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The correlation between the antioxidant and anti-aging activities of *Hydrangea serrata* Seringe extract

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Abstract

In this study, the correlation between antioxidant and anti-aging activities of *Hydrangea serrata* Seringe extract was confirmed. Total polyphenol and total flavonoid content were determined to be highest in 60% ethanol extract (11.45 ± 0.13 mg GAE/g) and 40% ethanol extract (19.27 ± 0.09 mg CE/g), respectively. In the reducing power experiment for transition metal ions, the 40% ethanol extract (285.96 ± 0.99 mM TE/g, 5.21 ± 0.99 mM TE/g) was the best for both. In the DPPH and ABTS radical scavenging activity, 80% ethanol extract (5.35 ± 0.00 mM TE/g) and 40% ethanol extract (969.14 ± 0.00 uM TE/g) were the best, respectively. In the ORAC assay, 60% ethanol extract showed the highest antioxidant activity (2.62 ± 1.22 mM TE/g). The RC_{50} values of the HR and HP experiments showed excellent activity in 20% ethanol extract (631.96 ± 0.6 μg/mL) and 40% ethanol extract (115.62 ± 0.9 μg/mL), respectively, and for active nitrogen evaluation of species elimination efficacy, the 40% ethanol extract (41.03 ± 3.7 μg/mL, 373.10 ± 5.1 μg/mL) displayed strong ($ONOO^-$) scavenging activity. In addition, 40% ethanol extract showed the highest anti-aging activity in the range of 16.40–42.54%. The correlation between reactive oxygen species (HR, HP) scavenging activity, active nitrogen species (NO, $ONOO^-$) scavenging activity, and anti-aging effect (collagenase inhibition) showed significantly high values. It was confirmed that the active nitrogen species scavenging rate has a high correlation with the anti-aging effect.

Keywords: *Hydrangea serrata* seringe; antioxidant; anti-aging; correlation.

Practical Application: Antioxidant and anti-aging effects were confirmed through experiments using Hydrangea serrata Seringe extract, and a correlation was confirmed between these evaluations. Based on these results, we aim to provide basic data for the development of natural antioxidant substitutes.

1 INTRODUCTION

More than 95% of the oxygen present in our body is reduced to water by combining with electrons generated during cellular metabolism. However, 2–3% of oxygen is incompletely reduced, leading to the formation of reactive oxygen species (ROS) (Alessio, 1993; Halliwell & Gutteridge, 1985). These ROS are highly reactive and possess potent oxidizing power, reacting quickly with other molecules and damaging cells and tissues. They also oxidize unsaturated fatty acids present in biological membranes, resulting in impaired membrane fluidity, inhibited enzyme and receptor activity, and damaged membrane proteins (Dean et al., 1993) (Figure 1). Therefore, the body has defense systems against ROS, including antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as natural antioxidants such as phenolic compounds, flavonoids, amino acids, peptides, and other substances obtained directly from the diet (Ames et al., 1981; Dalton et al., 1993; Namiki, 1990; Rice-Evans et al., 1996). Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole (BHA) have been widely used due to their effectiveness, economy, and safety, but their mutagenicity and toxicity to enzymes and lipids can lead to the development of cancer. Therefore, the development of natural antioxidant alternatives is necessary (Choe & Yang, 1982; Min et al., 2004; Ryu & Hwang, 1990). Various attempts have been made to enhance the body's antioxidant enzyme system and to obtain safe and natural antioxidants from natural sources (Cho et al., 2008).

Hydrangea, which was originally considered a high-value ornamental species and was used mainly for decoration, has recently gained attention for its medicinal properties, with the leaves, stems, and roots of the plant being used as herbal medicine. It contains various biologically active compounds such as dihydroisocoumarins, secoiridoids, and stilbenes (hydrangenol, phyllodulcin, macrophylloside, and glucosides). In particular, *Hydrangea serrata* Seringe, which belongs to the Hydrangea family and is used to make tea in Korea, has a sweet taste that is 1,000 times sweeter than sugar and is non-toxic compared to *Hydrangea aspera* DON from China and *Hydrangea macrophylla*

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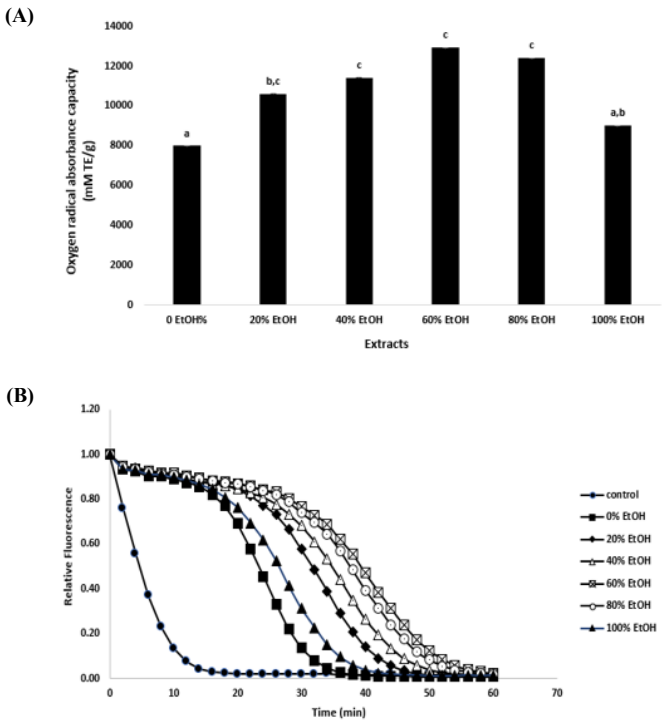


Figure 1. Oxygen radical absorbance capacity of *Hydrangea serrata* Seringe extracts. (A) Effect of *Hydrangea serrata* Seringe extracts on oxygen radical antioxidant capacity. Values are means ± SD. Means with different letters (a–d) in the same column are significantly different at p <0.05 (Tukey's test). (B) Effect of *Hydrangea serrata* Seringe extracts on fluorescein consumption induced by AAPH. Fluorescein (100 nM) was incubated in presence of AAPH (221 mM) and *Hydrangea serrata* Seringe extracts.

var. acumimata MAKINO from Japan. Its main sweetening component is phyllodulcin, which has been reported to have anti-allergic effects and inhibit microsomal lipid peroxidation induced by NADPH as well as phosphodiesterase in bovine adrenal cortical cells. Thunberginols and hydrangenols contained in *Hydrangea serrata* Seringe are reported to have anti-diabetic, anti-allergic, and antibacterial properties.

Although the biologically active compounds of *Hydrangea serrata* Seringe are known, research on their antioxidant and anti-aging activities is meager. Therefore, this study investigated the antioxidant and anti-aging activities of the native Jeju plant species *Hydrangea serrata* Seringe and confirmed the correlation between the two activities. Based on these results, we suggest *Hydrangea serrata* Seringe as a potential source for future natural antioxidant alternatives in pharmacy and food industries.

2 MATERIALS AND METHODS

2.1 Regents

Among the reagents used in this experiment, Folin-Ciocalteu reagent, gallic acid, catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), trolox, L-penicillamine, etc. were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and other extraction solvents and all reagents used were of guaranteed type. In addition, the equipment used in the experiment were a grinder (NFM-3561SN, NUC Co., Daegu, Korea), a reflux extractor (MS-DM, MISUNG Co., Seoul, Korea), a rotary vacuum concentrator (N-1000, EYELA, Japan), a spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea), and a microplate spectrophotometer (Thermo Fisher Scientific, Multiskan Sky, Boseong, Korea).

2.2 Preparation of Portulaca oleracea *L extracts*

The *Hydrangea serrata* Seringe sample used in this study was provided by the Jeonnam Bioindustry Promotion Agency. EtOH was selected as an extraction solvent through a preliminary experiment, and extraction conditions for each concentration were established. Solvent EtOH (100 mL) by concentration (0, 20, 40, 60, 80, and 100%) was added to 10 g of *Hydrangea serrata* Seringe and extracted under reflux for 1 h at 100°C. After filtration, it was concentrated using a rotary vacuum concentrator (N-1000, EYELA, Japan). Concentrated samples were stored in a refrigerated reagent cabinet at 4°C.

2.3 Antioxidant contents

2.3.1 Total polyphenol and total flavonoid contents

Total polyphenol content was determined by the Folin-Denis method using the Folin-Ciocalteu reagent (Folin & Denis, 1912). To 500 μL of each sample, 500 μL of Folin-Ciocalteu reagent was added, and it was incubated at room temperature for 3 min. After that, 500 μL of 10% Na_2CO_3 was added and incubated for 1 h in a dark room, and the absorbance was measured at 760 nm using a spectrophotometer (Optizen 2120 UV, Neogen, Sejong, Korea). According to the curve (y = 0.0548x + 0.1432, R^2 = 0.9992), the polyphenol content was calculated as the amount of gallic acid equivalent (GAE) per gram of extract.

The total flavonoid content was measured by partially modifying the method developed by Zhishen et al. (1999). To 200 μL of each sample, 800 μL of 80% ethanol and 60 μL of 5% $NaNO_2$ were added and incubated at room temperature for 5 min. Then, 60 μL of 10% $AlCl_3$ was added and reacted at room temperature for 5 min. After adding 400 μL of 1 N NaOH, the absorbance was measured at 415 nm using a spectrophotometer (Optizen 2120 UV, Neogen, Sejong, Korea). According to the standard calibration curve (y = 0.0025x + 0.0142, R^2 = 0.9992) prepared with catechin as a standard solution, the flavonoid content was calculated as CE (catechin equivalent) per gram of extract.

2.4 Reducing power activity

2.4.1 Ferric-reducing antioxidant power

The FRAP activity of *Hydrangea serrat*a Seringe was investigated using Pulido's method (Pulido et al., 2000). The FRAP solution was mixed with 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM $FeCl_3$ $6H_2O$ in a volume ratio of 10:1:1 at 37°C. After placing 35 μL of each sample in a tube and adding 1,050 μL of FRAP solution, it was incubated at 37°C for 30 min. Absorbance was measured at

595 nm using a UV-visible spectrometer (Optizen 2120 UV, Neogen, Sejong, Korea). According to the standard calibration curve (y = 0.0014x + 0.0628, R^2 = 0.9997) prepared with the standard solution trolox, the amount of trolox equivalent (TE) per gram of extract was expressed.

2.4.2 Phosphomolybdenum antioxidant activity

The PMA activity of *Hydrangea serrata* Seringe was evaluated by a phosphomolybdenum antioxidant assay by Prieto et al. (1999). Notably, 100 μL of the sample and 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were added to the test tube. The test tube was wrapped with parafilm and incubated for 90 min at 90°C in a water bath. After cooling the test tube at room temperature, the absorbance was measured at 695 nm using a UV-visible spectrometer (Optizen 2120 UV, Neogen, Sejong, Korea) and the standard calibration curve (y = 0.0021x - 0.0165, R^2 = 0.9910) and expressed as ascorbic acid equivalent (AAE) amount per gram of extract.

2.5 Radical scavenging activity

2.5.1 DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity test is a quick and simple measurement method that is widely used to measure the antioxidant activity of plant extracts or single compounds (Shi et al., 2009). A volume of 950 μL of 0.2 mM DPPH solution was added to 50 μL of each sample in a tube and mixed. The test tube was wrapped with parafilm to block the light and incubated at 25°C for 30 min. The absorbance at 515 nm using a spectrophotometer (Optizen 2120 UV, Neogen, Sejong, Korea) was measured using a standard calibration curve (y = -0.0011x + 1.3646, R^2 = 0.9997), expressed as the amount of TE per gram of extract. Ascorbic acid was used as a positive control.

2.5.2 ABTS radical scavenging activity

The ABTS radical scavenging activity test is a method to evaluate the antioxidant ability by using the characteristic of discoloration when the blue-green ABTS chromophore generated by the reaction of ABTS salt and potassium persulfate, a strong oxidizing agent, is reduced by a sample having excellent hydrogen donating ability (Hernandez-Rodriguez et al., 2019). To prepare the working solution for the ABTS assay, 7.4 mM ABTS and 2.45 mM potassium persulfate solutions were mixed in a 1:1 (v/v) ratio and incubated at 37°C overnight to generate radicals. The mixture was then diluted with distilled water to obtain a working solution with an absorbance of approximately 0.7 ($\pm$ 0.2) at 734 nm. A volume of 150 μL of each sample was placed in the tube, 1350 μL of ABTS solution was added, and the absorbance was measured at 734 nm after the light was blocked for 5 min. It was expressed in the amount of TE per extract G (y = -0.29 + 0.5841, R^2 = 0.9997) written in the standard solution trolox.

2.5.3 Oxygen radical absorbance capacity

The ORAC assay is a method for measuring the ability of antioxidant substances to inhibit the peroxyl radicals induced by peroxyl radical generators. It reflects the antioxidant capacity that occurs through hydrogen atom transfer (Ou et al., 2001). In a 96-well black microplate, 70 μL of 100 nM fluorescein solution was added, followed by 50 μL of the sample, and incubated at 37°C for 30 min. Then, 80 μL of 221 mM AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride) was added and mixed. The absorbance was measured every 2 min at 37°C for 1 h using a fluorescence microplate reader at excitation 480 nm and emission 520 nm wavelengths. The result was expressed as the amount of TE per gram of extract according to the standard calibration curve for Trolox (y = -0.3241x + 1.1656, R^2 = 0.9990), after calculating the net area under the curve (AUC) value by subtracting the AUC of the blank from the AUC of the sample fluorescence (Equations 1 and 2).

$$AUC = 1 + (RFU_1 \ldots RFU_{30})/RFU_0 \quad (1)$$

$$NET\ AUC = AUC\ (antioxidant) - AUC\ (blank) \quad (2)$$

2.6 Reactive oxygen species scavenging activity

2.6.1 Hydroxyl radical scavenging activity

The hydroxyl radical (OH) scavenging activity of the *Hydrangea serrata* Seringe extract was measured by modifying the method developed by Cho et al. (2015). A mixture of 1 mL of 9 mM Fe_2SO_4 and 1 mL of 9 mM salicylic acid was reacted in a conical tube. Then, 500 μL of 0.3% H_2O_2 and 1 mL of each sample were mixed and incubated in darkness for 30 min. The absorbance was measured at 510 nm using a UV-visible spectrometer (Optizen 2120 UV, Neogen, Sejong, Korea) (Equation 3).

$$\text{HR radical scavenging activity (\%)} = \frac{(A-B)}{A} \times 100 \quad (3)$$

A: Absorbance of control;

B: Absorbance of sample.

2.6.2 Hydrogen peroxide scavenging activity

The HP scavenging activity was measured by applying the method developed by Muller (1985), which utilizes the 2,2-azinobis (3-ethylbenzthiazolin)-6-sulfonic acid peroxidase system to measure the scavenging activity of H_2O_2. A sample (80 μL) was added to a 96-well plate, then mixed with 20 μL of 10 mM H_2O_2 and 100 μL of 0.1 M phosphate buffer (pH 5.0), and incubated for 5 min at 37°C. After that, 30 μL of 1.25 mM ABTS and 30 μL of 1 U/mL peroxidase were added, mixed, and then incubated for 10 min at 37°C. The absorbance was measured at 405 nm using a microplate spectrophotometer (Thermo Fisher Scientific, Multiskan Sky, Boseong, Korea) (Equation 4).

$$\text{HP radical scavenging activity (\%)} = \frac{(A-B)}{A} \times 100 \quad (4)$$

A: Absorbance of control;

B: Absorbance of sample.

2.7 Reactive nitrogen species scavenging activity

2.7.1 Nitric oxide scavenging activity

The NO scavenging activity of the extract from *Hydrangea serrata* Seringe was measured using the method developed by Nagata et al. (1999). Samples were added to a 96-well plate at various concentrations (10 μL per well) and mixed with 50 mM phosphate buffer (pH 7.4, 130 μL). Then, 10 μL of 40 mM SNP and 50 μL of DAF-2 were added to each well. The plate was incubated at room temperature for 10 min, and the fluorescence intensity was measured using a fluorescence microplate reader (SPECTRA MAX GEMINI EM, Molecular Devices Corp., California, USA) with excitation at 485 nm and emission at 525 nm (Equation 5).

$$\text{NO scavenging activity (\%)} = \left[1 - \frac{(A-B)}{A}\right] \times 100 \quad (5)$$

A: Absorbance of control;

B: Absorbance of sample.

2.7.2 Peroxynitrite ($ONOO^-$) scavenging activity

The scavenging activity of $ONOO^-$ was evaluated by measuring the fluorescence emitted from the oxidation of dihydrorhodamine 123 (DHR 123, Molecular Probes, Eugene, OR, USA) (Kooy et al., 1994). DHR 123 was prepared as a stock solution and used immediately before the experiment. Each sample (10 μL) was added to a 96-well black microplate, followed by the addition of rhodamine buffer (pH 7.4, 130 μL). DHR 123 (50 μL) was added and mixed, and then SIN-1 (10 μL) was added and allowed to react for 1 h at room temperature. The fluorescence intensity of $ONOO^-$ was measured using a fluorescence microplate reader (SPECTRA MAX GEMINI EM, Molecular Devices Corp., California, USA) at an excitation wavelength of 480 nm and an emission wavelength of 530 nm (Equation 6).

$$\text{Peroxynitrite } (ONOO^-) \text{ scavenging activity (\%)} = \left[1 - \frac{(A-B)}{A}\right] \times 100 \quad (6)$$

A: Absorbance of control;

B: Absorbance of sample.

2.8 Anti-aging effect from collagenase inhibition

To investigate the anti-aging effect, a collagenase inhibitory activity assay was conducted using the method developed by Wunsch and Heidrich (1963). A substrate solution was prepared by dissolving 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg at a concentration of 0.3 mg/mL in 0.1 M Tris-HCl buffer (pH 7.5) containing 4 mM $CaCl_2$. Prior to the experiment, each sample (100 μL) was added to a 15-mL Falcon tube, mixed with 250 μL of the substrate solution, and incubated with 150 μL of collagenase (prepared at a concentration of 0.2 mg/mL) at 37°C for 20 min. The reaction was stopped by adding 500 μL of 6% citric acid, followed by the addition of 1.5 mL of ethyl acetate for separation. The absorbance of the upper layer was measured at 320 nm using a spectrophotometer (Optizen 2120 UV, Neogen, Sejong, Korea). Epigallocatechin gallate was used as a control for collagenase (Equation 7).

$$\text{Collagenase Inhibition (\%)} = [1-(B/A)] \times 100 \quad (7)$$

A: Absorbance of control;

B: Absorbance of sample.

2.9 Statistics

All research data presented in this study were expressed as mean ± standard deviation (mean ± SD) from three independent experiments. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), and one-way analysis of variance (ANOVA) was conducted to test for statistical significance between each experimental group. If significance was found, a post hoc Tukey's test was conducted, and significance was determined at $p < 0.05$. Correlation was indicated using Pearson's correlation coefficient.

3 RESULTS AND DISCUSSION

3.1 Antioxidant contents

3.1.1 Total polyphenol contents

Polyphenols and flavonoids are known to have physiological effects, such as antioxidant activity, and are derived from natural sources (Bravo, 1998). The OH group in phenolic compounds is mainly responsible for the physiological effects, such as antioxidant, anticancer, and antibacterial effects (Droge, 2001; Halliwell et al., 1995). It has been reported that as the total polyphenol content increases, the physiological effects also increase (Halliwell et al., 1995; Imai et al., 1994; Rice-Evans et al., 1997). Moreover, it has been reported that over 4000 flavonoid-containing plants that produce yellow pigments also display antioxidant, anti-inflammatory, anti-allergic, antibacterial, antiviral, lipid-lowering, immune-boosting, and blood vessel-strengthening effects (Jeon et al., 2008; Kim et al., 2004).

In this study, the total polyphenol and flavonoid contents of lotus tea were measured by extracting it with various concentrations of ethanol solvent (Table 1). The polyphenol content was highest in the 60% ethanol extract (11.45 ± 0.13 mg GAE/g) and the 40% ethanol extract (10.38 ± 0.05 mg GAE/g). The order of polyphenol content from highest to lowest was as follows: 80% ethanol extract (9.51 ± 0.06 mg GAE/g), 20% ethanol extract (8.73 ± 0.05 mg GAE/g), 0% ethanol extract (8.59 ± 0.08 mg GAE/g), and 100% ethanol extract (5.61 ±

0.17 mg GAE/g). The flavonoid content was highest in the 40% ethanol extract (19.27 ± 0.09 mg CE/g) and the 60% ethanol extract (14.93 ± 0.00 mg CE/g). The order of flavonoid content from highest to lowest was as follows: 0% ethanol extract (12.24 ± 0.09 mg GAE/g), 20% ethanol extract (11.53 ± 0.00 mg GAE/g), 100% ethanol extract (10.65 ± 0.09 mg GAE/g), and 80% ethanol extract (10.33 ± 0.15 mg GAE/g). Based on these results, the ethanol solvent conditions that showed the highest polyphenol and flavonoid contents were determined to be the 60% and 40% ethanol extracts, respectively.

3.2 Reducing power

3.2.1 Ferric-reducing antioxidant power (FRAP)

Fe^{2+} promotes the generation of hydroxyl radicals (·OH) and superoxide anions ($\cdot O^2$), and the chelating activity toward these transition metal ions is used as an indicator of the ability to reduce the catalytic activity in oxidative reactions (Lewoyehu & Amare, 2019).

In this study, the FRAP assay was conducted on different ethanolic extracts of *Hydrangea serrata* Seringe (Table 1). The results showed that the FRAP of the extracts with 20% ethanol (285.96 ± 0.00 mM TE/g), 40% ethanol (271.79 ± 0.00 mM TE/g), 60% ethanol (271.79 ± 0.00 mM TE/g), and 80% ethanol (256.79 ± 0.00 mM TE/g) were similar, except for the 0% ethanol extract (253.46 ± 0.75 mM TE/g) and the 100% ethanol extract (169.29 ± 0.00 mM TE/g).

3.2.2 Phosphomolybdenum antioxidant activity

The phosphomolybdenum assay is a method of evaluating antioxidant activity by utilizing the characteristic of the formation of a green phosphate molybdenum (V) complex, where Mo (VI) is reduced to Mo (V) by the sample at an acidic pH (Alam et al., 2013).

In this study, the phosphomolybdenum assay was conducted on *Hydrangea serrata* Seringe extracts with varying concentrations of ethanol (Table 1). The total antioxidant activity values of *Hydrangea serrata* Seringe extracts under different solvent conditions showed that the highest antioxidant activity was observed in the 40% ethanol extract (5.21 ± 1.86 mg AAE/g) and the 60% ethanol extract (4.90 ± 3.02 mg AAE/g). The order of antioxidant activity from highest to lowest was as follows: 20% ethanol extract (4.87 ± 4.39 mg AAE/g), 80% ethanol extract (4.72 ± 1.81 mg AAE/g), 100% ethanol extract (4.57 ± 1.30 mg AAE/g), and 0% ethanol extract (3.90 ± 2.87 mg AAE/g). Based on these results, it was confirmed that the extract with the highest antioxidant activity was the 40% ethanol extract.

3.3 Radical scavenging activity

3.3.1 DPPH radical scavenging activity

DPPH radical scavenging activity refers to the ability to inhibit the activity of active oxygen by donating electrons or hydrogen to active radicals, which hinders their activity. DPPH appears purple in its free radical state and turns yellow when it is reduced to a non-radical state by gaining electrons or hydrogen from an antioxidant. The degree of color change from purple to yellow is measured to determine the antioxidant activity. Although DPPH is known to be sensitive to temperature, pH, and light, the DPPH radical scavenging assay is widely used to measure the antioxidant activity of natural product extracts (Imai et al., 1994; Park et al., 2011; Seo et al., 2010).

The DPPH radical scavenging activity of extracts of *Hydrangea serrata* Seringe with varying ethanol concentrations was measured, and the 80% ethanol extract (1,119.64 ± 1.85 uM TE/g) and 60% ethanol extract (1,046.30 ± 2.44 uM TE/g) showed the highest scavenging activity, followed by the 100% ethanol extract (1,085.70 ± 1.02 uM TE/g), 40% ethanol extract (1,034.18 ± 1.56 uM TE/g), 20% ethanol extract (1,028.7 ± 2.09 uM TE/g), and 0% ethanol extract (949.64 ± 1.28 uM TE/g), in that order (Table 2). From these results, it was established that the DPPH radical scavenging activity of *Hydrangea serrata* Seringe varied depending on the extraction solvent, with the highest value observed in the 80% ethanol extract; however, there was not a significant difference between the values of the 40% and 60% ethanol extracts, which showed high antioxidant activity. Moreover, all the *Hydrangea serrata* Seringe extracts showed higher values than the positive control, L-ascorbic acid, at 800 ppm (896.38 ± 1.85 uM TE/g) (data not shown). Therefore, it can be concluded that the *Hydrangea serrata* Seringe extracts exhibit excellent radical scavenging activity.

Table 1. Total polyphenol (TPC), total flavonoid content (TFC), ferric-reducing antioxidant power (FRAP), and phosphomolybdenum antioxidant activity (PMA) of *Hydrangea serrata* Seringe extracts.

Sample	TPC (mg GAE/g[1])	TFC (mg CE/g[2])	FRAP (mM TE/g[3])	PMA (mM AAE/g[4])
0% EtOH	8.59 ± 0.08[d5]	12.24 ± 0.09[d]	253.46 ± 0.00[c]	3.90 ± 2.87[a]
20% EtOH	8.73 ± 0.05[c]	11.53 ± 0.00[c]	285.96 ± 0.00[d]	4.87 ± 4.39[d]
40% EtOH	10.38 ± 0.05[e]	19.27 ± 0.09[f]	271.79 ± 0.00[d]	5.21 ± 1.86[e]
60% EtOH	11.45 ± 0.13[f]	14.93 ± 0.00[e]	271.79 ± 0.00[d]	4.90 ± 3.02[d]
80% EtOH	9.51 ± 0.06[d]	10.33 ± 0.15[a]	256.79 ± 0.00[b]	4.72 ± 1.81[c]
100% EtOH	5.61 ± 0.17[a]	10.65 ± 0.09[b]	169.29 ± 0.00[a]	4.57 ± 1.30[b]

[1]GAE: gallic acid equivalent; [2]CE: catechin equivalent; [3]TE: trolox equivalent; [4]AAE: ascorbic acid equivalent; [5]Values are means ± SD; Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey's test).

Table 2. DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS), and oxygen radical absorbance capacity (ORAC) of *Hydrangea serrata* Seringe extracts.

Sample	DPPH (μM TE/g[1])	ABTS (μM TE/g)	ORAC (mM TE/g)
0% EtOH	949.64 ± 1.28[a2]	713.51 ± 3.86[c]	1.38 ± 0.66[a]
20% EtOH	1028.7 ± 2.09[e]	651.24 ± 3.44[d]	0.66 ± 1.34[b]
40% EtOH	1034.18 ± 1.56[c]	843.82 ± 2.86[a]	2.24 ± 1.22[b]
60% EtOH	1046.30 ± 2.44[d]	732.46 ± 2.24[b]	2.62 ± 1.62[b]
80% EtOH	1119.64 ± 1.85[a]	800.79 ± 3.25[a]	2.49 ± 1.41[b]
100% EtOH	1085.70 ± 1.02[b]	627.14 ± 3.11[e]	1.64 ± 1.08[a]

[1]TE: trolox equivalent; [2]Values are means ± SD; Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey's test).

3.3.2 ABTS radical scavenging activity

ABTS radical scavenging activity is a method of measuring antioxidant capacity by using the decolorization of ABTS+ generated by reaction with potassium persulfate, which is removed by antioxidant substances in the sample (Lewoyehu & Amare, 2019).

The ABTS radical scavenging activity of extracts of *Hydrangea serrata* Seringe at different concentrations of ethanol was measured, and the results showed that the 40% ethanol extract (843.82 ± 2.86 uM TE/g) and 80% ethanol extract (800.79 ± 3.25 uM TE/g) had the highest scavenging activity, followed by the 60% ethanol extract (732.46 ± 2.24 uM TE/g), 0% ethanol extract (713.51 ± 3.86 uM TE/g), 20% ethanol extract (651.24 ± 3.44 uM TE/g), and 100% ethanol extract (627.14 ± 3.11 uM TE/g) (Table 2). The results of the DPPH radical scavenging activity experiment in this study showed relatively higher activity than the ABTS radical scavenging activity, which is consistent with the reports that the types and amounts of polyphenols and flavonoids present in the extract can affect the DPPH and ABTS radical scavenging activities (Wang et al., 1998).

3.3.3 Oxygen radical absorbance capacity

The ORAC assay measures the fluorescence decay induced by the presence of peroxyl radicals and is a method for evaluating the antioxidant reaction to short-lived radicals such as hydroxyl or peroxyl radicals (Cronin, 2004).

The ORAC values of the different solvent extracts of *Hydrangea serrata* Seringe were measured, and the 60% ethanol extract (2.62 ± 1.22 mM TE/g) and 80% ethanol extract (2.49 ± 1.41 mM TE/g) showed the highest antioxidant activity. The ORAC values for the other extracts were in the following order: 40% ethanol extract (2.24 ± 1.22 mM TE/g), 100% ethanol extract (1.64 ± 1.08 mM TE/g), 0% ethanol extract (1.38 ± 0.66 mM TE/g), and 20% ethanol extract (0.66 ± 1.34 mM TE/g) (Table 2). Additionally, as shown in Figure 1, all the solvent extracts of *Hydrangea serrata* Seringe showed higher free radical scavenging abilities induced by AAPH than the negative control. The solvent extracts with the highest free radical scavenging abilities were in the following order: 60, 80, 40, 20, 100, and 0% ethanol extracts. These results indicate that the ethanol extract with the highest ORAC value was the 60% ethanol extract.

3.4 Reactive oxygen species scavenging activity

3.4.1 HR scavenging activity

HRs are known as the most reactive among the active oxygen radicals, initiating lipid oxidation and causing DNA damage or mutations. They are the most toxic free radicals generated in the metabolic processes of lipids or HP in the presence of Fe^{2+} or Cu^{2+} ions (Kim et al., 2011).

The results of measuring the HR scavenging activity of *Hydrangea serrata* Seringe showed that the scavenging activity was dependent on the concentration of ethanol in the *Hydrangea serrata* Seringe extracts. As shown in Table 3, the RC_{50} values of the ethanol concentration-dependent extracts of *Hydrangea serrata* Seringe were as follows: 20% ethanol extract (631.96 ± 0.6 μg/mL), 0% ethanol extract (808.23 ± 7.6 μg/mL), 40% ethanol extract (1,027.38 ± 5.6 μg/mL), 60% ethanol extract (1,433.47 ± 9.1 μg/mL), 100% ethanol extract (1,923.08 ± 8.1 μg/mL), and 80% ethanol extract (3,162.60 ± 9.3 μg/mL). The scavenging activity of all *Hydrangea serrata* Seringe extracts was relatively low compared to the positive control, ascorbic acid (83.95 ± 6.8 μg/mL).

Table 3. RC_{50} (μg/mL) values of reactive oxygen species from *Hydrangea serrata* Seringe extracts.

Sample	RC_{50}[1]	
	HR[2]	HP[3]
BHA[4]	–	143.59 ± 1.2[a]
L-Ascorbic acid[5]	83.95 ± 6.8[a6]	–
0% EtOH	808.23 ± 7.6[a]	195.75 ± 2.5[a]
20% EtOH	631.96 ± 0.6[c]	2,055.17 ± 2.6[c]
40% EtOH	1027.38 ± 5.6[a]	115.62 ± 0.9[a]
60% EtOH	1,433.47 ± 9.1[a]	267.22 ± 2.0[a]
80% EtOH	3,162.60 ± 9.3[d]	3,672.62 ± 2.1[d]
100% EtOH	1,923.08 ± 8.1[b]	540.86 ± 4.0[b]

[1]The half reduction concentration; [2]Hydroxyl radical scavenging activity; [3]Hydrogen peroxide scavenging activity; [4]Positive control of HP; [5]Positive control of HR; [6]Values are means ± SD. Means with different letters (a–d) in the same column are significantly different at $p<0.05$ (Tukey's test).

3.4.1 HP scavenging activity

HP, a non-radical ROS, is known to cause serious damage to organs and cells in the body, leading to diseases or aging (Kim et al., 2006; Ko et al., 2010).

The results of measuring the HP scavenging activity of extracts of *Hydrangea serrata* Seringe showed that the scavenging activity of HP increased in a concentration-dependent manner. The RC_{50} values of *Hydrangea serrata* Seringe at different ethanol concentrations were as follows: 40% ethanol extract (115.62 ± 0.9 μg/mL), 0% ethanol extract (195.75 ± 2.5 μg/mL), 60% ethanol extract (267.22 ± 2.0 μg/mL), 100% ethanol extract (540.86 ± 4.0 μg/mL), 20% ethanol extract (2,055.17 ± 2.6 μg/mL), and 80% ethanol extract (3,672.62 ± 2.1 μg/mL). The 40% ethanol extract showed higher activity than the positive control, BHA (145.92 ± 1.2 μg/mL).

3.5 RNS scavenging activity

3.5.1 NO scavenging activity

NO is a highly reactive free radical generated from L-arginine by the catalytic action of NO synthase in the body (David & Ljubuncic, 2001), and this RNS oxidizes molecules such as carbohydrates, lipids, proteins, and DNA, which are the main components of cell membranes and nucleic acids, causing serious cell damage such as apoptosis (Brune et al., 2003).

As a result, the NO radical scavenging activity of the extracts of *Hydrangea serrata* Seringe varied in a concentration-dependent manner, and the RC_{50} values for the scavenging activity according to the solvent conditions of the *Hydrangea serrata* Seringe extract are shown in Table 4. The values were 41.03 ±

3.7 μg/mL for the 40% ethanol extract, 60.77 ± 2.1 μg/mL for the water extract, 79.51 ± 0.3 μg/mL for the 60% ethanol extract, 112.50 ± 0.8 μg/mL for the 100% ethanol extract, 720.68 ± 1.2 μg/mL for the 20% ethanol extract, and 889.79 ± 6.5 μg/mL for the 80% ethanol extract. The 40% ethanol extract showed higher activity than the positive control, BHA (55.91 ± 0.5 μg/mL), while the water and 60% ethanol extracts showed similar activity as that of BHA. Therefore, it can be concluded that the NO radical scavenging activity of the *Hydrangea serrata* Seringe extract is superior to that of BHA.

3.5.2 Peroxynitrite ($ONOO^-$) scavenging activity

RNS is a term used to describe nitrogen compounds such as NO, NO_2, HNO_2, and $ONOO^-$, which are highly reactive and powerful oxidants. Among these, $ONOO^-$ is a relatively stable molecule compared to other free radicals and is known to induce protein and lipid peroxidation, oxidative damage to DNA, and cell toxicity, as well as being involved in chronic diseases such as Alzheimer's disease, rheumatoid arthritis, cancer, atherosclerosis, and diabetes (Lin et al., 1997; Virag et al., 2003).

The scavenging activity of lotus tea extracts was found to be concentration-dependent. The RC_{50} values of the lotus tea extracts according to the solvent conditions were as follows: 373.10 ± 5.1 μg/mL for 40% ethanol extract, 515.60 ± 5.7 μg/mL for 0% ethanol extract, 570.60 ± 3.2 μg/mL for 20% ethanol extract, 1,041.82 ± 7.9 μg/mL for 60% ethanol extract, 1,221.44 ± 6.0 μg/mL for 80% ethanol extract, and 2,859.11 ± 6.8 μg/mL for 100% ethanol extract, respectively. It was found that the 40% ethanol extract had a scavenging activity of $ONOO^-$ radicals similar to that of the positive control L-penicillamine (328.89 ± 5.8 μg/mL).

Table 4. RC_{50} (μg/mL) values of reactive nitrogen species from *Hydrangea serrat*a Seringe extracts.

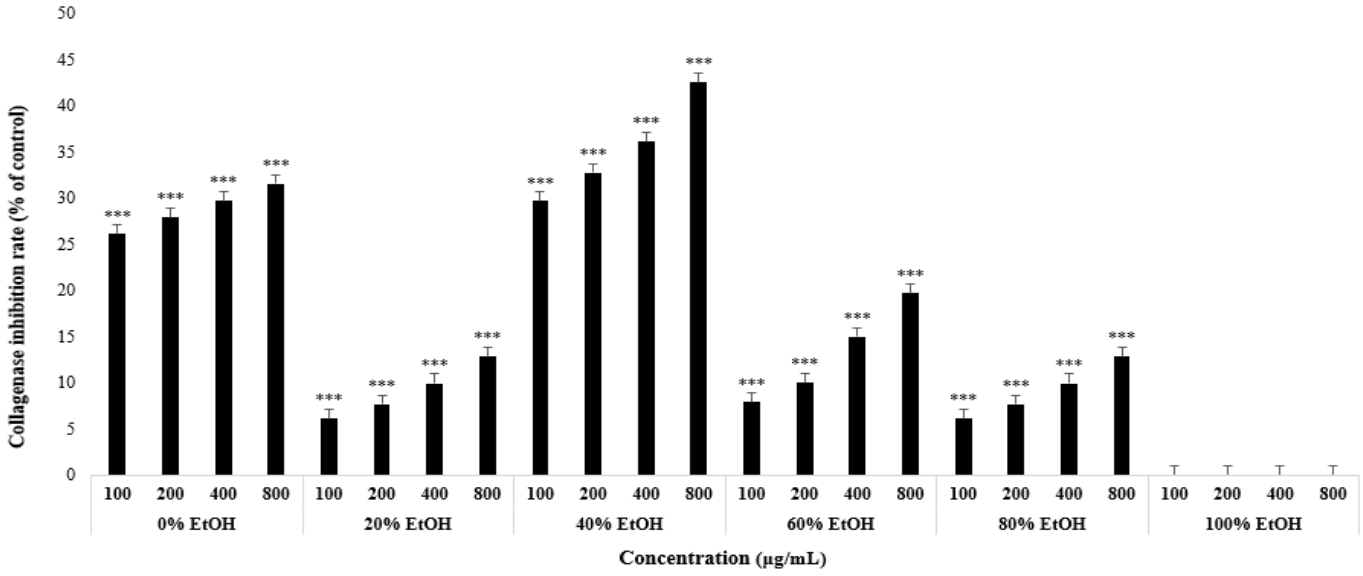
Sample	RC_{50}[1]	
	NO[2]	$ONOO^-$[3]
L-Penicillamine[4]	–	328.89 ± 5.8[b]
BHA[5]	55.91 ± 0.5[b6]	–
0% EtOH	60.77 ± 2.1[a]	515.60 ± 5.7[a]
20% EtOH	720.68 ± 1.2[d]	570.60 ± 3.2[d]
40% EtOH	41.03 ± 3.7[b]	373.10 ± 5.1[b]
60% EtOH	79.51 ± 0.3[c]	1,041.82 ± 7.9[c]
80% EtOH	889.79 ± 6.5[f]	1,221.44 ± 6.0[f]
100% EtOH	112.50 ± 0.8[d]	2,859.11 ± 6.8[d]

[1]The half reduction concentration; [2]Nitric oxide radical scavenging activity; [3]Peroxinitrile ($ONOO^-$) radical scavenging activity; [4]Positive control of $ONOO^-$; [5]Positive control of NO; [6]Values are means ± SD. Means with different letters (a–d) in the same column are significantly different at $p < 0.05$ (Tukey's test).

3.6 Anti-aging effect

3.6.1 Collagenase inhibition

Collagen in the dermis layer of the skin is known as an important extracellular matrix that protects the skin from external stimuli and pressure through its binding with elastin and also prevents wrinkles and skin aging. However, excessive generation of intracellular ROS can increase the activity of collagenase, a type of matrix metalloproteinase, leading to collagen degradation and subsequent wrinkle formation and loss of elasticity in the skin (Cho, 2017; Gu et al., 2018; Kang et al., 2010). Therefore, we investigated the collagenase inhibitory activity of the *Hydrangea serrata* Seringe extracts at different concentrations and found that all extracts showed concentration-dependent inhibition of collagenase except for the 100% ethanol extract, with the 40% ethanol extract (16.40–42.54% inhibition) exhibiting the most potent inhibitory activity at a concentration of 800 μg/mL, as shown in Figure 2.



***$p < 0.001$ significance for the comparison with the control.

Figure 2. Collagenase inhibition activity according to extraction solvent of *Hydrangea serrata* Seringe. The values are indicated as mean ± standard deviation of three independent experiments.

3.7 Correlation

The correlation between antioxidant activity and anti-aging activity was analyzed based on the extracts obtained from different ethanol concentrations of *Hydrangea serrata* Seringe. The results, presented in Table 5, showed a significant positive correlation ($r = 0.847$, $p < 0.01$) between HR scavenging activity and HP scavenging activity for ROS elimination. Moreover, a significant positive correlation was observed between HR scavenging activity and NO radical scavenging activity ($r = 0.956$, $p < 0.01$), as well as between HR scavenging activity and $ONOO^-$ radical scavenging activity ($r = 0.890$, $p < 0.01$). Similarly, HP scavenging activity showed a significant positive correlation with NO radical scavenging activity ($r = 0.953$, $p < 0.01$) and $ONOO^-$ radical scavenging activity ($r = 0.923$, $p < 0.01$). These findings suggest that ROS elimination activity has a strong association with RNS elimination activity. Furthermore, a significant positive correlation was observed between HR scavenging activity and collagenase inhibition activity ($r = 0.950$, $p < 0.01$), as well as between HP scavenging activity and collagenase inhibition activity ($r = 0.965$, $p < 0.01$), indicating a high correlation between ROS elimination activity and anti-aging effect.

The correlation analysis between NO radical scavenging activity and $ONOO^-$ radical scavenging activity showed a significant positive correlation ($r = 0.922$, $p < 0.01$), which suggests that RNS elimination activity has a strong association with anti-aging effect. In addition, a significant positive correlation was observed between NO radical scavenging activity and collagenase inhibition activity ($r = 0.986$, $p < 0.01$), as well as between $ONOO^-$ radical scavenging activity and collagenase inhibition activity ($r = 0.963$, $p < 0.01$), indicating a high correlation between RNS elimination activity and anti-aging effect.

Based on these results, it can be concluded that the extracts obtained under conditions with higher ROS scavenging activity from different ethanol concentrations of *Hydrangea serrata* Seringe exhibit superior RNS scavenging activity (reactive nitrogen scavenging activity) and anti-aging effect (Figures 3 and 4).

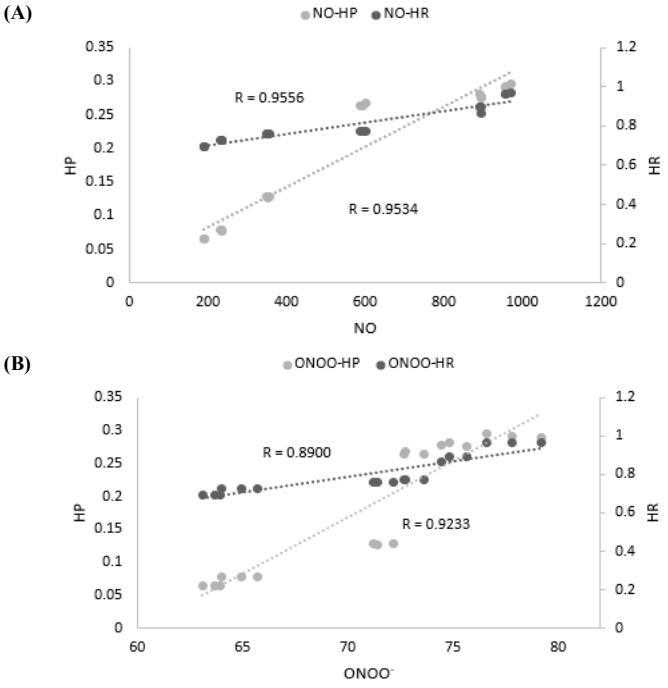


Figure 3. Correlation between reactive oxygen scavenging activity and reactive nitrogen scavenging activity of *Hydrangea serrata* Seringe extracts. (A) Correlation between reactive oxygen scavenging activity and nitric oxide (NO) scavenging activity. (B) Correlation between reactive oxygen scavenging activity and peroxynitrite ($ONOO^-$) scavenging activity.

Table 5. Correlation coefficient between antioxidant contents, reducing power, radical scavenging activity, reactive oxygen scavenging activity, reactive nitrogen scavenging activity, and anti-aging effect of *Hydrangea serrata* Seringe extracts.

Factors	Antioxidant contents		Reducing power		Radical scavenging activity			Reactive oxygen scavenging activity		Reactive nitrogen scavenging activity		Anti-aging effect
	TPC[1]	TFC[2]	FRAP[3]	PMA[4]	DPPH[5]	ABTS[6]	ORAC[7]	HR[8]	HP[9]	NO[10]	ONOO[11]	Collagenase[12]
TPC	1	-0.064	0.832	-0.976	-0.972	-0.064	0.997*	-0.855	-0.582	-0.830	0.415	0.896
TFC		1	0.500	-0.156	0.297	1.000**	0.013	-0.463	-0.774	-0.504	0.881	-0.500
FRAP			1	-0.933	-0.679	0.500	0.872	-0.999*	-0.935	-1.000**	0.850	0.500
PMA				1	0.897	-0.156	-0.990	0.948	0.746	0.932	-0.604	-0.778
DPPH					1	0.297	-0.951	0.709	0.375	0.675	-0.189	-0.975
ABTS						1	0.013	-0.463	-0.774	-0.054	0.881	-0.500
ORAC							1	-0.892	-0.643	-0.870	0.484	0.859
HR								1	0.847**	0.956**	0.890**	0.950**
HP									1	0.953**	0.923**	0.965**
NO										1	0.922**	0.986**
ONOO											1	0.963**
Collagenase												1

[1]Total polyphenol contents; [2]Total flavonoid contents; [3]Ferric-reducing antioxidant power; [4]Phosphomolybdenum antioxidant activity; [5]DPPH radical scavenging activity; [6]ABTS radical scavenging activity; [7]Oxygen radical absorbance capacity (ORAC); [8]Hydroxyl radical scavenging activity; [9]Hydrogen peroxide (HP) scavenging activity; [10]NO radical scavenging activity; [11]Peroxynitrite ($ONOO^-$) scavenging activity; [12]Collagenase inhibition; Correlation is significantly different at $*p < 0.05$, $**p < 0.01$ (Pearson's test).

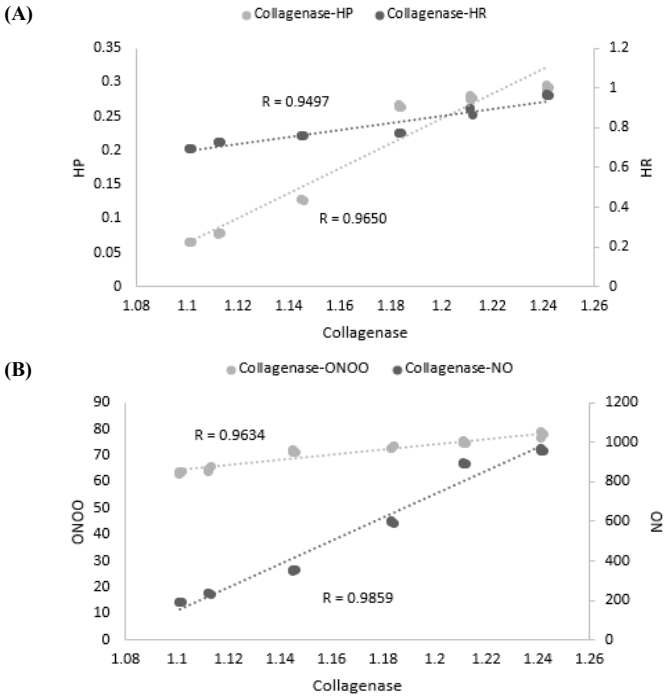


Figure 4. Correlation between anti-aging effect and reactive oxygen scavenging activity and reactive nitrogen scavenging activity of *Hydrangea serrata* Seringe extracts. (A) Correlation between collagenase inhibition and reactive oxygen scavenging activity. (B) Correlation between collagenase inhibition and reactive nitrogen scavenging activity.

4. CONCLUSION

In this study, we investigated the antioxidant contents (total polyphenol and total flavonoid contents), reducing power for transition metal ions (FRAP and PMA), radical scavenging activity (DPPH, ABTS, and ORAC), reactive oxygen scavenging activity (HR and HP), and reactive nitrogen scavenging activity (NO and $ONOO^-$) of *Hydrangea serrata* Seringe using solvent extracts to confirm the correlation between their physiological activities. The highest total polyphenol content was found in the 60% ethanol extract (11.45 ± 0.13 mg GAE/g), while the highest total flavonoid content was observed in the 40% ethanol extract (19.27 ± 0.09 mg CE/g). The 40% ethanol extract showed the highest FRAP value (3637.80 ± 0.99 mM TE/g) and PMA value (5.21 ± 0.99 mM TE/g). The 80% ethanol extract exhibited the highest DPPH radical scavenging activity (5.35 ± 0.00 mM TE/g), while the 40% ethanol extract showed the highest ABTS radical scavenging activity (969.14 ± 0.00 μM TE/g). The 60% ethanol extract had the highest ORAC value (2.62 ± 1.22 mM TE/g). The RC_{50} values for HR and HP scavenging activities were the lowest in the 20% and 40% ethanol extracts, respectively (631.96 ± 0.6 μg/mL and 115.62 ± 0.9 μg/mL). The RC_{50} values for NO and $ONOO^-$ scavenging activities were also the lowest in the 40% ethanol extract (41.03 ± 3.7 and 373.10 ± 5.1 μg/mL). These findings were confirmed by the correlations between HR and HP scavenging activities ($r = 0.847$, $p < 0.01$), HR scavenging activity and NO/$ONOO^-$ scavenging activities ($r = 0.956$, $p < 0.01$), (r = 0.890, p<0.01), and HP scavenging activity and NO/$ONOO^-$ scavenging activities ($r = 0.956$, $p < 0.01$), ($r = 0.890$, $p < 0.01$), as shown in Table 5 and Figure 3. In addition, the collagenase inhibition activity was highest in the 40% ethanol extract at concentrations of 25–800 μg/mL, with a range of 16.40–42.54%. This finding was confirmed by the correlations between collagenase inhibition activity and HR/HP scavenging activities ($r = 0.950$, $p < 0.01$), ($r = 0.965$, $p < 0.01$), and NO/$ONOO^-$ scavenging activities ($r = 0.986$, $p < 0.01$), ($r = 0.963$, $p < 0.01$), as shown in Table 5 and Figure 4.

These results suggest that the scavenging activities of reactive oxygen and nitrogen species are highly correlated with the anti-aging (collagenase inhibition) activity.

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