Correlation between Solid Content and Antioxidant Activities in Umbelliferae Salad Plants

Jin-Sun Kim¹ and Je-Hyuk Lee²

¹Major in Food and Nutrition, Department of Integrated Life Science and Technology and ²Department of Food and Nutrition, Kongju National University, Chungnam 32439, Korea

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 mg TE/g, respectively. The TPC had a superior linear correlation (r²=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 (Cherng 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 (Wiart, 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, Dacus 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, flavonoids, 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).
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-aminodipropane) 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-disolved 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) Na2CO3 was added to the mixture. After 10 min at room temperature, 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) FeCl3. The absorbance of the mixture was measured at 750 nm using a spectrophotometer (Libra S22, Biochrom Ltd.). The TFC content of extracts was expressed as mg of catechin equivalents (CE)/g 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) NaNO2 (75 mL), and the mixture was incubated for 5 min. Then, 0.15 mL of 10% (w/v) AlCl3·6H2O 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 Fe3+ 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) K3Fe(CN)6. The mixture was 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) FeCl3. The absorbance of the mixture was measured at 700 nm using UV-spectrophotometry (Libra S22, Biochrom Ltd.).

**ABTS radical scavenging assay:** The ABTS⁺ 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⁺ cation decolorization reaction (the blue-green color). To generate ABTS⁺ 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⁺ 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⁺ solution was prepared for each assay. Umbelliferae extracts (10 μL) were incubated with 190 μL of the ABTS⁺ 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 (Čič et al., 2010), with a few modifications. The ORAC assay measures the peroxyl 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 peroxyl 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, every 5 min for 1 h; the microplate was automatically shaken prior to each reading. Phosphate buffer solution 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 plant 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. decorus*, *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 TFC (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...
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, isoflavonoids, flavanones, anthocyanidins, flavan-3-ols, and dihydrochalcones (Tsao, 2010; Ghasemzadeh and Ghasemzadeh, 2011; Del Rio et al., 2013). The main subclasses of flavonoids are flavonols, flavones, isoflavonoids, 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, TFC, 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 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 antioxidative 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. longiradiatum*, *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 scavenging activities, exhibiting IC50 values of 39, 48, 49, and 50 μg DW/mL, respectively.

The antioxidative 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 ± 0.36 μg TFC/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*...
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 μg/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 A700). In contrast, C. sativum, C. japonica, and D. carota showed the lowest reducing capacity (0.11 on A700).

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 A700 (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 2. DPPH radical scavenging activity of Umbelliferae plant extracts

| Umbellifera | % Control | IC50 | TPC | TFC |
|-------------|-----------|------|-----|-----|
| Angelica decursiva | 49.5±3.2 | NT | NT | NT |
| Angelica fallax | 17.3±0.3 | 65.8 | 21.40 | 1.38 |
| Bupleurum longiradiatum | 17.4±0.4 | 79 | 28.35 | 1.12 |
| Coriandrum sativum | 62.4±3.4 | NT | NT | NT |
| Cryptotaenia japonica | 23.0±0.6 | 61 | 22.25 | 1.47 |
| Daucus carota var. sativa | 53.6±1.1 | NT | NT | NT |
| Glehnia littoralis | 15.9±1.0 | 94 | 33.93 | 0.86 |
| Ostericum koreanum | 18.4±0.3 | 102 | 36.78 | 0.36 |
| Ostericum sieboldii | 35.2±0.6 | 179 | 38.11 | 0.97 |
| Pimpinella brachycarpa | 20.1±1.0 | 122 | 22.46 | 1.09 |
| Sanicula chinensis | 26.4±1.7 | 48 | 28.20 | 1.51 |
| Sanicula rubiflora | 17.6±0.9 | 50 | 20.07 | 1.33 |
| Torilis japonica | 18.0±0.0 | 39 | 14.08 | 0.86 |
| Torilis scabra | 17.6±1.0 | 49 | 15.78 | 0.98 |

1) IC50 for DPPH radical scavenging activity were measured in 0∼300 μg/mL of extract.
2) DPPH radical (100 mM) was quenched by extracts (300 μg/mL).
3) IC50 of TPC and TFC were derived from IC50 of extract.

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

The 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 A700 (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 | ABTS radical scavenging activity |
|-----------------------|-------------------|----------------------------------|
|                       | (A<sub>200</sub>) | Extract (mg TE/g DW) | TPC (mg TE/g) | TFC (g TE/g) |
| Angelica decursiva    | 0.16±0.00         | 517.5±10.3<sup>a</sup> | 4,579.94±90.82<sup>b</sup> | 1,991.28±39.49<sup>a</sup> |
| Angelica fallax       | 0.52±0.06         | 850.9±43.9<sup>ab</sup> | 2,615.64±135.03<sup>d</sup> | 125.15±6.46<sup>b</sup> |
| Bupleurum longermanatum| 0.32±0.03         | 914.2±55.2<sup>ab</sup> | 2,547.23±153.93<sup>de</sup> | 179.38±10.84<sup>a</sup> |
| Coriandrum sativum    | 0.11±0.00         | 381.5±10.15<sup>d</sup> | 5,333.14±209.95<sup>b</sup> | 533.61±20.99<sup>c</sup> |
| Cryptotaenia japonica | 0.11±0.00         | 900.2±98.2<sup>ab</sup> | 2,467.65±269.31<sup>de</sup> | 102.39±11.17<sup>e</sup> |
| Daucus carota var. sativa | 0.11±0.01     | 367.5±12.9<sup>f</sup> | 2,430.78±85.04<sup>de</sup> | 84.5±15.0<sup>f</sup> |
| Glehnia littoralis     | 0.35±0.01         | 746.9±51.6<sup>ef</sup> | 2,068.88±143.01<sup>de</sup> | 224.88±15.54<sup>f</sup> |
| Ostericum koreanum     | 0.35±0.05         | 865.5±11.6<sup>b</sup> | 2,400.26±32.02<sup>de</sup> | 685.79±9.15<sup>b</sup> |
| Ostericum sieboldii    | 0.19±0.00         | 520.9±71.1<sup>f</sup> | 2,446.53±333.77<sup>de</sup> | 453.06±61.81<sup>d</sup> |
| Pimpinella brachycarpa | 0.26±0.02         | 536.9±20.4<sup>e</sup> | 2,916.17±110.97<sup>f</sup> | 327.66±12.47<sup>f</sup> |
| Sanicula chinensis     | 0.63±0.03         | 986.2±58.4<sup>ef</sup> | 1,678.64±99.43<sup>de</sup> | 540.17±18.90<sup>f</sup> |
| Sanicula rubiflora     | 0.78±0.05         | 924.9±40.9<sup>de</sup> | 2,304.68±101.81<sup>ef</sup> | 86.64±3.83<sup>f</sup> |
| Torilis japonica       | 0.45±0.03         | 863.5±35.0<sup>de</sup> | 2,392.06±96.81<sup>de</sup> | 102.4±4.38<sup>de</sup> |
| Torilis scabra         | 0.38±0.03         | 619.5±22.7<sup>de</sup> | 1,924.02±70.64<sup>de</sup> | 96.20±3.53<sup>de</sup> |

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 µM TE/100 g DW (82 µM TE/100 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 potential 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 to 171.91 mg GAE/g TPC and 0.09 to 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 µmol TE/g (Gorinstein et al., 2009); for Peucedanum japonicum Thunberg roots were reported as 42.24–50.55 µmol TE/g and 58.10–133.37 µmol/g, respectively (Lim et al., 2019); and for Chrysanthemum boreale extract were reported as 0.47 mmol/mg and 94.34 µmol 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; Čič 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

| Extract | ORAC (mg TE/g DW) | TPC (mg TE/g) | TFC (g TE/g) | HORAC (mg TE/g DW) | TPC (mg TE/g) | TFC (g TE/g) |
|---------|------------------|---------------|--------------|-------------------|---------------|--------------|
| Angelica archangelica | 25.84±1.23 | 228.37±10.92 | 11.22±5.04 | 1.70±0.44 | 6.70±2.2 |
| Angelica archangelica | 29.54±1.39 | 90.82±11.99 | 1.41±0.19 | 26.93±1.06 | 1.20±0.10 |
| Bupleurum longifolium | 32.66±1.48 | 91.01±9.69 | 2.30±0.24 | 19.09±5.94 | 5.20±4.44 |
| Coriandrum sativum | 11.44±1.36 | 159.99±19.04 | 1.14±0.14 | ND | ND |
| Cryptotaenia japonica | 17.29±1.31 | 19.40±3.60 | 0.72±0.05 | 3.26±1.31 | 8.93±0.39 |
| Daucus carota var. sativa | 36.32±2.72 | 240.19±18.01 | 8.07±0.61 | 25.99±2.29 | 5.78±0.51 |
| Glechoma hederacea | 25.53±2.32 | 70.73±6.42 | 2.78±0.25 | 12.28±1.53 | 3.04±0.25 |
| Ostericum koreanum | 20.35±1.68 | 56.43±4.67 | 5.81±0.48 | 15.78±1.25 | 7.43±0.58 |
| Ostericum sieboldii | 30.15±2.89 | 141.61±13.55 | 5.58±0.33 | 15.78±1.25 | 7.43±0.58 |
| Pimpinella brachycarpa | 20.08±0.62 | 109.09±3.38 | 2.26±0.07 | 7.33±0.71 | 3.89±0.38 |
| Sanicula chinesis | 41.54±2.79 | 70.87±4.76 | 1.33±0.09 | 37.90±2.30 | 6.45±3.92 |
| Sanicula rubiflora | 42.88±1.75 | 106.84±9.34 | 1.61±0.14 | 38.05±1.13 | 9.84±2.81 |
| Torilis japonica | 35.15±2.23 | 97.37±6.17 | 1.59±0.10 | 34.80±2.49 | 9.64±1.90 |
| Torilis scabra | 30.86±5.47 | 95.84±14.18 | 1.54±0.23 | 18.36±2.23 | 5.71±0.92 |

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.

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 (r²=0.820) with ABTS values (Fig. 1A). Additionally, shown in Fig. 1. TPC had a superior linear correlation with ORAC and HORAC, r²=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 TPC 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 peroxyl 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-
Correlation between Solid Content and Antioxidant Activities in Umbelliferae

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  | 0.693**          | 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’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); TE, Trolox equivalent; 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 reliable. 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.

**REFERENCES**

Cherng JM, Chiang W, Chiang LC. Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids. Food Chem. 2008. 106:944-950.

Číž M, Čížová H, Denev P, Kratchanova M, Slavov A, Lojek A. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control. 2010. 21:518-523.

Croft KD. The chemistry and biological effects of flavonoids and phenolic acids. Ann NY Acad Sci. 1998. 854:435-442.

Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges
G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal. 2013. 18:1818-1892.

Di Majo D, La Guardia M, Giammanco S, La Neve L, Giammanco M. The antioxidant capacity of red wine in relationship with its polyphenolic constituents. Food Chem. 2008. 111:45-49.

Duh PD. Antioxidant activity of burdock (Arctium lappa Linné): its scavenging effect on free-radical and active oxygen. J Am Oil Chem Soc. 1998. 75:455-461.

Fernandes RP, Trindade MA, Tonin FG, Lima CG, Pugine SM, Munekata PE, et al. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J Food Sci Technol. 2016. 53:451-460.

Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal. 2011. 24:1043-1048.

Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: role and biochemical activity in plants and human. J Med Plants Res. 2011. 5:6697-6703.

Gomes A, Fernandes E, Lima JLF. Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods. 2005. 65:45-80.

Gorinstein S, Jastrzebski Z, Leontowicz H, Leontowicz M, Namiesnik J, Najman K, et al. Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions. Food Control. 2009. 20:407-413.

Gao J, Wang MH. Antioxidant and anti-diabetic activities of Ulmus davidiana extracts. Food Sci Biotechnol. 2007. 16:55-61.

Kim SY. Fluctuations in phenolic content and antioxidant capacity of green vegetable juices during refrigerated storage. Prev Nutr Food Sci. 2015. 20:169-175.

Kim YS, Hwang JW, Park PJ, Jeong JH. Antioxidant activity and protective effects of extracts from Chrysanthemum boreale on tert-BHP induced oxidative stress in Chang cells. J Korean Soc Food Sci Nutr. 2014. 43:60-66.

Lee EJ, Jang KH, Hussain M, Lee DJ. Estimation of antioxidant and tyrosinase inhibition activities in different species of Apiaceae family. Korean J Intl Agri. 2011a. 23:382-388.

Lee EJ, Kim KS, Jung HY, Kim DH, Jang HD. Antioxidant activities of garlic (Allium sativum L) with growing districts. Food Sci Biotechnol. 2005. 14:123-130.

Lee JH, Kim GH. Evaluation of antioxidant activity of marine algae extracts from Korea. J Aquat Food Prod Technol. 2015. 24:227-240.

Lee JI, Lee JH. Antioxidant and inhibitory activities of thiolflavonones against nitric oxide production. Food Sci Biotechnol. 2014. 23:957-963.

Lee YM, Baeh JH, Jung HY, Kim JH, Park DS. Antioxidant activity in water and methanol extracts from Korean edible wild plants. J Korean Soc Food Sci Nutr. 2011b. 40:29-36.

Lim H, Kim I, Jeong Y. Antioxidant activities of Peucedanum japonicum Thunberg root extracts. J Korean Soc Food Sci Nutr. 2019. 48:32-39.

Lim H, Shin S. Study on the essential oils from the roots of Angelica decursiva and Peucedanum praetextatum. Korean J Pharmacogn. 2012. 43:291-296.

Liu W, Yin D, Li N, Hou X, Wang D, Li D, et al. Influence of environmental factors on the active substance production and antioxidant activity in Potentilla fruticosa L and its quality assessment. Sci Rep. 2016. 6:28591. https://doi.org/10.1038/srep28591.

Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed. 2012. 2:960-965.

Moore J, Liu JG, Zhou K, Yu LL. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. J Agric Food Chem. 2006. 54:5313-5322.

Oh PS, Lee SJ, Lim KT. Antioxidative activity of 90 kDa glycoprotein isolated from Opuntia ficus-indica var. saboten Makino. Food Sci Biotechnol. 2004. 13:781-789.

Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986. 44:307-315.

Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008. 4:89-96.

Prior RL, Cao G, Martin A, Sofic E, McEwen J, O’Brien C, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J Agric Food Chem. 1998. 46:2686-2693.

Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radic Biol Med. 1999. 27:1173-1181.

Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003. 51:2866-2887.

Sasidharan S, Chen Y, Saravananas D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants’ extracts. Afr J Tradit Complement Altern Med. 2011. 8:1-10.

Sayed-Abdoh B, Talou T, Saad Z, Hijazi A, Merah O. The Apiaceae: ethnomedicinal family as source for industrial uses. Ind Crops Prod. 2017. 109:661-671.

Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition. 2005. 21:207-213.

Sun T, Tanumihardjo SA. An integrated approach to evaluate food antioxidant capacity. J Food Sci. 2007. 72:R159-R165.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Sun T, Tanumihardjo SA. An integrated approach to evaluate food antioxidant capacity: comparison of products of browning reaction prepared from guava fruit. Food Chem. 2006. 102:1173-1181.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006. 19:669-675.

Tsaio R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010. 2:1231-1246.

Wiart C. Medicinal plants of China, Korea, and Japan: bioresources for tomorrow’s drugs and cosmetics. CRC Press, Boca Raton, FL, USA. 2012. p 354-373.

Yao Y, Sang W, Zhou M, Ren G. Phenolic composition and scavenging effects on superoxide radicals. Food Chem. 1999. 64:555-559.