

Correlation between Solid Content and Antioxidant Activities in Umbelliferae Salad Plants

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ABSTRACT: The aim of this study is to evaluate the antioxidant properties of 70% methanolic extracts and the correlation between several antioxidant activities in selected Umbelliferae plants, based on total phenolic content (TPC) and total flavonoid content (TFC). For Umbelliferae plants extracts, the IC₅₀ of DPPH radical (100 μM) quenching activities for extract, TPC, and TFC were 39~179 μg dry weight (DW)/mL, 14.08~38.11 μg TPC/mL, and 0.36~1.51 μg TFC/mL, respectively. The oxygen radical absorbance capacity (ORAC) of extracts ranged from 11.44 to 42.88 mg Trolox equivalent (TE)/g DW extract, whereas ORAC for TPC and TFC was 47.40~240.19 mg TE/g and 0.72~11.22 g TE/g, respectively. The TPC had a superior linear correlation ($r^2=0.817$) with 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) values. Of the 14 Umbelliferae plant extracts, *Sanicula rubiflora*, *Sanicula chinensis*, *Torilis japonica*, *Torilis scabra*, and *Angelica fallax* showed the strongest antioxidant activity.

Keywords: antioxidant activity, correlation, total flavonoid content, total phenolic content, Umbelliferae

INTRODUCTION

Umbelliferae (Apiaceae) is widely distributed throughout the world, from polar regions to subtropical regions, and is particularly abundant in temperate zones of the northern hemisphere (Sayed-Ahmad et al., 2017). Approximately 3,780 species in Umbelliferae include caraway, carrot, celery, chervil, coriander, cumin, dill, fennel, hemlock, parsley, parsnip, and sea holly, which are important in the production and consumption in food industry (Chereng et al., 2008). Various Umbelliferae plants, including *Angelica dahurica* (Fisch.) Benth. & Kook.f., *Angelica decursiva* (Miq.) Franch. & Sav., *Bupleurum chinense* DC., *Cnidium monnieri* (L.) Cusson, and *Oenanthe javanica* (Blume) DC inhabit in Korea (Wiar, 2012).

Traditional Korean food is primarily prepared using a variety of vegetables; in particular, this includes the fermented food *Kimchi* and the non-fermented salad *Namul*. *Angelica decursiva*, *Bupleurum longiradiatum*, *Coriandrum sativum*, *Cryptotaenia japonica*, *Daucus carota* subsp. *sativus*, *Ostericum koreanum*, and *Sanicula chinensis* belong to the family Umbelliferae, and have been used in various *Kimchis* and *Namuls*. In addition, the several Umbelliferae plants contain the potent anti-oxidants carotenoids, fla-

vonoids, and various polyphenols, which have several physiological activities (Lee et al., 2011a; Sayed-Ahmad et al., 2017).

Excessive amounts of extracellular and intracellular reactive oxygen species (ROS) are produced during metabolism and can modify DNA/RNA in the cell; these modifications can lead to mutations or cancer. ROSs have also been implicated in the early stages as well as in the progression of diseases (Pham-Huy et al., 2008). To maintain optimal ROS levels is considered an important factor for maintaining health and preventing diseases. Many investigations have been carried out to understand the antioxidant efficacy of several plant extracts for removing ROS (Mahdi-Pour et al., 2012; Fernandes et al., 2016). Most antioxidative studies are based on total phenolic content (TPC) and total flavonoid content (TFC) in plant extracts, and the scavenging activities of free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS), oxygen radical absorbance capacity (ORAC), and hydroxyl oxygen radical averting capacity (HORAC). However, there has been very few reports on the effects of TPC and TFC on the antioxidant activity of extracts (Mahdi-Pour et al., 2012; Fernandes et al., 2016).

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Antioxidant activity of plants is mostly due to the amount and type of polyphenolic and flavonoid compounds contained in them (Prior et al., 1998; Di Majo et al., 2008). The specificity of antioxidant activity in plant resources is determined by the amount and type of polyphenolic and flavonoid compounds due to climate and soil conditions in certain habitats (Moore et al., 2006; Liu et al., 2016). Therefore, it is imperative to show that the antioxidant activity of a plant extract depends on the amount of polyphenols or flavonoids.

The purpose of this study is to measure the amount of TPC and TFC and to evaluate the antioxidant activities of Korean 14 Umbelliferae plants by a correlation between TPC (or TFC) and the antioxidant activities. These results could be applied to develop health enhancing foods and cosmetics containing antioxidative substances from Umbelliferae plants.

MATERIALS AND METHODS

Chemicals and reagents

Dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, DPPH, ascorbic acid, pyrogallol, ABTS, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and fluorescein disodium salt were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ethanol and other reagents were used as first grade reagents.

Plant materials

The extracts of 14 Umbelliferae plants were obtained from the Korea Plant Extract Bank, Daejeon, Korea. For preparation of extracts, plants were washed, freeze dried, and crushed. Crushed plants were extracted with 70% methanol (in water). Extracts were filtered through Whatman No. 1 filter paper and concentrated using an evaporator under reduced pressure. Extracts were re-dissolved in DMSO to a concentration of 100 mg/mL, stored at -20°C , and used as a stock solution.

Measurement of TPC and TFC

TPC was determined using the Folin-Ciocalteu method (Oh et al., 2004), with modifications. Each Umbelliferae extract was dissolved in 1 mL of 1 N Folin-Ciocalteu reagent and was incubated for 5 min. Then, 2 mL of 20% (w/v) Na_2CO_3 was added to the mixture. After 10 min at room temperature, the mixture was centrifuged at 15,000 g for 1 min, and the absorbance of the supernatant was measured on 765 nm using a spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK). Gallic acid was used to plot a standard calibration curve. The TPC content of extracts was expressed as mg of gallic acid equivalents (GAE)/g dry weight (DW).

Analysis of TFC was performed using the colorimetric Dowd method (Zhishen et al., 1999), with modifications. Extracts were added to a test tube containing distilled water (1.25 mL) and 5% (w/v) NaNO_2 (75 mL), and the mixture was incubated for 5 min. Then, 0.15 mL of 10% (w/v) $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added to the mixture. After 6 min at room temperature, 0.5 mL of 1 M NaOH was added, and the mixture was diluted with 0.275 mL distilled water. The absorbance of the mixture was measured immediately at 510 nm using a UV-spectrophotometer (Libra S22, Biochrom Ltd.). The TFC content of extracts was expressed as mg of catechin equivalents (CE)/g DW.

Antioxidant assay

DPPH radical scavenging assay: The DPPH radical scavenging activity of Umbelliferae extracts was measured by Lee and colleagues' method with modifications (Lee et al., 2005). Methanolic extracts (0.2 mL) were mixed with 4 mL of methanol, and then methanolic solution of DPPH (1 mM, 0.5 mL) was added. This mixture was vortexed for 15 s, incubated at room temperature for 30 min, and the absorbance was measured on 517 nm using UV-spectrophotometry (Libra S22, Biochrom Ltd.).

Measurement of reducing capacity: The reducing capacity of Umbelliferae extracts was determined by Fe^{3+} reduction (Oyaizu, 1986). Extracts (1 mg/mL) in distilled water were first mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) $\text{K}_3\text{Fe}(\text{CN})_6$. The mixture were then incubated at 50°C for 20 min. Next, 2.5 mL of 10% (w/v) trichloroacetic acid was added and the mixture was centrifuged at 2,090 g for 10 min. Supernatant solution (2.5 mL) was then added to 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) FeCl_3 . The absorbance of the mixture was measured at 700 nm using UV-spectrophotometry (Libra S22, Biochrom Ltd.).

ABTS radical scavenging assay: The ABTS $^{+\cdot}$ free radical scavenging activity of Umbelliferae extracts was measured using the methods of Thaipong et al. (2006), with some modifications. Assays were performed using the ABTS $^{+\cdot}$ cation decolorization reaction (the blue-green color). To generate ABTS $^{+\cdot}$ in phosphate-buffered saline (pH 7.4), the stock solution was prepared to have 7 mM ABTS and 2.45 mM potassium persulfate and was allowed to react for 24 h at room temperature in the dark. Then, the dark blue-green colored ABTS $^{+\cdot}$ radical solution was diluted to obtain an absorbance of 0.70 (± 0.02) on 732 nm using the spectrophotometer (Libra S22, Biochrom Ltd.). Fresh ABTS $^{+\cdot}$ solution was prepared for each assay. Umbelliferae extracts (10 μL) were incubated with 190 μL of the ABTS $^{+\cdot}$ solution for 30 min in the dark. The absorbance of the reactant was determined at 734 nm using a spectrophotometer. Trolox was used as a standard for the ABTS assay. Results were expressed as mg of Trolox equivalents (TE)/g DW by comparing the slope for

ABTS^{•+} scavenging to Trolox.

ORAC assay: ORAC assays were performed based on a previous method (Čiž et al., 2010), with a few modifications. The ORAC assay measures the peroxy radical antioxidant scavenging activity induced by AAPH at 37°C. Fluorescein was used as a fluorescent probe. Loss of the fluorescence of fluorescein indicates the peroxy radical production (Gomes et al., 2005). Fluorescein (70 nM) and other reagents were prepared in phosphate buffer (75 mM, pH 7.4). One-hundred and seventy microliters of fluorescein solution (60 nM final concentration) and 10 µL of sample were placed in wells of a microplate (clear bottom, black plate) and incubated at 37°C for 30 min. AAPH (20 µL, 50 mM final concentration) was then added using a multichannel pipette to initiate the reaction. The fluorescence was recorded at 460 and 550 nm for excitation and emission, respectively, at every 5 min, and the microplate was automatically shaken prior to each reading in an enzyme-linked immunosorbent assay plate reader (Spark[®] 10M, Tecan, Grödig, Austria) at 37°C. For the blank, phosphate buffer was used instead of sample, and Trolox was used as an antioxidant standard for plotting the calibration curve to quantitate oxygen radical absorbance capacity in each assay. The final ORAC values were calculated using a regression equation between the Trolox concentration and the net area under the curve (AUC). The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as mg TE/g DW of Umbelliferae extract (Lee and Lee, 2014).

HORAC assay: The HORAC assay (Lee and Lee, 2014) measures the metal-chelating activity of antioxidants under conditions of Fenton-like reactions using a Co(II) complex; hence, HORAC assays measure the ability to protect against hydroxyl radical formation. A hydrogen peroxide solution (0.55 M) was prepared in distilled water. Co(II) was prepared as follows: 15.7 mg of CoF₂·4H₂O and 20 mg of picolinic acid were dissolved in 20 mL of distilled water. Fluorescein 170 µL (60 nM, final concentration) and 10 µL of sample were incubated for 10 min at 37°C in the dark. Then, 10 µL of H₂O₂ (27.5 mM, final concentration) and 10 µL of Co(II) were added to each well. The fluorescence was recorded at 460 and 550 nm for excitation and emission, respectively, every 5 min for 1 h; the microplate was automatically shaken prior to each reading. Phosphate buffer solution was used as a blank. Gallic acid solutions were used to plot a standard curve. AUC values were calculated in the same manner as for the ORAC assay. Final HORAC values were calculated using regression analysis between the gallic acid concentration and the net area under the curve. The HORAC of the sample was expressed as mg of GAE/g DW of Umbelliferae plant extract.

Statistical analysis

SPSS 24.0 (IBM SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All results were expressed as mean ± standard deviation (SD) in at least triplicate and were analyzed using one way analysis of variance (ANOVA) and Duncan's multiple comparison test for individual comparisons. The correlation between TPC, TFC, and antioxidant activity is presented by Pearson correlation coefficient. Results were considered statistically significant when *P*-values were below 0.05.

RESULTS AND DISCUSSION

TPC and TFC in Umbelliferae plant extracts

The TPC contents of the extracts of the selected 14 Umbelliferae plants cultivated in Korea, are tabulated in Table 1. Extracts of *S. chinensis* and *Sanicula rubiflora* had the highest TPC contents of the selected Umbelliferae extracts (587.5 and 401.3 mg GAE/g DW, respectively). The TPC content of *S. chinensis*, *S. rubiflora*, *Angelica fallax*, *B. longiradiatum*, *C. japonica*, *Glehnia littoralis*, *Ostericum koreanum*, *Torilis japonica*, and *Torilis scabra* extracts showed more than 300 mg GAE/g DW. However, *C. sativum* had the lowest TPC content (71.5 mg GAE/g DW). The TPC contents of *A. decursiva*, *B. longiradiatum*, *C. japonica*, *D. carota*, *O. koreanum*, *Pimpinella brachycarpa*, and *T. japonica* have been reported in several previous studies (Croft, 1998; Robbins, 2003; Tsao, 2010; Ghasemzadeh and Ghasemzadeh, 2011; Del Rio et al., 2013). However, the comparison of TPC contents between the present study and earlier studies is not possible since the methods used to prepare the plant extracts, extraction solvents, and equivalent compounds for quantification are different in each other.

More than 8,000 phenolic plant compounds exist as secondary metabolites in plants and have various physiological activities (Tsao, 2010), many of which have been developed as pharmaceuticals. Phenolic compounds are considered to be natural substances with health-improving functions, which eliminate active oxygen free radicals and reduce the risk of inflammation and cancers (Ghasemzadeh and Ghasemzadeh, 2011). Phenolic compounds have at least one aromatic ring with one or more hydroxyl groups and are classified as flavonoids and non-flavonoids (Del Rio et al., 2013). Therefore, the TPC values observed in the present study indicate the total amount of flavonoids and non-flavonoids phenolic compounds in each Umbelliferae plant extract.

The TFC contents of the 14 Umbelliferae plant extracts are shown in Table 1. *S. chinensis* extract had the highest TPC (587.5 mg GAE/g DW) and TFC (31.4 mg CE/g DW) contents. The TFC values of *A. fallax*, *C. japonica*, *S. chinensis*, *S. rubiflora*, *T. japonica*, and *T. scabra* extracts were

Table 1. Total phenolic and flavonoid contents of Umbelliferae plant extracts

Umbelliferae	Korean name	Voucher no. ¹⁾	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	Flavonoids ratio ²⁾
<i>Angelica decursiva</i>	Badi-namul	PB4036.1	113.0±12.0 ^{de}	2.3±0.5 ^g	2.0
<i>Angelica fallax</i>	Sayakchae	PB4038A.1	325.3±11.4 ^b	20.9±2.8 ^d	6.4
<i>Bupleurum longerradiatum</i>	Gaesihoh	PB3994.1	358.9±15.2 ^b	14.2±1.6 ^e	4.0
<i>Coriandrum sativum</i>	Gosuh	PB4005.1	71.5±1.8 ^e	10.0±4.3 ^f	14.0
<i>Cryptotaenia japonica</i>	Paddeuk-namul	PB4006.2	364.8±73.0 ^b	24.1±1.8 ^{bc}	6.6
<i>Daucus carota var. sativa</i>	Carrot	PB4059.1	151.2±17.7 ^{de}	4.5±0.0 ^g	3.0
<i>Glehnia littoralis</i>	Gaekbangpung	PB4027.4	361.0±29.3 ^b	9.2±0.3 ^f	2.5
<i>Ostericum koreanum</i>	Gangwhal	PB4048.1	360.6±37.0 ^b	3.5±0.2 ^g	1.0
<i>Ostericum sieboldii</i>	Muecminari	PB4049.1	212.9±11.4 ^{cd}	5.4±0.8 ^g	2.5
<i>Pimpinella brachycarpa</i>	Cham-namul	PB4010.2	184.1±32.9 ^d	8.9±0.5 ^f	4.8
<i>Sanicula chinensis</i>	Chambandi	PB3996.1	587.5±12.5 ^a	31.4±1.3 ^a	5.3
<i>Sanicula rubiflora</i>	Red chambandi	PB3997.1	401.3±67.6 ^b	26.6±0.6 ^b	6.6
<i>Torilis japonica</i>	Sasangza	PB4003.2	361.0±39.2 ^b	22.1±2.0 ^{cd}	6.1
<i>Torilis scabra</i>	Gaexasangza	PB4004.2	322.0±5.3 ^{bc}	20.0±1.2 ^d	6.2

¹⁾Korea Plant Extract Bank (KPEB), Daejeon, Korea.

²⁾Flavonoids ratio = (TFC/TPC) × 100.

TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; DW, dry weight; CE, catechin equivalent. Values followed by the different letters (a-h) in the same column are significantly different ($P < 0.05$).

higher than 20 mg CE/g DW. However, the TFC levels were lowest in the *A. decursiva* and *O. koreanum* extracts (2.3 and 3.5 mg CE/g DW, respectively). Flavonoids are a group of phenolic compounds synthesized through the phenylpropanoid pathway from phenylalanine, and consist of approximately 4,000 compounds, such as quercetin, lutein, apigenin, and baicalein (Tsao, 2010). All flavonoids have a basic C6-C3-C6 structural skeleton, which is composed of two aromatic C6 rings and one heterocyclic ring with one oxygen atom (Tsao, 2010; Ghasemzadeh and Ghasemzadeh, 2011; Del Rio et al., 2013). The main subclasses of flavonoids are flavonols, flavones, isoflavones, flavanones, anthocyanidins, flavan-3-ols, and dihydrochalcones (Tsao, 2010; Ghasemzadeh and Ghasemzadeh, 2011; Del Rio et al., 2013).

C. sativum extracts had the highest TFC/TPC ratio (14.0%), and *A. fallax*, *C. japonica*, *S. rubiflora*, *T. japonica*, and *T. scabra* extracts all showed TFC/TPC ratios >6.0%. On the other hand, *O. koreanum* and *A. decursiva* had the lowest TFC/TPC ratios (1.0 and 2.0%, respectively). TFC/TPC ratios indicate comparative levels of flavonoids to total phenolic acid in the extracts. Therefore, TPC, excluding TFC, indicates only non-flavonoids, such as gallic acid, chlorogenic acid, and cinnamic acid. In 14 Umbelliferae plant extracts, the ratio of the maximum to minimum TPC was 8.21, and the ratio of the maximum to minimum TFC was 13.6. The difference for the amount of TPC and TFC in extracts results in variation in the ABTS radical scavenging activity, ORAC, and HORAC of Umbelliferae plant extracts.

Antioxidative activities of Umbelliferae plant extracts

DPPH radical scavenging activity: To evaluate the antioxidant activity of the Umbelliferae plant extracts, DPPH

radical scavenging activity was measured (Table 2). *G. littoralis* extracts (300 µg/mL) were able to quench DPPH radicals (100 mM) by 84.1%. Several of the extracts (300 µg/mL) were able to quench DPPH radical (100 mM) by over 80%; in decreasing order of magnitude: *G. littoralis* (84.1%) > *A. fallax* (82.7%) > *B. longerradiatum*, *S. rubiflora*, and *T. scabra* (82.4%) > *T. japonica* (82.0%) > *O. koreanum* (81.6%). In contrast, *D. carota* and *C. sativum* showed the weakest DPPH radical scavenging activity (46.4% and 37.6%, respectively). Umbelliferae plant extracts exhibited half-maximal inhibitory concentration (IC₅₀) values for quenching DPPH radicals (100 mM) in the range of 39~179 µg DW/mL. IC₅₀ values for *A. decursiva*, *C. sativum*, and *D. carota* were not analyzed because their DPPH radical scavenging activities were <50%. *T. japonica*, *S. chinensis*, *T. scabra*, and *S. rubiflora* extracts showed the most potent DPPH radical quenching activities, exhibiting IC₅₀ values of 39, 48, 49, and 50 µg DW/mL, respectively.

The antioxidant activities of plant extracts are dependent on various phenolic compounds, including flavonoids. The IC₅₀ values observed for the DPPH radical scavenging activity are estimated differently by the levels of TPC and TFC in the Umbelliferae plant extracts; these contents were calculated to be 14.08~38.11 µg TPC/mL and 0.36~1.51 µg TFC/mL, respectively (excluding *A. decursiva*, *C. sativum*, and *D. carota*) (Table 2). *T. japonica* showed the most potent DPPH radical scavenging activity with an IC₅₀ of 14.08 µg TPC/mL. On the other hand, *G. littoralis*, *O. koreanum*, and *Ostericum sieboldii* extracts showed relatively low anti-oxidative activities, with IC₅₀ values of 33.93, 36.78, and 38.11 µg TPC/mL, respectively. In addition, *O. koreanum* had the lowest IC₅₀ (0.36 µg TFC/mL), which suggests that the flavonoids in *O. koreanum*

Table 2. DPPH radical scavenging activity of Umbelliferae plant extracts

Umbelliferae	DPPH radical scavenging activity ¹⁾			
	% Control ²⁾	IC ₅₀		
		Extract ($\mu\text{g DW/mL}$)	TPC ³⁾ ($\mu\text{g TPC/mL}$)	TFC ³⁾ ($\mu\text{g TFC/mL}$)
<i>Angelica decursiva</i>	49.5 \pm 3.2 ^f	NT	NT	NT
<i>Angelica fallax</i>	17.3 \pm 0.3 ^{ab}	65.8	21.40	1.38
<i>Bupleurum longiradiatum</i>	17.4 \pm 0.4 ^{ab}	79	28.35	1.12
<i>Coriandrum sativum</i>	62.4 \pm 3.4 ^h	NT	NT	NT
<i>Cryptotaenia japonica</i>	23.0 \pm 0.6 ^c	61	22.25	1.47
<i>Daucus carota var. sativa</i>	53.6 \pm 1.1 ^g	NT	NT	NT
<i>Glehnia littoralis</i>	15.9 \pm 1.0 ^a	94	33.93	0.86
<i>Ostericum koreanum</i>	18.4 \pm 0.3 ^{ab}	102	36.78	0.36
<i>Ostericum sieboldii</i>	35.2 \pm 0.6 ^e	179	38.11	0.97
<i>Pimpinella brachycarpa</i>	20.1 \pm 1.0 ^b	122	22.46	1.09
<i>Sanicula chinensis</i>	26.4 \pm 1.7 ^d	48	28.20	1.51
<i>Sanicula rubiflora</i>	17.6 \pm 0.9 ^{ab}	50	20.07	1.33
<i>Torilis japonica</i>	18.0 \pm 0.0 ^{ab}	39	14.08	0.86
<i>Torilis scabra</i>	17.6 \pm 1.0 ^{ab}	49	15.78	0.98

¹⁾IC₅₀ for DPPH radical scavenging activity were measured in 0~300 $\mu\text{g/mL}$ of extract.

²⁾DPPH radical (100 mM) was quenched by extracts (300 $\mu\text{g/mL}$).

³⁾IC₅₀ of TPC and TFC were derived from IC₅₀ of extract.

IC₅₀, the half maximal inhibitory concentration; DW, dry weight; TPC, total phenolic content; TFC, total flavonoid content; NT, not tested.

Values followed by the different letters (a-h) in the same column are significantly different ($P < 0.05$).

have potent DPPH radical scavenging activity. Thus, the IC₅₀ expressed as TPC or TFC, is much lower than that expressed as whole extract contents and indicates that total polyphenol or total flavonoid in the extract is the major substance with potent anti-oxidative DPPH radical scavenging activity. Since many flavonoids and phenolic compounds in plant extracts mediate antioxidant activity, the DPPH radical scavenging activity might be more appropriately expressed as IC₅₀ of TPC or TFC rather than that of total extract content.

Several earlier studies have reported the DPPH radical scavenging activity of Umbelliferae plant extracts. In a previous study, *B. longiradiatum*, *C. sativum*, *O. koreanum*, *P. brachycarpa*, and *T. japonica* have been shown to have DPPH radical scavenging activities (IC₅₀) of 96.5, 161, 70, 257, and 58 $\mu\text{g/mL}$, respectively (Lee et al., 2011a; Lee et al., 2011b). Considering the total DPPH radical scavenging activities and IC₅₀ values in the previous and present studies (Table 2), *S. chinensis*, *S. rubiflora*, *T. japonica*, and *T. scabra* have comparatively more potent antioxidant activities.

Reducing capacity and ABTS radical scavenging activity: The reducing capacity of Umbelliferae plant extracts is shown in Table 3; the pattern of reducing capacity was similar to that of DPPH radical scavenging activity. In 14 Umbelliferae plant extracts, *S. rubiflora*, *S. chinensis*, and *A. fallax* showed the highest reducing capacity (> 0.5 on A_{700}). In contrast, *C. sativum*, *C. japonica*, and *D. carota* showed the lowest reducing capacity (0.11 on A_{700}).

The reducing capacity of plant extracts indicate the presence of reductones, which have an antioxidant effect through donating hydrogen atoms and breaking free radical chains (Duh, 1998). Reductones are involved in a reaction with peroxide precursors, thereby preventing formation of peroxides (Guo and Wang, 2007). In a previous study, the essential oil of *A. decursiva* has been reported to have a reducing capacity of 0.35 on A_{700} (Lim and Shin, 2012), which is higher than the reducing capacity observed in this study. One of the reasons for the difference may be the variation between the composition of the essential oil and extract used in the antioxidant experiments.

The ABTS radical scavenging activities of the Umbelliferae plant extracts were calculated using plots on a straight line derived from the Trolox standard (Table 3). Umbelliferae plant extracts had ABTS radical scavenging activities of 367.5~986.2 mg TE/g DW. In this study, the Umbelliferae plant extracts with >900 mg TE/g DW for ABTS radical scavenging activity were (in decreasing order of magnitude): *S. chinensis* $>$ *S. rubiflora* $>$ *B. longiradiatum* $>$ *C. japonica*. With respect to the TPC and TFC, the Umbelliferae plant extracts had ABTS radical scavenging activity in the range of 1,678.64~5,336.13 mg TE/g and 53.46~1,991.28 g TE/g, respectively.

ABTS scavenging activity of Umbelliferae plant extracts have been estimated in diverse units in previous studies. In one study, the ABTS scavenging activity for *A. decursiva*, *B. longiradiatum*, and *D. carota* were expressed in percent

Table 3. Reducing capacity and ABTS radical scavenging activity of Umbelliferae plant extracts

Umbelliferae	Reducing capacity ¹⁾ (A ₇₀₀)	ABTS radical scavenging activity		
		Extract (mg TE/g DW)	TPC (mg TE/g)	TFC (g TE/g)
<i>Angelica decursiva</i>	0.16±0.00 ^{hi}	517.5±10.3 ^e	4,579.94±90.82 ^b	1,991.28±39.49 ^a
<i>Angelica fallax</i>	0.52±0.06 ^c	850.9±43.9 ^b	2,615.64±135.03 ^d	125.15±6.46 ^h
<i>Bupleurum longiradiatum</i>	0.32±0.03 ^f	914.2±55.2 ^{ab}	2,547.23±153.93 ^{de}	179.38±10.84 ^g
<i>Coriandrum sativum</i>	0.11±0.00 ⁱ	381.5±15.0 ^f	5,336.13±209.95 ^a	533.61±20.99 ^c
<i>Cryptotaenia japonica</i>	0.11±0.00 ⁱ	900.2±98.2 ^b	2,467.65±269.31 ^{de}	102.39±11.17 ^h
<i>Daucus carota var. sativa</i>	0.11±0.01 ⁱ	367.5±12.9 ^f	2,430.78±85.04 ^{de}	540.17±18.90 ^c
<i>Glehnia littoralis</i>	0.35±0.01 ^{ef}	746.9±51.6 ^c	2,068.88±143.01 ^{fg}	224.88±15.54 ^f
<i>Ostericum koreanum</i>	0.35±0.03 ^{ef}	865.5±11.6 ^b	2,400.26±32.02 ^{de}	685.79±9.15 ^b
<i>Ostericum sieboldii</i>	0.19±0.00 ^h	520.9±71.1 ^e	2,446.53±333.77 ^{de}	453.06±61.81 ^d
<i>Pimpinella brachycarpa</i>	0.26±0.02 ^g	536.9±20.4 ^e	2,916.17±110.97 ^c	327.66±12.47 ^e
<i>Sanicula chinensis</i>	0.63±0.03 ^b	986.2±58.4 ^a	1,678.64±99.43 ^h	53.46±3.17 ⁱ
<i>Sanicula rubiflora</i>	0.78±0.05 ^a	924.9±40.9 ^{ab}	2,304.68±101.81 ^{ef}	86.64±3.83 ^{hi}
<i>Torilis japonica</i>	0.45±0.03 ^d	863.5±35.0 ^b	2,392.06±96.81 ^{de}	108.24±4.38 ^h
<i>Torilis scabra</i>	0.38±0.03 ^e	619.5±22.7 ^d	1,924.02±70.64 ^{gh}	96.20±3.53 ^h

¹⁾Reducing capacity was measured using 1 mg/mL of extract.

TPC, total phenolic content; TFC, total flavonoid content; TE, Trolox equivalent; DW, dry weight.

Values followed by the different letters (a-i) in the same column are significantly different ($P<0.05$).

(36%, 60%, and 83%, respectively) (Lee et al., 2011a), whereas in another study the ABTS scavenging activity of *C. japonica* was expressed in $\mu\text{M TE}/100\text{ g DW}$ (82 $\mu\text{M TE}/100\text{ g DW}$) (Yao et al., 2010). The ABTS radical scavenging activities of the Umbelliferae plants in this study (Table 3) were observed to be higher than those reported previously.

ORAC and HORAC of Umbelliferae plant extracts: The peroxyl radical scavenging activities of the Umbelliferae plant extracts were measured using ORAC and HORAC assays. The ORAC values ranged from 11.44 to 42.88 mg TE/g DW extract (Table 4), as follows; *S. rubiflora* (42.88 mg TE/g DW) > *S. chinensis* (41.64 mg TE/g DW) > *D. carota* (36.32 mg TE/g DW) > *T. japonica* (35.12 mg TE/g DW) > *B. longiradiatum* (32.66 mg TE/g DW) > *T. scabra* (30.86 mg TE/g DW) > *O. sieboldii* (30.15 mg TE/g DW). With respect to the TPC and TFC contents, the Umbelliferae plant extracts had ORAC values in the range of 47.40~240.19 mg TE/g and 0.72~11.22 g TE/g, respectively.

The HORAC values of the Umbelliferae plant extracts (excluding *C. sativum*, which was not detected in the assay) ranged from 1.55 to 38.05 mg GAE/g DW extract (Table 4). *S. chinensis*, *S. rubiflora*, and *T. japonica* had potent HORAC values of 38.05, 37.90, and 34.80 mg GAE/g DW extract, respectively. With respect to the TPC and TFC content, the HORAC values ranged from 8.93~171.91 mg GAE/g TPC and 0.09~5.78 g GAE/g TFC, respectively. Judging by the ORAC and HORAC values observed, *S. chinensis* and *S. rubiflora* had the highest antioxidant activity of the Umbelliferae plant extracts tested in this study. From the studies on the antioxidant activity of plant foods, the ABTS activity and ORAC for 27 kinds

of vegetables was reported as 4.1~49.7 $\mu\text{mol TE/g}$ (Gorinstein et al., 2009); for *Peucedanum japonicum* Thunberg roots were reported as 42.24~50.55 $\mu\text{mol TE/g}$ and 58.10~133.37 $\mu\text{mol/g}$, respectively (Lim et al., 2019); and for *Chrysanthemum boreale* extract were reported as 0.47 mmol/mg and 94.34 $\mu\text{mol TE/g}$, respectively (Kim et al., 2014). In this study, the ABTS activity of *S. chinensis* was 3.94 mmol TE/g, indicating a higher antioxidant activity the vegetables and plants mentioned above. However, the ORAC of *S. rubiflora* was higher (0.22 mmol GAE/g), but it should not be directly compared due to the use of different standards.

Generally, the ABTS assay has been widely used for analysis of the total antioxidant capacity of plant extracts, but it is inadequate for assessing antioxidant activity *in vivo* (Scalzo et al., 2005; Lee and Kim, 2015). ORAC and HORAC methods can measure antioxidant activity according to the concentration of the antioxidant and the reaction time, are more sensitive than other antioxidant assays, and thus more accurately reflecting antioxidant activity *in vivo* (Prior and Cao, 1999; Číž et al., 2010). Therefore, the ORAC method has been extensively applied to and approved for use to evaluate the antioxidant activity of food ingredients, medicines, and plant extracts. There are numerous reports of free radical scavenging activities of food and plant-derived substances. Comparisons with antioxidant activities are reported regularly in the same study, but the antioxidant activities are rarely compared with those from other studies. This is because although the principles and basic methods of antioxidant analysis, such as ABTS and ORAC assays, are similar, the reagent concentrations and reaction times vary between studies. In addition, it is not meaningful to numer-

Table 4. Oxygen radical absorbance capacity (ORAC) and hydroxyl oxygen radical absorbance capacity (HORAC) of Umbelliferae plant extracts

	ORAC			HORAC		
	Extract (mg TE/g DW)	TPC (mg TE/g)	TFC (g TE/g)	Extract (mg GAE/g DW)	TPC (mg GAE/g)	TFC (g GAE/g)
<i>Angelica decursiva</i>	25.81±1.23 ^d	228.37±10.92 ^a	11.22±0.54 ^a	1.55±0.50 ^h	13.70±4.43 ^h	0.67±0.22 ^f
<i>Angelica fallax</i>	29.54±3.90 ^{cd}	90.82±11.99 ^e	1.41±0.19 ^f	26.93±1.06 ^c	79.78±6.54 ^c	1.24±0.10 ^{de}
<i>Bupleurum longiradiatum</i>	32.66±3.48 ^{bc}	91.01±9.69 ^e	2.30±0.24 ^e	19.09±1.59 ^d	53.20±4.43 ^e	1.34±0.11 ^{cd}
<i>Coriandrum sativum</i>	11.44±1.36 ^e	159.99±19.04 ^b	1.14±0.14 ^{fg}	ND	ND	ND
<i>Cryptotaenia japonica</i>	17.29±1.31 ^e	47.40±3.60 ^g	0.72±0.05 ^g	3.26±1.31 ^h	8.93±3.59 ^h	0.09±0.05 ^g
<i>Daucus carota var. sativa</i>	36.32±2.72 ^b	240.19±18.01 ^a	8.07±0.61 ^b	25.99±2.29 ^c	171.91±15.13 ^a	5.78±0.51 ^a
<i>Glehnia littoralis</i>	25.53±2.32 ^d	70.73±6.42 ^f	2.78±0.25 ^d	12.28±1.53 ^f	34.02±4.25 ^{fg}	1.34±0.17 ^{cd}
<i>Ostericum koreanum</i>	20.35±1.68 ^e	56.43±4.67 ^{fg}	5.81±0.48 ^c	9.70±0.76 ^f	26.89±2.10 ^g	2.77±0.22 ^b
<i>Ostericum sieboldii</i>	30.15±2.89 ^c	141.61±13.55 ^c	5.58±0.53 ^c	15.78±1.25 ^e	74.13±5.88 ^c	2.92±0.23 ^b
<i>Pimpinella brachycarpa</i>	20.08±0.62 ^e	109.09±3.38 ^d	2.26±0.07 ^e	7.33±0.71 ^g	39.81±3.88 ^f	0.82±0.08 ^f
<i>Sanicula chinensis</i>	41.64±2.79 ^a	70.87±4.76 ^f	1.33±0.09 ^f	37.90±2.30 ^a	64.51±3.92 ^d	1.21±0.07 ^{de}
<i>Sanicula rubiflora</i>	42.88±3.75 ^a	106.84±9.34 ^{de}	1.61±0.14 ^f	38.05±1.13 ^a	94.83±2.81 ^b	1.43±0.04 ^{cd}
<i>Torilis japonica</i>	35.15±2.23 ^b	97.37±6.17 ^{de}	1.59±0.10 ^f	34.80±2.49 ^b	96.41±6.90 ^b	1.57±0.11 ^c
<i>Torilis scabra</i>	30.86±4.57 ^c	95.84±14.18 ^{de}	1.54±0.23 ^f	18.36±2.23 ^d	57.01±6.92 ^{de}	0.97±0.06 ^{ef}

Values followed by the different letters (a–h) in the same column are significantly different ($P<0.05$).

ORAC (or HORAC) of TPC and TFC were derived from ORAC (or HORAC) value of extract.

TPC, total phenolic content; TFC, total flavonoid content; TE, Trolox equivalent; GAE, gallic acid equivalent; DW, dry weight; ND, not detected.

ically compare antioxidant results because the conditions of the samples and the standards used as quantitative criteria vary from study to study. For example, Floegel et al. (2011) reported the ABTS activity and ORAC of onion as 29.6 mg vitamin C equivalents/100 g and 1,034 $\mu\text{mol TE}/100\text{ g}$, respectively; however, in another study, the ABTS activity and ORAC of onion were reported as 3.55 $\mu\text{mol TE}/\text{g}$ wet weight and 0.50 $\mu\text{mol TE}/\text{g}$ wet weight, respectively (Sun and Tanumihardjo, 2007).

The relative antioxidant potentials of Umbelliferae plant extracts varied depending on how the antioxidants are analyzed. In 14 Umbelliferae plant extracts, the highest antioxidant activities (as determined by DPPH, reducing capacity, ABTS, ORAC, and HORAC assays) were shown for (in order of magnitude) *S. rubiflora*, *S. chinensis*, *T. japonica*, *T. scabra*, *A. fallax*, and *G. littoralis*.

Correlation between TPC and various antioxidant activities of Umbelliferae plant extracts

The relationship between antioxidant activity and TPC is shown in Fig. 1. TPC had a superior linear correlation ($r^2=0.817$) with ABTS values (Fig. 1A). Additionally, there was a strong linear correlation between ORAC and HORAC, $r^2=0.820$ (Fig. 1B). This relationship is consistent with the Pearson correlation between TPC, TFC and various antioxidant activities (Table 5). Since total phenolic compounds contain flavonoids and non-flavonoid polyphenols, the TPC and TFC contents of 14 Umbelliferae plant extracts showed a strong positive correlation ($r=0.747$, $P<0.01$), indicating that TFC contributes a significant amount of TPC. TPC (and TFC) showed a strong positive correlation to ABTS radical scavenging

activity ($r=0.904$, $P<0.01$), reducing capacity ($r=0.737$, $P<0.01$) and HORAC ($r=0.630$, $P<0.05$). The DPPH radical scavenging activity also showed a strong negative correlation with TPC ($r=-0.725$, $P<0.01$). DPPH radical quenching activity is indicated by a negative correlation since the radical content decreases as activity increases. Therefore, TPC in Umbelliferae plant extracts may play a major role in the increase in DPPH radical scavenging activity.

The TPC (or TFC) of Umbelliferae plant extracts showed a stronger correlation with ABTS radical scavenging activity ($r=0.904$, $P<0.01$) than with DPPH radical scavenging activity ($r=-0.725$, $P<0.01$). Methanol, a polar solvent used to prepare Umbelliferae plant extracts, removes hydrophilic, lipophilic, and highly pigmented compounds from samples (Sasidharan et al., 2011). The antioxidant activities of the hydrophilic and lipophilic compounds are sensitively analyzed by the ABTS assay, while the DPPH radical scavenging assay is appropriate for analysis of hydrophobic compounds (Floegel et al., 2011; Kim, 2015). Therefore, ABTS activity have a stronger correlation with TPC content than DPPH activity. The reducing capacity of Umbelliferae plant extracts was positively correlated with the ABTS radical scavenging activity and ORAC ($r=0.695$, $P<0.01$ and $r=0.683$, $P<0.01$, respectively). ORAC and HORAC assays measure peroxy radical scavenging activity and are therefore strongly correlated ($r=0.905$, $P<0.01$). DPPH radical scavenging activity showed a strong correlation with ABTS radical scavenging activity and reducing capacity ($r=-0.770$, $P<0.01$ and $r=-0.608$, $P<0.05$, respectively). In addition, the reducing capacity showed a strong positive cor-

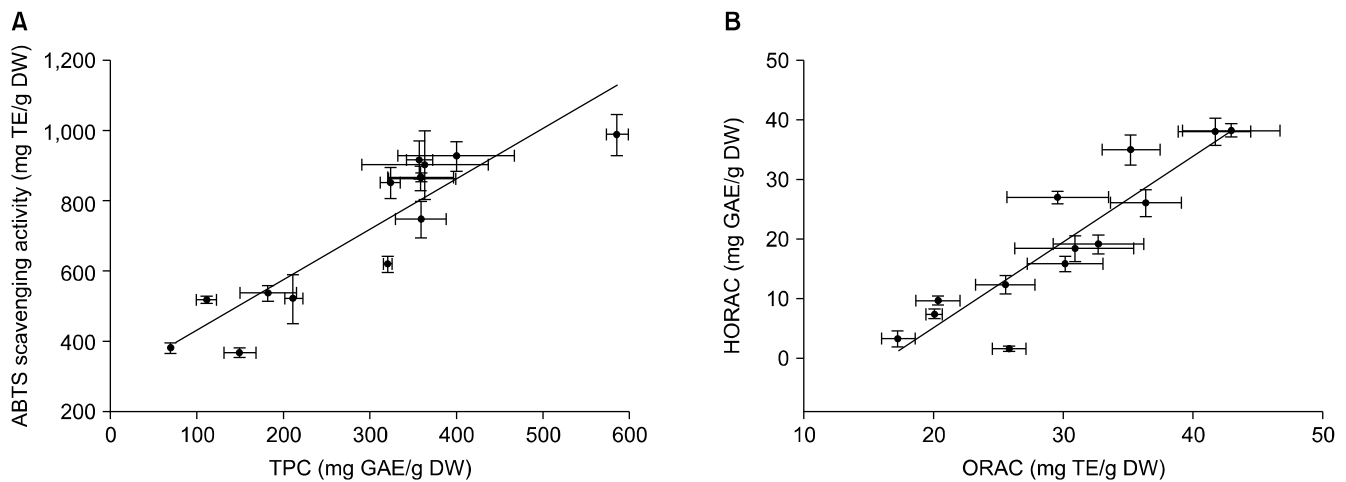


Fig. 1. Correlations between TPC content and various antioxidant activities of Umbelliferae plant extracts. (A) TPC versus ABTS: $y = 288.7 + 1.426x$, $r^2 = 0.817$; (B) ORAC versus HORAC: $y = -23.40 + 1.430x$, $r^2 = 0.820$. TPC, total phenolic content; GAE, gallic acid equivalent; DW, dry weight; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TE, Trolox equivalent; ORAC, oxygen radical absorbance capacity; HORAC, hydroxyl oxygen radical absorbance capacity.

Table 5. Pearson's correlations between antioxidant activities measured using different assays and total phenolic/flavonoid contents

	TPC	TFC	DPPH	ABTS	Reducing capacity	ORAC	HORAC
TPC	1.000						
TFC	0.747**	1.000					
DPPH	-0.725**	-0.473	1.000				
ABTS	0.904**	0.696**	-0.770**	1.000			
Reducing capacity	0.737**	0.685**	-0.608*	0.695**	1.000		
ORAC	0.532	0.465	-0.275	0.375	0.683**	1.000	
HORAC	0.545	0.630*	-0.172	0.384	0.764**	0.905**	1.000

* $P < 0.05$, ** $P < 0.01$.

TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ORAC, oxygen radical absorbance capacity; HORAC, hydroxyl oxygen radical absorbance capacity.

relation with HORAC ($r = 0.764$, $P < 0.01$). The reliability for the antioxidant activity results are confirmed by strong correlations between results from different assays.

The different types of polyphenols present in plant extracts exert several antioxidant effects and properties through numerous mechanisms, including free radical scavenging and transition metal chelation and singlet oxygen quenching (Prior and Cao, 1999). Using one type of detection method provides only limited information about the antioxidant activity of plants. Therefore, it is more appropriate to carry out analyses of the antioxidant activity of plant extracts using a variety of methods. Using multiple analytical methods is a more comprehensive approach to determine the antioxidant activity of plant extracts (Prior and Cao, 1999).

To conclude, the results of this study indicate that the TPC contents and antioxidant activities of Umbelliferae plant extracts are closely correlated, and that there is a significant correlation between the antioxidant activities measured by different assays. Therefore, the TPC contents and potent antioxidant activities of 14 Umbelliferae plant extracts calculated in this study are considered re-

liable. Umbelliferae plant extracts with potent antioxidant activities (including *S. rubiflora*, *S. chinensis*, *T. japonica*, *T. scabra*, *A. fallax*, and *G. littoralis*) are expected to play a role in adding antioxidant functions to foods and cosmetics.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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