0.05$) more potent ABTS radical scavenging activity than their counter solvent extracts. Likewise, the methanolic, ethanolic, and water extracts of *M. stenopetala* leaf and *R. coreanus* fruit exhibited markedly stronger ($P<0.05$) ABTS radical scavenging activity than their counter chloroform extracts. The effectiveness of the solvents in ABTS radical scavenging activities was determined by the number of their extracts giving rise to significantly strong activity from the 18 selected plants and ranked as methanol> ethanol extract> water extract> chloroform by having 9, 8, 7, and 1 active extracts, respectively.

Table 4. ABTS radical scavenging activity of various plant solvent extracts (unit: mg BHT equivalents/g)

Explored plant	Various solvent extract				SEM
	Methanol	Ethanol	Chloroform	Water	
Brassica juncea seed	84.34^{Bh}	40.88^{Ck}	98.57^{Ad}	96.09^{Ae}	4.38
Caragana sinica leaf	95.08^{Bgh}	110.78^{Ag}	ND	37.54^{Ch}	3.75
Cornus officinalis fruit	73.45^{Bi}	79.54^{Ai}	ND	ND	2.71
Cudrania tricuspidata fruit	213.87^{Ab}	56.25^{Bj}	28.46^{Dh}	48.76^{Cgh}	3.25
Lepidium sativum seed	163.62^{Ae}	118.57^{Cfg}	38.52^{Dg}	146.35^{Bcd}	4.43
Liriope platyphylla stem	5.96^{Bk}	7.53^{Am}	ND	ND	0.76
Lonicera japonica leaf	96.45^{Bg}	122.79^{Aef}	55.92^{Cf}	50.17^{Dg}	2.37
Mentha piperascens leaf	129.02^{Bf}	134.62^{Bde}	33.11^{Cgh}	219.07^{Aa}	7.24
Moringa stenopetala leaf	87.47^{gh}	91.45^{h}	ND	85.20^{e}	5.20
Morus bombycis fruit	182.65^{Ad}	129.94^{Cdef}	103.78^{Dd}	155.44^{Bcd}	10.23
Paeonia lactiflora root	233.49^{Aa}	192.65^{Bc}	117.96^{Cc}	ND	7.30
Petasites japonicus root	155.84^{Be}	138.69^{Cd}	3.45^{Di}	185.82^{Ab}	4.55
Pinus deniflora needle	195.45^{Ac}	137.26^{Cd}	64.55^{D}	179.20^{Bc}	4.45
Poncirus trifoliata fruit	50.93^{ABj}	56.82^{Aj}	33.10^{Cgh}	44.30^{Bgh}	3.74
Rubus coreanus fruit	239.46^{Aa}	232.30^{Aa}	124.32^{Bb}	228.61^{Aa}	13.85
Scutellaria baicalensis root	119.91^{Df}	212.34^{Ab}	181.84^{Ba}	136.93^{Cd}	8.43
Taraxacum coreanum flower	55.04^{Bj}	55.18^{Bj}	1.61^{Ci}	70.07^{Af}	3.89
Zanthoxylum piperitum seed	66.02^{Ai}	23.85^{Bl}	ND	67.18^{Af}	2.82
SEM	6.20	6.81	3.63	6.92	

Means in a row (different solvent extracts of the same target plant) followed by uppercase letters (A-D) are significantly different ($P<0.05$).
Means in a column (the same solvent extracts of different target plants) followed by lowercase letters (a-m) are significantly different ($P<0.05$).
ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); BHT, butylated hydroxytoluene; SEM, mean standard error (n=3); ND, not detected.

Based on these results, we identified seven effective plants in the ABTS radical scavenging ability from methanolic extract: *R. coreanus* fruit and *P. lactiflora* root> *C. tricuspidata* fruit> *P. deniflora* needle> *M. bombycis* fruit> *L. sativum* seed and *P. japonicus* root exhibiting the scavenging values of 239.46, 233.49, 213.87, 195.45, 182.65, 163.62, and 155.84 mg BHT equivalents/g, respectively. The *L. platyphylla* stem extract exhibited the least scavenging activity of 5.96 mg BHT equivalents/g, and the variation among methanolic extracts was approximately 40-fold. Among the ethanolic extracts, the most effective ($P<0.05$) plants exhibiting ABTS radical scavenging ability were: *R. coreanus* fruit> *S. baicalensis* root> *P. lactiflora* root> *P. japonicus* root> *P. deniflora* needle corresponding to 232.30, 212.34, 192.65, 138.69 and 137.26 scavenging activates in mg BHT equivalents/g, respectively. The least scavenging ability of <30 mg BHT equivalents/g was observed in the *Z. piperitum* seed and *L. platyphylla* stem extracts. Furthermore, the chloroform extracts exhibited significant ABTS radical scavenging activity ($P<0.05$) in the following five plants and ordered as *S. baicalensis* root> *R. coreanus* fruit> *P. lactiflora* root, *M. bombycis* fruit, and *B. juncea* seed with scavenging activates of 181.84, 124.32, 117.96, 103.78, and 98.57 mg BHT equivalents/g, respectively. However in this study, the *C. sinica* leaf, *C. officinalis* fruit, *L. platyphylla* stem, *M. stenopetala* leaf, and *Z. piperitum* seed chloroform extracts did not show ABTS radical scavenging activity. The five noticeably ($P<0.05$) potent extracts in ABTS radical scavenging capacity from water extracts were as follows *R. coreanus* fruit and *M. piperascens* leaf> *P. japonicus* root> *P. deniflora* needle> *M. bombycis* fruit, *L. sativum* seed, and S. *baicalensis* root with the scavenging values of 228.61, 219.07, 185.82, 179.20, 155.44, 146.35, and 136.93 mg BHT equivalents/g, respectively, and *P. lactiflora* root, *L. platyphylla* stem, and *C. officinalis* fruit showed no scavenging activity for ABTS radical.

FRAP antioxidant activity of explored plants

The FRAP assay is a simple and cost effective technique for determining the total antioxidant activity of samples. After quantifying the change in absorbance at 600 nm and comparing it to the standard, the antioxidant capacity of a given sample can be determined (Halvorsen et al., 2002). The FRAP assay determines whether an extract can donate an electron to Fe(III). The higher the FRAP value, the more potent the antioxidant activity of the sample. Dennog et al. (1999) demonstrated that FRAP can be used to determine the antioxidant status of individuals undergoing hyperbaric oxygen therapy. Table 5 summarizes the FRAP (mg QE/g) antioxidant activity of various solvent extracts of functional plants used in this study. Four of the solvents used in extraction caused a significant ($P<0.05$) variation in FRAP in eight of the 18

Table 5. Ferric reducing antioxidant power of various plant solvent extracts (unit: mg QE/g)

Explored plant	Various solvent extract				SEM
	Methanol	Ethanol	Chloroform	Water	
Brassica juncea seed	21.04^{ef}	20.64^{cde}	17.93^{b}	18.56^{e}	2.37
Caragana sinica leaf	18.85^{f}	19.41^{de}	18.57^{b}	18.99^{e}	2.10
Cornus officinalis fruit	69.20^{b}	71.54^{a}	68.83^{a}	ND	2.78
Cudrania tricuspidata fruit	21.79^{Aef}	18.77^{ABde}	18.54^{Bb}	19.04^{ABe}	1.56
Lepidium sativum seed	23.80^{Ae}	23.37^{Acd}	18.27^{Bb}	20.03^{ABe}	2.13
Liriope platyphylla stem	68.85^{b}	69.99^{a}	69.92^{a}	ND	3.09
Lonicera japonica leaf	4.13^{g}	3.82^{f}	4.08^{c}	3.84^{f}	0.19
Mentha piperascens leaf	71.87^{ABab}	72.97^{ABa}	69.00^{Ba}	77.87^{Aa}	3.87
Moringa stenopetala leaf	20.11^{ef}	20.52^{cde}	17.86^{b}	19.61^{e}	1.57
Morus bombycis fruit	21.29^{ef}	19.81^{de}	19.17^{b}	19.88^{e}	2.28
Paeonia lactiflora root	30.00^{Ad}	24.42^{Bc}	18.36^{Cb}	ND	1.68
Petasites japonicus root	73.71^{a}	74.30^{a}	68.26^{a}	74.71^{a}	3.78
Pinus deniflora needle	29.98^{Ad}	24.80^{Bc}	18.98^{Cb}	25.56^{Bd}	1.89
Poncirus trifoliata fruit	18.42^{f}	18.23^{e}	18.10^{b}	18.29^{e}	2.11
Rubus coreanus fruit	46.32^{Bc}	52.05^{Ab}	18.40^{Db}	40.44^{Cc}	2.05
Scutellaria baicalensis root	21.21^{ef}	19.10^{de}	17.91^{b}	22.19^{de}	2.16
Taraxacum coreanum flower	18.90^{f}	19.43^{de}	18.25^{b}	20.58^{e}	1.78
Zanthoxylum piperitum seed	69.66^{ab}	70.20^{a}	68.59^{a}	67.44^{b}	3.28
SEM	2.45	2.47	2.29	2.43	

Means in a row (different solvent extracts of the same target plant) followed by uppercase letters (A–D) are significantly different (P<0.05).
Means in a column (the same solvent extracts of different target plants) followed by lowercase letters (a–g) are significantly different (P<0.05).
QE, quercetin equivalents; SEM, standard mean error (n=3); ND, not detected.

plant materials examined.

Subsequently, we considered the FRAP between various solvent extracts of each plant studied. The methanolic extracts of *C. tricuspidata* fruit, *P. lactiflora* root, and *P. deniflora* needle, ethanolic extract of *R. coreanus* fruit, and water extract of *M. piperascens* leaf had higher FRAPs (P<0.05) (mg QE/g) than their counter solvent extracts. Similarly, strong (P<0.05) FRAP activity was exhibited in three methanolic, ethanolic, and water extracts of *L. sativum* seed than in the chloroform extract. Likewise, plant materials of *C. officinalis* fruit and *L. platyphylla* stem, methanolic, ethanolic, and chloroform extracts had an equivalently strong (P<0.05) FRAP than water extracts that did not exhibit FRAP antioxidant activity. In contrast, the FRAP assay results revealed that there was no significant (P>0.05) difference among various solvent extracts of *B. juncea* seed, *C. sinica* leaf, *L. japonica* leaf, *M. stenopetala* leaf, *M. bombycis* fruit, *P. japonicus* root, *P. trifoliata* fruit, *S. baicalensis* root, *T. coreanum* flower, and *Z. piperitum* seed. In addition, solvents that resulted in a significant variation in FRAP antioxidant activity in the plants were further analyzed. We observed an increase in the variation where methanol> ethanol> chloroform and water had 6, 4, 2, and 2 active extracts, respectively.

In this study, the highest absorbance (>50.00 mg QE/g) of FRAP from methanolic extracts was observed in the following five plants: *P. japonicus* root> *M. piperascens* leaf and *Z. piperitum* seed> *C. officinalis* fruit and *L. platyphylla* stem with a reducing power of 73.71, 71.87, 69.66, 69.20, and 68.85 mg QE/g, respectively. Furthermore, the lowest antioxidant activity (<20 mg QE/g) in methanolic extracts was observed in *C. sinica* leaf, *L. japonica* leaf, *P. trifoliata* fruit, and *T. coreanum* flower. The range in ethanolic extract FRAP values was 74.30 to 3.82, which had an almost 19-fold variation. Likewise, from ethanolic extracts, substantial (P<0.05) FRAP antioxidant ability was exhibited in the following five plants: *P. japonicus* root, *M. piperascens* leaf, *C. officinalis* fruit, *Z. piperitum* seed, and *L. platyphylla* stem with corresponding values of 74.30, 72.97, 71.54, 70.20, and 69.99 mg QE/g, respectively; however, *L. japonica* leaf extract had the lowest performance of all the ethanolic extracts. The FRAP antioxidant capacity of chloroform extracts ranged from 69.92 to 4.08 mg QE/g, with an approximately 17-fold variation. Among the plants explored, an extensive (P< 0.05) FRAP antioxidant capacity from chloroform extracts was exhibited in the following five plant materials: *L. platyphylla* stem, *M. piperascens* leaf, *C. officinalis* fruit, *Z. piperitum* seed, and *P. japonicus* root with FRAP values of 69.92, 69.00, 68.83, 68.59, and 68.26 mg QE/g respectively, and the lowest FRAP activity was presented by *L. japonica* leaf extract. From water extracts, several plant materials had noticeably (P<0.05) potent FRAPs in the order: *M. piperascens* leaf and *P. japonicus* root> *Z. piperitum* seed> *R. coreanus* fruit> *P. deniflora* needle corresponding with 77.87, 74.71, 67.44, 40.44, and 25.56 mg QE/g, re-

spectively. The water extract of *L. japonica* leaf exhibited the lowest FRAP value of 3.840 mg QE/g, while *P. lactiflora* root, *L. platyphylla* stem, and *C. officinalis* fruit had no FRAP antioxidant activity.

In this study, methanol and ethanol were found to be more effective than chloroform and water in isolating total phenolic content and total flavonoid active compounds and active antioxidant compounds such as ABTS radical scavenging activity and FRAP. The DPPH radical scavenging activity of ethanol and water extracts was notably high. *M. piperascens* leaf extracts exhibited superior total phenolic content (chloroform and water extracts), total flavonoid content (all solvent extracts), DPPH radical scavenging activity (methanol, ethanol, and water extracts), ABTS radical scavenging activity (water extract), and FRAP radical scavenging activity (water extract) (in all solvent extracts). Similarly, *Petasites japonicas* root was another extract with a high total flavonoid content (in all solvent extracts), DPPH and ABTS radical scavenging activity (in methanol, ethanol, and water extracts), and FRAP activity (in methanol, ethanol, and water extracts) (in all solvent extracts). *R. coreanus* fruit with the highest total phenolic content (methanol, ethanol, and water extracts), DPPH and ABTS radical scavenging activate (in all solvent extracts), and FRAP in its water extract were the two most effective functional plants. Due to the abundance of antioxidant chemical constituents and the most potent antioxidant activities demonstrated in this study, extracts from *M. piperascens* leaf, *P. japonicas* root, and *R. coreanus* fruit are recognized as promising functional plants for meeting the current demand for natural preservatives in the food and pharmaceutical industries.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SNK, SS. Analysis and interpretation: SNK, SS. Data collection: SS, AA. Writing the article: all authors. Critical revision of the article: SNK, SS, Final approval of the article: all authors. Statistical analysis: SNK, SS. Obtained funding: SNK. Overall responsibility: SNK.

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