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Comparison of the Antioxidant Activities of Organic Solvent Fractions of Leaf and Root Extracts of *Peucedanum insolens* Kitagawa

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This study compared and analyzed the antioxidant activities of various organic solvent fractions from the leaves and roots of *Peucedanum insolens* Kitagawa. For this study, the dried leaves and roots of *P. insolens* Kitagawa were first extracted using 70% ethanol. The extracts were sequentially sub-fractionated in the order of hexane, chloroform, ethyl acetate, n-butanol, and water. The results revealed that the distribution of total phenolic contents by organic solvent fractions showed the same pattern in both the leaves and roots, with the highest in the ethyl acetate fraction (101.1±1.0 mg vs 71.2±3.4 mg of GAE/mg), but the lowest content in the hexane fraction (9.5±0.2 mg vs 7.5±2.1 mg of GAE/mg). The distribution of total flavonoid content in the organic solvent fractions showed the same pattern as that of total phenolic content. The results of DPPH, ABTS, and FRAP assays showed that the leaf and root extracts exhibited free radical scavenging activity in the same pattern, particularly, the ethyl acetate fraction had the highest activity. These results indicate that not only the roots of *P. insolens* Kitagawa but also the leaves possess potential substances that exhibit strong antioxidant activity. Significant correlations (R=0.903, p<0.0001, DPPH radical; R=0.891, p<0.001, ABTS radical; R=0.745, p<0.05, FRAP radical) between total phenolics and radical scavenging activities, but also significant correlations (R=0.867, p<0.001, DPPH vs. ABTS radicals; R=0.882, p<0.0001, DPPH vs. FRAP radicals; R=0.973, p<0.0001, ABTS vs. FRAP radicals) between radical scavenging activities were found in the organic solvent fractions. Therefore, as in the roots of *P. insolens* Kitagawa, the leaves possess strong antioxidant capacity and can be used as the main antioxidant material.

Key words : ABTS, DPPH, FRAP, *Peucedanum insolens* Kitagawa, total polyphenol

Introduction

Peucedanum insolens Kitagawa, a Koean endemic umbelliferous plant, is a perennial herb distributing in middle Korea and chiefly related to *P. cervaria* (L.) Cusson. This plant is also called “Wangsan Bang Poong” or “Deokwoo Oil herb” because it was first discovered in around Mountain Deokwoo in Wangsan-myeon, Myeongju-gun, Gangwon Province, South Korea [8]. The plant mainly grows in the limestone areas of Gangwon Province, and also grows naturally in nearby areas of Mungyeong and Andong. Korean umbelliferous plant, “Bang Poong”, is an important herbal medicine used in oriental medicine for antipyretic and pain relief, colds, chills, and sore throats [10]. *Siler divaricatum* in China and *Glehnia littoralis* in Korea are used as a substitute of “Bang Poong” in fork medicine. The dried roots “wang-san-fang-feng” have been used for medicinal purposes in Korea for the treatment of diaphoresis, sedation and antipyresis [8, 9, 26].

Reactive oxygen species (ROS) such as superoxide anion (O_2^{-}.), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) can be generated not only endogenously by metabolic processes and physical stress, but also exogenously such as from environmental pollutants, radiation, chemicals, and toxins. These highly reactive species are responsible for inducing the formation of abnormal proteins, leading to the depletion of antioxidants in the immune system [1]. Excess production

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of ROS leads to oxidative stress, which can cause number of diseases, including atherosclerosis, Alzheimer disease, cardiovascular diseases [CVDs], chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer. In such conditions, dietary intake of antioxidant compounds is needed in assisting the body to neutralize the free radicals to remove the harmful effects of oxidative stress. Many natural resources such as fruits, vegetables, grains and medicinal plants are known to contain number of phenolic compounds with strong antioxidant potential. The generation of highly ROS with a lone unpaired electron induce oxidative stress and plays a key role in the pathogenesis of numerous physiological conditions [23, 24]. For this reason, antioxidant behavior in plant extracts is one of the most commonly applied biological activities for the prevention of chronic diseases [13]. The most commonly used antioxidant assays to determine the antioxidant activity of plant extracts are ones being based on the scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) radicals.

Even though there are a number of endogenous antioxidant enzymes, such as glutathione peroxidase, catalase and superoxide dismutase, which can deactivate free radicals and therefore maintain optimal cellular functions, endogenous antioxidants may not be sufficient to maintain optimal cellular functions under increased oxidative stress. Therefore, additional dietary antioxidants may be necessary [30].

Phenolic compounds are thought to be the active ingredients in many dietary plants and traditional medicines used for the treatment of disorders related to oxidative stress and inflammation [39]. Natural phenolic and flavonoid compounds are plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group [38]. Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action [4]. According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease [27]. In the present study, we have analyzed and compared antioxidant capacity of the five different organic solvent-fractions from the leaves and roots of *P. insolens* Kitagawa.

Materials and Methods

Reagents and chemicals

ABTS, diethylene glycol, DPPH, ferrous sulfate, Folin-Ciocalteu reagent, gallic acid, quercetin standards and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Aluminum chloride hexahydrate, methanol, potassium persulfate and sodium carbonate were obtained from Fisher Scientific (Fair Lawn, NJ, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (Trolox) was purchased from Tokyo Chemical Industry Co (Tokyo, Japan). Water was purified using a Milli-Q system (Millipore, Billerica, USA). All chemicals and solvents used were of analytical grade.

Preparation of organic solvent fractions of *P. insolens* Kitagawa leave and root

P. insolens Kitagawa plants were collected in August 2020 from Jeongseon area, Gangwon-do. Sik-Je Cho, a specialist for plant classification, formally identified roots and leaves of *P. insolens* Kitagawa. The collected leaves and roots of *P. insolens* Kitagawa were dried in the shade at room temperature for a sufficient period. The dried roots or leaves were cut to about 0.5 cm or less in length. Five different organic solvent (hexane, chloroform, ethyl acetate, n-butanol, and water)-fractions of *P. insolens* Kitagawa leave or root were prepared according to the scheme shown in Fig. 1. Briefly, the dried cut samples, i.e, 570 g leaves or 600 g of roots, were extracted three times with a total 25 l of 70% ethanol for 5 days at room temperature with frequent agitation. The extract was filtered using a Buckner funnel and Whatman No. 1 filter paper. The filtrate was concentrated to dryness in a rotary vacuum evaporator (EYELA N-1200 Tokyo Rikakikai Co., Ltd., Japan) under reduced pressure and controlled temperature (40~50℃). As shown in Fig. 1, five organic solvent fractions of the leaves or roots of *P. insolens* Kitagawa were prepared by sequentially fractionating hexane, chloroform, ethyl acetate and butanol in the ethanol extract. The ethanol extracts and their organic solvent-fractions prepared from both leaf and root were stored at -20℃ in an airtight container until further use.

Determination of total phenolic contents in the organic solvent-fractions of *P. insolens* Kitagawa

The total phenolic contents of the organic solvent-fractions were determined by Folin-Ciocalteu method as described by Singleton and Rossi [33] with slight modifications. Briefly, 5 ml of the fraction (1 mg/ml) was mixed with 100 ml of 2% (w/v in water) sodium carbonate solution in a 96-well plate. After 5 min, 5 μl of 50% Folin-Ciocalteu reagent was

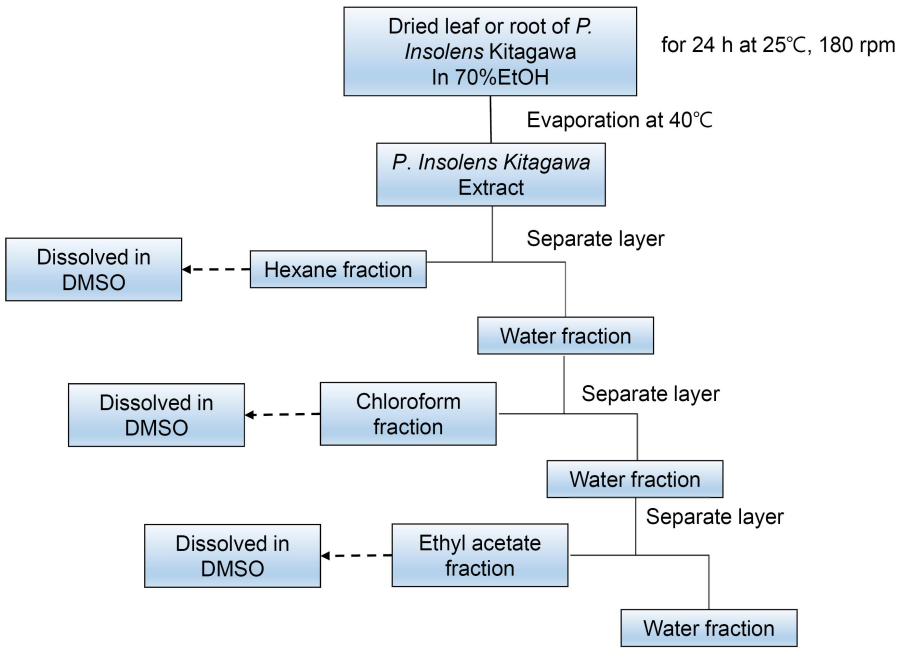

Fig. 1. Scheme for preparation of organic solvent-fractions of *P. insolens* Kitagawa leaf or root.

added to the mixture and allowed to stand in the dark for 30 min at room temperature. After centrifuging, the absorbance of blue color from the reaction mixtures was measured using a microplate reader (Biotek EL808, Winooski, VT, USA) at 630 nm against the reagent blank without extract. The content of total phenolic compounds was expressed as μg of gallic acid equivalents per mg dry plant extract (μg GAE/mg DE) based on a standard curve of gallic acid (0~20 mg/ml). All the experiments were run in triplicate. The mean values and standard deviations were calculated using the Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA).

Determination of total flavonoid contents in the organic solvent-fractions of *P. insolens* Kitagawa

Total flavonoid content was determined in all the fractions using the colorimetric Davis method [11] with slight modifications. Briefly, after mixing of 10 ml of the fraction and 100 ml of diethylene glycol in a 96-well plate, 10 μl of 1 N NaOH was added to the mixture and reacted in a water bath at 37℃ for 1 hr. Afterwards, the mixture was cooled and the absorbance was measured at 405 nm against the reagent blank without extract using a microplate reader (Biotek EL808, Winooski, VT, USA). In this method, fractions reacting with diethylene glycol in alkaline solution produce a yellow chalcone, which was measured at a wavelength of 405 nm. The measurement was compared with a standard curve of prepared quercetin solution. The content of total flavonoid in the organic solvent-fractions was expressed as mg of quercetin equivalents per g of dry plant extract (μg QE/mg DE) based on a standard curve of quercetin (0~2 mg/ml). All the experiments were run in triplicate. The mean values and standard deviations were calculated using the Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA).

Measurement of DPPH radical scavenging activity of organic solvent-fractions of *P. insolens* Kitagawa

The antioxidant activity of the extracts, based on the scavenging activity of the stable DPPH free radical, was determined by the method described by Brand-Williams et al, [6] with slight modifications. Briefly, 2 ml of sample solution was mixed with 198 ml of 0.15 mM DPPH solution in a 98-well plate, and then reacted for 30 min at room temperature while blocking the light. Absorbance was measured at 540 nm using a microplate reader (Biotek EL808, Winooski, VT, USA). Ascorbic acid solution (0.0-10.0 μg/ml) was used as a standard. The radical scavenging capacity using the free DPPH radical was evaluated by measuring the decrease of absorbance at 540 nm. When the reading was complete, the percentage of DPPH radical scavenging activity of samples was calculated using the equation: The antioxidant activity of the extracts was expressed in terms of μg Ascorbic acid/mg sample. All the experiments were performed in triplicate.

Measurement of ABTS Radical Scavenging Activity of organic solvent-fractions of *P. insolens* Kitagawa

Free radical scavenging ability of the extracts was determined using the modified ABTS radical cation decolorization assay [31]. For ABTS cation radical formation reaction, 7.4 mM ABTS in water and 2.4 mM potassium persulfate were mixed at a ratio of 14:1 (v/v) and leave it in the dark at room temperature for 20 hr. ABTS cation radical solution was then diluted with distilled water to obtain an absorbance of 0.700 at 730 nm. After getting the stable absorbance, the antioxidant plant extract was added to the reaction medium and the antioxidant power was measured by studying decolorization as described below. After the addition of 1.5 µl of plant extract to 150 µml of diluted $ABTS^{\cdot+}$ solution, the absorbance was measured at 5 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. The relative antioxidant ability to scavenge the radical $ABTS^{+}$ has been compared by using Trolox as a standard material. The ABTS radical scavenging activity was expressed in terms of mM Trolox eq/mg sample.

Measurement of FRAP radical scavenging activity of organic solvent-fractions of *P. insolens* Kitagawa

The antioxidant capacity of the medicinal plants using FRAP assay was determined spectrophotometrically by the method of Benzie and Strain (1996)[5]. The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+}-tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The FRAP reagent as a working reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the proportion of 10:1:1 at 37℃. Freshly prepared working FRAP reagent (190 µl) was mixed with 10 µl of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric (Fe^{3+}) TPTZ complex was reduced to ferrous (Fe^{2+}) form. After keeping in dark for 30 min for reaction at 37℃, the absorbance at 593 nm was recorded against a reagent blank (190 µl FRAP reagent + 10 µl distilled water). All the measurements were performed in triplicates. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. Different aliquots of Trolox were treated as standard. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant Trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mM of Trolox eq/mg sample.

Statistical analysis

Experimental data were expressed as means ± standard deviation (SD), and the results were obtained from at least three independent experiments performed in triplicate. Student's *t*-test and one-way ANOVA were used to determine the statistical significance of the difference between values for the various experimental and control groups. A *p*-value of 0.05 or less was considered statistically significant. Non-parametric Spearman correlation analysis was used to determine the correlation between three antioxidant activities, and between antioxidant activities and the total phenolic content.

Results

Preparation of organic solvent fractions of the leaves or roots of *P. insolens* Kitagawa

Five kinds of organic solvent (hexane, chloroform, ethyl acetate, n-butanol and water) fractions from 70% ethanol extracts of leaves or roots of *P. insolens* Kitagawa root were prepared as shown in Fig. 1. For this, 70% ethanol extract was first prepared from the 570 g of dried leaves or 600 g of dried roots of *P. insolens* Kitagawa, respectively. Concentrated contents of the 70% ethanolic extracts were 98 g in the leaves and 85 g in the roots, respectively. From the 70% ethanol extracts, fractions of hexane, chloroform, ethyl acetate, n-butanol and water were sequentially prepared and the recovered yields were 8.5, 1.0, 2.0, 3.5 and 79 g from the leaves and 1.0, 0.8, 0.4, 1.0 and 53 g from the roots, respectively.

Analysis of total phenolic and total flavonoid contents in the organic solvent-fractions from leaves and roots of *P. insolens* Kitagawa

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity [34]. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics found in plants with known properties, which include free radical scavenging activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. As a basis, phenolic content was measured using the Folin-Ciocalteu reagent in each extract. The results were derived from a calibration curve ($y = 0.0345x + 0.0235$, $R^2 = 0.9931$) of gallic acid (0–20 mg/ml) and expressed in µg

Table 1. Comparison of total phenolic contents in organic solvent fractions from leaves and roots of *P. insolens* Kitagawa

Organic solvent-Fractions	Total polyphenol[a] (μg GAE/mg extracts)	
	Leaf extract	Root extract
Hexane (LH)	9.5±0.2	7.5±2.1
Chloroform (LC)	26.4±7.8	59.7±7.3
Ethyl acetate (LE)	101.1±1.0	71.2±3.4
n-Butanol (LB)	45.3±3.4	45.8±3.5
Water (DW)	24.7±0.9	5.3±1.0

[a]Total phenolic content expressed in μg of gallic acid equivalent (GAE) per mg of dry weight of plant extracts.

Table 2. Comparison of total flavonoid contents in organic solvent fractions from leaves and roots of *P. insolens* Kitagawa

Organic solvent-Fractions	Total flavonoid[a] (μg QE/mg extracts)	
	Leaf extract	Root extract
Hexane (LH)	1.2±0.1	0.8±0.1
Chloroform (LC)	10.6±0.2	108.7±3.7
Ethyl acetate (LE)	100.3±13.1	128.8±15.5
n-Butanol (LB)	12.7±4.4	57.9±23.7
Water (DW)	48.4±0.4	4.3±0.9

[a]Total flavonoid content expressed in μg of quercetin equivalent (QE) per mg of dry weight of plant extracts.

GAE/mg DE (Table 1). The contents of total phenolic compounds in the hexane-, chloroform- ethyl acetate-, n-butanol- and water-fractions of the leaf extracts were determined as 9.5±0.2, 26.4±7.8, 101.1±1.0, 45.3±3.4 and 24.7±0.9 mg GAE/mg DE, respectively, suggesting that the ethyl acetate-fraction possess the remarkably high contents of total phenolic compounds. The contents of total phenolic compounds in the organic solvent-fractions of the root extracts were also determined as 7.5±2.1, 59.7±7.3, 71.2±3.4, 45.8±3.5 and 5.3±1.0 mg GAE/mg DE, respectively, showing the same pattern of the contents of total phenolic compounds with that of the leaf extracts.

Total flavonoid content was also determined through a linear quercetin standard curve and expressed as mg QE/mg. As shown in Table 2, the contents of total flavonoids in the hexane-, chloroform-, ethyl acetate-, n-butanol- and water-fractions of the leaf extracts were determined as 1.2±0.1, 10.6±0.2, 100.3±13.1, 12.7±4.4 and 48.4±0.4 mg QE/mg, respectively, suggesting that the content of total flavonoids was the remarkably high at the ethyl acetate-fraction. Total flavonoid contents in the organic solvent-fractions of the root extracts were also determined as 0.8±0.1, 108.7±3.7, 128.8±15.5, 57.9±23.7 and 4.3±0.9 mg GAE/mg DE, respectively, showing the same pattern of the total flavonoid contents with that of the leaf extracts. Total flavonoid contents in the organic solvent fractions of *P. insolens* Kitagawa root showed a relatively similar pattern to the total flavonoid content in the leaf extract. However, in the chloroform fraction, a specific result showing a high content similar to that in the ethyl acetate fraction was obtained.

Phenolic compounds in medicinal plants have been reported to be associated with antioxidant activity, anticancer effects, and other biological functions, and may prevent other diseases associated with aging [34, 37, 40]. Thus, our study results suggest that ethyl acetate-fraction of *P. insolens* Kitagawa root extracts with ethanol might have high antioxidant activities.

Total antioxidant capacities of the organic solvent-fractions of *P. insolens* Kitagawa

In the present study total antioxidant capacity of organic solvent-fractions from the leaves and roots of *P. insolens* Kitagawa were measured using three different methods namely DPPH, ABTS and FRAP assay. As shown in Fig. 2, The DPPH radical scavenging activities in ethyl acetate-fraction of leaf and root of *P. insolens* Kitagawa were found to be 985.3±28.9 vs 958.9±76.5 μg GAE/mg Sample, respectively, showing the strongest activity among the organic solvent fraction. The chloroform fraction of the root of *P. insolens* Kitagawa also showed strong DPPH radical scavenging activity at almost the same level as that of the ethyl acetate fraction. n-Butanol fraction in *P. insolens* Kitagawa leaf showed the second highest DPPH radical scavenging activity after ethyl acetate fractions, respectively. However, hexane- and water-fractions of the extracts from both leaf and root showed relatively low DPPH radical scavenging activities, compared to that of ethyl acetate-fraction. Therefore, it was confirmed that ethyl acetate-fraction of *P. insolens* Kitagawa root has the best electron donating ability among the organic solvent fractions. According to Hwang et al. [16], antioxidant activity highly correlates with the total phenolic content. Therefore, as observed in the DPPH assay in the present study, the excellent antioxidant activity of ethyl acetate-fraction of *P. insolens* Kitagawa root indicates high phenolic flavonoid contents of the extract.

In contrast to DPPH radical scavenging ability, ABTS radical scavenging activity is more reactive and involves an electron transfer process [17]. From Fig. 3, the trend for ABTS

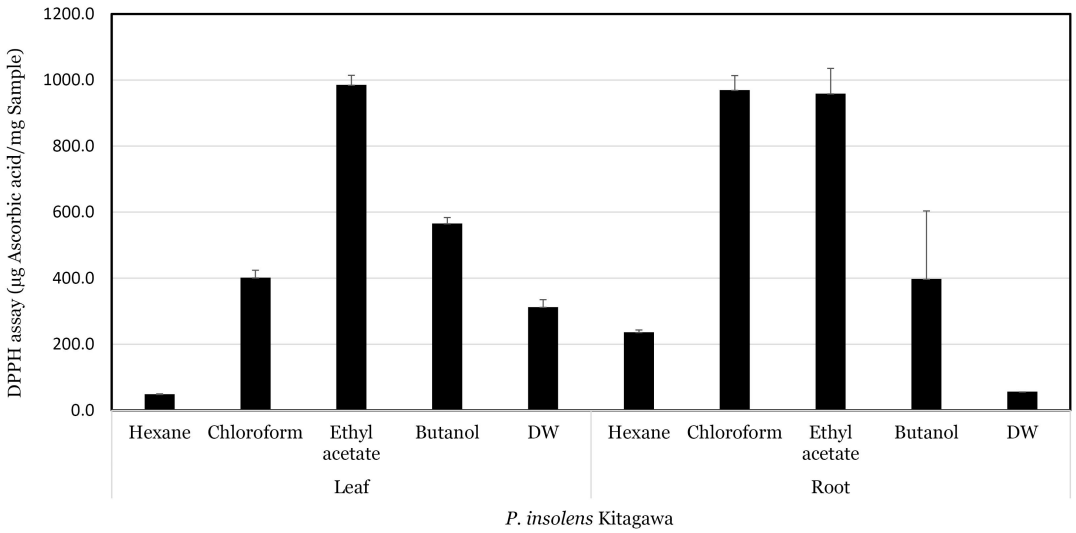

Fig. 2. Comparison of DPPH radical-scavenging activities in organic solvent-fractions from leaves and roots of *P. insolens* Kitagawa. Values are the mean ± SD of experiments in triplicate (n=3).

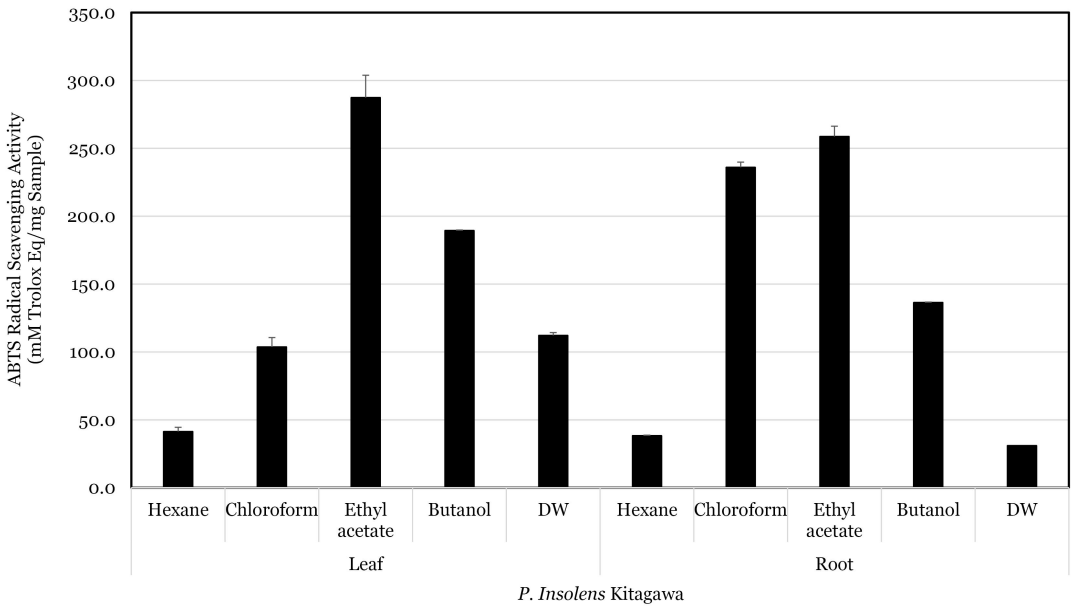

Fig. 3. Comparison of ABTS radical-scavenging activities in organic solvent-fractions from leaves and roots of *P. insolens* Kitagawa. Values are the mean ± SD of experiments in triplicate (n=3).

radical scavenging activity of organic solvent-fraction of *P. insolens* Kitagawa was similar to DPPH radical scavenging activity. The ethyl acetate fraction showed the strongest ABTS radical scavenging activity (287.5±16.4 vs 258.8±7.5 mM Trolox eq/mg sample) in both leaves and roots. The chloroform fraction of the root of *P. insolens* Kitagawa also showed strong ABTS radical scavenging activity at almost the same level as that of the ethyl acetate fraction. The hexane- or water-fraction showed the lowest ABTS scavenging activity (41.5±3.0 vs 31.2±0.6 mM Trolox eq/mg sample) in the leaves and roots of *P. insolens* Kitagawa. The ABTS radical scavenging activity in ethyl acetate fraction was 6.9 or 8.3 times higher than that in hexane or water fraction, respectively. This result indicated that the critical active components in both leaf and root of *P. insolens* Kitagawa have similar effects for these two indices. It is also known that ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain-breaking antioxidants. Therefore, the ABTS radical scavenging activity of ethyl acetate fraction of *P. insolens*

Kitagawa indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

Ferric reducing antioxidant power assay was also performed to evaluate and compare the antioxidant potential of different organic solvent fractions prepared from the leaves or roots of *P. insolens* Kitagawa. FRAP assay, which is relatively simple and easy to conduct, principally treats the antioxidants in the sample as reductant in a redox-linked colorimetric reaction [14]. FRAP assay measures the reducing potential of antioxidant to react on Fe^{3+}-TPTZ complex and produce blue color of ferrous form [5], which can be detected at absorbance 593 nm. Antioxidant compounds, which act as reducing agent, exert their effect by donating hydrogen atom to ferric complex and thus break the radical chain reaction. The higher the absorbance is, the higher is the antioxidant activity that is indicated by the high FRAP value. As shown in Fig. 4, among the organic solvent fractions, the ethyl acetate fractions prepared from the both leaf and root of *P. insolens* Kitagawa showed the highest (2,097.0±76.1 vs 1,345±16.9 mM Trolox eq/mg Sample) FRAP radical scavenging activity, respectively. These results indicate that not only the roots of *P. insolens* Kitagawa, but also the leaves possess potential substances that exhibit strong antioxidant activity. The chloroform- and n-butanol fractions also showed strong FRAP radical scavenging activities next to the ethyl acetate fraction.

In general, the antioxidant activity of medicinal plants is associated with total phenolic content [7, 22]. For spearman correlation analysis, it was found that there was a high correlation between the antioxidant capacities and total phenolic content in the both leaf and root of *P. insolens* Kitagawa. First, it was obtained that the total phenolic content correlates with DPPH assay. The correlation coefficient of DPPH method and total phenolic content was 0.903, (p<0.0001). It was also observed that the total phenolic contents correlates well with its ABTS and FRAP assays. The correlation coefficients of total phenolic content with ABTS and FRAP assays are 0.891 (p<0.001) and 0.745 (p<0.05), respectively, confirming that phenolic compounds are likely to contribute to radical scavenging activity of these plant extracts (data not shown). The significant correlation is also seen between DPPH assay and ABTS values (Correlation coefficient, R=0.867, p<0.001). The significant correlation is also seen between DPPH assay and FRAP values (Correlation coefficient, R=0.882, p< 0.0001). The significant correlation is also seen between ABTS assay and FRAP values (Correlation coefficient, R= 0.973, p<0.0001). Our results are in agreement with those reported by Zheng et al. [44] who found a strong correlation between total phenolic content and FRAP assay.

Discussion

The results of this investigation have demonstrated that screening for antioxidant activity of organic solvent fractions of *P. insolens* Kitagawa leaves as well as roots by DPPH, ABTS and FRAP assays gives essentially identical results. From this investigation, we found that not only root but also leaf of *P. insolens* Kitagawa possesses strong antioxidant capacity in vitro.

The various types of phytochemicals present in plants such

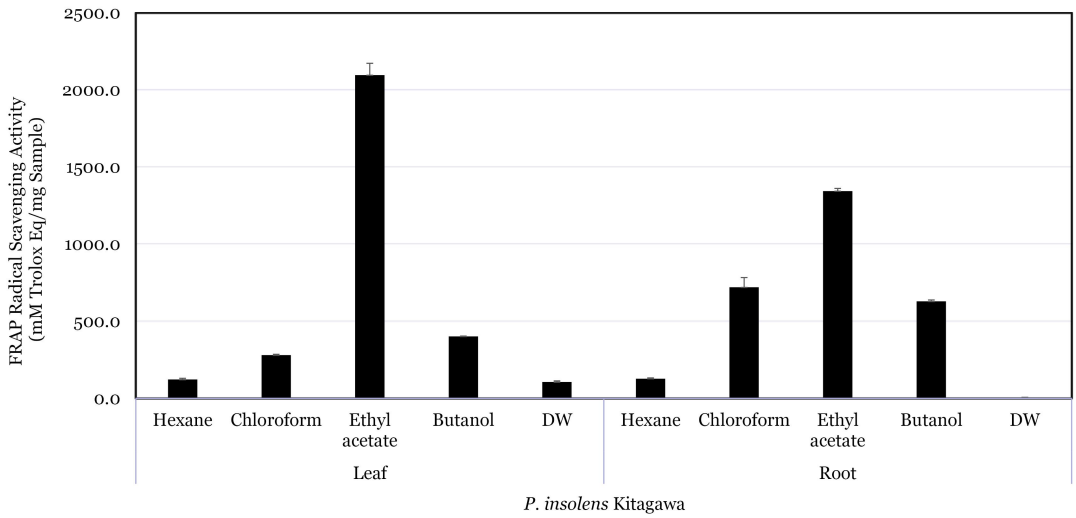

Fig. 4. Comparison of FRAP radical-scavenging activities in organic solvent-fractions from leaves and roots of *P. insolens* Kitagawa. All experiments were performed in triplicate and results are expressed as mean ± SD.

as phenols, flavonoids and alkaloids, are responsible for the effective biological activities of plants, including antioxidant and anti-inflammatory effects [25]. However, to the best of our knowledge, no previous studies have reported antioxidant and anti-inflammatory effects of organic solvent-extracts of *P. insolens* Kitagawa roots on macrophage cells for their biological activities. For the first time, our research team has identified that there are components that show strong antioxidant and anti-inflammatory activities in the root of *P. insolens* Kitagawa [18]. In this study, we confirmed that there was strong antioxidant activity not only in the roots of *P. insolens* Kitagawa but also in the leaves. From this study, we had demonstrated the total phenolic content, total flavonoids and total antioxidant capacity of *P. insolens* Kitagawa leaf as well as by three different free radical scavenging assays such as DPPH, ABTS and FRAP. The present study examined antioxidant capacities of organic solvent-extracts of *P. insolens* Kitagawa and compared the antioxidant capacities between the leaf and root extracts. Our results showed that ethyl acetate fraction of *P. insolens* Kitagawa leaf and root contained relatively high contents of total flavonoid (100.3±13.1 vs 128.8±15.6 mg QE/mg DE) and total polyphenol (101.1±1.0 vs 128.8±15.6 mg GAE/mg DE). DPPH assay result showed that *P. insolens* Kitagawa possessed strong free radical scavenging activity in the ethyl acetate fraction of both leaf and root of the plant. This result is thought to be because the ethyl acetate-fraction contains a high amount of total flavonoid and total polyphenol.

It was apparent that extracts displaying good antioxidant behavior in both the DPPH, ABTS and FRAP assays could be identified by high phenolics content. The finding that the results of the DPPH, ABTS and FRAP assays for plant extracts were highly correlated agrees with the work of others [12, 35, 41]. The three assays share a similar mechanistic basis, namely, transfer of electrons from the antioxidant to reduce an oxidant, as proposed by Huang et al., [15]. DPPH radical is a relatively stable free radical that is reduced by antioxidants, aromatic amines, etc., and is widely used to explore the antioxidant activity of various natural products [19]. ABTS radical scavenging activity can be measured in a short time, and it is a method that is widely used because it can measure not only hydrophilicity but also antioxidative activity of hydrophobic substances [3]. A number of papers have reported results from both DPPH, ABTS and FRAP (or other Fe^{3+}-reduction) assays on plant extracts [12, 29, 36, 42], presumably in the belief that using three assays improves the overall estimate of antioxidant capacity of the plant extracts. Total phenolic content assay is another assay that is commonly used in conjunction with either or both of the DPPH, ABTS and FRAP assays on a particular plant extract. The results presented in this study indicate that high antioxidant activity is associated with a high phenolic content, agreeing with a finding reported previously many times [12, 29, 41, 43]. Thus, it could be argued that the only virtue in performing the total phenolic content assay would either be as a screen to evaluate extracts further by the DPPH, ABTS or FRAP assay.

Several reports showed a close relationship between total phenolic content and high antioxidant activity [2, 21, 28]. The phenolic compounds exhibit extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties [32]. Li et al. [20, 21] have reported the existence of similar linear relationships between reducing power and total phenol content. Our Spearman correlation analysis also support that the phenolic content in the extracts of *P. insolens* Kitagawa showed a much higher correlation with reducing power than with the radical scavenging activity. From this analysis, we could estimate that the phenolic compounds present in the extracts of *P. insolens* Kitagawa act as an antioxidants directly through the mechanism of the reduction of oxidized intermediate in the chain reaction. Therefore, the present study provides the useful information about proximate composition, antioxidant properties and polyphenolic contents of *P. insolens* Kitagawa, which may be applicable for the therapeutic purposes.

In conclusion, from the results of this study, it was confirmed that there are many potentially active ingredients that show strong antioxidant activity not only in the roots of *P. insolens* Kitagawa but also in the leaves. By confirming strong antioxidant activity from *P. insolens* Kitagawa leaves, an important advantage secured from this study is that it is possible to supply antioxidant active materials easily without damaging *P. insolens* Kitagawa plants. To better understand their ability to control oxidative stress-related diseases, further investigation into the isolation and identification of responsible antioxidant components and their action mechanism of anti-oxidative stress is necessary.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 왕산방풍의 잎과 뿌리의 유기용매 분획물에서의 항산화 활성 비교

오명석[1] · 나란투야 난딘셋세그[1] · 박찬주[1] · 남길수[2] · 조식제[3] · 문자영[1]*
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본 연구는 *Peucedanum insolens* Kitagawa의 잎과 뿌리에서 추출한 용매 분획물들의 항산화 활성을 비교 분석하기 위해 수행되었다. 본 연구를 위해 *P. insolens* Kitagawa의 건조된 잎과 뿌리를 각각 70% 에탄올로 먼저 추출한 후 hexane, chloroform, ethyl acetate, n-butanol, 물의 순서로 분획하였다. 실험 결과 유기용매 분획별 총 폴리페놀 함량은 *P. insolens* Kitagawa의 잎과 뿌리 모두에서 동일한 분포 양상을 보였다. 유기용매 분획 중 총 폴리페놀 함량은 에틸아세테이트 분획(101.1±1.0 μg vs 128.8±15.6 μg GAE/mg)에서 가장 높게 나타났으며, 헥산 분획에서 가장 낮은 함량(9.51±0.2 μg vs 6.8±0.8 μg GAE/mg)을 보였다. *P. insolens* Kitagawa의 잎과 뿌리 모두에서 유기용매 분획에 의한 총 플라보노이드 함량 분포는 총 폴리페놀 함량과 동일한 양상을 보였다. *P. insolens* Kitagawa 잎과 뿌리의 유기용매 추출물을 DPPH, ABTS, FRAP assay를 통해 활성산소 소거 활성을 측정한 결과, 잎과 뿌리 추출물에서도 동일한 패턴으로 활성산소 소거 활성을 보였으며, 유기용매 분획 중 에틸아세테이트 분획이 가장 높은 자유 라디칼 소거 활성을 보였다. *P. insolens* Kitagawa의 잎과 뿌리의 유기용매 분획물에서 총 페놀 함량과 자유 라디칼 소거 활성 사이에 유의한 상관관계(R=0.903, p<0.0001, DPPH 라디칼, R=0.891, p<0.001, ABTS 라디칼, R=0.745, p<0.05, FRAP 라디칼)와 *P. insolens* Kitagawa의 잎 또는 뿌리의 용매 분획물에서 DPPH, ABTS 및 FRAP assay들 사이에서도 중요한 상관관계(R=0.867, p<0.001, DPPH vs ABTS 라디칼, R=0.882, p<0.0001, DPPH vs FRAP 라디칼, R=0.973, p<0.0001, ABTS vs FRAP 라디칼)가 있음이 확인되었다. 본 연구 결과에서 얻은 결론은 왕산방풍 식물의 뿌리에서와 마찬가지로 왕산방풍의 잎에서도 강한 항산화 활성을 가지고 있으며, 잎을 주요 항산화 물질의 소재로 사용할 수 있다.