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Correlation between antioxidant activity and anti-wrinkle effect of ethanol extracts of *Sanguisorba Officinalis* L.

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Abstract

Sanguisorba officinalis L., the dried roots of which are referred to as cucumber herb is a perennial herb belonging to the family Rosaceae. This study was showed the correlation between the antioxidant activity and its anti-wrinkle effects of ethanol extraction concentration of *S. officinalis* L. The extraction yield of the *S. officinalis* extracts was all 10% or more, which was judged to be economical. 80% ethanol extracts was the highest in total polyphenol and flavonoid contents and the best total antioxidant activity. The correlation between total polyphenol and flavonoid contents and antioxidant activity was 0.830 and 0.872, respectively, with a significant correlation was confirmed. In addition, in 80% ethanol extracts, superoxide anion and NO radical scavenging activities were the most effective. These results validated that the optimum extraction concentration for *S. officinalis* was 80% ethanol.

Keyword: *Sanguisorba officinalis* L.; ethanol extracts; antioxidant; anti-wrinkle; correlation.

Practical Application: Research about antioxidant activities and anti-wrinkling functional products of *Sanguisorba officinalis* L.

1 Introduction

The human body constantly needs oxygen when it gets the energy. About 2 to 3% of the oxygen inhaled during respiration is converted to toxic substances called free radicals, causing cell damage (Cho et al., 2008). Reactive Oxygen Species (ROS), hydroxyl radical, hydrogen peroxide and Superoxide anion etc., leads to DNA denaturation, lipid peroxidation, protein inactivation, and other cell dysfunctions. These cause the cancers, diabetes, atherosclerosis and Parkinson's disease (Halliwell et al., 1992; Thannickal & Fanburg, 2000). ROS and Reactive Nitrogen Species (RNS), which are directly responsible for oxidative stress, are unstable and highly reactive. Furthermore, these are easily reacted with biomaterials and attacked biomolecules, caused damage to cells and tissues (Lee & Im, 2012). In particular, ROS, was produced by free Radicals ($NO^{\cdot}$, $OH^{\cdot}$, $O_2^{\cdot}$), causes aging, inflammation, carcinogenesis, and atherosclerosis (Cho et al., 2008). The human body has biological defense systems such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione (GSH) and glutathione S-transferase (GST) to remove these free radicals (Kil et al., 2015).

It is being actively explored research about synthetic or natural antioxidants that eliminate ROS and increase antioxidant defense systems in human body (Kim et al., 2009). Highly reactive antioxidants remove harmful substances in the body, protect cells by preventing chain reactions caused by free radicals and prevent apoptosis of cells (Kil et al., 2015). Antioxidants can be classified as water-soluble antioxidants (glutathione, vitamin C, uric acid, etc.), fat-soluble antioxidants (flavonoid, tocopherol, carotenoids, etc.), synthetic antioxidants (tert-butylhydroxyanisole (BHA), tert-butylhydroxytoluene (BHT), etc.), and antioxidant enzyme (catalase, superoxide dismutase (SOD), glutathione peroxidase, etc.). Antioxidants can protect cells from oxidative damage by removing free radicals intermediates and prevent cell apoptosis (Lim, 2010). Synthetic antioxidants such as BHA and BHT have excellent antioxidant effects, however, showed various side effects caused by toxicity; thus, there is increasing interest in research on safe natural antioxidants (Park et al., 2012a).

Sanguisorba officinalis Linne. (*S. officinalis* L.) is a species of rose family, widely distributed throughout China, Japan, and Korea, and especially grows in places with moderate moisture on mountain slopes. It is a slightly curved spindle shape, 10–20 cm long and 5–20 mm in diameter. It has cold properties, tastes bitter and sour, and is non-toxic and fragrance-free (Kim et al., 2011). It has been mainly used in wound treatment and hemostasis in oriental medicine, and is known to be effectual in dermatitis, mucosal inflammation, eczema, and burns (Park et al., 2012b). The main ingredients are ziguglycoside I, II, pomlic acid, gallic acid, ellagic acid, and quercethin at the root; quercetin, kaempferol, ursolic acid, saponinat branches; vit C in leaf; chrysanthemin, cyanin in flower (Jang et al., 2013).

In this study, we extracted *S. officinalis* L. by various ethanol concentrations and investigated total polyphenol contents, total flavonoid contents, antioxidative activity, active nitrogen evaluation. In addition, we evaluated the ability to improvewrinkle with various *in vitro* models and identified the correlation between antioxidant capacity and wrinkle improvement. Furthermore,

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we intend to provide basic data to ascertain the availability of *S. officinalis* L. as a natural antioxidant material.

2 Materials and methods

2.1 Experimental materials

S. officinalis L. roots were purchased from Geohub (Yangju, Gyeonggi-do, Korea). Folin-Ciocalteu reagent, ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox, and L-ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The solvents and all reagents used special-grade reagents.

2.2 Sample extraction

Samples (10 g) pulverized with a grinder (NFM-3561SN, NUC Co., Daegu, Korea) were reflux extracted with 100 mL of distilled water, 20% ethanol, 40% ethanol, 80% ethanol, and 100% ethanol for 60 min at 100 °C and filtered. SpeedVac (COSMOS 660-50L, Kyungseo Machines Co., Incheon, Korea) was used to concentrate and obtain yields. The concentrated sample was stored at 4 °C before use.

2.3 Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Total polyphenol contents was measured using the Folin-Ciocalteu colorimetric method (Yun et al., 2018) and gallic acid was used as a standard solution. Each extracts (500 μL) was added to Folin-Coicalteu reagent (500 μL) for 3 min at room temperature, reacted to 500 μL of 10% Na_2CO_3 for 1 hour, block the light, and then measured at 760 nm with an UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). TPC (mg GAE/g) standard curve was obtainedwith gallic acid ($y = 0.0378x + 0.3719$, $R^2 = 0.997$).

Total flavonoid contents were determined using the Moreno method (Moreno et al., 2000), and catechin was used as a standard solution. Each sample was mixed with 5% $NaNO_2$ for 5 min at room temperature, then added 10% $AlCl_3$ for 5 min at room temperature. After adding 1 N NaOH, absorbance was measured at 415 nm using a spectrophotometer (Mecasys Co., Daejeon, Korea). TFC (mg CE/g) standard curve was obtained with catechin ($y = 0.0025x + 0.0142$, $R^2 = 0.999$).

2.4 Antioxidant activity

Phosphomolybdenum antioxidant assay (Total Antioxidant Capacity, TAC)

The total antioxidant activity was evaluated by phosphomolybdenum antioxidant assay as described by Prieto et al. (1999). Each sample was mixed with reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) at 90 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm using an UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea). The total antioxidant activity (mg AAE/g) standard curve was obtained with L-ascorbic acid ($y = 0.0021x - 0.0165$, $R^2 = 0.991$).

Ferric-Reducing Antioxidant Power (FRAP)

The ferric-reducing antioxidant power activity was measured by modifying method of Benzie & Strain (1996). Each sample was mixed with FRAP solution (300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM $FeCl_3 \cdot 6H_2O$) at 37 °C for 30 min, and then was measured an UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea) at 595 nm. The FRAP (mg TE/g) standard curve was obtained with Trolox ($y = 0.0021x - 0.0165$, $R^2 = 0.991$).

2.5 Free radical scavenging activity assay

DPPH radical scavenging activity

DPPH radical scavenging activity was measured by applying the experimental methods of Yamaguchi et al. (1998). The sample solution were added 0.2 mM DPPH (190 μL) at room temperature for 30 min, and measured at 515 nm using an ELISA reader (Thermo Fisher SCIENTIFIC, Multiskan Sky, Korea). The DPPH radical scavenging activity (mg TE/g) standard curve was obtained with Trolox ($y = 0.0015x + 2.3996$, $R^2 = 0.997$).

ABTS radical scavenging activity

ABTS radical scavenging activity was measured by applying the experimental method of Thaipong et al. (2006). A solution of 7.4 mM ABTS and 2.45 mM potassium persulfate was mixed at 37 °C for 24 h. Each sample and ABTS solution was reacted in the dark room for 5 min, and measured the UV/VIS spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea) at 734 nm. The ABTS radical scavenging activity (mg TE/g) standard curve was obtained with Trolox ($y = 0.0001x + 0.9179$, $R^2 = 0.996$).

Superoxide Anion Radical Scavenging Activity (SRSA)

Superoxide anion radical scavenging activity (SRSA) was measured by the nitroblue tetrazolium (NBT) reduction (Fridovich, 1970). Each sample was reacted with SASA solution (0.1 M phosphate buffer (pH 8.0), 0.05 U/mL xanthine oxidase, and substrate (0.4 mM xanthine, 0.24 mM nitroblue tetrazolium)) in the dark room at 37 °C. Then, the absorbance was measured at 510 nm. SRSA was calculated using the following equation: SRSA (%) = $(A - B)/A \times 100$, where A is the absorbance of the control and B is the absorbance of the test sample.

Nitric Oxide radical (NO·) scavenging activity

Nitric oxide radical scavenging activity was measured using the method of Nagata et al. (1999). Each sample was mixed with 50 mM phosphate buffer (pH 7.4), 40 mM soudium nitroprusside (SNP), and 4,5-Diaminofluorescein (DAF-2) in dimethyl Sulfoxide for at room temperature for 10 min, measured using a fluorescence microplate reader (Spectra Max Gemini EM, Molecular Devices Corp., USA) at excitation 485 nm and emission 525 nm. Nitric oxide radical scavenging activity (%) = $(A - B)/A \times 100$, where A is the fluorescence intensity of the control and B is the fluorescence intensity of the test sample.

2.6 Anti-wrinkling assay

Collagenase inhibition assay

The collagenase inhibitory effect was measured using Wünsch & Heidrich (1963) method. The substrate solution was prepared with 4 mM $CaCl_2$ and 0.3 mg/mL of 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg in 0.1 M Tris-HCl buffer (pH 7.5). Each sample were mixed with substrate solution and collagenase at 37 °C for 20 min and measured by UV/VIS spectrophotometer at 320 nm. Collagenase inhibition was calculated using the following equation: collagenase inhibition (%) = (A – B)/A × 100, where A is the absorbance of the control, Epigallocatechin gallate (EGCG) and B is the absorbance of the test sample.

Elastase enzyme inhibition assay

The effect of elastase inhibitory activity was measured by Kraunsoe et al. (1996) modifying method. Each sample was added the reaction mixture containing 3.2 mM N-succinyl-$(Ala)_3$-*p*-nitroanilide and 2.8×10^{-3} unit porcine pancreas elastase in 0.2 M Tris-HCl buffer (pH 8.0) at 37 °C for 30 min and measured at 410 nm. Elastase inhibitory activity (%) = (A – B)/A × 100, where A is the absorbance of the control (distilled water) and B is the absorbance of the test sample.

2.7 Statistical analysis

Experimental data of this study are expressed as mean ± standard deviation after three times experiments of each sample. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), and one-way analysis of variance (ANOVA) was performed to test the statistical significance between each experimental group. In cases where differences were observed, Tukey's test was conducted post-test, with $p < 0.05$ considered statistically significant. The correlation was indicated by Pearson's correlation coefficient.

3 Results and discussion

3.1 Extraction yield

Even if their excellent physiological activity, lower extracts yield is less economic feasibility. Extracts yield is an important factor to be considered in the various commercialization of functional extracts (Kim et al., 2019). After concentrating the extracts, the extraction yield was calculated. The highest extraction yields were 80% and 100% ethanol extracts (26.50%), and 20% ethanol extracts (13.37%) showed the lowest yield. The difference in extraction yields is thought to be due to the ratio of ethanol to water. If the extraction yield exceeds 10% in natural products, it is known to be economic feasibility (Park et al., 2003), so all extracts of *S. officinalis*are considered a commercially available plant material.

3.2 Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Many hydroxyl groups (-OH) in polyphenols are easily bonded to various compounds, so they have excellent antioxidant, anti-cancer, and anti-inflammatory effects (Kim et al., 2012). The highest total polyphenol content was in the 40% ethanol extracts and the 80% ethanol extracts at 121.96 ± 0.0 mg GAE/g, followed by the 100% ethanol extracts (108.02 ± 0.42 mg GAE/g), 20% ethanol extracts (76.56 ± 0.48 mg GAE/g), and distilled water extracts (59.29 ± 0.49 mg GAE/g) in that order, with a significant difference by Tukey's multiple range test at $p < 0.05$ (Table 1).

Flavonoids are known to have high antioxidant activity by effectively removing ROS. Like polyphenols, they are known to have anti-inflammatory, anticancer, and antiviral effects (Kim et al., 2012). In addition, flavonoids are also widely present in the plant system and are contained in all parts of the plant (Ham et al., 2018). Flavonoid contents showed the highest value of 101.19 ± 2.87 mg CE/g in the 80% ethanol extracts, followed by the 100% ethanol extracts (92.87 ± 0.64 mg CE/g), 40% ethanol extracts (89.82 ± 1.42 mg CE/g), 20% ethanol extracts (63.20 ± 0.66 mg CE/g), and distilled water extracts (52.37 ± 2.56 mg CE/g) in that order, with a significant difference by Tukey's multiple range test at $p < 0.05$ (Table 1). From these results, the highest polyphenol and flavonoid contents was found in 80% ethanol extracts.

3.3 Antioxidant activity

Total antioxidant capacity

The total antioxidant capacity experiment is based on the reduction of Mo (VI)-Mo (V) by the extracts and the subsequent formation of the green phosphate/Mo (V) complex at acidic pH (Saha et al., 2008). The best TAC was 80% ethanol extracts (34.66 ± 2.89 mg AAE/g), followed by 100% ethanol extracts (28.04 ± 0.39 mg AAE/g), and 40% ethanol extracts (28.04 ± 0.62), 20% ethanol extracts (18.83 ± 0.18 mg AAE/g), and distilled water extracts (16.34 ± 2.00 mg AAE/g), in that order (Table 2).

Ferric-reducing antioxidant power

FRAP can be measure the reducing power by measuring the blue wavelength at 593 nm that occurs when Fe^{3+} is reduced to Fe^{2+} (Lee et al., 2012; Ku et al., 2009). In this study, the 40% ethanol extracts showed the highest reducing power at 609.85 ± 5.25 mg TE/g followed by 80% ethanol extracts (565.13 ± 5.54 mg TE/g), and 100% ethanol extracts (543.86 ± 5.22 mg TE/g), 20% ethanol extracts (380.54 ± 3.36 mg TE/g), and distilled water extracts (331.50 ± 2.97 mg TE/g) in that order, with a significant difference

Table 1. Total polyphenol contents and total flavonoid contents in water and ethanol extracts of *Sanguisorba officinalis* L.

Sample	Total polyphenols contents (mg GAE/g[1)])	Total Flavonoid contents (mg CE/g[2)])
D.W	59.29 ± 0.49[a3)4)]	52.37 ± 2.56[a]
20% EtOH	76.56 ± 0.48[b]	63.20 ± 0.66[b]
40% EtOH	121.96 ± 0.00[d]	89.82 ± 1.42[c]
80% EtOH	121.96 ± 0.00[d]	101.19 ± 2.87[d]
100% EtOH	108.02 ± 0.42[c]	92.87 ± 0.64[c]

[1)]Gallic acid equivalent; [2)]Catechin equivalent; [3)]Values are mean ± standard deviation (n = 3); [4)]Values with different letters within the same row are significantly different by Tukey's multiple range test at $p < 0.05$.

Table 2. Measurement of antioxidant activity of water and ethanol extracts of *Sanguisorba officinalis* L.

Sample	TAC assay[1)] (mg AAE/g[3)])	FRAP assay[2)] (mg TE/g[4)])	DPPH (mg TE/g)	ABTS (mg TE/g)	SRSA[5)] RC_{50}
D.W	16.34 ± 2.00[a6)7)]	331.50 ± 2.97[a]	1,325.18 ± 1.04[b]	10,279.00 ± 0.00[d]	90.25 ± 0.68[a]
20% EtOH	18.83 ± 0.18[a]	380.54 ± 3.36[b]	1,338.62 ± 0.31[d]	10,165.67 ± 3.33[c]	88.44 ± 7.69[a]
40% EtOH	28.04 ± 0.62[b]	609.85 ± 5.25[e]	1,305.18 ± 0.29[a]	10,869.00 ± 0.00[e]	73.90 ± 6.31[a]
80% EtOH	34.66 ± 2.89[b]	565.13 ± 5.54[d]	1,333.40 ± 0.21[c]	9,845.67 ± 3.33[b]	70.89 ± 24.41[a]
100% EtOH	28.04 ± 0.39[b]	543.86 ± 5.22[c]	1,400.40 ± 0.24[e]	9,529.00 ± 5.77[a]	87.19 ± 1.37[a]

[1)]Total antioxidant capacity; [2)]Ferric-reducing antioxidant power; [3)]Ascorbic acid equivalent; [4)]Trolox equivalent; [5)]Superoxide anion radical scavenging activity; [6)]Values are mean ± standard deviation (n = 3); [7)]Values with different letters within the same row are significantly different by Tukey's multiple range test at $p < 0.05$.

(Table 2). These results showed that the 40% ethanol extracts had the highest reducing power when Fe^{3+} was reduced to Fe^{2+}.

3.4 Free radical scavenging activity assay

DPPH radical scavenging activity

DPPH is reduced in the presence of antioxidants to changes in color from deep purple to loss of color (Choi et al., 2003). The 100% ethanol extracts was the highest at 1,400.40 ± 0.24 mg TE/g, and the others were showed similar activity, at 1,300.00 mg TE/g or more (Table 2). Compared to ascorbic acid (100 μg/mL), which is used as a positive control, the activities of the all extracts of *S. officinalis* have been shown to be significantly better. These results confirmed that DPPH radical scavenging activity was the best in 100% ethanol extracts.

ABTS radical scavenging activity

When ABTS and potassium persulfate are reacted in a dark place, $ABTS^{\cdot+}$ is produced. This reacts with the antioxidants and the cations are eliminated. It is decolorized and its antioxidant capacity can be measured by its absorbance (Boo et al., 2009). The 40% ethanol extracts showed the highest ABTS activity at 10,869.00 ± 0.00 mg TE/g, followed by distilled water extracts (10,279.00 ± 0.00 mg TE/g), 20% ethanol extracts (10,165.67 ± 3.33 mg TE/g), 80% ethanol extracts (9,845.67 ± 3.33 mg TE/g), and 100% ethanol extracts (9,529.00 ± 5.77 mg TE/g) in that order (Table 2).

Superoxide anion radical scavenging activity

It is known that about 0.4 to 4% of the total consumed oxygen is converted into free radicals and superoxides during the normal oxidative phosphorylation process. Also the generated superoxide is known to be converted to other ROS, causing cell damage either directly or indirectly (Kim et al., 2009). The antioxidant effect of the extracts was confirmed through the ability to remove superoxide radiation directly. The SRSA was expressed as the RC_{50} value (Table 2). The RC_{50} value of the 80% ethanol extracts was the highest at 70.89 ± 24.4 μg/mL, followed by the 40% ethanol extracts (73.90 ± 6.31 μg/mL), 100% ethanol extracts (87.19 ± 1.37 μg/mL), 20% ethanol extracts (88.44 ± 7.69 μg/mL), and distilled water extracts (90.25 ± 0.68 μg/mL) in that order. From these results, all extracts of *S. officinalis* showed better scavenging activity than L-ascorbic acid, which showed a scavenging activity of 10 to 40% at 50 to 200 μg/mL.

Nitric Oxide radical (NO ·) scavenging activity

NO can easily diffuse cell membranes and react with other reactive oxygen species. In particular, it readily reacts with O_2^- to produce peroxynitrite ($ONOO^-$), a highly reactive oxidizing agent (Lee & Jeong, 2006). It is also said that mass-generated NO can further increase peroxynitrite production and cause cell and tissue damage, leading to aging and senile diseases (Jo & Jeong, 2008). The NO radical scavenging activity of the extracts were all higher than that of BHA used as a positive control (Figure 1A). Among them, 80% ethanol extracts showed the highest activity, ranging from 83.98 ± 0.53% to 92.04 ± 0.54%.

3.5 Assay for the estimation of anti-wrinkling

Collagenase enzyme inhibition assay

Collagen, along with elastin, is a key indicator material related to skin elasticity and wrinkles, and is known to occupy 90% of the skin dermis. Collagenase activity was increased by various factors such as ultraviolet rays or external environmental stress. Collagenase is known to cause skin aging by breaking down collagen causing wrinkles and loss of elasticity (Kim et al., 2018). All extracts of the *S. officinalis* had better collagenase enzyme inhibitory activity EGCG as a positive control. As the concentration increased, the collagenase enzyme inhibitory activity also increased (Figure 1B). The activity was the best in distilled water extracts, 21.11% to 46.32%; 20% ethanol extracts, 32.09% to 53.58%; 40% ethanol extracts, 23.53% to 48.64%; and 80% ethanol extracts, 25.95% to 46.32%, respectively. Among them, 80% ethanol extractsshowed the best collagenase enzyme inhibitory activity. These results were consistent with Jang et al. (2012) research that collagenase inhibitory activity of *S. officinalis* root fractions was effective.

Elastase enzyme inhibition assay

The occurrence of endogenous skin aging is another route of skin aging. Elastase is known to be the only enzyme that induces wrinkle formation by decomposing elastin in the stratum corneum to reduce skin elasticity (Hwang et al., 2016). All extracts of *S. officinalis* led to inhibition of elastase enzyme at concentrations of 50 to 400 μg/mL (Figure 1C) and these

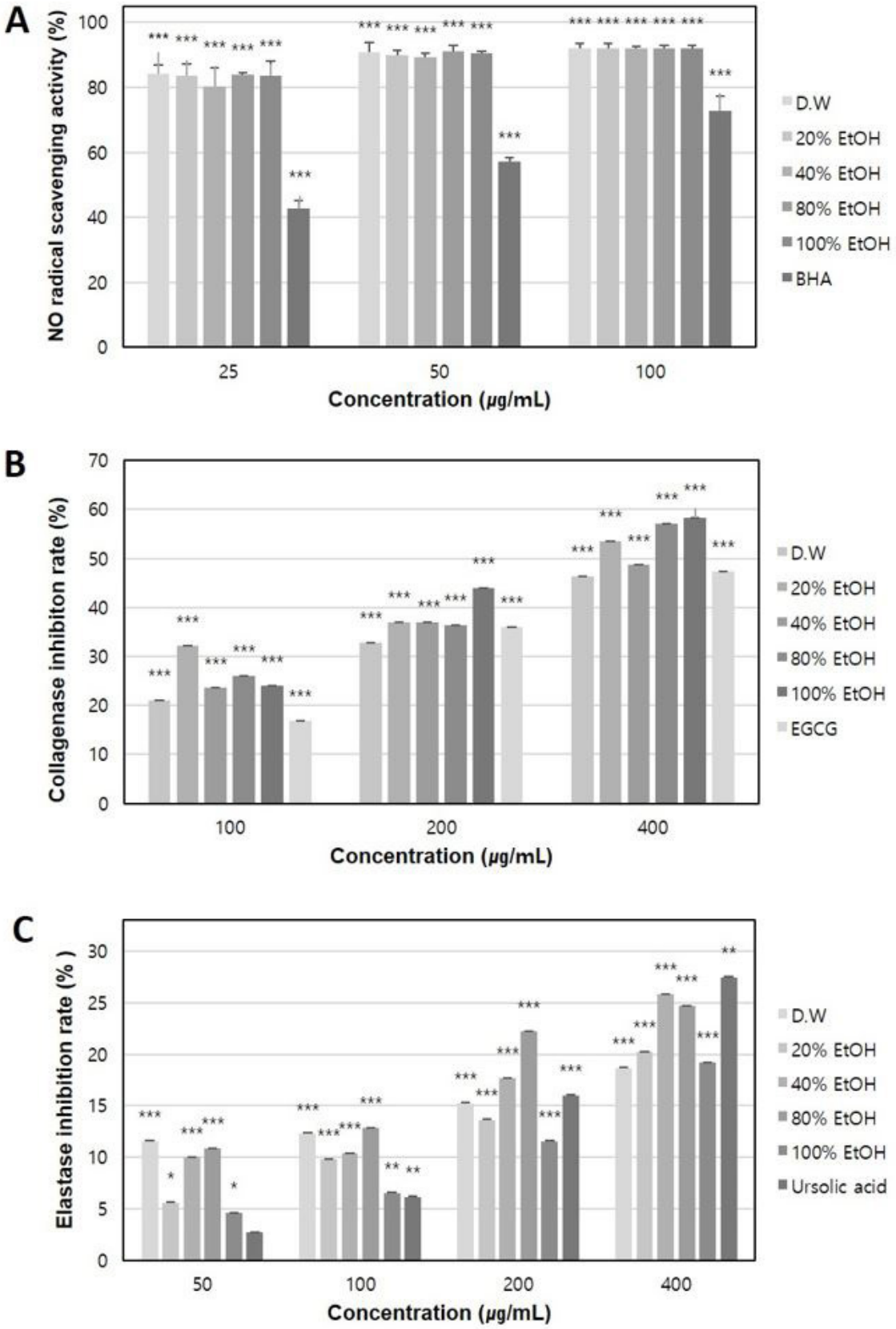

Figure 1. Effect of extracts from *S. officinalis* L. (A) NO radical scavenging activity; (B) Collagenase inhibition activity. Elastase inhibition activity. All the experiments were repeated three times. The values are indicated as mean ± standard deviation of three independent experiments. ***$p < 0.001$ significance for the comparison with the control.

Table 3. Correlation coefficient between antioxidant, reactive nitrogen species and anti-wrinkle of extracts by *Sanguisorba officinalis* L. condition

Factors	TPC[1)]	TFC[2)]	TAC[3)]	FRAP[4)]	DPPH	ABTS	SARS[5)]	Elastase	Collagenase	NO[6)]
TPC	1	0.958**	0.830**	0.983**	-0.054	0.316	-0.316	-0.012	-0.286	-0.129
TFC		1	0.872**	0.932**	-0.241	0.320	-0.224	0.136	-0.262	-0.140
TAC			1	0.822**	-0.120	0.140	-0.053	0.230	-0.142	0.035
FRAP				1	-0.062	0.293	-0.389*	-0.110	-0.312	-0.181
DPPH					1	0.282	0.152	-0.240	-0.046	0.212
ABTS						1	-0.122	-0.080	-0.307	-0.290
SARS							1	0.421**	-0.385*	0.542**
Elastase								1	0.227*	0.499**
Collagenase									1	0.450**
NO										1

[1)]Total polyphenol contents; [2)]Total flavonoid contents; [3)]Total antioxidant capacity; [4)]Ferric-reducing antioxidant power; [5)]Superoxide anion radical scavenging activity; [6)]NO radical scavenging activity; *P < 0.05; **P < 0.01 (Pearson).

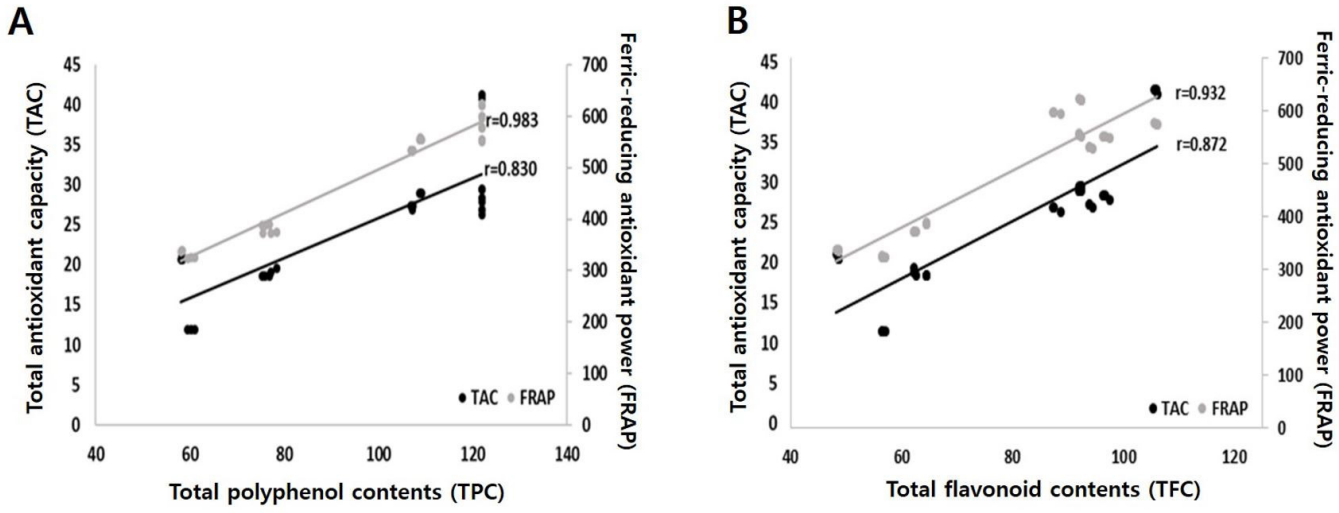

Figure 2. Correlation between antioxidant activity and anti-wrinkle of *S. officinalis* L. extracts. (A) Correlation between Total polyphenol contents and antioxidant capacity; (B) Correlation between Total flavonoid contents and antioxidant capacity. All the experiments were repeated three times.

results were in concentration-dependent manner and were better than the positive control of ursolic acid at concentrations of 50 to 100 μg/mL. The best was 80% ethanol extracts showing 10.90 ± 0.01% to 24.69 ± 0.01%.

3.6 Correlation between antioxidant activity assay and anti-wrinkling

Table 3 showed the results of analyzing the correlation between antioxidant and anti-aging in the samples. Depending on the ethanol concentrations of the *S. officinalis* extracts, total polyphenol contents showed high positive correlation with total antioxidant activity ($r = 0.830$, $p < 0.01$) and FRAP ($r = 0.983$, $p < 0.01$) (Figure 2A), and flavonoids showed a high positive correlation with total antioxidant activity ($r = 0.872$, $p < 0.01$) and FRAP ($r = 0.932$, $p < 0.01$) (Figure 2B). These results were confirmed that the higher the concentrations of polyphenols and flavonoids, the higher the total antioxidant activity. For example, the polyphenol content and flavonoid content was the highest in 80% ethanol extracts, and total antioxidant activity were also the highest in 80% ethanol extracts at 121.96 ± 0.00 mg GAE/g and 101.19 ± 2.87 mg CE/g, respectively (Table 1, Table 2).

Superoxide anion radical scavenging activity showed elastase inhibitory activity ($r = 0.421$, $p < 0.01$), collagenase enzyme inhibitory activity ($r = 0.385$, $p < 0.05$), and No radical scavenging activity ($r = 0.542$, $p < 0.01$) (Table 3). NO radical scavenging activity showed elastase inhibitory activity ($r = 0.499$, $p < 0.01$) and collagenase enzyme inhibitory activity ($r = 0.450$, $p < 0.01$) (Table 3).

4 Conclusions

To evaluate the physiological activity of *S. officinalis* and to confirm the correlations between the physiologically active substances, the extraction was performed using distilled water, 20%, 40%, 80%, and 100% ethanol. Among them, the highest extraction yield were 80% and 100% ethanol extracts, which was judged to be economical as it showed an extraction yield of

10% or more. The total polyphenol contents and total flavonoid contents were the highest in the 80% ethanol extracts, and the total antioxidant activity (TAC) was also the best in the 80% ethanol extracts. The total antioxidant activity, polyphenol contents ($r = 0.830$, $p < 0.01$), and flavonoid contents ($r = 0.872$, $p < 0.01$) were correlated with each other. In superoxide anion radical scavenging activity, 80% ethanol extracts had the highest. These results validated that the optimum extraction concentration for *S. officinalis* was 80% ethanol.

Conflict of interest

The authors declare that they have no conflict of interest.

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