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Antimicrobial activity and protective effect of *Geranium thunbergii* against oxidative DNA damage via antioxidant effect

Tae-Hyung Kwon[1], Su-Jin Lee[2], Jae-Ho Park[3], Taewan Kim[4], Jung-Ja Park[5], Nyun-Ho Park[6]*

[1]*Eco-friendly Certification Support Center, Chuncheon Bioindustry Foundation, Chuncheon 24232, Korea*
[2]*Department of Herbal Medicine Resource, Kangwon National University, Dogye 25913, Korea*
[3]*Department of medicinal plant science, Jungwon University, Goesan 28024, Korea*
[4]*Department of Food Science and Biotechnology, Andong National University, Andong 36729, Korea*
[5]*Department of Food and Biotechnology, Hankyong National University, Ansung 17579, Korea*
[6]*Gyeongbuk Institute for Marine Bio-Industry, Uljin 36315, Korea*

현초의 항산화 활성에 의한 산화적 DNA 손상 보호효과 및 항균활성

권태형[1] · 이수진[2] · 박재호[3] · 김태완[4] · 박정자[5] · 박년호[6]*

[1](재)춘천바이오산업진흥원, [2]강원대학교 생약자원학과, [3]중원대학교 생약자원학과,
[4]안동대학교 식품생명공학과, [5]한경대학교 식품생명공학과, [6](재)경북해양바이오산업연구원

Abstract

This study aimed to investigate the various biological activities of *Geranium thunbergii* such as antimicrobial activity and protective effect against oxidative damage. To evaluate its antioxidant and antimicrobial activities, we first performed methanol extraction; this methanol extract was further partitioned using various solvents. And then, its antioxidant activity was measured using various assays including total phenolic content and protection against oxidative DNA damage, and antimicrobial activities were examined using minimum inhibiting concentration (MIC) test, and paper disc method. In addition, high-performance liquid chromatography was performed to analyze the major chemical components of ethyl acetate fraction. The *G. thunbergii* fraction with ethyl acetate exhibited higher antioxidant and antimicrobial activities than the other fractions. The results showed that *G. thunbergii* ethyl acetate fraction at 50 μg/mL had strong DPPH and ABTS radical scavenging activities of 80.88% and 80.12%, respectively. In addition, the ethyl acetate fraction protected DNA from the oxidative damage induced by ferrous ion and hydroxyl radicals and showed high antimicrobial activity with diameter of inhibition zones ranging from 13.33 to 15.67 mm. High-performance liquid chromatography analysis revealed the major phenolic compounds of *G. thunbergii* to be ellagic acid and gallic acid. These results suggest that *G. thunbergii* might protect DNA against oxidative stress induced by reactive oxygen species and can be utilized as a natural source of antioxidant and antimicrobial agent in the food industry.

Key words : antioxidant, antimicrobial, plant, *Geranium thunbergii*, DNA damage

Introduction

Oxidation is a biochemical reaction that occurs during food processing and storage, and it can cause rancidity, discoloration and deterioration shortening the shelf life (1). In addition, oxidation can negatively affect human health owing to its involvement in various diseases including odontoblast, cancer, cardiovascular diseases, and osteoporosis (2,3). Excessive levels of reactive oxygen species can lead

*Corresponding author. E-mail : pnh863660@gimb.or.kr
Phone : 82-54-780-3452; Fax : 82-54-780-3469
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to oxidative DNA damage which results in DNA double strands breaks, mutation, and cancer (3). Antioxidants are commonly employed in food industry and various fields to delay the initiation or propagation of oxidation and protect against oxidative damage induced by excessive free radicals in cells (4). There are synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone, and propyl gallate used in processing foods to improve quality and stability (5). However, these compounds are toxic, carcinogenic in animal models, and may cause liver damage (6). Hence, many studies have focused on natural antioxidants derived from plants, seaweeds, vegetables, and fruits which are considered beneficial for human health and have wide applications in food industry (7,8).

Conventional physical and chemical methods are commonly used to prevent food contamination and spoilage. However, the high temperature may result in poor product quality; and chemical preservatives such as ascorbic acid, sodium metabisulphate, and nitrite have safety concerns and tend to be bioaccumulated (9). Moreover, consumers are increasingly concerned about the potential risk of chemical residues in food (10). Hence, several studies have focused on naturally-derived preservatives with potential for application in food industry (9,11,12).

Plants are good sources of medicine and food, traditionally used for prevention of various diseases, and rich in secondary metabolites including vitamins, polyphenol, and minerals. In addition, many studies have reported their antimicrobial and antioxidant activities in food industry (13,14). *G. thunbergii* also called *Ijilpul,* or *Hyuncho*, is a perennial plant belonging to the Geraniaceae family. It is used as an oriental medicinal plant in East Asia, for diarrhea, constipation and gastrointestinal disorders (15). It is a well known α -glucosidase inhibitor (16), functional material in seasoning sauce for pork meat (15), and anti-inflammatory effect via inhibition of IL-6 production (17), and antioxidant effect in RAW 264.7 macrophage or HS-68 normal skin fibroblast cells were reported (18). However, its antioxidant and antimicrobial effects still remains to be demonstrateds and only few studies have been performed.

Therefore, the purpose of this study was to investigate the antioxidant and antimicrobial activities of *G. thunbergii*. To evaluate its antioxidant and antimicrobial activities, we first performed methanol extraction; this methanol extract was further partitioned using various solvents. And then, its antioxidant activity was measured using various assays including total phenolic content and protection against oxidative DNA damage, and antimicrobial activities of solvent-partitioned fractions against foodborne pathogens were measured using paper disc method and minimum inhibitory concentration. In addition, high-performance liquid chromatography was performed to analyze the major chemical components of ethyl acetate fraction (Alliance, Waters, USA).

Materials and methods

Materials and Chemicals

The *G. thunbergii* was purchased from Omniherb of Gyeongsan (Gyeongbuk province, Korea). The samples were pulverized to 80 mesh size using Sung Chang Machine (ACM10INCH, Namyangju, Korea) and kept at -20℃ until assayed. Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, sodium carbonate, dimethyl sulfoxide (DMSO), potassium ferricyanide, butylated hydroxy anisole (BHA), trolox, trichloroacetic acid, ferric chloride and potassium persulfate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 2,2-Azino-bis (3-ethyl benzthiazoline- 6-sulfonic acid) (ABTS) was purchased from Wacko chemical (Tokyo, Japan). All other reagents and chemicals used in this study were analytical grade.

Extraction of *G. thunbergii*

The dried sample (100 g) was extracted with 1.0 L of 95% methanol overnight at room temperature for 2 days. The methanol extract was then filtered through filter paper Advantec 5C (Advantec, Toyo Roshi Kaisha Ltd., Tokyo, Japan) and by vacuum rotary evaporator (BÜCHI Rotavapor R-210, Flawil, Switzerland). The methanol extract was partitioned with different solvents (H; n-hexane C; chloroform E; ethyl acetate B; butanol, W; water). Five fractions were completely evaporated and dried and then their yields were calculated. The yield (g/dry) of solvent-partitioned fractions was hexane fraction (1.21 g), chloroform fraction (0.36 g), ethyl acetate fraction (0.58 g), butanol fraction (1.52 g), and water fraction (4.15 g), respectively. The solvent-partitioned fractions were stored at -20℃ prior to use.

Total phenolic content (TPC)

The TPC of the extract was measured using the Folin-Denis method (19). 0.5 mL of Folin Ciocalteu's phenol reagent was added to 0.5 mL sample and the reaction mixture incubated for 3 min. After this, 0.5 mL of Na_2CO_3 (10%, w/v) was added and further incubated at room temperature (25℃) for

60 min. Absorbance of the reaction mixture was then measured at 760 nm using spectrophotometer (Jasco, Tokyo, Japan). TPC was expressed in terms of mg gallic acid equivalents (GAE)/g.

DPPH radical scavenging activity

The antioxidant activity of *G. thunbergii* was measured using DPPH radical scavenging assay (20). Fifty microliters of the sample was added to 100 μL of 0.2 mM DPPH solvent, vortex-mixed, and allowed to stand at room temperature for 10 min. Absorbance was measured at 517 nm. All determinations were carried out in triplicate. BHA was used as a positive control and the capability of scavenging DPPH radicals was calculated using the following equation:

Radical scavenging activity=$[1-(A_{sample}/A_{control})]\times100$

ABTS radical scavenging activity

ABTS radical cation decolorization assay was performed using the Re method (21). 7.0 mM 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) was added to 2.45 mM potassium-persulfate and the mixture was incubated at room temperature in the dark for 24 h. Absorbance of the ABTS radical cation solution was measured at 734 nm using ethanol. Fifty microliters of the sample was added to 100 μL ABTS radical cation solution and the reaction mixture was incubated at room temperature for 10 min. Its absorbance was measured at 734 nm. All determinations were carried out in triplicate. Trolox was used as a positive control and the capability scavenging ABTS radicals was calculated using the following equation:

Radical scavenging activity=$[1-(A_{sample}/A_{control})]\times100$

Reducing power ability

Reducing power assay was performed using the Oyaizu method (22). One microliters of the sample was added to 1 mL of potassium ferricyanide (1%, w/v) and the reaction mixture was incubated in water bath at 50℃ for 20 min. And then 1 mL of trichloroacetic acid (10%, w/v) was added. 1 mL of this mixture was added with 1 mL of distilled water and 0.1 mL of ferric chloride (0.1%, w/v). Absorbance was measured at 700 nm.

Protective effects of oxidative DNA damage

Conversion of the supercoiled form of plasmid DNA to the open-circular and linear forms has been used as index of DNA damage. Reaction mixtures (20 μL) contained 5 μL of ΦX-174 RF I plasmid DNA, 10 μL of varying concentrations of the ethyl acetate fraction, 5 μL of 1 mM $FeSO_4$ or 1 mM hydrogen peroxide were incubated at 37℃ for 30 min. After 30 min, 5 μL of a solution containing 50% glycerol (v/v), 40 mM EDTA and 0.05% bromophenol blue was added to stop the reaction. The mixtures were electrophoresed on 1% agarose gel (w/v). DNA in the gel was visualized and photographed under ultraviolet light after ethidium bromide staining.

Bacteria strains and their cultures

To determine antimicrobial activity, *Bacillus subtilis* (KCTC 1666), *Bacillus cereus* (KCTC 1012), *Staphylococcus epidemidis* (KCTC 1917), *Staphylococcus aureus* (KCTC 1916), *Listeria monocytogenes* (KCTC 3569), *Escherichia coli* (KCTC2643) were used. These microorganisms were kept frozen at -80℃ in broth containing glycerol (40%, v/v). These cultures were grown in liquid and solid media at optimal temperature of microorganisms, respectively. All media were purchased from Difco Co. (Sparks, MD, USA).

Determination of antimicrobial activity

Antimicrobial activity of solvent-partitioned fraction was performed using the paper disc method (15). The fractions were dissolved in DMSO. The culture of each strain was inoculated with colony on agar plate, and incubated at 37℃ for 24 h and the 100 μL of each strain culture was spread on agar plate. The sample 40 μL added to the paper disk (Advantec, Toyo Roshi Kaisha, Tokyo, JAPAN) was placed on the inoculated plate. The plates were incubated at 37℃ for 24 h and diameter of the inhibition zones was measured. All analyses were conducted in triplicate for each extract.

Minimum inhibitory concentration (MIC) test

The MIC of ethyl acetate fraction from *G. thunbergii* methanol extract was measured using paper disc method. The concentrations of ethyl acetate fraction were from 0.025 to 1.6 mg/mL. All analyses were conducted under same conditions as paper disc method, and MIC was defined as the lowest concentration of ethyl acetate fraction required for inhibition of bacteria. All analyses were conducted in triplicate for each extract.

Analysis of phenolic compounds using high performance liquid chromatography

Analysis of major phenolic compounds in *G. thunbergii* ethyl acetate fraction was conducted using high performance liquid chromatography (Alliance, Waters, USA). An Eclipse

plus C_{18} column (Agilent Technologies Ltd., 4.6×250 mm, particle size 5 μm) was used to analyze gallic acid and ellagic acid. The mobile phases were 0.1% formic acid (v/v) in 10% acetonitrile (solvent A) and, 0.1% formic acid (v/v) in 90% acetonitrile (solvent B). All reagents used were HPLC grade. The gradient followed the order: 0-6 min 0% B, 6-31 min 10-90% B, 31-41 min 20-80%, 41-45 min 50-50% B, 45-50 min 0% B. The separated phenolic compounds were detected with photodiode array detector at 280 nm.

Statistical analysis

All analyses were conducted in triplicate. Statistical comparisons were carried out using SPSS 18.0 statistical software (SPSS, Chicago, IL, USA) and significance was determined by one-way ANOVA followed by Duncan multiple range test for multiple comparisons. Means with different letters in groups one significantly different ($p<0.05$).

Results and Discussion

Yields of solvent-partitioned fractions and their total polyphenolic content (TPC)

In the present study, *G. thunbergii* was extracted twice with methanol at room temperature, followed by progressive partitioning in different solvents. The yield of solvent-partitioned fractions followed the order: water fraction > butanol fraction > n-hexane fraction > ethyl acetate fraction > chloroform fraction and was found to be 49.52%, 18.16%,14.51%, 6.94%, and 4.35%, respectively. TPC has been used to evaluate the function of various materials as a measure of biological activity (8). TPC of solvent-partitioned fractions from *G. thunbergii* methanol extract are shown in Table 1. Also, five fractions had high phenolic content, which ranged from 83.72 to 604.28 mg/g with the ethyl acetate fraction being highest 604.28 mg/g. In addition, the butanol fraction (465.65 mg/g) had higher TPC than the hexane, chloroform, and water fractions. Lee et al. (15) also reported TPC of *G. thunbergii* ethanol extract (96.51 mg/g) and investigated its correlation with antioxidant potential using radical scavenging assays. The phenolic compounds in herbs, plants and seaweeds have been regarded as antioxidants owing to their involvement in oxidation-reduction reactions and their important role in various functions such as antimicrobial, DNA damage inhibition and anti-inflammatory activities (23). Several studies have shown that high amounts of TPC in the sample extract may correlate with their antioxidant activities (24). Zeng et al. (6) showed that the antioxidant activity of *Rhizoma homalomenae* extract according to total polyphenolic content increases as also shown by this study. The correlation values were estimated as DPPH/ABTS radical scavenging activity and reducing power with correlation coefficients of $r^2=0.9804$, $r^2=0.9828$, and $r^2=0.9875$, respectively (data not shown).

DPPH and ABTS radical scavenging activities and reducing power of solvent-partitioned fractions

The DPPH assay provides basic information on the antiradical reaction of numerous extracts because of theformation of stable free radicals and follows a radical-radical interaction to form a stable molecule (25). The

Table 1. Total phenolic contents and radical scavenging activity of *Geranium thunbergii* solvent-partitioned fractions

	TPC[2] (mg/g)	DPPH radical scavenging activity (%, μg/mL)				ABTS radical scavenging activity (%, μg/mL)			
		25	50	100	200	25	50	100	200
HF[1]	83.72±5.04[4)E5)]	7.24±1.01^{Ec}[6)]	13.43±0.67^{Ec}	25.03±0.67^{Db}	45.41±1.57^{Ea}	0.50±1.09^{Ed}	6.63±0.33^{Gc}	19.65±1.36^{Db}	42.76±0.91^{Da}
CF	148.83±1.40^{C}	12.60±0.67^{Dd}	26.24±1.01^{Dc}	42.27±1.07^{Cb}	71.93±1.83^{Da}	6.82±1.28^{Ed}	21.36±1.90^{Ec}	38.16±2.71^{Bb}	74.33±1.22^{Ba}
EF	604.28±1.95^{A}	54.64±0.35^{Bc}	80.88±1.34^{Bb}	93.31±0.25^{Aa}	93.98±0.10^{Ba}	47.14±1.58^{Bc}	80.12±2.41^{Cb}	99.50±0.17^{Aa}	99.99≥^{Aa}
BF	465.65±4.88^{B}	43.70±0.10^{Cc}	73.48±1.15^{Cb}	92.21±0.60^{Aa}	92.76±0.10^{Ba}	38.53±0.50^{Cd}	70.72±1.28^{Dc}	97.37±1.14^{Ab}	99.99≥^{Aa}
WF	98.52±1.18^{D}	8.78±3.37^{DEc}	13.76±3.80^{Ec}	28.01±2.64^{Db}	45.80±3.48^{Ea}	10.43±7.19^{Dc}	15.54±6.58^{Fc}	33.66±5.68^{Cb}	57.69±5.10^{Ca}
BHA[3]		9.94±3.39^{DEd}	27.02±3.57^{Dc}	64.86±2.23^{Bb}	87.85±0.83^{Ca}	54.87±4.02^{Ac}	87.70±2.94^{Bb}	99.99≥^{Aa}	99.99≥^{Aa}
AA[3]		76.85±3.68^{Ab}	96.52±0.29^{Aa}	96.57±0.25^{Aa}	96.91±0.25^{Aa}	50.84±4.51^{ABb}	99.05±0.92^{Aa}	99.99≥^{Aa}	99.99≥^{Aa}

[1)]HF, n-hexane fraction; CF, chloroform fraction; EF, ethyl acetate fraction; BF, butanol fraction; WF, water fraction; BHA, butylated hydroxyl anisole; AA, ascorbic acid.
[2)]TPC was determined with reference to the standard curve of gallic acid content of samples using spectrophotometer.
[3)]Positive controls were used butylated hydroxyl anisole and ascorbic acid.
[4)]Data represent the means±SD of 3 determinations.
[5)A-G]Means of the different superscripts within same column are significantly different at $p<0.05$.
[6)a-d]Means of the different superscripts within same row are significantly different at $p<0.05$.

DPPH scavenging activity of *G. thunbergii* solvent-partitioned fractions significantly increased in a dose dependent manner at concentrations from 25 to 200 μg/mL. The DPPH scavenging activity of *G. thunbergii* solvent-partitioned fractions at 50 μg/mL concentration was as following order ethyl acetate fraction > butanol fraction > chloroform fraction > water fraction > n-hexane fraction, with the following values 80.88%, 73.48%, 26.24%, 13.76%, and 13.43%, respectively. The DPPH radical scavenging activities of the positive controls (butylated hydroxyanisole and ascorbic acid) were 27.02% and 96.52%, respectively (Table 1).

ABTS assay is a commonly used method to evaluate the antioxidant capacity of various plants, seaweeds, and other sources. The ABTS radical scavenging activity of *G. thunbergii* solvent-partitioned fractions significantly increased in a dose-dependent manner at concentrations from 25 to 200 μg/mL. The ABTS radical scavenging activity of *G. thunbergii* solvent-partitioned fractions at 50 μg/mL concentration was following the order: ethyl acetate > butanol > chloroform > water > n-hexane with values of 80.12%, 70.72%, 21.36%, 15.54%, and 6.63%, respectively. The ABTS radical scavenging activities of the positive controls (butylated hydroxyanisole and ascorbic acid) were 87.70% and 99.05%, respectively (Table 1).

Also the reducing power assay is used to screen for antioxidant in plants, foods, and various fields (26,27). The reducing power of *G. thunbergii* solvent-partitioned fractions at 50 μg/mL concentration showed following the order: ethyl acetate fraction > butanol fraction > chloroform fraction > water fraction > n-hexane fraction with values of 0.28, 0.23, 0.13, 0.09, and 0.08, respectively. The reducing powers of the positive controls butylated hydroxyanisole and ascorbic acid, were 0.36 and 0.34, respectively (Fig. 1). According to their report, *G. thunbergii* ethanol extract at 1,000 μg/mL concentration had DPPH radical scavenging activity of 56.79%, TPC (96.51 mg/g) *G. thunbergii* ethanol extract of was higher than that of other plant extracts (24,28).

Plants are known for having various biological activities such as antioxidant, anti-inflammatory, anti-obesity, and antimicrobial effects owing to the presence of diverse phenolic compounds (18,29). Kim et al. (18) reported the antioxidant activity and cell toxicity of pressurized liquid extracts of 20 selected plant species. The extracts of these species had high total phenolic content, antioxidant activities, and inhibitory activity against nitric oxide production in lipopolysaccharide-induced RAW 264.7 macrophages. In addition, Singh et al. (29) demonstrated the antioxidant and antimicrobial activities of Trigonella foenum-gracum, and its protective effect against oxidative DNA damage. Thus, various plants including *G. thunbergii* can be utilized as a good source of antioxidant in food industry and medicine.

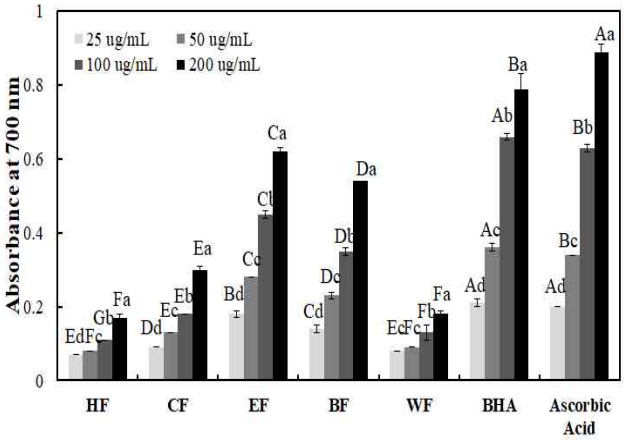

Fig. 1. Reducing power ability of *Geranium thunbergii* solvent-partitioned fractions. Butylated hydroxyl anisole and ascorbic acid were used as positive controls.

Data represent the means±SD of three determinations. Means with different letters on the bar are significantly different at $p<0.05$.

Protective effects of *G. thunbergii* ethyl acetate fraction (GTEF) against oxidative DNA damage

Figure 2 shows gel electrophoretogram demonstrating the protective effect of *G. thunbergii* ethyl acetate fraction against cleavage of plasmid DNA by ferrous ion-induced oxidative damage. Addition of ferrous ion in the absence of GTEF converted the supercoiled form of DNA into open-circular form. DNA was not detected after the addition of ferrous ion in absence of GTEF. Recently, the protective effect of plant extracts with antioxidant activity has been reported. Cheng et al. (30) have reported the protective effect of extract of *Crataegus pinnatifida* pollen against DNA damage response to oxidative stress. According to the results, *C. pinnatifida* pollen showed antioxidant activity, protective effect against DNA damage, and their main phenolic compound was examined. Further, Abbas et al. (31) showed the phenolic profile, antioxidant potential, and protective effect on DNA of sugarcane (*Saccharum officinarum*), and their results showed that these cultivars can be used not only as accessible source of natural antioxidants but also as an ingredient of functional food for the control of degenerative diseases. Thus, various plants including *G. thunbergii* may be useful as ingredients for prevention of oxidative damage-mediated diseases.

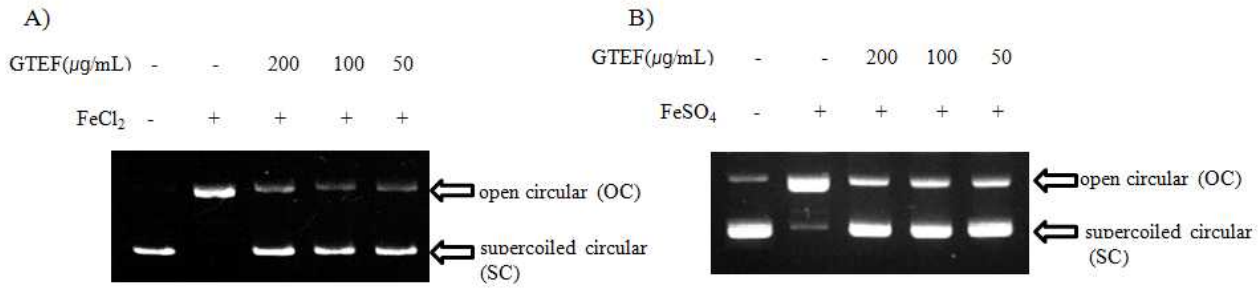

Fig. 2. Protective effect of *G. thunbergii* ethyl acetate fraction against DNA cleavage induced by $FeCl_2$ (A) and hydroxyl radical (B) using phiX-174RFI plasmid DNA.

Control was treated with nothing, and another control was treated with $FeCl_2$ and hydroxyl radical pre-reaction without *G. thunbergii* ethyl acetate fraction. Sample lane was treated with varying concentrations of the *G. thunbergii* ethyl acetate fraction (50, 100, and 200 μg/mL).

Antimicrobial activities of solvent-partitioned fractions

Table 2 shows the antimicrobial activities of all fractions. The ethyl acetate fraction showed remarkable antimicrobial activity with diameter of inhibition zones ranging from 13.33 to 15.67 mm. In addition, the MIC values of the ethyl acetate fraction ranged from 0.2 to 0.8 mg/mL. Previous reports on studies with *G. thunbergii* and other plant extracts had shown similar antimicrobial activities. Lee et al. (15) reported the antimicrobial activity of four herbal ethanol extracts. Among the samples, *G. thunbergii* ethanol extract had good antimicrobial activity against *L. monocytogenes, S. aureus, B. cereus*, and *E. coli* and their activities remained relatively high heat treatment. Shen et al. (32) demonstrated the antimicrobial effect of blueberry (*Vaccinium corymbosm* L.) extracts against *L. monocytogenes* and *Salmonella enteritidis* and showed four active phenolic products in blueberry extract. Quercetin was ineffective at 8 μg/mL, but ellagic acid, quercetin, and quercetin-3-galctoside at 200 μg/mL, showed significant ($p<0.05$) inhibitory effect when compared to the control. These antimicrobial activities have been attributed to phenolic compounds and flavonoids.

Analysis of phenolic compounds using high performance liquid chromatography

To analyze the contents of phenolic compounds in *G. thunbergii* ethyl acetate fraction, we compared it with standard substances (gallic acid, chlorogenic acid, caffeic acid, ellagic acid, myricetin, icariin, quercetin, and kampferol). From HPLC analysis, ellagic acid and gallic acid were detected in the ethyl acetate fraction and their contents were 55.14 and 5.42 mg/g, respectively (Fig. 3). Other reports have indicated significant roles for these and other phenolic and flavonoid compounds with the antioxidant, neuroprotective,

Table 2. Antimicrobial activity of *Geranium thunbergii* solvent-partitioned fractions on tested bacteria strains

Strains		Diameter of inhibition zone (mm)[1)]					
		HF[2)]	CF	EF	BF	WF	EF MIC[3)] (mg/mL)
Gram positive	*B. subtilis*	ND[4)]	9.50±0.01[5)b6)]	13.17±1.04^{ab}	12.67±0.58^{abc}	ND	0.4
	B. cereus	9.33±0.58^{b}	9.50±0.00^{b}	13.83±1.26^{a}	14.00±1.80^{a}	9.67±0.58^{a}	0.2
	L. monocytogenes	ND	ND	14.83±0.29^{ab}	13.33±0.29^{ab}	ND	0.4
	S. aureus	ND	ND	15.67±1.04^{bc}	14.67±0.58^{bc}	ND	0.4
	S. epidermidis	10.17±0.29^{a}	10.67±0.29^{a}	13.33±0.58^{c}	12.17±0.29^{c}	9.50±0.00^{a}	0.4
Gram negative	*E. coli*	ND	ND	13.33±0.58^{abc}	13.50±0.50^{abc}	ND	0.8

[1)]Included the disc diameter of 8 mm.
[2)]HF, n-hexane fraction; CF, chloroform fraction; EF, ethyl acetate fraction; BF, butanol fraction; WF, water fraction.
[3)]MIC, minimum inhibitory concentrations.
[4)]ND, not detected.
[5)]Data represent the means±SD of 3 determinations.
[6)a-d]Means of the different superscripts within same column are significantly different at $p<0.05$.

and anticarcinogenic effects of plant extracts. Choi et al. (16) reported α-glucosidase inhibitory effect of active compounds isolated from *G. thunbergii* ethyl acetate fraction and confirmed *p*-hydroxybenzoic acid, geraniin, and ellagic acid to be the active components. Liu et al. (17) also reported inhibitory activity against IL-6 production of flavonoids and phenolic compounds from *G. thunbergii* and confirmed kampferol-3-rhamnoside, protocatechuic acid, and gallic acid to be the active components. Xiong et al. (33) reported that gallic acid was expressed as 7.99 mg/g in flower. They demonstrated that gallic acid and chlorogenic acid have attracted considerable attention for their antioxidant activity, neuroprotective effects, and anticarcinogenic properties.

요 약

본 연구에서는 현초의 식품소재 적용과 기능성 소재의 개발을 위해 현초 에틸 아세테이트 분획물을 이용하여 활성산소종의 소거활성과 산화적 DNA 손상 보호효과 및 항균

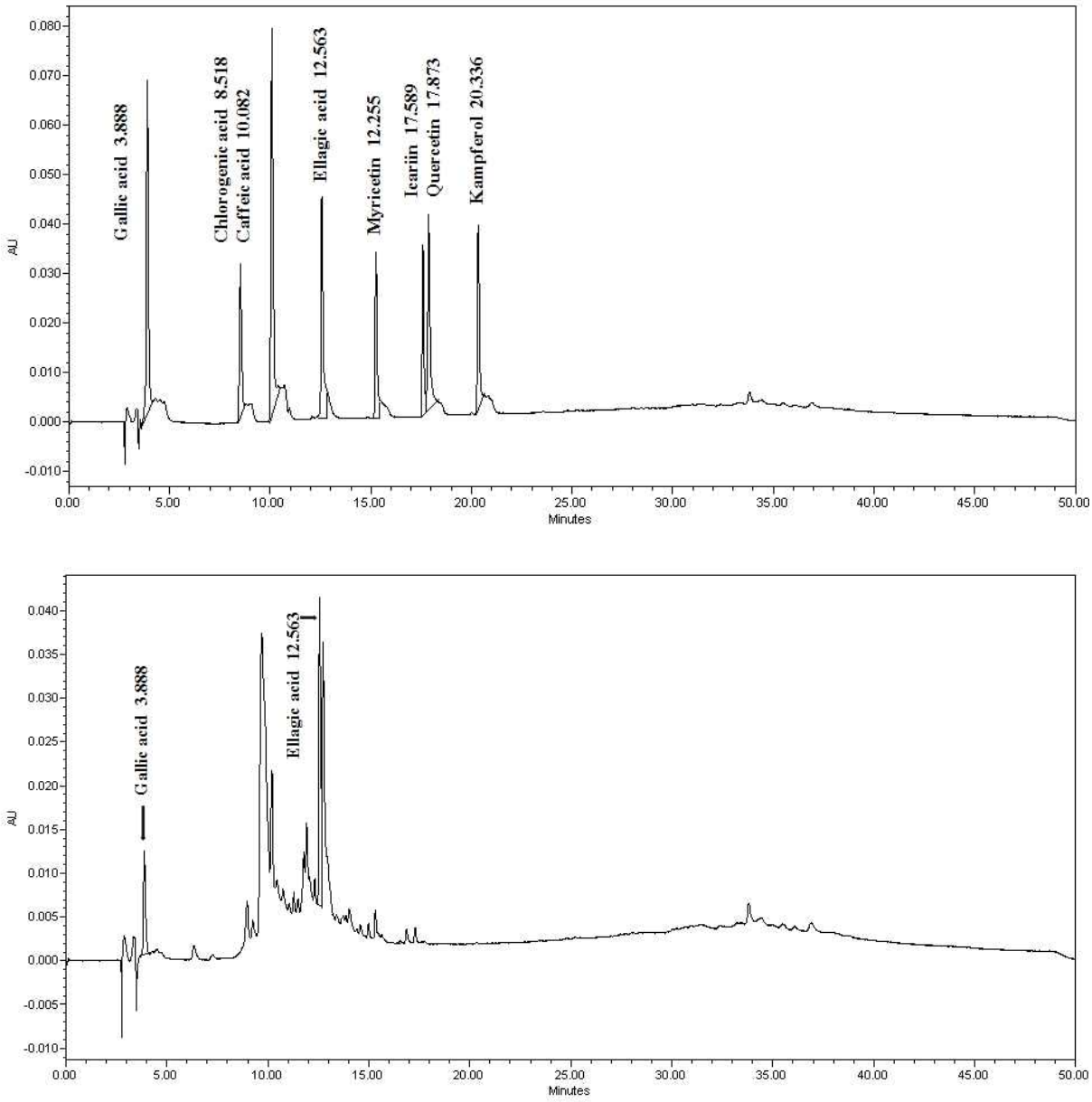

Fig. 3. HPLC chromatogram of ellagic acid and gallic acid in *G. thunbergii* ethyl acetate fraction.

The separated phenolic compounds were detected with a photodiode array detector at 280 nm.

활성에 대해 검증하였다. 현초를 메탄올로 추출하여 얻어진 추출물에 대해 n-hexane, chloroform, ethyl acetate, n-butanol, water의 용매를 이용하여 순차분획을 실시하였고, 얻어진 결과물에 대하여 다양한 항산화 측정 방법을 통하여 항산화 효능을 측정한 결과 에틸 아세테이트 분획물의 경우 DPPH 라디칼 소거능, ABTS 라디칼 소거능 및 환원력에서 효과가 높게 측정 되었으며, 50 μg/mL의 농도에서 각각 80.88%, 80.12%, 28%를 저해하는 것으로 측정되었다. 이러한 항산화 효능과 함께 산화적 DNA 손상 보호 효과를 검증하였고 농도별로 억제하는 경향을 나타냈다. 또한 식품 소재 및 다양한 첨가 소재로 이용하기 위하여 항균활성을 측정하였으며, 에틸 아세테이트 분획물에서 연구에 사용된 모든 균주에 대하여 저해 활성을 보였다. 이러한 활성을 가진 현초 에틸 아세테이트 분획물의 활성물질을 검증하기 위하여 phenolic compound 및 flavonoid 대조군을 이용하여 LC 분석을 하였다. 그 결과 ellagic acid와 gallic aicd가 검출 되었으며 각각 55.14 mg/g, 5.42 mg/g 측정 되었다. 이는 결과적으로 현초는 다양한 식품소재로서의 활용될 수 있으며, 본 논문은 기능성 물질로 활용을 위한 기초자료가 될 것으로 사료 된다.

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