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Potential Antioxidant Components and Characteristics of Fresh *Polygonum multiflorum*

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ABSTRACT

The leaf, stem and root of fresh *Polygonum multiflorum* plant were investigated. The contents of bioactive compounds and their antioxidant potential were studied by several radicals scavenging assay, and the antioxidative substances measured. The total phenolic contents were 179.0, 70.3 and 118.0 mg/g, whereas flavonoids contents were 7.3, 8.0 and 37.5 mg/g for water extracts from root (R), stem (S) and leaf (L), respectively. Further HPLC analysis revealed that the leaf and stem of this functional plant contained quercetin at 13,469 and 1,095 mg/kg, respectively. Furthermore, the root and stem contained rich emodin-related compounds that were 6,620 and 1,245 mg/kg, respectively. And different concentrations of emodin-related compounds and quercetin in root, stem and leaf showed different antioxidative ability. We observed that the DPPH scavenging activity, total antioxidant activity (TEAC), reducing power and NO scavenging ability of the three parts of the plant samples and found that the activities were R > L > S except that was S > L > R on superoxide anion scavenging effect. Our results indicated that the radical scavenging abilities of the three sample parts were more potent than that of emodin and quercetin. *P. multiflorum* contains rich emodin-related compounds and quercetin, indicating that these compounds played important roles in antioxidative effect. We suggested that the antioxidative effect of the fresh *P. multiflorum* plant correlate-well with emodin-related compounds and quercetin.

Key words: *Polygonum multiflorum*, emodin, quercetin, antioxidant activity

INTRODUCTION

Polygonum multiflorum is a widely used traditional Chinese medicine. It has been used to treat liver diseases and age-related diseases. Various parts of the plants were utilized for different medicinal purposes. The leaves and root tuber of this plant have been used as tonic and antiaging agents whereas the stem is used to alleviate insomnia. Active components included emodin (1,3,8-trihydroxy-6-methylantraquinone), chrysophanol, rhein, 6-OH-emodin, emodin-8-β-D-glucoside, polygonimitin B, 2,3,5,4'-tetrahydroxystilbene-2-β-D-glucoside, gallic acid and an unknown glycoside in this herb have been reported[(1)]. In addition, the antioxidation of stilbene glycoside from *P. multiflorum* was investigated[(2)]. Furthermore, a study showed that bioactive substances from four *Polygonum* species, and the root, stem and leaf of *P. cuspidatum* contained rich tannins, emodin, emodin-I-glucoside, quercetin-3-galactoside, quercetin-3-glucuronide and quercetin-3-rhamnoside[(3)]. However, the functional antioxidative substances of fresh *P. multiflorum* are unclear.

Generally, flavonoids and phenolic acids are major types of phenolic compounds that possess potent antioxidant activity. Quercetin, the key representative in the family of flavonoids, has been shown to scavenge free radical[(4)]. In addition, emodin, an anthraquinone derivative from *P. multiflorum* is also well-known to exhibit antioxidant activity. Emodin has been shown to reduce lipid peroxidation in mitochondria of heart tissue, inhibit leukemia cell line K562 proliferation and regulate immune responses[(5-7)].

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This study aims to investigate the contents of antioxidative compounds of the leaf, stem and root of fresh *P. multiflorum* and their potential antioxidant activities. The relationships between the bioactive components and their antioxidant activities at different part of *P. multiflorum* extracts were also evaluated.

MATERIALS AND METHODS

I. *Standards and Reagents*

1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiasoline-6-sulfonic acid) (ABTS), aluminum nitrate, butylated hydroxyl anisole (BHA), ferrous chloride, falin-ciocalteu's reagent, gallic acid, peroxidase, nitro blueterazolium (NBT), phenazine methosulfate (PMS), dihydronicotinamid-adenin dinucleotide (NADH), sodium nitroprusside (SNP), sulfanilamide, H_3PO_4 and N-(1-naphthyl)-ethylenediamine dihydrochloride were purchased from Sigma Corporation (St. Louis, MO, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich Corporation (Milwaukee, USA), whereas potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck Corporation (Darmstadt, Germany).

II. *Sample Preparation*

Fresh *P. multiflorum* were supplied from the Abi-Er farm at Tainan, Taiwan. The fresh *P. multiflorum* were cut into root, stem and leaf. The root (4 mm slice thickness), stem and leaf were freeze-dried and sealed in package. Subsequently, the samples were stored at -20°C and prepared for analyses. For water extraction, the 25 g sample was added with 250 mL water and underwent 40 minutes of ultrasonic extraction. Then, the sample was filtered with Whatman no. 1 filter paper. Finally, the filtrate was freeze-dried and stored at -20°C.

III. *Determination of Antioxidant Components*

The flavonoids contents were determined according to the modified method of Jia[8] *et al*. One half milliliter sample was mixed with 1.5 mL water, 0.1 mL $Al(NO_3)_3$ and 0.1 mL CH_3COOK (1 M). Then, the mixture was left in the dark room for 40 minutes. Finally, the absorbance was measured against blank at 415 nm using spectrophotometer. Quercetin was used as the standard calibration curve and the flavonoids contents in the sample were calculated using the linear equation based on the calibration curve.

Phenolic compounds were estimated using the method described by Singleton and Rossi[9] with modifications, using gallic acid as a standard phenolic compound. Briefly, 0.2 mL of sample at systematic concentration of 100-1000 mg/kg was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. Then, 0.8 mL of sodium carbonate solution (7.5%) was added to the mixture and left for 90 minutes. Thereafter, the absorbance was measured at 760 nm (Analytikijena 200-2004 spectrophotometer). The results were expressed as milligram of gallic acid equivalents (GAEs) per gram of extract.

For High Performance Liquid Chromatography (HPLC) analysis, water extract of the sample was dissolved in 250 mL of methanol by ultrasonic extraction. Then, the sample was filtered with Whatman no. 1 filter paper. The volume of filtrate was measured and 5 mL HCl (25%) was added. After hydrolysis at 80°C, the sample was evaporated at 37°C under vacuum. Thereafter, the volume of the sample was adjusted with methanol to 50 mL and finally stored at -20°C.

Analysis of emodin was carried out as Yao and Kong[10]. HPLC analysis (L-7420, Hitachi Ltd., Japan) was perform with a Mightysil-RP-18 column (250 × 4.6 mm, 25 μm) at room temperature. The mobile phase was methanol: water in gradient mode as follows: 5:95 to 100:0 (v/v) in 60 minutes. The effluent was monitored at 254 nm (Hitachi L-7420 UV-Vis detector) and the flow rate was set at 0.5 mL/min constantly. The injected volume was 20 μL. The chromatographic peaks of the analytes were confirmed by comparing the retention time and UV spectra with the emodin standards.

Analysis of quercetin was performed by the method of Zu *et al.*[11] with modification. Sample was analyzed using HPLC (L-7420, Hitachi Ltd., Japan) and compared to quercetin as standard. HPLC analysis was carried out by Mightysil-RP-18 column (250 × 4.6 mm, 25 μm) and Hitachi L-7420 UV-Vis detector (365 nm). The mobile phase was acetonitrile: 3% acetic acid (35:65, v/v). Flow rate and injection volume were 0.8 mL/min and 20 μL, separately. Compounds identification has been further confirmed with Photo Diode Array (PDA) spectra.

IV. *Analysis of Quercetin by HPLC/MS/MS*

Compound separation was performed using a 14.6 × 250 mm C18 column, containing 5-μm bead size, 120-Å pore size Acclaim 120 media (Dionex, Sunnyvale, CA) run at 700 μL/min. The mass spectrum was acquired using a ThermoFinnigan model LXQ (San Jose, CA, USA) ion-trap mass spectrometer equipped with an ESI source interface and controlled by Xcalibur 2.06. The instrument was coupled with a Surveyor HPLC binary pump and a sample volume of 10 μL was loaded. The isocratic condition was used at 35% water with 0.1% acetic acid and 65% acetonitrile over a period of 15 min. High purity nitrogen was used as nebulizing gas. The mass spectra were acquired in the negative ion mode: a sheath gas and auxiliary gas at the flow rate of 240 mL/min and 60 mL/min, respectively, capillary temperature of 300°C, capillary voltage of -17 V, and tube lens of -60.95 V. The single reaction monitoring

(SRM) experiment was set up with 20% collision energy. Quercetin was used as standard compound and detected using the same procedure as for samples.

V. *Antioxidant Activity Assay by DPPH*

The scavenging effect of *P. multiflorum* extract on DPPH was estimated using the modified method of Shimada[12] *et al*. The 5 mL of DPPH (100 μM) radical in methanol was added to 1 mL of sample at a systematic concentration of 100-1000 mg/kg. The mixture was shaken vigorously and was allowed to stand for 50 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm (Analytikijena 200-2004 spectrophotometer). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Trolox and BHA were used for comparison. The scavenging ability was calculated as follows:

Scavenging effect (%) = $[1 - (A_{517nm, Sample})/(A_{517\ nm, Blank})] \times 100$

VI. *Antioxidant Activity Assay by ABTS*$^+$

The capacity of the samples to scavenge $ABTS^+$ radicals was evaluated according to ABTS method as described by Miller[13] *et al*. and Arnao[14] *et al*., and expressed as percentage of the absorbance of the uninhibited radical cation solution and quantified in terms of Trolox Equivalent Antioxidant Capacity (TEAC). One and half milliliter of deionized water, 0.25 mL peroxidase (4.4 unit/mL), 0.25 mL H_2O_2 (50 μM) and 0.25 mL ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt)] (100 μM) were mixed and allowed to stand in the dark at room temperature for 1 hour before use. Then, test samples at systematic concentration of 100-1000 mg/kg were added and the mixture was allowed to stand in the dark at room temperature for 1 hour. Then, samples were measured at 734 nm (Analytikijena 200-2004 spectrophotometer). Trolox and BHA were adopted as antioxidant standards and the antioxidant activity was calculated using the following formula:

Trolox equivalent antioxidant capacity (%)
= $[1- (A_{734\ nm, Sample} - A_{734\ nm, Blank})] \times 100$

VII. *Reducing Power (FRAP)*

The reducing power was determined according to the method of Oyaizu[15] with slight modification. One milliliter of sample at systematic concentration of 100-1000 mg/kg was mixed with 0.8 mL of 200 μM sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After 1 mL of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 30000 rpm for 10 minutes. Then, the upper layer (1 mL) was mixed with 1 mL of 0.1% ferric chloride. After shaking, the mixture was left at room temperature for 10 minutes. The absorbance was measured at 700 nm and compared to standards (Trolox and BHA). Higher absorbance indicates higher reducing power.

VIII. *Superoxide Anion Radical Scavenging Activity (SRSA)*

The modified method was used to determine the superoxide anion radical scavenging capacity of the test samples[2]. One milliliter of sample at systematic concentration of 100-1000 mg/kg was added to an equal volume of 120 μM phenazine methosulphate (PMS), 936 μM dihydronicotinamide dinucleotide (NADH) and 300 μM nitro-blue tetrazolium (NBT) in 0.1 M phosphate buffer (pH 7.4). The mixture was left at room temperature for 5 minutes. Then, the optical density was read at 560 nm. Gallic acid was used as standards. The degree of scavenging of the superoxide anion radical was calculated by the following equation:

Scavenging effect % (capacity to scavenging the superoxide anion radical)
= $[1-(A_{Sample\ at\ 560\ nm}-A_{Blank\ at\ 560\ nm})/(A_{Control\ at\ 560\ nm}-A_{Blank\ at\ 560\ nm})] \times 100$.

IX. *Nitrite Oxide Radical Scavenging Activity (NRSA)*

The ability of the samples to scavenge nitric oxide free radicals was determined using the modified method described by Marcocci[16] *et al.* One milliliter of sodium nitropruside (SNP) (25 mM) in phosphate-buffered saline (PBS) was mixed with 0.1 mL sample at systematic concentration of 100-1000 mg/kg and left at room temperature for 120 minutes. Thereafter, the sample was reacted with 0.3 mL of Greiss reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride). Then, the absorbance was taken at 570 nm. Trolox and BHA were used as standards.

Scavenging effects % (capacity to scavenging the nitrite oxide radical)
= $[1-(A_{Sample\ at\ 570\ nm}-A_{Blank\ at\ 570\ nm})/(A_{Control\ at\ 570nm}-A_{Blank\ at\ 570\ nm})] \times 100$.

X. *Statistical Analysis*

All values obtained are expressed as mean ± SD. Analysis of variance was performed by one-way ANOVA. Significant differences ($p < 0.05$) among different groups were determined by Duncan's post-hoc *t*-test.

RESULTS AND DISCUSSION

The yields of water extracts from root, stem and leaf of *P. multiflorum* were in descending order of root (25.1

g/100.0 g) > leaf (23.5 g/100.0 g) > stem (6.3 g/100.0 g).

The antioxidant activities of plant or herb extracts are often explained with respect to their total phenolic and flavonoid contents. The total phenolic content of *P. multiflorum* root extract (179.0 ± 11.1 mg/g) was significantly higher than those of leaf extract (118.0 ± 6.0 mg/g) and stem extract (70.3 ± 1.4 mg/g). It seemed that the total phenolic content of *P. multiflorum* was higher than *Polygonum lapathifolium L*. The total phenolic content of *P. lapathifolium L*. was 43.5 ± 1.0 mg/g[17]. However, the total flavonoid content of *P. multiflorum* stem extract and root extract were not significantly different, but both were significantly different from that of leaf extract. The total flavonoid content of *P. multiflorum* leaf extract was 37.5 ± 0.4 mg/g. The content of flavonoid in *P. multiflorum* leaf extract was generally 4-5 times higher than that detected for stem and root extracts (Table 1).

The HPLC analysis revealed that emodin was

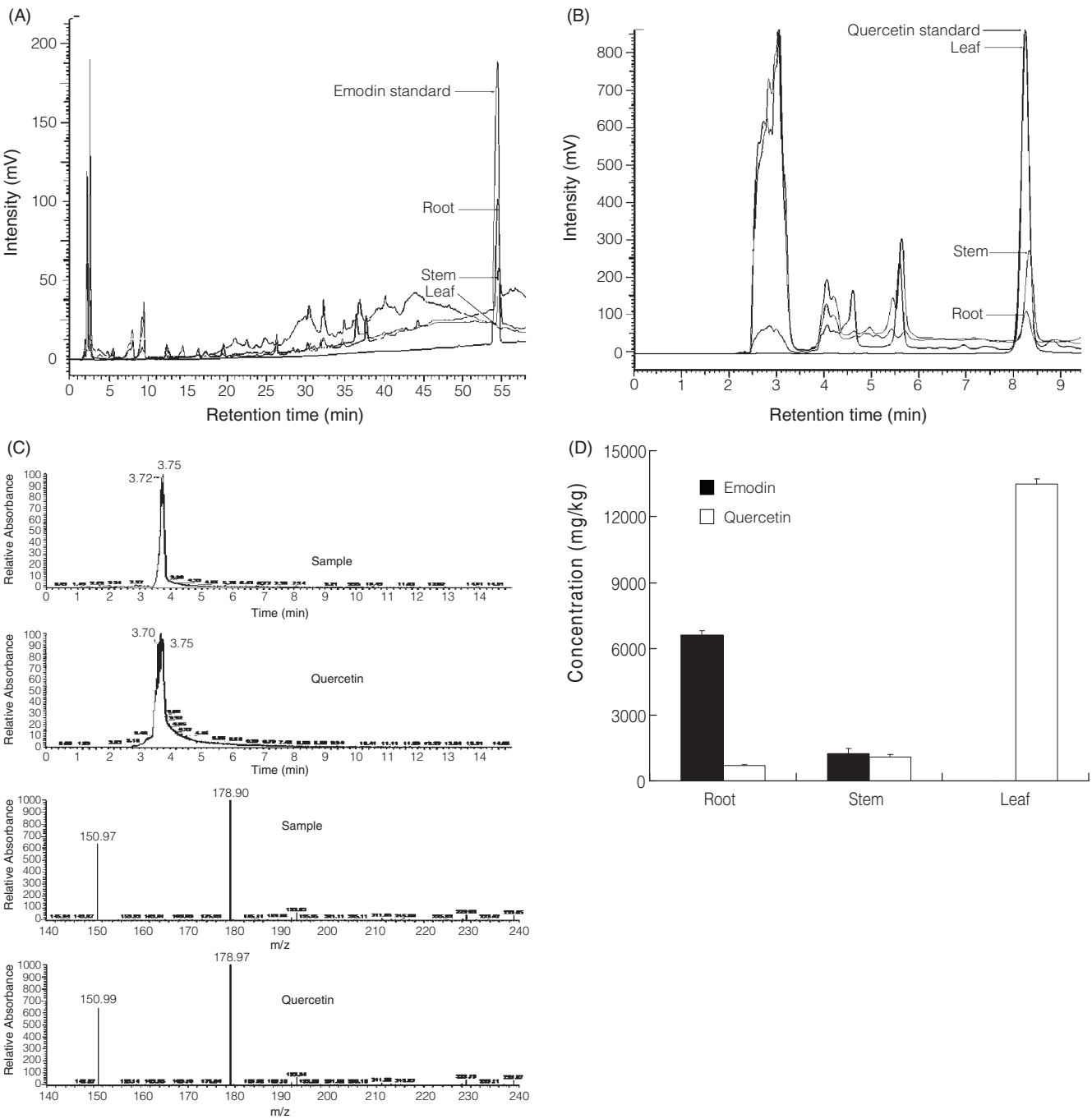

Figure 1. (A) the HPLC chromatogram of emodin and samples; (B) the HPLC chromatogram of quercetin and samples; (C) the LC/MS/MS spectrum of quercetin and samples; (D) the concentrations of emodin and quercetin in the water extracts obtained from different parts of *Polygonum multiflorum*. The concentration of emodin and quercetin standard were using the 100 ppm and 150 ppm, respectively.

Table 1. The total phenolics and flavonoids levels at different parts of *Polygonum multiflorum*

Part of sample	Total phenolics (mg/g, extract)	Flavonoids (mg/g, extract)
Root	179.0 ± 11.1[a]	7.3 ± 0.1[b]
Stem	70.3 ± 1.4[c]	8.0 ± 0.6[b]
Leaf	118.0 ± 6.0[b]	37.5 ± 0.4[a]

[a,b,c]Each value was tested in triplicate. Values (means ± standard deviation) in the same column with different superscripts indicate significant differences, $p < 0.05$.

found in root (6620 mg/kg) and stem (1245 mg/kg) of *P. multiflorum* (Figure 1). However, emodin was absent in the leaf of *P. multiflorum*. On the other hand, quercetin content in leaf of *P. multiflorum* (13469 mg/kg) was the highest, and quercetin content in stem and root of *P. multiflorum* were 1095 mg/kg and 692 mg/kg, respectively. Figure 1(C) illustrated the chromatogram and LC/MS spectra of the quercetin standard and the active compound collected from the leaf and stem of fresh *Polygonum multiflorum*. Their spectra were found to be comparable to each other, showing that the active compound present in the leaf and stem of the fresh *P. multiflorum* plant was same as quercetin. Quercetin content of other common plants such as *Cuscuta chinensis*, *Chassalia grandifolia* and *Trochetia boutoniana* at the levels of 55, 115 and 817 mg/kg, respectively, have been demonstrated.[18] However, further research should be done to investigate whether the antioxidant activities of fresh *P. multiflorum* was due to the presence of quercetin, emodin and its glycosides composition.

The profile of scavenging activity of *P. multiflorum* on DPPH showed that the radical scavenging activity of root, leaf and stem extracts from *P. multiflorum* on DPPH increased with increasing concentration (Figure 2). Generally, the root of *P. multiflorum* had higher hydrogen hydrogen-donating capacity compared to the leaf, while stem rendered the weakest effect. The scavenging effect of water extracts from *P. multiflorum* root was 92.5% at the concentration of 100 mg/kg. However, the scavenging abilities of the leaf and stem were 30.9 and 18.8% at the concentration of 100 mg/kg, respectively. These results showed that water extracts of the *P. multiflorum* root contained considerable amounts of free radical inhibitors, as well as primary antioxidants that react with free radicals. For TEAC assay, the effect on $ABTS^+$ of water extracts of *P. multiflorum* root, stem and leaf were shown in Figure 3. Total antioxidant of all samples increased with increasing concentrations. The standards, BHA and Trolox, showed high scavenging abilities. At the concentration of 250 mg/kg, the water extract of *P. multiflorum* root showed highest antioxidant activity (94.1%), the *P. multiflorum* leaf next (76.7%) and the *P. multiflorum* stem had the lowest activity (40.4%). On the other hand, at the concentration of 1000 mg/kg, *P. multiflorum* root, stem and leaf showed high scavenging abilities (98.7, 92.3 and 96.1%, respectively), whereas *Potentilla freynIana* showed lower scavenging ability of 61.7%[19]. The antioxidant activity of herbal plants depends largely on their chemical composition, i.e., phenolic and flavonoid content. *P. multiflorum* root revealed a higher content in phenolic compounds. In fact, the root of *P. multiflorum* was the most efficient part of the plant concerning antioxidant activity, while stem presented lower antioxidant properties which are compatible to its lower phenols content (70.3 ± 1.4 mg/g).

The reducing power of the test samples correlated well with increasing concentration (Figure 4). The reducing power of water extract of *P. multiflorum* root was relatively more pronounced than stem and leaf. At concentration of 750 mg/kg, the optical density (OD) of root, leaf and stem were 2.3, 0.9 and 0.7, respectively. The reducing capacity of the extracts depend on the amount and activity of reducers (antioxidants), which is

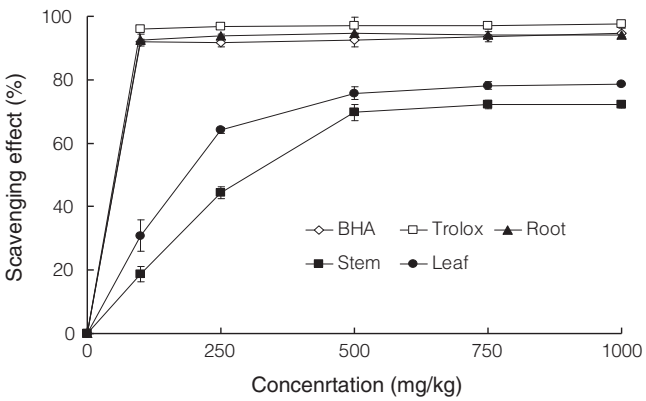

Figure 2. DPPH radical scavenging effects of the water extracts from different parts of *Polygonum multiflorum*. Each value is expressed as mean ± SD (n = 3).

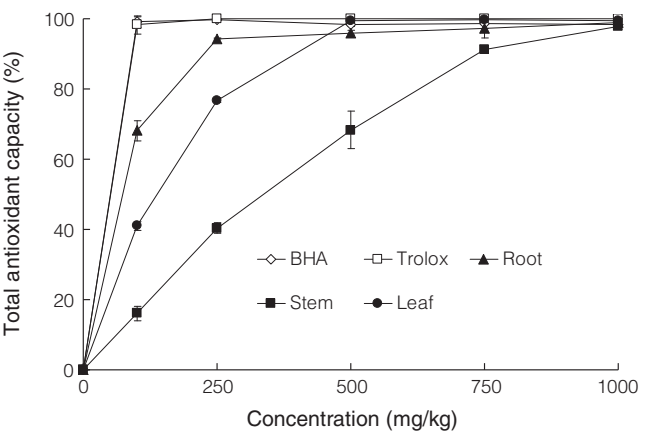

Figure 3. Total antioxidant activity (%) of the water extracts from different parts of *Polygonum multiflorum*. Each value is expressed as mean ± SD (n = 3).

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Table 2. The correlation between the antioxidant properties and bioactive compounds of water extracts obtained from the root, stem and leaf of *Polygonum multiflorum*

Scavenging activity*	Correlation values*				
	DPPH	$ABTS^{\cdot+}$	Reducing power	NO	O^{2-}
Total phenolics	1.000	0.900	0.995	0.997	1.000
Flavonoids	0.652	0.907	1.000	0.995	0.944
Emodin	0.665	0.549	0.984	0.832	0.950
Quercetin	0.971	0.634	0.876	0.948	1.000

*Correlation values were performed with statistical software (SPSS, version 11, SPSS Inc.).

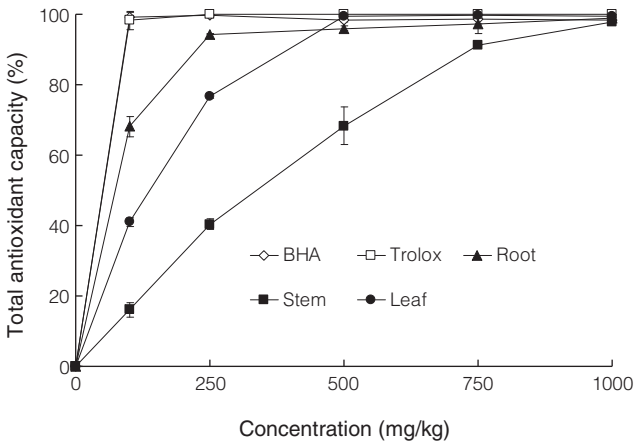

Figure 4. Reducing power of the water extracts from different parts of *Polygonum multiflorum*. Each value is expressed as mean ± SD (n=3).

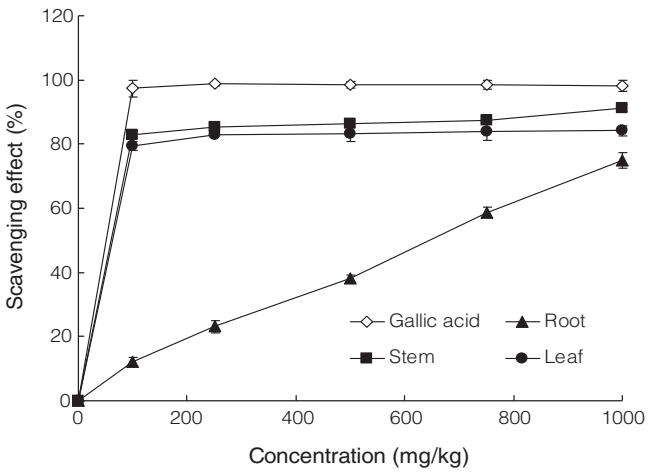

Figure 5. Superoxide anion scavenging effects of the water extracts from different parts of *Polygonum multiflorum*. Each value is expressed as mean ± SD (n = 3).

determined by their capacity to reduce Fe(III) to Fe(II). The reducing properties could be attributed mainly to the bioactive compounds associated with antioxidant activity[20]. Accordingly, *P. multiflorum* root contained higher amounts of bioactive phenolic compounds (179.0 ± 11.1 mg/g), which could react with free radicals to stabilize and block radical chain reactions.

Meantime, all of the extracts exhibited potent scavenging activity against O_2^- in a concentration dependent manner (Figure 5). Robak and Gryglewski[4] reported that the antioxidant properties of some flavonoids were effective mainly via scavenging of superoxide anion radicals. Studies have discovered that the superoxide anion scavenging effects of phytochemicals in descending order of quercetin > aspalathin > orientin > catechin > rutin > isoquercitrin > iso-orientin > luteolin >chrysoeriol[21,22]. Water extracts of *P. multiflorum* stem and leaf had comparable superoxide anion scavenging effects. At the concentration of 100 mg/kg, the superoxide anion scavenging effects of *P. multiflorum* stem and leaf extracts were greater than 80%. The O_2^- scavenging data indicates that the *P. multiflorum* stem and leaf extracts do possess the ability to scavenge reactive oxygen species but it is less efficient than gallic acid. This result is intriguing because quercetin and flavonoids are most rich in the leaf (almost 10-fold more than stem) (Table 1 and Figure 1). However, the O_2^- scavenging activity of stem was higher than leaf and root. That may be because the emodin or other specific compounds of stem showed superoxide anion scavenging effect other than quercetin. Albeit leaf contained the most quercetin but it had no emodin-related compounds, thereby showed weak ability on O_2^- scavenging activity. But the root contained several emodin-related compounds that effectively scavenged ABTS, DPPH and NO radicals (Figures 2, 3 and 6). However, the water extract of *P. multiflorum* root exhibited a relatively low level of radical-scavenging activity. Proportionally, the water extract of *P. multiflorum* root also demonstrated low level of quercetin. The flavonoid and quercetin considered to be a potent superoxide radical scavenger. Therefore, scavenging abilities of the leaf extracts on superoxide anion radicals might be due to quercetin alone; and several emodin-related and specific compounds were interacting to scavenge superoxide anion radicals with quercetin in stem extracts.

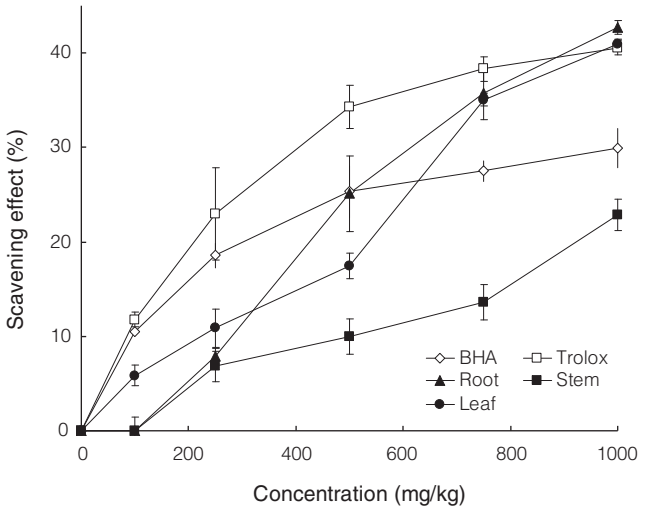

Figure 6. NO scavenging effects of the water extracts from different parts of *Polygonum multiflorum*. Each value is expressed as mean ± SD (n = 3).

Table 3. The scavenging radical effects of emodin and quercetin

Concentration*	Scavenging effects (%)		
	DPPH	Superoxide anion	NO
Emodin (6.6 mg/kg)	34.6	10.8	9.2
Quercetin (13.469 mg/kg)	67.3	81.9	21.2

*6.6 mg/kg of emodin was used which was equivalent to the concentration of emodin-related compounds in root extracts; 13.469 mg/kg of quercetin was used which was equivalent to the concentration of root extracts-contained.

The nitric-oxide (NO) scavenging activities of *P. multiflorum* water extracts were enhanced with increasing concentration (Figure 6). The extract of *P. multiflorum* root, with higher polyphenol content compared to leaf and stem, showed better ability in inhibiting nitrite accumulation. At concentration of 1000 mg/kg, the water extracts of *P. multiflorum* root and *P. multiflorum* leaf exhibited 42.7 and 40.9% of NO radical scavenging activity, which were significantly ($p < 0.05$) higher than those of the Trolox, BHA and stem extract.

Correlation analyses (Table 2) showed an apparent correlation between total phenolic contents and all antioxidant assays (DPPH, $r^2 = 1.000$; ABTS, $r^2 = 0.900$; reducing power, $r^2 = 0.995$; NO, $r^2 = 0.997$; superoxide anion, $r^2 = 1.000$). The results indicate that phenolic compounds in *P. multiflorum* extracts maybe responsible for the antioxidant activities. A significant correlation was also observed between reducing power and quercetin contents ($r^2 = 0.876$), and also NO scavenging activity and emodin contents ($r^2 = 0.832$). The emodin contents were weakly correlated with ABTS radical-scavenging activity ($r^2 = 0.549$).

From the above findings, this study showed that the various parts of *P. multiflorum* contained considerable amount of bioactive compounds of emodin and quercetin and contribute to the antioxidant activities. Various parts of *P. multiflorum* such as leaf, stem and root contained these bioactive compounds to different extents. Results from Table 3 compared to Figures 2, 5 and 6 indicated that the radical scavenging abilities of emodin and quercetin were lower than the leaf, stem and root extracts. However, these compounds were most observed in root and leaf by HPLC assay, indicating they played key roles in antioxidative ability. These findings demonstrated that the radical scavenging activities of the root, stem and leaf were from various substances as well as emodin-related compounds and quercetin. The presence of these compounds adds to the meaningfulness of their antioxidant activities. Furthermore, a study indicated that the mixed extracts of stem and leaf from *P. multiflorum* had weaker antioxidative abilities such as TEAC, OH^- and Fe^{2+} chelating capacity[3] than the root, stem and leaf extracts from fresh *P. multiflorum* in this study. These results indicated fresh *P. multiflorum* may be more useful as functional medicine.

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