Current Research on Agriculture and Life Sciences (2013) 31(3) : 170-176
ISSN 2287-271×(Print)
ISSN 2288-0356(Online)

Original Article

Antioxidant and Antimicrobial Activity of *Rosa multiflora* Thunberg Fruits Extracts

Young-Je Cho*

School of Food Science & Biotechnology, Food & Bio-Industry Research Institute, Kyungpook National University, Daegu 702-701, Korea

Abstract

In this study, we selected some material to have potential bioactivity from natural plants, confirmed as basic data for industrializing and tried to develope the food materials using them. DPPH, ABTS, antioxidant protection factor, TBARs and antimicrobial activity of extracts from *Rosa multiflora* Thunberg fruits were determined. The total phenolics extracted from *Rosa multiflora* were 12.08, 11.82, 11.1 and 12.6 mg/g when using water, 70% ethanol, 70% methanol and 70% acetone as the solvent, respectively. The optimum conditions for extracting the phenolic compounds were 70% ethanol over for 12 hrs(11.82 mg/g). The electron donating ability and inhibition rate on ABTS of the 70% ethanol extracts were 97% and 92.2%, respectively while the antioxidant protection factor(PF) of the water extracts and 70% ethanol extracts were 1.79 and 1.34 PF, respectively. The TBAR (thiobarbituric acid reactive substance) value were 1.3 μM for the control and 0.15 μM for the 70% ethanol extracts. The inhibitory activity against α-amylase was 26% for the 70% ethanol extracts. The 70% ethanol extracts from *Rosa multiflora* Thunberg fruits exhibited antimicrobial activity against *H. pylori, S. epidermidis, S. aureusand* and *E. coli* with clear zone diameters of 14, 25, 14 and 13 mm, respectively when using 200 μg/mL of the phenolic compounds. An HPLC analysis identified 6 major phenolic metabolites in the *Rosa multiflora* Thunberg fruits extracts: rosmarinic acid, caffeic acid, chlorogenic acid, courmaric acid, protocatechuic acid and quercetin. In particular, the content of rosmarinic acid was the highest in the 70% ethanol extracts. Therefore these results indicate that 70% ethanol extracts from *Rosa multiflora* Thunberg fruits can be useful as a natural antioxidant and in functional foods.

Keywords : Antimicrobial activity, Antioxidant activity, α-Amylase inhibitory activities, *Rosa multiflora* Thunberg fruits

Introcuction

Prooxidants and antioxidants are normally balanced in the human body yet this balance can break under certain conditions, resulting in oxidative stress that can lead to potent cell damage or bacterial disease (Videla and Fernandez 1998). The direct cause of such oxidative stress is reactive oxygen species (ROS) that are unstable and highly reactive, meaning they react easily with various biological substances and, attack cells and tissues, damaging them irreversibly or inducing mutation, cell poisoning and carcinogenic activity. Extensive studies have already been conducted to find antioxidants in food and nature, resulting in the development of many functional foods and cosmetics promoting human health(Cho et al. 2008; Choi et al. 2003). The fruits of *Rosa multiflora* Thunberg is used in Oriental medicine to cure diuresis, edema, constipation and boils. In addition, it has been reported to help improve energy levels, unblock the coronary arteries, strengthen protein and lipid metabolism, and inhibit the occurrence the bamboo arteries (Park et al. 2008). The key components include a quercetin derivative (multiside A, multinoside B, quercitrin), kaempferol derivative (multiflorin A, multiflorin B, afzelin, astragalin), methyl gallate from the tannin group, and lycopene that is the red pigment in the fruit (Higasi 2000; Kang et al. 1996).

With the recent growing interest in healthy life styles, there is also a growing interest in the development of functional foods and functional substances with natural bioactivity. Accordingly, this study, selected various natural plants with potential bioactivity and confirmed their basic data for future application in food materials.

Materials and Methods

Chemicals

The ABTS[2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], yeast extracts, BHT, beef extracts, pyruvic acid, β-carotene, α,α-diphenyl-β-picrylhydrazyl (DPPH), pancreatin α-amylase, α-glucosidase, ρ-nitrophenol-α-D-glucopyranoside (PNPG) were all special grade and purchased from Sigma Co.

Received: August 20 2013 / Revised: September 23 2013 / Accept: September 27 2013
*Corresponding Author: Young-Je Cho, Tel. 82-53-950-7755, Fax. 82-53-950-7762, Email. yjcho@knu.ac.kr
©2012 College of Agricultural and Life Science, Kyungpook National University
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org.1:censes/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, Provided the Original work is Properly cited.

Sample Preparation
The *Rosa multiflora* fruits was purchased at an oriental herb market dried in a drying oven at 50℃ mashed using a 40 mesh screen, and stored at 4℃until use.

Extract preparation
Water extracts :1 g of the sample was added to 200 mL of distilled water in a flask boiled until the volume was reduced to 100 mL, and then cooled and shaken for 24 hrs. Ethanol extracts : 1 g of the *Rosa multiflora* Thunberg fruits powder was added to 100 mL of various concentrations of ethanol, shaken at room temperature for 24 hrs, and then filtered using Whatman No.1 filter paper. The ethanol extracts were also concentrated using rotary vacuum evaporator (Eyela NE, Japan).

Total phenolic assay
The total phenolic was measured according to the Folin-Denis' method (Joslyn 1970). Briefly, 1 mL sample of the water extracts and ethanol extracts were added to test tubes and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. Thereafter 0.5 mL of a 1N Folin-ciocalteu reagent was added, then 5 min later, 1 mL of 5% Na_2CO_3 was added and the absorbance read at 725 nm. The total phenolic content was calculated using gallic acid equivalents.

DPPH radical scavenging assay
The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was measured using Blois method (Brand-Williams et al.1995). Briefly, 3 mL of 60 μM DPPH in ethanol was added to 1 mL of each sample and incubated at room temperature for 15 min. The absorbance was then read at 517 nm. The activity was calculated as follows:

$$Activity(\%) = 1 - \frac{Abs517nm(control) - Abs517nm(sample)}{Abs517nm(control)} \times 100$$

Measurement of ABTS radical cation decolorization
The measurement of the ABTS[2,2'-azinobis(3-ethylbenzothiazoline-6- sulfonic acid)] radical cationdecolorization was determined using the Pellegrin et al. method (Pellegrinet al. 1998). Briefly, 5 mL of 7 mM ABTS and 88 μL of 140 mM $K_2S_2O_8$ were mixed and left to stand in a dark place for 14~16 hrs before use. Prior to the assay, the ABTS stock solution was diluted with absolute ethanol (ratio 1:88) to give an absorbance at 734 nm of 0.7±0.002 and equilibrated to 30℃ Next 1 mL of ABTS was added to glass test tubes containing 50 μL of each extract. The contents were then mixed using a vortex mixer for 30 sec and incubated for 2.5 min. The absorbance was read at 734 nm. The percentage inhibition was calculated as follows:

$$Radical\,scavenging\,activity(\%) = 1 - \frac{Abs734nm(sample)}{Abs734nm(control)} \times 100$$

Antioxidant Protection Factor (PF)
The antioxidant protection factor (PF) was evaluated using the assay described by Andarwulan and Shetty (Andarwulan and Shetty 1999). Briefly, 10 mg of β-carotene was dissolved in 50 mL of spectroscopic grade chloroform. 1.0 mL of this solution was then evaporated to dryness in a round flask using a rotary evaporator at 40℃ Next, 20 μL of linoleic acid, 184 μL of Tween 40, and 50 mL of H_2O_2 were added to the β-carotene flask and mixed to make an emulsion. 5 mL of the emulsion solution was then added to glass test tubes containing 100 μL of each extract and mixed using a vortex mixer. Finally, the tubes were incubated at 50 ℃ for 30 min and cooled. The absorbance was read at 470 nm using ethanol as the blank. The antioxidant activity was expressed as the protection factor (PF) and calculated as follows:

$$Antioxidant\,protection\,factor(PF) = \frac{Abs470nm(sample)}{Abs470nm(control)} \times 100$$

Measurement of Thiobarbituric acid substances(TBARs)
The thiobarbituric acid substances(TBARs) were measured using the Buege and Aust' method (Buege and Aust 1978). Briefly, emulsions were prepared using 1% linoleic acid and 1% Tween 40 in 100 mL of distilled water. 0.8 mL of the emulsions was then added to glass tubes containing 0.2 mL of each extract, and the tubes incubated in a water bath at 50℃for 10 hrs. Thereafter, 1.0 mL of the reacted solution was added to a tube containing 2 mL of the TBA reagent, vortexed and heated in a boiling water bath for 15 min. After cooling for 10 min, the solution was centrifuged for 15 min at 1,000 rpm and, the absorbance measured at 532 nm. The TBAR value (absorbance×0.0154) was determined as the μM of 1,1,3,3-tetraethoxypropane (TEP) product from 1 mL of the reacted mixture.

Measurement of pancreatin α-amylase inhibition activity
The inhibition activity of pancreatin α-amylase was measured using an agar diffusing method (Cavidson and Parish 1989). Briefly, the plates was prepared using 1% agar and 1% soluble starch that were, solved, boiled, and sterilized at 121℃ for 15 min in a flask and then 15 mL plated on petri dishes. After cooling the petri dishes, 0.8 μL of the sample and 0.2 μL of α-amylase were mixed and plated on the petri dishes using

a disc paper. The control was plated with a mixture of distilled water and α-amylase. The petri-dishes were then incubated at 37℃ or 3 days, followed by the addition of 5 mL of I_2/KI (inhibition rate calculated as 5 mM I_2 in 3% KI) and standing for 15 min for coloring.

$$Inhibition\ Rate(\%) = \frac{(Area\ of\ control - Area\ of\ samples)}{Area\ of\ control} \times 100$$

Measurement of antimicrobial activity

This study used *Staphylococcus aureus* (standard colony KCTC 1039), *Candida albicans* (standard colony KCTC 7965), *Escherichia coli* (standard colony KCTC 1039) and *Streptococcus mutans* (standard colony KCTC 3065) that cause cavities. The microorganisms were incubated on agar plates using an nutrient medium, Brain Heart medium, YM medium and Tryptic soy medium in a 37℃ incubator and CO_2 incubator while maintaining air and nonair conditions for 24~48 hrs. The antimicrobial activity measurements were used to confirm a clear zone around the disc of the plate medium prepared using 100 μL of the stock microorganism, then filter paper(Φ 8mm) discs containing 50~200 μg/mL phenolics were applied to the surface (Ju and Cho 2009).

HPLC analysis

The HPLC chromatographic analysis of the *Rosa multiflora* extracts was conducted using a modified version of the Whitaker' method (Vattem and Shetty 2002; Whitaker et al. 1984). Briefly, 2 mL of the *Rosa multiflora* extracts was filtered through a 0.2 mm filter. 5 mL of the sample was then injected using an by Agilent ALS 1100 autosampler into an Agilent 1100 series HPLC equipped with a VWD 1100 variable wavelength detector. The solvents used for the gradient were (A) 10 mM phosphoric acid (pH 2.5) and (B) methanol. The methanol concentration was increased to 60% during the first 8 min and to 100% during the next 7 min, then decreased to 0% during 3 min and maintained at 0% for the last 7 min (total run time, 25 min). The analytical column was an Agilent Zorbax SB-C18, 250 ×4.6 mm i.d., including packing material with a 5 mm particle size at a flow rate of 1 mL/min at an ambient temperature. During each run the chromatogram was recorded at 306 nm and integrated using an Agilent Chemstation enhanced intergrator. Pure procatechuic acid, caffeic acid, coumaric acid, rosmarinic acid and quercetin in 100% methanol were used to calibrate the standard curve and retention times.

Results and Discussion

Total content of phenolics

Phenolic compounds are known as secondary metabolic products whit various structures and molecular weights, plus they exhibit

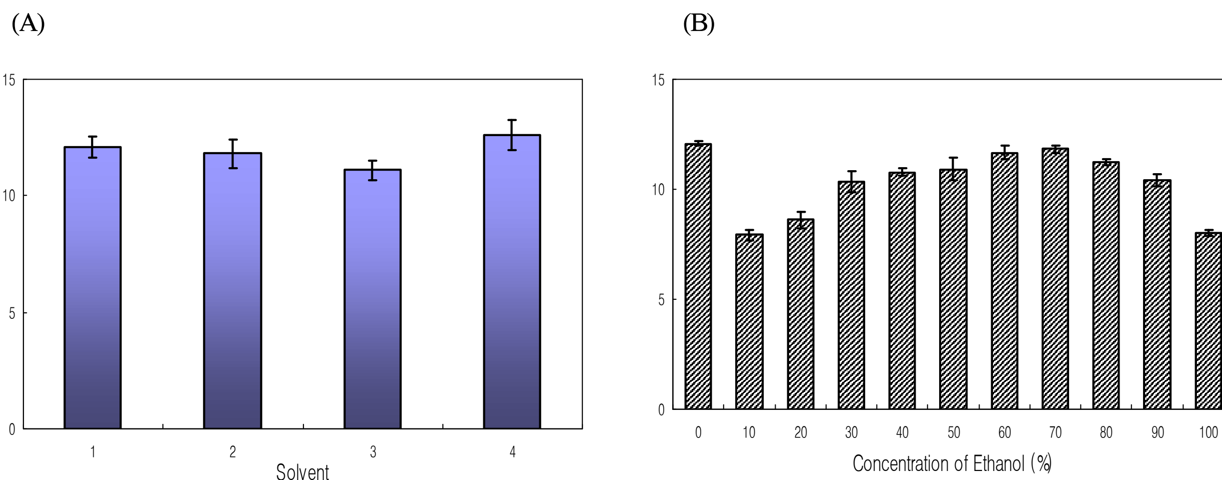

Figure 1. Effect of different solvents(A) and ethanol concentrationons(B) on extraction of phenolics from *Rosa multiflora* Thunberg.

1: Water extracts
2: 70% Ethanol extracts
3: 70% Methanol extracts
4: 70% Acetone extracts
* Each value represents mean±SD(n=3)

antioxidant activities based on compounding protein and huge molecules due to their phenolic hydroxyl group (Jung et al. 2004). To determine the optimal conditions for extracting samples, the total phenolics extracted using the water, 70% ethanol, 70% methanol and 70% acetone solvent were 12.08 mg/g, 11.82 mg/g, 11.1 mg/g, and 12.6 mg/g, respectively (Figure. 1-A). To apply *Rosa multiflora* extracts to food products, the total phenolics were prepared using 10~100% ethanol, which is harmless and highly solubile with phenolic substances. As a result, the highest total phenolics were extracted when using 70% ethanol (Figure. 1-B). In addition, the total phenolics were compared when using different extraction times (Figure. 2). In the case of 70% ethanol, the total phenolics increased during 6~24 hrs, yet remained unchanged after 24 hrs. Thus, the optimal extraction conditions for *Rosa multiflora* were 70% ethanol for 24 hrs. Clark et al. (Clark and Tl-Feraly 1981) previously reported that phenolic substances in plants exhibit antimicrobial activity, thus it was expected that the phenolics in the 60% and 70% ethanol extracts would be a natural antimicrobial substance and physiologically active substance.[4)]

(superoxide) (Torelet al. 1986). The DPPH radical scavenging ability of the *Rosa multiflora* extracts (200 µg/mL) was remarkable at over 90% over, and the 70% ethanol extracts were the highest at 97% (Table 1). When compared with Park's report that 70% water extracts of *Cornus officinalis, Glycyrrhiza uralensis* and *Astragali radix* had a DPPH radical scavenging ability of more than 70% (Park et al. 2008), the antioxidant ability of the *Rosa multiflora* extracts is clearly excellent, plus the present study used less total phenolics. Kang et al.(1996) reported that thehigher the ability of electron donation, the higher the reducing ability of phenolic substances. To measure the hydrophilic antioxidant ability, the ABTS radical cation decolorization was assayed and no differences were found between the water extracts and the 70% ethanol extracts, as both exhibited high inhibition rates at 91.6% and 92.2%, respectively [Table 1]. Therefore it is anticipated that the water extracts and 70% ethanol extracts can both be developed as hydrophilic and lipophilic agents. To determine the lipophillic antioxidant activity, the antioxidant protection factor (PF) was measured and the results for the water extracts (200 µg/mL) and 70% ethanol extracts (200 µg/mL) were 1.79 PF and 1.34 PF, respectively, as shown in Table 1. These results indicate very high antioxidant activities, as 1.2 PF is commonly considered high antioxidant activity. Furthermore, the TBAR values were measured to confirm the inhibitory effect of the *Rosa multiflora* extracts against lipid oxidation, where the value for the water extracts (200 µg/mL) was 0.21±0.06 µM and that for the 70% ethanol extracts (200 µg/mL) was 0.15±0.04 µM.

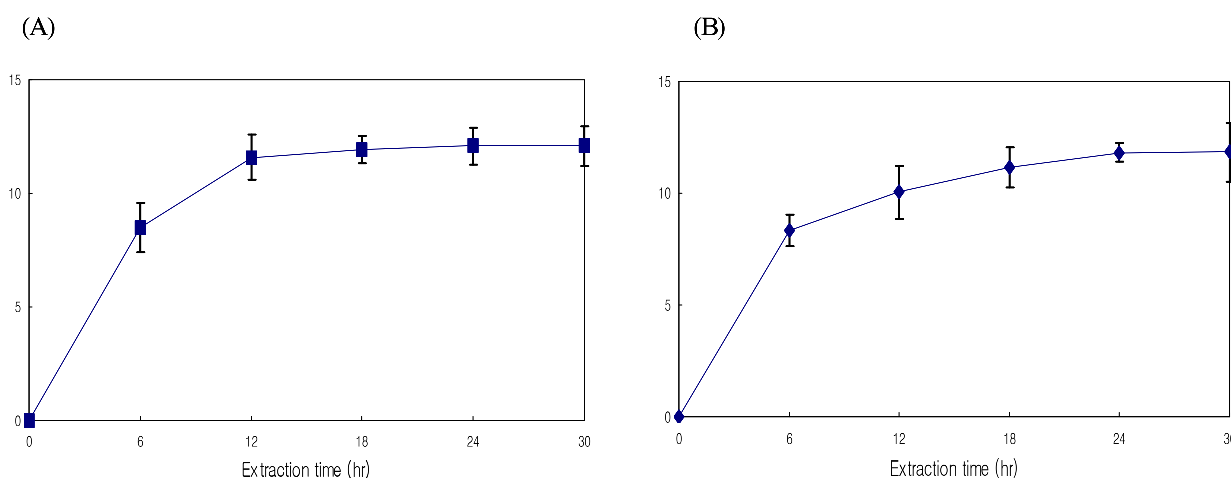

Figure 2. Effect of extraction time on water extraction(A) and 70% ethanol extraction(B) of phenolics from *Rosa multiflora* Thunberg.

Each value represents mean±SD(n=3)

A antioxidant effects

A DPPH radical scavenging assay of antioxidant activities is an effective method for measuring antioxidant ability using decoloring anioic acids, like cysteine and glutathione that contain sulfur, aromatic amine, and ascorbic acid. These substances are also used to measure aging inhibition in the body against free radicals in terms of reducing or offsetting free radicals

Table 1. Antioxidant activities of water and 70% ethanol extracts from *Rosa multiflora* Thunberg

Antioxidant assay	Antioxidant activity						
	Control	Water extracts		70% Ethanol extracts		Vitamin C	
		Phenolic content(μg/mL)		Phenolic content(μg/mL)		Content(μg/mL)	
		100	200	100	200	100	200
DPPH(%)	-	41.0±1.2	96.0±1.5	43.0±2.1	97.0±2.4	66.0±2.9	87.0±4.7
ABTS(%)	-	39.1±2.1	91.6±3.9	41.2±2.7	92.2±3.8	76.4±6.4	98.2±3.7
Antioxidant protection factor(PF)	-	0.9±0.5	1.9±0.2	0.7±0.3	1.3±0.1	1.1±0.2	1.2±0.3
TBARS(X10^2 μM)	1.3±0.1	0.7±0.1	0.2±0.1	0.5±0.1	0.2±0.1	0.6±0.2	0.2±0.1

*Phenolic content was 100 and 200(μg/mL)
*Each value represents mean±SD(n=3)

These values were lower than the control which was 1.3±0.02 μM. Therefore, the results indicated that *Rosa multiflora* extracts had a high inhibitory ability against oxidation. Thus, the antioxidant activities of the phenolic compounds in the *Rosa multiflora* extracts match previous reports of phenoilc compounds and flavonoids in plants acting, as natural antioxidants (Bors and Saran 1987; Fitzpatrick et al.1993; Sato et al.1996). However, even though the water extracts included more total phenolic compounds than the 70% ethanol extracts, the antioxidant activity of the water extracts was lower than that of 70% ethanol extracts, meaning the 70% ethanol extracts included more antioxidant properties influencing antioxidant activity. Notwithstabding, the water extracts and 70% ethanol extracts can both still be used as antioxidant agents due to their remarkable activities against oxidation.

Measurement of pancreatin α-amylase inhibition activity

Pancreatin α-amylase is a liquefactive enzyme that randomly hydrolyzes polysaccharides, and the *Rosa multiflora* extracts were examined to confirm their ability to inhibit the α-amylase enzyme. The enzyme inhibition effects of the water extracts and 70% ethanol extracts were both high at 40% and 61%, respectively (Figure. 3). Yet, the current results were remarkably low when compared with those previously reported for Rubifructus extracts (84%) and *Schizandrae fructus* extracts (100%). Iwuoha and Aina (Iwuoha and Aina 1997) reported that, phenolic matter reduces the ability to resolve starch, thereby inhibiting the activity of α-amylase. Thus, while the *Rosa multiflora* extracts exhibited excellent antioxidant activities, their ability to inhibit α-amylase activity was low.

Measurement of antimicrobial activity

The antimicrobial activities of the *Rosa multiflora* extracts were measured gainst *S. aureus, E. coli, S. epidermidis, S. mutans* and *C. albicans* based on Disc's method (Higasi 2000; Chun et al. 2005). Table 2 and Figure. 4 show that the water extracts did not show any inhibitory effect against skin microorganisms, yet the ethanol extracts showed a remarkable inhibitory effect against *S. epidermidis* with a clear zone of 11, 16, 20, and 25 mm in case of a phenolic content of 50, 100, 150, and 200 μg/mL, respectively. While the inhibitory effect against *S. aureus* and *E. coli*, was with a low phenolic content of 100 μg/mL, both microorganisms were inhibited by phenolic contents of 150 and 200 μg/mL. In the case of *C. albicans*, weak antimicrobial activity was exhibited with a phenolic content over 150 μg/mL. No antimicrobial activity was exhibited against *S. mutans*.

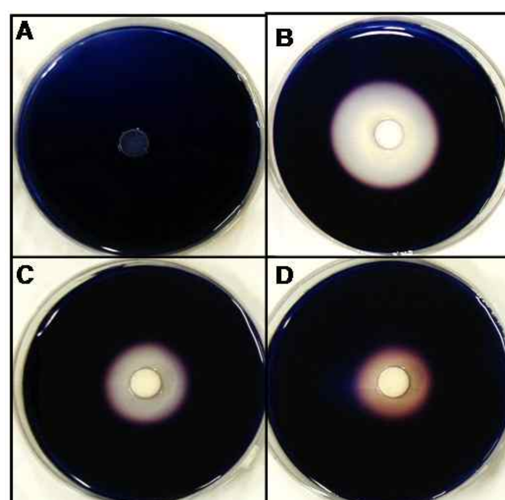

Figure 3. Inhibition of α-amylase activity by water and 70% ethanol extracts from *Rosa multiflora* Thunberg.

A: blank, B: control, C: water extracts, D: ethanol extracts

Table 2. Antimicrobial activity of water and 70% ethanol extracts from *Rosa multiflora* Thunberg with according to concentration of phenolic compounds

Sample	Diameter of clear zone (mm)							
	Water extracts				70% Etanol extracts			
	Phenolic content(µg/mL)				Phenolic content(µg/mL)			
	50	100	150	200	50	100	150	200
S. epidermidis	ND[1)]	ND	ND	ND	11	16	20	25
S. aureus	ND	ND	ND	ND	trace	trace	11	14
E. coli	ND	ND	ND	ND	ND	trace	12	13
C. albicans	ND	ND	ND	ND	ND	ND	ND	trace
S. mutans	ND	ND	ND	ND	ND	ND	ND	ND

*[1)]ND : not detected
*Each value represent mean±SD(n=3)

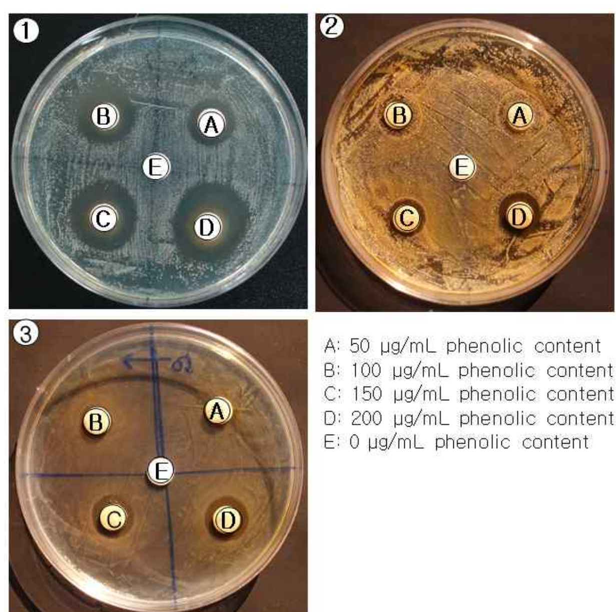

Figure 4. Antimicrobial activity of 70% ethanol extracts from *Rosa multiflora* Thunberg.
① *Staphylococcus epidermidis*, ② *taphylococcus aureus*, ③ *Escherichia coli*

HPLC analysis of extracts

An HPLC analysis was carried out to identify the phenolic profiles of the *Rosa multiflora* extracts. The 6 major phenolic metabolites identified were rosmarinic acid, caffeic acid, chlorogenic acid, coumaric acid, protocatechuic acid and quercetin (Table 3). The contents of rosmarinic acid, courmaric acid and quercetin were higher in the ethanol extracts than in the water extracts. Conversely, the contents of protocatecuic acid, caffeic acid and chlorogenic acid were higher in water extracts at 9.86, 2.14 and 8.62 mg/g dry weight, respectively, than in the ethanol extracts at 6.49, 1.69 and 5.37 mg/g dry weight, respectively. Among the 6 phenolics, the rosmarinic acid content was highest in the 70% ethanol extracts at 15.04 mg/g dry weight campare to 8.53 mg/gdry weight in the water extracts. The second highest content of a specific phenolic was related to protocatecuic acid, where the highest content was found in the ethanol extracts at 6.49 mg/g dry weight compared to 9.86 mg/g dry weight in the water extracts. Two other major phenolics of significance were chlorogenic acid and quercetin which were found at levels of 8.62 and 5.37 mg/g dry weight in the water and ethanol extracts, respectively. The other phenolics such as courmarinic acid and caffeicacid, are generally found in lower concentrations in oriental medicinal plant extracts.

In conclusion, the *Rosa multiflora* extracts were identified and characterized with a high phenolic profile and high antioxidant and antimicrobial activity. Furthermore, the high phenolic profile and high antioxidant activity were clearly correlated with a high antimicrobial and α-amylase inhibitory activity. The development of these phenolics many now faciliatte the development dietary phenolic ingredients with antioxidant potential that can enhance the protective host defence response in humans (Akyon 2002), while also inhibiting prokaryotic pathogenic bacteria, such as *Helicobacter pylori* linked to gastric infections, *Staphylococcus epidermidis* linked to skin infections, and *Staphylococcus aureus* and *Escherichia coli* linked to food poisoning infections. Innovative strategies can also be used to develop functional foods against chronic bacterial infections.

Table 3. Phenolic profiles of *Rosa multiflora* extracts

Standard	Phenolic content (μg/mL)	
	Water extracts	70% Ethanol extracts
Protocatecuic acid	9.86±0.28	6.49±0.40
Caffeic acid	2.14±0.19	1.69±0.18
Chlorogenic acid	8.62±1.36	5.37±1.10
Courmaric acid	1.32±0.22	2.47±0.50
Rosemarinic acid	8.53±0.06	15.04±0.43
Quercetin	ND[1)]	4.26±0.15

[1)]ND: Not detected

References

Akyon Y (2002) Effects of antioxidants on the immune response of *Helicobacter pylori. Cli. Microbial Infect* 8: 438-441.

Andarwulan N and Shetty K (1999) Phenolic content in different plated tissue cultures of untransformed and *Agrobacterium* transformed roots of anise (*Pimpinella anisum* L.). *J Agric Food Chem* 47: 1776-1780.

Bors W and Saran M (1987) Radical scavenging by flavonoid antioxidants. *Free Rad Res Comm* 2: 289-294.

Brand-Williams W, Cuvelier ME and Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Food Sci Tech* 28: 25-30.

Buege JA and Aust SD (1978) Microsomal lipid peroxidation. *Method Enzymol* 105: 302-310.

Cavidson PH and Parish ME (1989) Methods of testing the efficacy of food antimicrobials. *Food Technol*, 43: 148-150.

Cho YJ, Ju IS, Chun SS, An BJ, Kim JH, Kim MU and Kwon OJ (2008) Screening of biological activities of extracts from *Rhododendron mucronulatum* Turcz flowers. *J Kor Soc Food Sci Nutr* 37: 276-281.

Choi SI, Lee YM and Heo TR (2003) Screening of hyaluronidase inhibitory and free radical scavenging activity in vitro of traditional herbal medicine extracts. *Kor J Biotech Bioeng* 18: 282-288.

Chun SS, Vattem DA, Lin YT, Shetty K (2005) Phenolic antioxidants from clonal oregano(*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori. Process Biochemistry* 40: 809-816.

Clark AM and Tl-Feraly FS (1981) Antimicrobial activity of phenolic constituents of *Magnolia grandiflora* L. *J. Pharm Sci.,* 70: 951-952.

Fitzpatrick DF, Hirschfield SL, Coffey RG (1993) Endo-thelium-dependent vasorelaxing activity of wine and other grape products. *Am J Physiol* 265: 774-778.

Joslyn MA (1970) Methods in food analysis. Acad press, New York. 710-711.

Higasi GS (2000) Appraisement of antioxidative activity from vegetables. *Jpn J Food Ind* 57: 56-64.

Iwuoha CI and Aina JO (1997) Effects of steeping condition and germination time on the alpha-amylase activity, phenolics content and malting loss of Nigerian local red and hybrid short kaura sorghum malt. *Food Chem* 58: 289-295.

Ju IS and Cho YJ (2009) Purification and identification of phenol compounds with inhibitory activity on *Helicobacter pylori* from *Rhododendron mucronulatum* Flos extracts. *Kor J Life Sci* 19: 1125-1131.

Jung MS, Lee GS, Chae HJ (2004) In vitro biological activity assay of ethanol extract of Radish. *J Kor Soc Appl Biol Chem* 47: 67-71.

Kang YH, Park YK, Lee GD (1996) The nitrite scavenging and electron donating ability of phenolic compounds. *Kor J Food Sci Technol* 28: 232-236.

Park CS, Kim DH, Kim ML (2008) Biological activities of extracts from Cornifructus, Astragalusmembranaceus and *Glycyrrhiza uralensis. Kor J Herbology* 23: 93-101.

Pellegrin N, Re R, Yang M, Rice-Evans C (1998) Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activites applying 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay. *Method Enzymol* 299: 379-389.

Sato M, Ramarathnam N, Suzuki Y, Ohkubo T, Takeuchi M, Ochi H (1996) Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *J Agric Food Chem* 44: 37-41.

Torel J, Gillard J, Gillard P (1986) Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochem* 25: 383-385.

Vattem DA and ShettyK (2002) Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus. Food Biotechnol* 16: 189-210.

Videla LA and Fernandez V (1998) Biochemical aspects of cellular oxidative stress. *Arch Biol Med Exp* 21: 85-92.

Whitaker RJ, Hashimoto T, Evans DA (1984) Production of the secondary metabolite, rosemarinic acid, by plant cell suspension cultures. *Ann NY Acad Sci* 435: 364-366.