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Antioxidant and Antimicrobial Activities of Various Stachy Sieboldii Miq Extracts for Application in Meat Product

KEYWORDS: Stachy sieboldii MIQ, antioxidant activity, polyphenol, flavonoid, radical scavenging activity, antibacterial activity

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ABSTRACT *In order to analyze bioactive functionalities for application in meat product depending on different parts of leaf, stem and tuber in Stachy sieboldii MIQ (SSM) and extraction solvents, we investigated the antioxidant and antibacterial activities of the various SSM extracts. The leaf methanol and ethanol extracts from SSM maintained more polyphenol and flavonoid contents as well as higher antioxidant activities than those of the stem and tuber extracts. DPPH, ABTS•+ and nitrite radical scavenging activities from the methanol extracts maintained a higher or similar value compared with the ethanol extracts. DPPH, ABTS•+ and FRAP radical scavenging activities were proportional to total polyphenol and flavonoid contents in the various SSM extracts, whereas nitrite radical scavenging activity was partially related to these materials, which the methanol extracts were observed by the coincident activity with their materials, but the ethanol extracts were not depending on their materials. The SSM leaf and tuber methanol extracts or the tuber ethanol extract were showed by strong antibacterial activities against S. typhimurium. In addition, the SSM leaf methanol extract was showed by strong antibacterial activity against S. aureus. It is assumed that the antioxidant and antibacterial activities of SSM are related to total polyphenol and flavonoid contents extracted by methanol and ethanol. Therefore, we suggest that the SSM extracts have a high possibility for application as a bioactive material in meat product.*

Introduction

In vivo defense mechanisms against active oxygen include antioxidant enzymes such as superoxide dismutase (SOD), catalase, and peroxidase, as well as antioxidant materials such as vitamin C, vitamin E, and glutathione (Indo et al., 2015). Natural antioxidants include phenolic compounds, flavone derivatives, tocopherols, ascorbic acid, carotenoids, glutathione, and amino acids. On the other hand, artificial antioxidants including 2-tert-butyl-4-methoxyphenol (BHA), 2,6-di-butylated hydroxytoluene (BHT), 2,4,6-tri-tert-butyphenol (TBP), alkyl (propyl, octyl, lauryl), and gallates (PG, OG, LG) have been developed for applications in food processing due to their improved efficacy and economic advantages (Murakami et al., 2015; Peschel et al., 2006; Wang and Liu, 2004). However, artificial antioxidants are known to cause cancer, interfere with body energy production, cell metabolism, and respiration, and have strong toxicity (Murakami et al., 2015).

When a large amount of food containing nitrite is ingested, the nitrite reacts with amine compounds in food to create a nitrosamine carcinogen and forms methemoglobin via the oxidation of hemoglobin in blood; the accumulation of methemoglobin causes methemoglobinemia (Hare et al., 2012; Hord, 2011). Therefore, various studies on the suppression of diseases involving nitrite-scavenging activity have been performed using natural materials (Chen et al., 2015; Gammone et al., 2015; Taghvaei and Jafari, 2015).

Stachy is a large genus consisting of about 300 species distributed in temperate and tropical regions (Evans, 1996; Salmaki et al., 2012). The genus *Stachy* biosynthesizes various secondary metabolites including flavonoids, iridoids, fatty acids, phenolic acids, and essential oils, which are associated with anti-inflammatory, cytotoxic, antitoxic, antibacterial, and antioxidant activities (Tundis et al., 2014). The amount of these biologically active substances differs depending on countries and regions within the same country (Tundis et al., 2014). Although the genus *Stachy* has been promoted as containing various physiologically active substances, SSM has been examined by relatively few studies, and most studies have focused on stachyose and acteoside (Greutert and Keller, 1993; Hayashi et al., 1996; Yin et al., 2006). Recently, studies on the antioxidant activity of ethanol extracts from SSM have been performed by Feng et al (2015).

SSM has been used in studies on antioxidant and antibacterial activities and for the development of meat-processed products. Therefore, to obtain basic data on the development of functional meat products, we examined antioxidant and antibacterial activities of methanol and ethanol extracts from the leaf, stem, and tuber of SSM.

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Materials and Methods

Preparation of SSM extracts

SSM was purchased from a traditional market (South Korea), separated into leaf, stem, and tuber after washing, and dried for 6 h at 40°C. The dried component (50 g) was mixed with 70% ethanol or methanol at a 1:9 ratio (w/v), and then extracted for 24 h in the dark room. Each extract was filtered with Whatman No.1 filter paper, concentrated using an evaporator (RW-0252G 4000/G1, Heidolph, Germany), and dried by lyophilization (PVTFD10R, Ilsinbiobase, Korea). The lyophilized powder was used at 0~1 mg/mL for the assay.

Total polyphenol content

Total polyphenolic compounds were measured as described by Peschel et al (2006). Briefly, the reaction solution was mixed with 0.1 mL of properly diluted samples from 1 mg/mL extract, 7.9 mL distilled water, and 0.5 mL Folin–Ciocalteu's reagent, after which 1.5 mL of a 20% sodium carbonate anhydrous solution was added 2 min after the addition of Folin–Ciocalteu's reagent. The treated solution was reacted for 2 h and measured at a wavelength of 765 nm with a microplate reader (Multiscan GO, Thermo Scientific co. ltd., USA). Total polyphenol content was presented as mg galic acid (GAE)/g equivalent.

Total flavonoid content

Total flavonoid content was evaluated as described by Chang et al (2002). The reaction solution was mixed with 0.5 mL of properly diluted samples from 1 mg/mL extract, 1.5 mL 95% ethanol, 0.1 mL 10% aluminum chloride, 0.1 mL 1 M potassium acetate, and 2.8 mL distilled water. The mixed solution was reacted for 30 min at room temperature and then measured at a wavelength of 415 nm with a microplate reader (Multiscan GO, Thermo Scientific Co. Ltd., USA). Total flavonoid content was indicated as mg quercetin (QE)/g equivalent.

DPPH radical scavenging activity

DPPH scavenging activity was examined as described by Brand-Williams et al (1995), with minor modifications based on the reducing power in a sample represented by the free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl. Briefly, the reaction mixture was prepared with the same volumes of DPPH solution (D9132, Sigma, USA) and the extract (0~1 mg/mL) was well mixed and reacted for 20 min at room temperature. The reacted solution was measured at a wavelength of 525 nm with a microplate reader (Multiscan GO, Thermo Scientific Co. Ltd., USA). Ascorbic acid was employed as a positive control. Free radical scavenging activity was presented based on the absorbance ratio between the sample and control, and calculated as follows: DPPH radical scavenging capacity (%) = (1 − (AS − AS0)/A0) × 100% where A0 is the absorbance of the negative control group without sample, AS0 is the absorbance of the sample solution, and AS is the absorbance of the treatment group with sample.

$ABTS^{+}$ radical scavenging activity

$ABTS^{+}$ (2,2′ -azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid) radical scavenging activity was measured as described by Re et al (1999). ABTS was dissolved in water to a concentration of 7 mM. ABTS radical cation ($ABTS^{+}$) was obtained by reacting for 12–16 h in the dark at room temperature after mixing with 7 mM ABTS and 2.45 mM potassium persulfate. The reaction solution was composed of the properly diluted samples of 1 mg/mL extract and $ABTS^{+}$ solution, reacted for 10 min, and then measured at a wavelength of 732 nm with a microplate reader Multiscan GO (Thermo Scientific Co. Ltd., USA). ABTS activity was indicated as mg ascorbic acid (AA)/g equivalent.

FRAP activity

Ferric-reducing antioxidant power (FRAP) activity was measured as described by Benzie and Strain (1996). The reaction solution was prepared by the extract (0~1 mg/mL) and FRAP solution, which immediately before the assay was prepared at a ratio of 10:1:1 with 0.3 M sodium acetate buffer (pH 3.6), 10 mM TPTZ [2,4,6-tris (2-pyridyl)-s-triazine] and 20 mM $FeCl_3 \cdot 6H_2O$. The mixed solution was measured at a wavelength of 595 nm with a microplate reader (Multiscan GO, Thermo Scientific Co. Ltd., USA). Ascorbic acid, BHA, catechine, and quercetin were employed as positive controls. FRAP activity was presented as mg ascorbic acid (AA)/g equivalent.

Nitrite scavenging activity

Nitrite scavenging activity was evaluated as described by Gray and Dugan (1975). The reaction mixture was prepared by the properly diluted samples of 1 mg/mL extract and 1 mM sodium nitrite, and reacted for 1 h at 37°C. The treated solution was mixed with 2% acetic acid and griess reagent (1% sulfanilic acid in 30% acetic acid and 1% naphthylamine in 30% acetic acid at a ratio of 1:1). The absorbance of the reaction mixture was measured at a wavelength of 520 nm with a microplate reader (Multiscan GO, Thermo Scientific Co. Ltd., USA). Nitrite activity was indicated as mg ascorbic acid (AA)/g equivalent.

Antibacterial activity

Antibacterial activity was evaluated using the dispersion method with 8 mm discs (Barry, 1976). The applied extract was adjusted to 20 mg/mL. The employed bacteria were allocated from the Korean Collection for Type Cultures (KCTC) as follows: Gram-negative bacteria including pathogenic *Escherichia coli* (ATCC11775), *Salmonella typhimurium* (ATCC14028), and *Vibrio parahaemolyticus* (ATCC 17802D-5); Gram-positive bacteria including *Bacillus cereus* (ATCC1178IFO), *Clostridium perfringe ns* (ATCC13124), *Listeria monocytogenes* (ATCC 19114), and *Staphylococcus aureus* (ATCC112692); and the yeast *Candida albicans* (00432 ATCC 1023 IFO 1594). The allocated bacteria were subcultured in nutrient broth (Difco, USA). The subcultured bacteria were smeared on Nutrient agar and positioned on an 8 mm disc with 0.2, 1, 2, and 4 mg/mL of each extract, and then incubated for 24 h at 37°C. After incubation, antibacterial activity was evaluated based on the size of the clear zone around the disc.

Statistical analysis

When significant differences were detected, the mean values were separated by the probability difference option. The results are presented as least square means with standard deviations. Duncan's multiple range tests (MRT) were employed to verify significant differences ($P<0.05$) between sample types. All of the analyses were performed within the SAS statistical software package (version 9.1, SAS Inst., Inc., USA, 1999), and differences were considered significant at $P<0.05$.

Results and Discussion

Total polyphenol and flavonoid contents

Among the physiologically active substances identified from *Stachy* sp. (Tundis et al., 2014), antioxidant activity associated with polyphenolic compounds was detected from a methanol extract of the flowering parts of *S. alpine* subsp. Dinarica, *S. anisochila*, *S. beckeana*, and *S. plumose* (Kukic et al., 2006). Furthermore, *Stachys* genus maintains

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various flavonoids known to have antioxidant and antibacterial activities as follows: apigenin, chrysosplenetin, apigenin-7-*O*-(6′′ -*O*-*E*-*p*-coumaroyl)-′ -D-glucopyranoside, 5-hydroxy-3,6,7,4′ -tetramethoxyflavone, and salvigenin (Tundis et al., 2014). Therefore, we measured total polyphenol and flavonoid contents to analyze the association between polyphenols or flavonoids in SSM and antioxidant or antibacterial activity.

Total polyphenolic content was 3.21, 0.86, and 0.58 mg GAE/g from leaf, stem, and tuber methanol extracts, respectively, and 2.34, 0.77, and 0.27 mg GAE/g from ethanol extracts, respectively (Table 1). On the other hand, total flavonoid content was 1.01, 0.05, and N.D. mg QE/g from leaf, stem and tuber methanol extracts, respectively, and 0.81, 0.03 and N.D. mg QE/g from the ethanol extracts, respectively. Total polyphenolic content from the differential parts of SSM was extracted at higher levels in methanol than in ethanol. In particular, the SSM leaf methanol extract maintained the most total polyphenolic compounds. In addition, total flavonoid content from the leaves was detected at higher level in methanol than in ethanol. The flavonoid content extracted in the leaves was the highest value, and at least 20-fold greater than the other parts. Therefore, since the methanol and ethanol extracts from the leaves maintained high polyphenol and flavonoid contents, it is assumed that the extracts have high antioxidant activity.

DPPH radical scavenging activity

Ascorbic acid used as a positive control showed better DPPH activity than those of SSM extracts, with IC_{50} value of 9.7 μg/mL, where the IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100% of samples (de Oliveira et al., 2013). The leaf, stem, and tuber extracts of SSM were examined for DPPH radical scavenging activity (Fig. 1). DPPH radical scavenging activities of the SSM methanol extracts were 422.0, 493.0 and 958.0 μg/mL as IC_{50} values of the leaf, stem, and tuber, respectively, whereas the ethanol extracts were 188.0, 864.6, 837.5 μg/mL, respectively. The leaf ethanol extract showed the highest activity among the extracts, whereas the tuber methanol extract revealed the lowest activity.

A fraction named the partial purified SSP II-a among the 80% ethanol extraction of SSM tuber showed 37% DPPH activity compared to the maximal activity of ascorbic acid (Feng *et al.*, 2015). However, in the present study, DPPH activities from the leaf ethanol and methanol extracts showed lower activities of 22 and 44 folds, respectively, compared to ascorbic acid. Furthermore, the tuber methanol and ethanol extracts showed relatively less IC_{50} values of 99 and 86 folds, respectively, compared to ascorbic acid. Therefore, the tuber extracted with 70% ethanol maintained a similar activity with SSP II-a extracted with 80% ethanol extract (Feng et al., 2015). It is assumed that high levels of polyphenol and flavonoid contents in the leaf extracts induce the high values of DPPH activity. In addition, the DPPH activities in this study were proportional to the total polyphenol and flavonoid contents in the extracts. However, comparing the extracts between methanol and ethanol solvents, the methanol extracts showed higher polyphenol and flavonoid contents than the ethanol extracts, but lower or similar levels for DPPH activities. Therefore, we suggest that DPPH activities are partially associated with polyphenol and flavonoid contents based on these extracts, but the activities in the methanol and ethanol extracts were related with different chemical properties or materials.

$ABTS^{\cdot+}$ radical scavenging activity

Total phenolic content extracted from *Antidesma bunius* showed a strongly positive correlation with ABTS activity, but total flavonoid content showed weakly positive correlations (Jorjong et al., 2015). In this study, $ABTS^{\cdot+}$ radical scavenging activities were 68.39, 4.46, and 12.28 mg AA/g from the leaf, stem and tuber methanol extracts of SSM, respectively, whereas the ethanol extracts were 46.38, 20.15, and 31.37 mg AA/g, respectively (Fig. 2). The leaf methanol extract showed the highest activity among the extracts, whereas the stem methanol extract exhibited the lowest activity.

A fraction of a partial purified SSP II-a among the 80% ethanol extract of SSM tuber showed 68% ABTS activity compared to maximal activity of ascorbic acid (Feng et al., 2015). This activity maintained a higher value than DPPH activity in the same fraction. In this study, IC50 value of ABTS for ascorbic acid was 950.7 mg/L. Therefore, the 70% ethanol and methanol extracts in the present study showed lower activities of ABTS compared to the SSP II-a fraction among the 80% ethanol extract fraction (Hare et al., 2012). The ABTS activities in this study were partially proportional to the total polyphenol and flavonoid contents in the extracts (Fig. 2). Therefore, we suggest that ABTS activities in the SSM extracts are partially associated with total polyphenol and flavonoid contents.

FRAP activity

Total phenolic content extracted from *Antidesma bunius* showed a strongly positive correlation with FRAP activity, but total flavonoid content showed weakly positive correlation (Jorjong et al., 2015). In the present study, FRAP activities were 19.79, 8.79 and 6.18, mg AA/g from the leaf, stem and tuber methanol extracts of SSM, respectively, whereas the ethanol extracts were 17.60, 9.77 and 6.02 mg AA/g (Fig. 3). The leaf methanol extract showed the highest activity among the extracts, whereas the tuber ethanol extract showed the lowest activity.

The FRAP activities in this study, as a different result from the extracts of *Antidesma bunius* (Jorjong et al., 2015), were proportional to the total polyphenol and flavonoid contents in the extracts (Fig. 2). Therefore, we suggest that FRAP activities in the SSM extracts are associated with total polyphenol and flavonoid contents.

Nitrite scavenging activity

Total phenols and flavonoids included in the extracts of *Rhodiola rhizomes* were associated with nitrite radical scavenging activity (Cui et al., 2015). Therefore, we examined nitrite scavenging activity to analyze the association between total phenols or flavonoids and nitrite radical scavenging activity. Nitrite scavenging activities (%) were 33.00, 21.84, and 9.57 mg AA/g from the leaf, stem and tuber methanol extracts of SSM, respectively, whereas the ethanol extracts were 7.24, 4.37 and 6.36 mg AA/g (Fig. 4). The leaf methanol extract of SSM showed the highest activity than those of the others. Nitrite scavenging activities were associated with total phenols and flavonoids in the methanol extracts, but were not associated with those in the ethanol extracts.

The IC_{50} values from nitrite radical scavenging activity according to the methanol- extracted components of *Lantana camara* showed the order of tuber>leaves>allopur inol>flower>stem>fruit (Mahdi-Pour et al., 2012). In the present study, the activities exhibited the differential order

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of leaves>stem or tuber. Therefore, we suggest that the nitrite activities in the extracts are related with different chemical properties or materials

Antibacterial activity

Generally, total phenols and flavonoids are known to be associated with antibacterial activity (Daglia, 2012). Therefore, we examined antibacterial activity to explore the association between total phenols or flavonoids in SSM extracts and antibacterial activity. The methanol extracts from the measured results of the clear zone around the 8 mm disc showed stronger activities than the ethanol extracts (Table 2). In particular, strong antibacterial activity was observed from the leaf and tuber methanol extracts or the tuber ethanol extract against *S. typhimurium* and the leaf methanol extract against *B. cereus*. Intermediate activities were observed from the leaf methanol extract against pathogenic *E. coli*, the stem methanol extract or the leaf and stem ethanol extracts against *S. typhimurium*, the leaf and stem methanol extracts against *C. perfringens*, and all the methanol extracts against *S. aureus*. The other bacteria or extracted fractions showed weak or no antibacterial activities.

Antibacterial activities were not observed from ethanol, light petroleum, dichloromethane, or ethyl acetate *n*-butanol extracts of *S. cretica* subsp. lesbiaca and *S. cretica* subsp. Trapezuntica (Serbetci et al., 2010). However, the flavonoids in methanol extracts of several *Stachy* genera including *S. byzantina*, *S. inflata*, *S. lavandulifolia*, and *S. laxa* showed antifungal, antiviral, and antibacterial activities (Cushnie and Lamb, 2005). In the present study, as in previous reports (Cushnie and Lamb, 2005), the methanol extracts showed antibacterial activities against all the employed bacteria, but did not show antifungal activity towards *C. albicans*. The antibacterial activity towards *E. coli* was directly associated with polyphenol and flavonoid contents, whereas the activities towards *S. typhimurium* and *S. aureus* were partially associated with polyphenol and flavonoid contents. As opposed to previous reports, the antibacterial activity of the ethanol extract was a specific feature of the present study. As with the methanol extracts, the leaf ethanol extract was detected at a broader range and showed higher antibacterial activities than those of the stem and tuber ethanol extracts. Therefore, we suggest that antibacterial activities of the SSM extracts are partially associated with polyphenol and flavonoid contents.

Conclusion

Total polyphenol and flavonoid contents were superior to the leaf extracts of SSM depending on methanol and ethanol extraction (p<0.05). The antioxidant activities were detected by relatively high levels from SSM leaf extracts, but were observed at low levels from SSM tuber extracts. The leaf and tuber methanol extracts or the tuber ethanol extract showed strong antibacterial activities against *S. typhimurium* and the leaf methanol extract exhibited strong antibacterial activities against *S. aureus*. The antioxidant and antibacterial activities of the SSM extracts were partially associated with polyphenol and flavonoid contents.

Acknowledgments

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Figure legends

Fig. 1.

A

AA	LM	SM	TM	LE	SE	TE
0.7±0.3	422.0±5.0	491.0±16.5	991.0±7.0	181.0±0.1	864.6±80.2	837.5±50.1

B

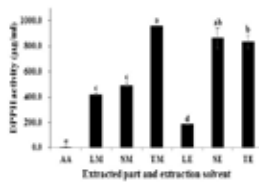

Fig. 1. DPPH radical scavenging activities of the SSM extracts depending on the extracted parts and extraction solvent. An aliquot of the extracts (0~1,000 μg/mL) was applied for assay of DPPH. X- and Y-axes indicate the extracted parts and extraction solvent and DPPH radical scavenging activity, respectively. LM; leaf methanol extract, SM; stem methanol extract, TM; tuber methanol extract, LE; leaf ethanol extract, SE; stem ethanol extract, TE; tuber ethanol extract, respectively. [a-e]Values indicate significantly different among the samples (P<0.05).

Fig. 2

A

LM	SM	TM	LE	SE	TE
68.39±14.19	4.46±1.96	12.28±6.38	46.38±6.38	20.15±6.86	31.37±7.76

B

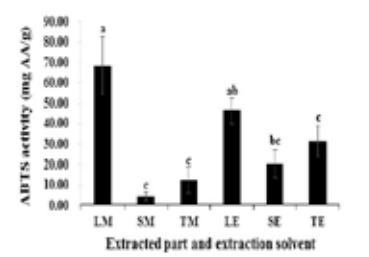

Fig. 2. ABTS^{+} radical scavenging activities of the SSM extracts depending on the extracted parts and extraction solvent. An aliquot of the extracts (0~1,000 μg/mL) was applied for assay of ABTS$^{\cdot+}$ activity. X- and Y-axes indicate the extracted parts and extraction solvent and ABTS$^{\cdot+}$ radical scavenging activity, respectively. LM; leaf methanol extract, SM; stem methanol extract, TM; tuber methanol extract, LE; leaf ethanol extract, SE; stem ethanol extract, TE; tuber ethanol extract, respectively. [a-c]Values indicate significantly different among the samples (P<0.05).

Fig. 3.

A

LM	SM	TM	LE	SE	TE
19.79±0.60	8.79±0.69	6.18±0.53	17.60±0.87	9.77±0.61	6.02±0.37

B

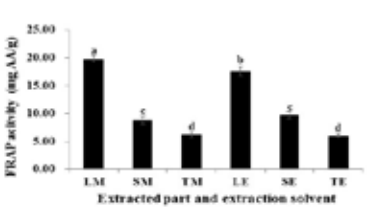

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Fig. 3. Ferric reducing ability of power activities of the SSM extracts depending on the extracted parts and extraction solvent. An aliquot of the extracts (0~1,000 μg/mL) was applied for assay of FRAP activity. X- and Y-axes indicate the extracted parts and extraction solvent and FRAP activity, respectively. LM; leaf methanol extract, SM; stem methanol extract, TM; tuber methanol extract, LE; leaf ethanol extract, SE; stem ethanol extract, TE; tuber ethanol extract, respectively. [a-d]Values indicate significantly different among the samples (P<0.05).

Fig. 4.

A

LM	SM	TM	LE	SE	TE
33.00±3.36	21.84±2.17	9.57±1.13	7.24±1.89	4.37±1.42	6.36±1.47

B

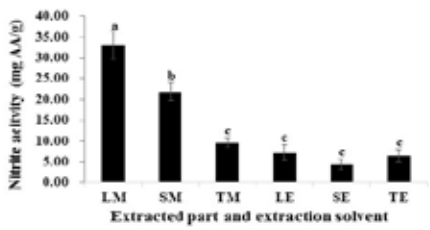

Fig. 4. Nitrite scavenging activities of the SSM extracts depending on the extracted parts and extraction solvent. An aliquot of the extracts (0~1,000 μg/mL) was applied for assay of nitrite scavenging activity. X- and Y-axes indicate the extracted parts and extraction solvent and nitrite activity, respectively. LM; leaf methanol extract, SM; stem methanol extract, TM; tuber methanol extract, LE; leaf ethanol extract, SE; stem ethanol extract, TE; tuber ethanol extract, respectively. [a-c]Values indicate significantly different among the samples (P<0.05).

Table 1. Total phenol and flavonoid contents in S. sieboldii MIQ extracts

Extraction type[1)]	Total polyphenol (mg GAE/g)[2)]	Total flavonoid mg QE/g)[3)]
LM	3.21±0.07[a]	1.01±0.02[a]
SM	0.86±0.02[c]	0.05±0.02[c]
TM	0.58±0.05[d]	N.D.[4)]
LE	2.34±0.02[b]	0.81±0.01[b]
SE	0.77±0.02[c]	0.03±0.02[c]
TE	0.27±0.04[e]	N.D.

[1)]LM; leaf methanol extract; SM; stem methanol extract, TM; tuber methanol extract, LE; leaf ethanol extract, SE; stem ethanol extract, TE; tuber ethanol extract. [2)] GAE; gallic acid. [3)]QE; quercetin. 4)N.D.; not detected. [a-e]Means±SD were significantly different within the same column (p<0.05).

Table 2. Antibacterial activities of the *S. sieboldii* MIQ extracts

Extraction solvent		Methanol												Ethanol											
extraction part		Leaf				Stem				Tuber				Leaf				Stem				Tuber			
concentration (mg)		0.2	1	2	4	0.2	1	2	4	0.2	1	2	4	0.2	1	2	4	0.2	1	2	4	0.2	1	2	4
Microorganisms	pathogenic *E. coli*	-	-	++	++	-	-	+	++	-	-	-	-	-	-	+	+++	-	-	-	+	-	-	-	-
	S. typhimurium	-	++++	++++	++++	-	-	++++	++++	-	++++	++++	++++	-	-	++++	++++	-	-	++++	++++	-	++++	++++	++++
	V. parahaemolyticus	-	-	-	++	-	-	-	++	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	B. cereus	-	++	+++	+++	-	-	+++	+++	-	-	-	++	-	-	++	+++	-	-	+++	+++	-	-	-	+++
	C. perfringens	-	-	+++	+++	-	-	++	+++	-	-	-	+++	-	-	-	++++	-	-	-	+	-	-	-	-
	L. moncytogenes	-	-	-	++	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	S. staphylococcus	-	-	++	+++	-	-	+++	+++	-	-	+++	++++	-	-	-	+	-	-	-	-	-	-	-	-
	C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-; No antibacterial activity, +; very slight antibacterial activity inhibition zone (I.Z) of 8.1-9.0 mm; ++; Moderate antibacterial activity I.Z of 9.1-10.9 mm, +++; Clear antibacterial activity I.Z of 11.0-12.9 mm, ++++; strong antibacterial activity I.Z of 13.0-15.9 mm, +++++; very strong antibacterial activity I.Z. of >16.0 mm.

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