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Correlation of antioxidant and radical scavenging activity in *Hydrangea macrophylla* L. extract from various cultivars

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Abstract

In this study, the polyphenol content [total polyphenol content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC)] and antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric ion reducing antioxidant power (FRAP), phosphomolybdenum complex assay (PMA), and oxygen radical absorbance capacity (ORAC)] of hydrangea leaf extract, collected based on the cultivation method of each variety, were confirmed, and the correlation between them was evaluated. To confirm the polyphenol content, TPC, TFC, and TAC contents were measured. The TPC was highest in Morning Star (open field) at 4.65 ± 0.00 mg tannic acid equivalent/g, while the TFC was highest in *Hydrangea serrata* (open field) at 6.44 ± 0.00 mg catechin equivalent/g. The anthocyanin content was highest in *H. serrata* (open field) at 1.56 ± 0.00 mg CGE/100 g DW. The antioxidant activity (PMA and FRAP) using the reducing power of transition metal ions was 6.17 ± 0.00 mg ascorbic acid equivalent/g in *H. serrata* (greenhouse) and 140.76 ± 0.00 mM Trolox equivalent (TE)/g in *H. serrata* (open field). The antioxidant activities measured by radical scavenging assays were highest in White ari (open field) for ABTS at 9.97 ± 0.00 mM TE/g, *H. serrata* (open field) for DPPH at 28.48 ± 0.00 mM TE/g, and *H. serrata* (open field) for ORAC at 81.09 ± 0.31 mM TE/g. Our study established a significant quadruple correlation between TPC and radical scavenging activity (ABTS, DPPH, and ORAC) and between transition metal ion (FRAP) and radical scavenging activity (ABTS, DPPH, and ORAC).

Keywords: *Hydrangea macrophylla* L.; total phenolic contents; antioxidant activity; correlation; extract.

Practical Application: A Study on the Antioxidant Activity of *Hydrangea macrophylla* L. Extract.

1 INTRODUCTION

As medical care and living standards improve, there will be an increase in human life expectancy, desire for a healthy life, awareness of health and well-being, as well as disease prevention and aging (Joo et al., 2015; Park & Ryu, 2022). One of the causes of aging and diseases is the accumulation of harmful substances produced during the metabolic process and the production of lipid peroxides in cells by free radicals *in vivo* (Woo et al., 2010). In addition, free radicals cause skin inflammation, lipid and membrane oxidation, and suppression of skin immune function, destroying the protective membrane of the skin (Nam & Ko, 2020; Park & Lee, 2019). Reactive oxygen, which is continuously produced during biometabolism, is a reducing metabolite of oxygen and is formed by the metabolism of mitochondria or the action of enzymes in the cytoplasm. Over-generated active oxygen breaks the balance of the antioxidant defense system in the body, damaging the structure and membrane in the cell and causing oxidative stress (Jo et al., 2020). Within the human body, non-enzymatic antioxidants such as vitamin E and reduced glutathione help protect cells and tissues from damage caused by free radicals and reactive oxygen species. Additionally, enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase are also found (Kim et al., 2012). Research is actively underway to develop natural antioxidants derived from plants that can indirectly increase or directly eliminate reactive oxygen species without side effects on the human body (Ryu et al., 2017). The development of safe and highly effective natural antioxidants that can replace the existing antioxidants is an urgent need (Kim et al., 2022).

Hydrangea macrophylla is a deciduous shrub belonging to the family *Hydrangeaceae*. It blooms with 10–15-cm-sized flowers from June to July. At first, the flowers start off white but gradually turn blue and then become reddish, eventually changing to purple. The color varies depending on the soil composition, as the acidity of the soil affects the color of the flowers. It is known that there are physiological characteristics that give a bluish color to flowers in strongly acidic soil and a reddish color in alkaline soil (Jo et al., 2020). Hydrangea plants, originally esteemed for their ornamental value, have been predominantly used for decoration. However, the leaves, stems,

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and roots of hydrangea plants are also used for herbal remedies, drawing attention to their medicinal properties and effects (Lee & Kim, 2011). Leaf extract of hydrangea has been reported to possess various biological activities, including antimicrobial (Jung et al., 2016), anti-ulcer, and anti-allergic effects and anti-inflammatory, hepatoprotective, and anti-malarial activities (Shin et al., 2019). It has been reported that many bioactive compounds such as dihydroisocoumarins, secoiridoids, stilbenes, hydrangenol, phyllodulcin, macrophylloside, and their glucosides are present in hydrangea species (Na et al., 2021; Shin et al., 2019). According to pharmacological research, these compounds have been shown to have biological functions such as antidiabetic and antibacterial activity (Yang et al., 2012), but research on the antioxidant comparison of different varieties of hydrangea leaves based on cultivation conditions and other correlation studies is limited.

Therefore, our objective was to assess the total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), and antioxidant activities [1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric ion reducing antioxidant power (FRAP), phosphomolybdenum complex assay (PMA), and oxygen radical absorbance capacity (ORAC)] of different varieties of hydrangea leaves according to the cultivation methods. Furthermore, we aimed to examine the correlations between these factors.

2 MATERIALS AND METHODS

2.1 Materials and reagents

The reagents used in this study, including Folin-Ciocalteu reagent, gallic acid, catechin, DPPH, ABTS, and (±)-6-hydroxy-2,5,7,8-etramethylchromane-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and all other solvents and reagents used were of guaranteed grade.

The equipment used included a grinder (NFM-3561SN, NUC Co., Daegu, Korea), an extraction apparatus (MS-DM, MISUNG Co., Seoul, Korea), a rotary vacuum concentrator (N-1000, EYELA, Japan), a spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea), a microplate spectrophotometer (Thermo Fisher Scientific, Multiskan Sky, Boseong, Korea), and a fluorescence microplate reader (Spectra Max Gemini EM, Molecular Devices Co., California, USA).

2.2 Samples and extraction

In this experiment, a total of three hydrangea leaf cultivars were utilized for extraction, including *Hydrangea serrata* (greenhouse, open field), as well as the cultivars Morning Star (greenhouse, open field) and White ari (greenhouse, open field), which have undergone varietal improvement, provided by the Jeonnam Agricultural Technology Institute. The six hydrangea leaf samples were subjected to heat drying at 70°C for 18 h and then ground into powder. The ground powder (2 g) was mixed with 70% EtOH (100 mL), refluxed, cooled at 70°C for 5 min, and then filtered using a 185-mm Whatman filter paper (GE Healthcare Life Sciences, California, USA). The extraction process was repeated three times. The extracted solution was filtered through a 0.45-μm Whatman polyethersulfone sterilized syringe filter (Navimro, Seoul, Korea) and stored in a refrigerated reagent storage at 4°C for use in the experiments.

2.3 Total polyphenol contents

The TPC of the hydrangea leaf extract was measured using the Folin-Ciocalteu colorimetric method (Folin & Denis, 1912). Each extract (500 μL) was mixed with 500 μL of the Folin-Ciocalteu reagent and allowed to react at room temperature for 3 min. Then, 10% Na_2CO_3 (500 μL) was added, and the mixture was allowed to react for 1 h while shielded from light. The absorbance was measured at 760 nm using a spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). Tannic acid was used as a standard solution. The results for the six samples were calculated as tannic acid equivalent (TAE) per gram of the extract based on the tannic acid standard calibration curve ($y = 0.0548x + 0.1432$, $R^2 = 0.999$).

2.4 Total flavonoid contents

The TFC of the hydrangea leaf extract was measured using the Saleh and Hameed's method (Abdel-Hameed, 2009). A volume of 200 μL of each extract was mixed with 800 μL of 80% ethanol. Then, 60 μL of 5% $NaNO_2$ was added, and the mixture was allowed to react for 5 min. Next, 60 μL of 10% $AlCl_3$ was added, and the mixture was allowed to react for 5 min at room temperature. Finally, 400 μL of 1 N NaOH was added and the absorbance was measured at 510 nm using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). Catechin was used as a standard solution. The results for the six samples were calculated as catechin equivalent (CE) per gram of extract based on the catechin standard calibration curve ($y = 0.0068x - 0.0799$, $R^2 = 0.999$).

2.5 Total anthocyanin content

The TAC of hydrangea leaf extract was performed by using the Benvenuti's analysis method (Benvenuti et al., 2004). pH 1.0 buffer (0.025 M potassium chloride) and pH 4.5 buffer (0.4 M sodium acetate) were prepared, and the extracts were diluted with each buffer. The diluted samples were measured at 510 and 700 nm using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea) to measure the absorbance at both wavelengths. Cyanidin 3-*O*-glucoside was used as a standard solution, and anthocyanin content was calculated using the measured absorbance values (Equation 1):

$$A*MW*D*100/\varepsilon \quad (1)$$

Where:

*A (absorbance value): $[(A_{510nm}-A_{700nm})\ pH1.0-[(A_{510nm}-A_{700nm})\ pH4.5]$;

*MW (cyanidin 3-*O*-glucoside molecular weight): 449.2;

*D (dilution factor): dilution factor;

*ε (cyanidin 3-*O*-glucoside molar absorptivity): 26.900.

2.6 Ferric ion reducing antioxidant power

The FRAP analysis was conducted by applying the method developed by Benzie and Strain (1996). To conduct the experiment, FRAP working solution [300 mM acetate buffer (pH 3.6): 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ): 20 mM $FeCl_3 \cdot 6H_2O$ = 10:1:1] was prepared and incubated at 37°C for 10 min. A 50 μL aliquot of the extract was mixed with 1,050 μL of the FRAP working solution, and the mixture was incubated at 37°C for 30 min. The absorbance at 595 nm was measured using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). Trolox was used as a standard solution, and the Trolox equivalent (TE) values of each extract were calculated and expressed as TE per gram of extract according to the Trolox standard calibration curve (y = 0.3515x + 0.0568, R^2 = 0.999) obtained from the measurement of six extracts.

2.7 Phosphomolybdenum complex assay

The PMA was evaluated by the phosphomolybdenum antioxidant assay method developed by Prieto et al. (1998). Each extract was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in 100 μL aliquots, and the mixture was incubated at 90°C for 90 min in a water bath. After incubation, the mixture was cooled to room temperature (25°C). The absorbance was measured at 695 nm using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). L-Ascorbic acid was used as the standard substance, and the ascorbic acid equivalent (AAE) values of each extract were calculated and expressed as AAE per gram of extract according to the L-ascorbic acid standard calibration curve (y = 0.0021x + 0.0165, R^2 = 0.991) obtained from the measurement of extracts.

2.8 ABTS radical scavenging activity

The ABTS radical scavenging activity, which is one of the methods for measuring the antioxidant activity of a sample, was measured using the method developed by Pellegrini (Re et al., 1999). To measure the ABTS radical scavenging activity, a 7.4 mM ABTS solution and a 2.45 mM potassium persulfate solution were mixed in a 1:1 ratio, and the mixture was kept at room temperature for about 24 h to form ABTS radicals. The mixture was then diluted with distilled water to obtain a working solution with an absorbance of approximately 0.7 (± 0.2) at 734 nm. Notably, 150 μL of each extract was mixed with 1,350 μL of the working solution and incubated in the dark for 5 min. The absorbance at 734 nm was measured using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea), and the results were expressed as TE per gram of extract, based on the Trolox standard curve (y = -0.0029x + 0.05841, R^2 = 1.000).

2.9 DPPH radical scavenging activity

The DPPH radical scavenging activity is a method of measuring antioxidant activity that is based on the principle that the DPPH radical is decolorized from a deep purple to a yellow color when it reacts with substances possessing antioxidant activity, resulting in a decrease in absorbance (Yen & Chen, 1995). To conduct the experiment, a working solution was prepared by mixing 0.2 mM DPPH with 50 mL of methanol. Then, 950 μL of the working solution was added to each 50 μL of extract, and the mixture was allowed to react for 30 min. The absorbance at 515 nm was measured using the spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). The results for the six samples were expressed as TE per gram of extract, based on the Trolox standard curve (y = -0.0035x + 2.8883, R^2 = 0.999).

2.10 Oxygen radical absorbance capacity

The ORAC analysis method used to measure the antioxidant activity of the samples was based on the experimental procedures developed by Talcott and Lee (2002) with some modifications. Trolox was used as the standard solution. Each extract was mixed with 100 nM fluorescein (70 μL) and incubated for 30 min at 37°C, followed by the addition of 80 μL of 221 mM AAPH to initiate the reaction. The reaction mixture was then measured for absorbance every 2 min for 1 h at 37°C using a fluorescence microplate reader at 480 and 520 nm wavelengths, and the TE per gram of extract was calculated based on the Trolox standard curve (y = 0.3241x + 1.1656, R^2 = 0.999) (Equations 2 and 3).

$$\text{AUC (area under the curve)} = 1 + (RFU_1 + RFU_2 + \ldots RFU_{29} + RFU_{30})/RFU_0 \quad (2)$$

$$\text{Net AUC} = \text{AUC (Antioxidant)} - \text{AUC (Blank)} \quad (3)$$

2.11 Statistical analysis

All analytical values presented in this study were expressed as the mean ± standard deviation (Mean ± SD) of three repeated measurements. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), and a one-way analysis of variance (ANOVA) was conducted to test the statistical significance. If there was significance, Duncan's multiple range test was conducted, and significance was determined when $p < 0.05$. Correlations were indicated with significance using Pearson's correlation coefficient.

3 RESULTS

3.1 Total polyphenol, flavonoid, and anthocyanin content

In this study, the TPC, TFC, and TAC in the hydrangea leaf extract were measured and compared using tannic acid, catechin, and cyanidin 3-*O*-glucoside as standard substances. The TPC was highest in the *H. serrata* (3.24 ± 0.01 mg TAE/g) and Morning Star (4.65 ± 0.00 mg TAE/g) cultivars in both greenhouse and open-field conditions. The TFC was highest in the *H. serrata* (2.21 ± 0.00 mg CE/g and 6.44 ± 0.00 mg CE/g) cultivars in both greenhouse and open-field conditions, and the TAC was highest in the White ari (1.42 ± 0.00 mg CGE/100 g DW) and *H. serrata* (1.56 ± 0.00 mg CGE/100 g DW) cultivars in both greenhouse and open-field conditions (Table 1).

Table 1. Total polyphenol contents, total flavonoid contents, and total anthocyanin contents of *Hydrangea* L. extract.

Sample	Total Polyphenol Contents (mg TAE/g)	Total Flavonoid Contents (mg CE/g)	Total Anthocyanin Contents (mg CGE/100 g DW)
Hydrangea serrata (GH)	3.24 ± 0.01^{c}	2.21 ± 0.00^{d}	0.96 ± 0.01^{a}
White ari (GH)	1.52 ± 0.00^{a}	1.37 ± 0.00^{a}	1.42 ± 0.00^{e}
Morning star (GH)	2.47 ± 0.01^{b}	1.74 ± 0.00^{c}	0.98 ± 0.00^{d}
Hydrangea serrata (OF)	2.40 ± 0.00^{f}	6.44 ± 0.00^{b}	1.56 ± 0.00^{e}
White ari (OF)	2.40 ± 0.00^{e}	4.58 ± 0.00^{b}	0.63 ± 0.00^{c}
Morning star (OF)	4.65 ± 0.00^{d}	3.41 ± 0.00^{e}	0.32 ± 0.00^{b}

TAE: tannin acid equivalent; CE: catechin equivalent; CGE: cyanidin-3-*O*-glucoside. Values are mean ± SD. Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey). Group according to cultivation method: greenhouses (GH) and open fields (OF).

3.2 Antioxidant activity of transition metal ions

The PMA method is based on the principle that the extract reduces Mo (VI) to Mo (V) and forms a green phosphate/Mo (V) complex at an acidic pH. The PMA antioxidant activity results of the greenhouse and open-field cultivars showed the highest values in the *H. serrata* (6.17 ± 0.00 mg AAE/g and 6.00 ± 0.00 mg AAE/g) cultivars, respectively (Table 2).

The FRAP method is a technique for measuring the total antioxidant capacity of a sample by analyzing the process in which the colored tripyridyltriazine complex reduces ferric ions to ferrous ions. This method was devised based on the principle that most antioxidants have reducing power, as the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to ferrous tripyridyltriazine (Fe^{2+}TPTZ) by a reducing agent at a low pH (Benzie & Strain, 1996). The FRAP antioxidant activity results showed the highest values in the greenhouse and open-field cultivars of *H. serrata* (40.85 ± 0.00 mM TE/g and 140.76 ± 0.00 mM TE/g, respectively) (Table 2).

3.3 Free radical scavenging activity

The ABTS radical scavenging activity was measured based on the principle that the greenish-blue ABTS cation radical (ABTS+) generated by the reaction between 2,2'-azino-bis diammonium salt (ABTS) and potassium persulfate is reduced by substances with antioxidant activity, leading to a decrease in absorbance as the color fades (Lee & Kim, 2011). The ABTS radical scavenging activity results showed the highest values in the greenhouse cultivar of *H. serrata* (3.24 ± 0.00 mM TE/g) and in the open-field cultivar of Morning Star (4.65 ± 0.00 mM TE/g) (Table 3).

The DPPH radical scavenging activity is determined by measuring the color change (from deep purple to bright yellow) resulting from the reduction of DPPH radical upon reaction with antioxidant compounds (Garcia et al., 2012). *H. serrata* (21.38 ± 0.00 mM TE/g and 28.48 ± 0.00 mM TE/g) had the highest antioxidant activity in greenhouse and open-field varieties (Table 3).

3.4 Oxygen radical absorbance capacity measurement

The ORAC is a method that indicates the extent of free radical damage based on changes in fluorescence intensity. In the presence of antioxidants, this principle measures antioxidant activity by the reduction of fluorescent material through AAPH

Table 2. Phosphomolybdenum complex assay and ferric ion reducing antioxidant power of *Hydrangea* L. extract.

Sample	PMA (mg AAE/g)	FRAP (mM TE/g)
Hydrangea serrata (GH)	6.17 ± 0.00^{f}	40.85 ± 0.00^{c}
White ari (GH)	5.42 ± 0.00^{d}	16.81 ± 0.00^{a}
Morning star (GH)	5.06 ± 0.00^{c}	29.14 ± 0.00^{b}
Hydrangea serrata (OF)	6.00 ± 0.00^{e}	140.76 ± 0.00^{f}
White ari (OF)	4.02 ± 0.00^{b}	109.04 ± 0.00^{e}
Morning star (OF)	3.71 ± 0.00^{a}	81.82 ± 0.00^{d}

PMA: phosphomolybdenum complex assay; FRAP: ferric ion reducing antioxidant power; AAE: ascorbic acid equivalent; TE: Trolox equivalent. Values are mean ± SD. Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey).

Table 3. ABTS, DPPH radical scavenging activity, and oxygen radical absorbance capacity of *Hydrangea* L. extract.

Sample	ABTS (mM TE/g)	DPPH (mM TE/g)	ORAC (mM TE/g)
Hydrangea serrata (GH)	9.43 ± 0.00^{c}	21.38 ± 0.00^{d}	45.90 ± 0.96^{a}
White ari (GH)	3.20 ± 0.00^{a}	18.56 ± 0.00^{a}	23.68 ± 0.82^{e}
Morning star (GH)	6.46 ± 0.00^{b}	17.22 ± 0.00^{c}	33.66 ± 1.08^{d}
Hydrangea serrata (OF)	9.87 ± 0.00^{f}	28.48 ± 0.00^{b}	81.09 ± 0.31^{e}
White ari (OF)	9.97 ± 0.00^{e}	26.18 ± 0.00^{b}	52.17 ± 0.91^{c}
Morning star (OF)	9.86 ± 0.00^{d}	23.10 ± 0.00^{e}	30.33 ± 0.16^{b}

ABTS: ABTS radical scavenging activity; DPPH: DPPH radical scavenging activity; ORAC: oxygen radical absorbance capacity; TE: Trolox equivalent. Values are mean ± SD. Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey).

(Gokarn & Tamoli, 2021). In this study, the antioxidant activity of hydrangea leaf extract was expressed as a fluorescence value and a TE value that decreased over time (Figure 1 and Table 3). ORAC antioxidant activity results showed that the highest values were obtained from the greenhouse and open-field varieties of *H. serrata* (45.90 ± 0.96 mM TE/g and 81.09 ± 0.31 mM TE/g, respectively).

3.5 Correlation between antioxidant content and antioxidant activity

The correlation between antioxidant activity (PMA, FRAP, ABTS, DPPH, and ORAC) and total phenol content (TPC, TFC, and TAC) of each variety of hydrangea leaves was analyzed and shown (Table 4 and Figure 2). As for the zero-based correlation value, values closer to 1 indicate a positive correlation and values closer to -1 indicate a negative correlation.

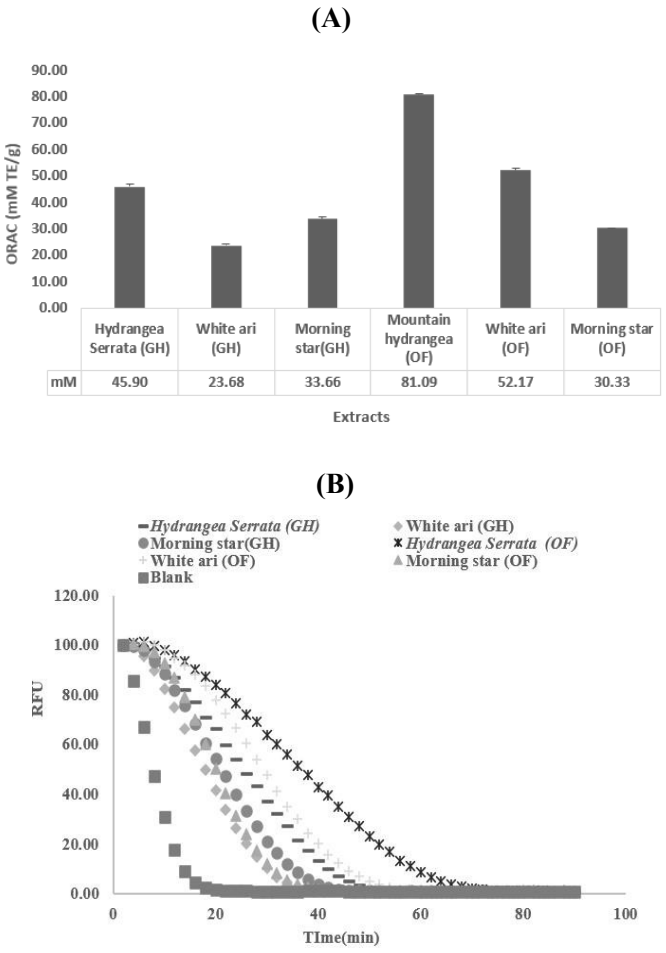

Figure 1. Oxygen radical absorbance capacity (ORAC) of *Hydrangea* L. extract. (A) ORAC by *Hydrangea* L. extract. Values are mean ± SD. (B) Effect of *Hydrangea* L. extract on fluorescein consumption induced by AAPH.

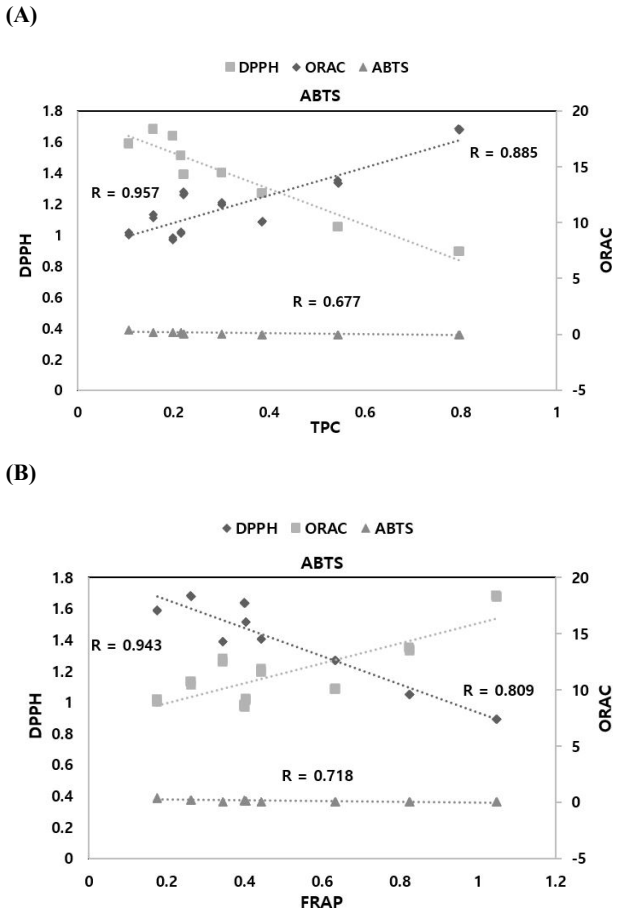

Figure 2. Antioxidant activity correlation between total polyphenols and transition metal ions and antioxidant activity of *Hydrangea* L. extract. (A) Correlation between TPC and radical scavenging activity (ABTS, DPPH, and ORAC). (B) Correlation between FRAP and radical scavenging activity (ABTS, DPPH, and ORAC).

Table 4. Correlation between polyphenol contents and antioxidants of *Hydrangea* L. extract.

Factors	Antioxidant contents			Reducing power		Radical scavenging activity		
	TPC	TFC	TAC	FRAP	PMA	ABTS	DPPH	ORAC
TPC	1	0.080	0.109	0.986**	0.026	0.677**	0.957**	0.885**
TFC		1	0.446*	0.142	0.350	0.634**	0.154	0.068
TAC			1	0.112	0.096	0.439*	0.058	0.082
FRAP				1	0.133	0.718**	0.943**	0.809**
PMA					1	0.209	0.019	0.306
ABTS						1	0.711**	0.591**
DPPH							1	0.865**
ORAC								1

TPC: total polyphenol contents; TFC: total flavonoid contents; TAC: total anthocyanin contents; PMA: phosphomolybdenum complex assay; FRAP: ferric ion reducing antioxidant power; ABTS: ABTS radical scavenging activity; DPPH: DPPH radical scavenging activity; ORAC: oxygen radical absorbance capacity; Correlation is significantly different at *$p < 0.05$ and **$p < 0.01$ (Pearson).

The quadruple correlation between TPC and radical scavenging ability of six types of leaf hydrangea by cultivar, TPC, DPPH ($R = 0.957$, $p < 0.01$), ABTS ($R = 0.677$, $p < 0.01$), and ORAC ($R = 0.885$, $p < 0.01$) showed a significant correlation, indicating that the higher the TPC, the higher the correlation with the radical scavenging activity. Second, as a result of the quadruple correlation between transition metal ion reducing power and radical scavenging ability, FRAP and DPPH ($R = 0.943$, $p < 0.01$), ABTS ($R = 0.718$, $p < 0.01$), and ORAC ($R = 0.809$, $p < 0.01$), it can be seen that the higher the transition metal ion reducing power, the higher the correlation with the radical scavenging ability.

4 CONCLUSION

The polyphenol content (TPC, TFC, and TAC) and antioxidant activities (FRAP, PMA, ABTS, and ORAC) of each variety of hydrangea leaves were measured, and the correlation between them was analyzed to study their use as a natural antioxidant. The polyphenol content (TPC, TFC, and TAC) was the highest in Morning star (open field, 4.65 mg TAE/g), *H. serrata* (open field, 6.44 mg CE/g), and *H. serrata* (open field, 1.56 mg CGE/100 g DW), respectively. The reducing power of transition metal ions was high in *H. serrata* measured by PMA (greenhouse, 6.17 mg AAE/g) and FRAP (open field, 140.76 mM TE/g). The free radical scavenging activity (ABTS and DPPH) was the highest in Morning star (open field, 9.86 mM TE/g) and *H. serrata* (greenhouse, 28.48 mM TE/g). The correlation between TPC and radical scavenging activity (ABTS, DPPH, and ORAC) and the correlation between transition metal ions (FRAP) and radical scavenging activity (ABTS, DPPH, and ORAC) were confirmed. Through these results, *H. serrata* (open field) was the best among the six types of hydrangea leaves, and the possibility of developing natural antioxidants from six types of hydrangea leaves was confirmed.

REFERENCES

Abdel-Hameed, E. S. S. (2009). Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. *Food Chemistry*, *114*(4), 1271-1277. https://doi.org/10.1016/j.foodchem.2008.11.005

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, Anthocyanins, Ascorbic Acid, and Radical Scavenging Activity of Rubus, Ribes, and Aronia. *Journal of Food Science*, *69*(3), FCT164-FCT169. https://doi.org/10.1111/j.1365-2621.2004.tb13352.x

Benzie, I. F. F., Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Folin, O., & Denis, W. (1912). On phosphotungasticphosphomolybdic compounds as color reagents. *Journal Biological Chemistry*, *12*(2), 239-243. https://doi.org/10.1016/S0021-9258(18)88697-5

Garcia, E. J., Cadorin, O. T. L., Severino, M. A., Reis, A., Loguercio, A. D., & Grande, Rosa, H. M. (2012). Antioxidant activity by DPPH assay of potential solution to be applied on bleached teeth. *Brazilian Dental Journal*, *23*(1), 23-27. https://doi.org/10.1590/S0103-64402012000100004

Gokarn, V., & Tamoli, S. (2021). Comparative in vitro evaluation of lipophilic oxygen radical absorbance capacity of Standardized Curcuma longa extract vs standardized FMB CurcuZn extract. *International Journal of Herbal Medicine*, *9*(5), 42-45. https://doi.org/10.22271/flora

Jo, Y.-J., Lee, Y.-B., Hyun, J.-S., Kim, C.-Y., & Lee, N.-H. (2020). Anti-oxidative and Anti-inflammatory Constituents from the Extracts of Hydrangea macrophylla Flowers. *Journal of the Korean Applied Science and Technology*, *37*(5), 1356–65.

Joo, D.-H., Yoo, D.-H., Lee, S.-Y., & Lee, J.-Y. (2015). Whitening Effect of Astragalus Membranceus Bunge Water Extract and Development of Cosmeceutical. *Journal of the Korean Society of Cosmetology*, *21*(5), 888-894.

Jung, C.-H., Kim, Y., Kim, M.-S., Lee, S., & Yoo, S.-H. (2016). The establishment of efficient bioconversion, extraction, and isolation processes for the production of phyllodulcin, a potential high intensity sweetener, from sweet hydrangea leaves (Hydrangea macrophylla Thunbergii). *Wiley Analytical Science*, *27*(2), 140-147. https://doi.org/10.1002/pca.2609

Kim, H.-J., Kim, K.-S., Kim, D.-I. (2012). Inhibitory Effects of Lespedeza cuneata Ethanol Extract on Ultraviolet-Induced Photo Aging. *Journal of the Korean Society of Food Science and Nutrition*, *41*(11), 1540-1545.

Kim, J. H., Hwang, Y. S., Park, J. H., Kang, M. H., Oh, Y. S., & Kim, J. W. (2022). Determination of Antioxidant Activities and Bioactive Compounds from Rosa rugosa Extract. *Journal of Life Science*, *32*(11), 841-846.

Lee, K. J., & Kim, H. Y. (2011). The Physico-chemical and Sensory Characteristics of Kakdoogi Containing Hydrangea serrata Seringe Extract. *The Korean Journal of Community Living Science*, *22*(2), 211-222.

Na, C. S., Kim, H. J., Ahn, H. S., Shin, Y.-K., & Lee, S. H. (2021). In Vitro Evidence for Inhibitory Action of Hydrangea serrata (Thunb.) Ser. Leaf Extract on Urate Crystal-Induced inflammation. *Food Supplements and Biomaterial for Health*, *1*(3), 27. https://doi.org/10.52361/fsbh.2021.1.e27

Nam, S. S., & Ko, K.-S. (2020). Antioxidants of apple leaf extract. *Journal of the Korean Applied Science and Technology*, *37*(5), 1116-1124. https://doi.org/10.12925/jkocs.2020.37.5.1116

Park, J.-H., Lee, N.-H. (2019). Anti-oxidative, Anti-inflammatory and Whitening Constituents from Extract of Hydrangea etiolaris Stems [dissertation]. Graduate School of Jeju National University. Retrieved from http://dcoll.jejunu.ac.kr/common/orgView/000000008938

Park, R.-J., Ryu, M.-J. (2022). Antioxidant and Antimicrobial Effect of Rosemary, Parsley, Thyme, Chive, and Dill Extracts. *Asian Journal of Beauty and Cosmetology*, *20*(3), 305-314. https://doi.org/10.20402/ajbc.2022.0020

Prieto, P., Pineda, M., & Aguilar, M. (1998). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, *269*(2), 337-341. https://doi.org/10.1006/abio.1999.4019

Re, R., Pellegrinia, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Ryu, J.-Y., Song, Y.-W., Moon, J.-Y., Jun, N.-J., Kim, C. S. (2017). Antioxidant activities of soymilk added with green tea and rosemary extract. *Korean Journal of Food Preservation*, *24*(6), 871-878. https://doi.org/10.11002/kjfp.2017.24.6.871

Shin, J.-S., Han, H.-S., Lee, S.-B., Myung, D.-B., Lee, K., Lee, S. H., Kim, H. J., & Lee, K.-T. (2019). Chemical Constituents from Leaves of Hydrangea serrata and Their Anti-photoaging Effects on UVB-Irradiated Human Fibroblasts. *National Library of Medicine*, *42*(3), 424-431. https://doi.org/10.1248/bpb.b18-00742

Talcott, S. T., Lee, J.-H. (2002). Ellagic Acid and Flavonoid Antioxidant Content of Muscadine Wine and Juice. *Journal of Agricurtural and Food Chemical*, *50*(11), 3186-3192. https://doi.org/10.1021/jf011500u

Woo, J. H., Shin, S. L., & Lee, C. H. (2010). Antioxidant Effects of Ethanol Extracts from Flower Species of Compositae Plant. *Journal of Korean Society of Food Science and Nutrition*, *39*(2), 159-164. https://doi.org/10.3746/jkfn.2010.39.2.159

Yang, C.-J., Wang, Z.-B., Zhu, D.-L., Yu, Y., Lei, Y.-T., & Liu, Y. (2012). Two New Cyanogenic Glucosides from the Leaves of Hydrangea macrophylla. *Molecules*, *17*(5), 5365-403. https://doi.org/10.3390%2Fmolecules17055396

Yen, G.-C., & Chen, H.-Y. (1995). Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *Journal of Agriculture and Food Chemistry*, *43*(1), 27-32.