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Puerariae Flos extracts possess the potential antioxidant efficacy against oxidant stress

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Abstract

In this study, yield of Puerariae Flos extract showed 60% ethanol gave the highest yield of 26.34%. Total polyphenol contents and total flavonoid contents were highest in 40% ethanol extract (5.86 mg TAE/g) and hot water extract (3.06 mg CE/g), respectively. Evaluation of the reducing power of transition metal ions such as phosphomolybdenum and ferric tripyridyltriazine complex were high in 100% ethanol extract (4.41 mg AAE/g) and hot water extract (42.62 μM TE/g), respectively. Evaluation of free radical scavenging activity such as DPPH, ABTS and NO were excellent in 40% ethanol extract (95.74 μM TE/g, 17.78 μM TE/g, 175.5 μg/mL) and the HP radical scavenging activity was excellent in 80% ethanol extract (308.3 μg/mL), respectively. A positive correlation ($p < 0.01$) was showed between TFC and FRAP assay ($r = 0.866$) highest significant. The correlation between PMA and ABTS radical scavenging activity was significantly high with $r = 0.913$ ($p < 0.01$). The overall results of this work showed that Puerariae Flos is a candidate natural source of antioxidants.

Keywords: Puerariae Flos; total phenolic content; antioxidant activity.

Practical Application: Research about antioxidant activities and functional products of Puerariae Flos.

1 Introduction

Most commonly, products obtained from natural sources have been used by human beings as a leading source of medicinal agents so as to bring relief from many diseases, illnesses and frail (Yuan et al., 2016; Tournaire et al., 1993). The human use of plants as medicines may be traced back at least 60,000 years (Fabricant & Farnsworth, 2001). Use of indigenous drugs of natural origin forms a major part of such therapies; more than 1500 herbals are sold as dietary supplements or ethnic traditional medicines (Patwardhan et al., 2005; Kooy et al., 1994). There are about less than 1% of approximately 250,000 mores plants that have been explored in-depth for their phytochemistry or pharmacological potential (Katiyar et al., 2012; Choi et al., 2009).

The integrated antioxidant defense performs by the body's defense system against reactive oxygen species (ROS) and oxidative stress (Lobo et al., 2010; Yildirim et al., 2000). Anti-oxidants scavenging free radicals, ROS and reactive nitrogen species (RNS) from cells were prevented or reduced the damage caused by the oxidation of body tissues (Kalam et al., 2015). Especially, free radicals can adversely alter lipids, proteins and DNA, and it can trigger a number of human diseases (El-Beltagi & Mohamed, 2013). Oxidation leading to free radical formation in cell can be accelerated by stress, smoking, alcohol intake, unlight, air pollution and other factors (Lobo et al., 2010; Aktumsek et al., 2013).

Inflammation is a host defense mechanism to protect against pathogens, stresses and tissue damage, and is a major factor in the progression of many chronic diseases including ulcerative colitis, diabetes, atherosclerosis and arthritis (Nathan, 2002; Guldas et al., 2021). The inflammatory response involves a combination of different signaling elements such as cytokines, nitric oxide (NO) and two key transcription factors, nuclear factor-kappa B (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Kobayashi, 2010; Terao, 2009). Particularly, NO is an important inflammatory mediator produced by the nitric oxide synthases (NOSs) and it is implicated in the pathogenesis of chronic inflammatory diseases (Sharma et al., 2007; Yildiz et al., 2021). Increased production of NO ROS in body are implicated in many diseases and these are important targets for the treatment of inflammatory and oxidative stress mediated conditions (Kumaran & Karunakaran, 2007; Bak et al., 2013).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been used over the years, but these are causing the problem of toxicity (Qader et al., 2011; Altiner, 2021). It has been shown that natural products present in medicinal plants are inhibitory to the deleterious effects of oxidative stress, but their antioxidant activities of natural products are lower than those of synthetic antioxidants (Sabir & Rocha, 2008).

The objective of this study was to investigate the antioxidant properties of Puerariae Flos extracts. Total phenol content (TPC) and total flavonoid content (TFC) in the extracts-was determined spectrophotometrically. To compare the antioxidant activity, we used 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) radical scavenging assay, phosphomolybdenum complex assay

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(PMA), frerric reducing antioxidant power assay (FRAP), hydrogen peroxide (HP) scavenging activity and nitric oxide (NO) scavenging activity assay.

2 Materials and methods

2.1 Reagents

Dimethyl sulfoxide (DMSO), gallic acid, Folin-Ciocalteu reagent, ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), butylated hydroxyanisole (BHA), DPPH (1,1-diphenyl-2-picrylhydrazyl), 4,5-Diamino fluroprusside (DAF-2), sodium nitroprusside dehydrate are Sigma-Aldrich Co. (St. Louis, MO, USA) products were used, and other extraction solvents and all reagents used were special reagents. A spectrophotometer (Neogen, Optizen 2120 UV, Sejong, Korea), ELISA reader (Thermo Fisher SCIENTIFIC, Multiskan Sky, Seoul, KOREA) was used as the instrument.

2.2 Preparation of extracts

Extraction conditions according to the ethanol concentration were selected through preliminary tests of conditions for various solvent concentrations of Puerariae Flos. It was extracted with 10 vol (v/w) ethanol (0%, 20%, 40%, 60%, 80%, 100%) using a heating mantle at 100 °C for 4 h and concentrated by evaporator. The concentrated sample was frozen in a deep freezer at -70 °C for 24 h, then lyophilized and stored at 4 °C for use in the experiment (Figure 1).

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

TPC was determined with Folin–Ciocalteu reagent according to the method of Singleton et al. (1999). After creating a standard curve using tannic acid, the polyphenol amount was calculated as the tannic acid equivalent amount, and the equation was calculated as $y = 0.0444x + 0.1298$, $\left(r^2 = 0.9992\right)$.

The TFC of each sample was determined using the sodium borohyride/chloranil-based assay (He et al., 2008; Shahinuzzaman et al., 2020). A standard curve was prepared using Catechin to calculate the amount of flavonoids in terms of catechin equivalent, and the equation was calculated as $y = 0.0025x + 0.0142$ $\left(r^2 = 0.9992\right)$.

2.4 Determination of antioxidant activity

Phosphomolybdenum Complex Assay (PMA)

PMA was evaluated by Prieto et al. (1999) Phosphomolybdenum antioxidant assay. A standard curve was created using L-ascorbic acid, PMA was calculated as the equivalent amount of L-ascorbic acid, and the equation was calculated as $y = 0.0021x - 0.0165$ $\left(r^2 = 0.9910\right)$.

Ferric Reducing Antioxidant Power assay (FRAP)

FRAP was performed according to a previously reported method (Erel, 2004). A standard curve was created using Trolox, FRAP was calculated as the equivalent amount of Trolox, and the equation was calculated as $y = 0.0014x + 0.0628$ $\left(r^2 = 0.9933\right)$.

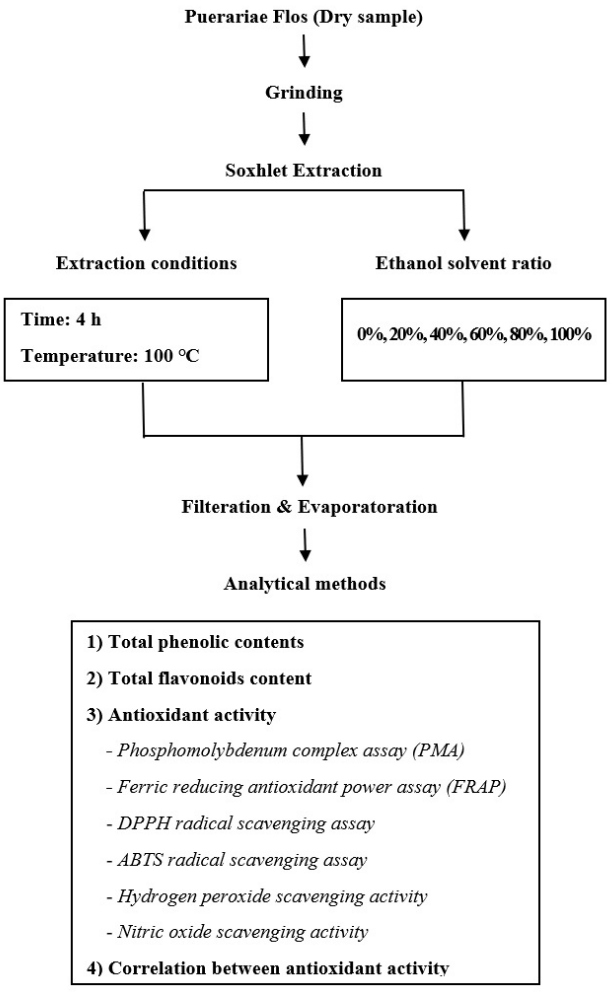

Figure 1. Antioxidant research flow chart from Puerariae Flos.

DPPH radical scavenging assay

DPPH radical scavenging activity was determined using a previously reported method (Yen & Chen, 1995). Ethanol was used as blank, and the sample without antioxidant was used as control. Trolox equivalent antioxidant capacity (TEAC) was calculated by preparing a standard Trolox curve from a standard Trolox solution. The outcomes were presented as mM Trolox equivalent (TE)/g sample.

ABTS radical scavenging assay

Spectrophotometric analysis of ABTS cation radical scavenging activity was determined using a previously reported procedure (Re et al., 1999; Wolfe & Liu, 2008). TEAC was calculated by preparing a Trolox curve for ABTS assay, and the results were presented as mM TE/g sample.

Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100, 200, 400, 800, and 1000 μg/mL) of the *P. thunbergiana* extracts

were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide (Chen et al., 1999; Khan & Akhtar, 2003). The hydrogen peroxide percentage scavenging activity was then calculated using the following Equation 1:

$$H_2O_2 scavenging\ effect\ (\%) = (A - B/A) \times 100 \quad (1)$$

Where A is the absorbance of the control reaction and B is the absorbance in the presence of the samples or standards.

Nitric oxide scavenging activity

The scavenging activity of nitric oxide forms a triazolo fluorescein that emits green fluorescence at an excitation wavelength of 490 ~ 495 nm by the specific NO indicator DAF-2 trapping NO between its two amino groups. DAF-2 solution was prepared by dissolving 1 mg of DAF-2 in 0.55 mL of Dimethyl sulfoxide and diluting it again to 400 times (v / v) using 50 mM phosphate buffer (pH 7.4). 10 μL of the sample was mixed with 130 μL of 50 mM phosphate buffer (pH 7.4), after which 10 μL of 40 mM SIN-1 and 50 μL of DAF-2 solution were added. The fluorescence intensity of triazolofluorescein produced by the reaction of DAF-2 and NO for 10 min at room temperature was measured at excitation 485 nm and emission 525 nm using a fluorescence microplate reader (Molecular Devices, Gemini EM, U.S.A).

2.5 Statistical analysis

All experiments were performed in triplicate. The results were subjected to an analysis of variance (ANOVA) using the Tukey test to analyze differences; $p < 0.05$ was considered significant.

3 Results and discussion

3.1 Extraction

The extraction yield of natural products acts as an important factor in measuring physiological activity, and even if the physiological activity is excellent, if the extraction yield is low, the economic feasibility of using natural products is low, so the extraction yield is an important factor to be considered for various commercialization (Ham et al., 2015). Table 1 shows the yield calculation of extracts after freeze-drying the extracts. The 60% ethanol extract of Puerariae Flos showed the highest at 26.34%, followed by 40% ethanol, 20% ethanol, and 80% ethanol extract, and the 100% ethanol extract showed the lowest extraction yield at 9.45%. This is considered to show a difference in yield depending on the mixing ratio of water and ethanol when extracting natural products, and it is known that there is economic feasibility when the extraction yield from natural products is 10% or more (Park et al., 2003). Puerariae Flos 60% ethanol, 40% ethanol, 20% ethanol, 80% ethanol, and hot water extract seem to be economically viable materials.

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

This study, total polyphenol content and flavonoid content, that is, polyphenol content, were expressed in terms of tannic acid and catechin standards (Table 1). As a result of analyzing the total polyphenol content of brown algae extracted under each ethanol concentration condition, the 40% ethanol extract showed the highest at 5.86 ± 0.01 mg TAE/g, followed by the 100% ethanol extract (5.82 ± 0.01 mg TAE/g) and 80% ethanol extract. (5.75 ± 0.01 mg TAE/g) 60% ethanol extract (5.43 ± 0.01 mg TAE/g), hot water extract (5.09 ± 0.01 mg TAE/g), 20% ethanol extract (4.94 ± 0.01 mg TAE/g) in that order It showed a high value and there was a significant difference. The flavonoid content showed the highest value in the hot water extract at 3.09 ± 0.02 mg CE/g, with 20% ethanol extract (3.02 ± 0.01 mg CE/g), 40% ethanol extract (2.79 ± 0.01 mg CE/g), 60% ethanol extract (2.59 ± 0.01 mg CE/g), 100% ethanol extract (2.43 ± 0.01 mg CE/g), and 80% ethanol (2.42 ± 0.01 mg CE/g) showed significant differences.

3.3 Antioxidant activity

Antioxidant activity of transition metal ions

Phosphomolybdenum complex assay

The PMA method is a principle in which Mo (VI) is reduced to Mo (V) by the extract and reacts with phosphate to form a green phosphate/Mo (V) complex (Prieto et al., 1999). In this study, the total antioxidant activity was expressed in terms of AAE/g using L-ascorbic acid as a standard material according to the extraction conditions for each ethanol concentration of brown algae (Table 2). 100% ethanol extract showed the highest at 4.41 ± 0.02 mg AAE/g, 80% ethanol extract (2.50 ± 0.03 mg AAE/g),

Table 1. Yield, total polyphenol contents and total flavonoid contents of Puerariae Flos extracts.

Sample	Yield[1)]	Polyphenol contents	Flavonoid contents
	(%, w/w)	(mg TAE/g[2)])	(mg CE/g[3)])
D.W	11.05	5.09 ± 0.01[b)4)]	3.06 ± 0.02[e)]
20% EtOH	22.30	4.94 ± 0.01[a)]	3.02 ± 0.01[d)]
40% EtOH	23.32	5.86 ± 0.01[f)]	2.79 ± 0.01[c)]
60% EtOH	26.34	5.43 ± 0.01[c)]	2.59 ± 0.01[b)]
80% EtOH	20.84	5.75 ± 0.01[d)]	2.42 ± 0.01[a)]
100% EtOH	9.45	5.82 ± 0.01[e)]	2.43 ± 0.01[a)]

[1)]Yield (%, w/w) = (dry weight of extracts / weight of dry Puerariae Flos) × 100. [2)]Tannic acid equivalent. [3)]Catechin equivalent. [4)]Values are mean ± SD (n=3). Means with different letters (a-f) in the same column are significantly different at $p < 0.05$ (Tukey).

Table 2. Antioxidant activity of Puerariae Flos extracts.

Sample	PMA[1)]	FRAP[2)]	DPPH	ABTS
	(mg AAE/g[3)])	(mM TE/g[4)])	(mM TE/g)	(mM TE/g)
D.W	1.54 ± 0.02[a)5)]	42.62 ± 0.19[f)]	92.66 ± 0.17[b)]	17.04 ± 0.02[c)]
20% EtOH	1.83 ± 0.00[b)]	34.84 ± 0.51[d)]	92.33 ± 0.17[b)]	17.34 ± 0.02[b)]
40% EtOH	1.82 ± 0.04[b)]	36.94 ± 0.19[e)]	95.74 ± 0.17[a)]	17.78 ± 0.02[a)]
60% EtOH	1.86 ± 0.03[b)]	24.07 ± 0.00[c)]	92.72 ± 0.14[b)]	15.46 ± 0.02[d)]
80% EtOH	2.50 ± 0.03[c)]	22.40 ± 0.00[b)]	91.86 ± 0.13[c)]	14.61 ± 0.03[e)]
100% EtOH	4.41 ± 0.02[d)]	14.96 ± 0.19[a)]	80.08 ± 0.21[d)]	12.15 ± 0.03[f)]

[1)]Phosphomolybdenum complex assay. [2)]Ferric-reducing antioxidant power. [3)]Ascorbic acid equivalent. [4)]Trolox equivalent. [5)]Values are mean ± SD (n=3). Means with different letters (a-f) in the same column are significantly different at $p < 0.05$ (Tukey).

60% ethanol extract (1.86 ± 0.03 mg AAE/g), 20% ethanol Extracts (1.83 ± 0.00 mg AAE/g), 40% ethanol extract (1.82 ± 0.04 mg AAE/g), and hot water extract (1.54 ± 0.02 mg AAE/g) were confirmed in this order. Through these results, the highest PMA antioxidant activity was confirmed with 100% ethanol extract.

Ferric-reducing antioxidant power

The FRAP method is a method to evaluate the reduction degree by directly donating electrons based on the basic principle that Fe^{3+} is reduced to Fe^{2+}. At low pH, depending on the reduction degree of the sample, the ferric tripyridyltriazine [Fe(III)-TPTZ] complex is ferrous by antioxidants. It uses the principle of reduction to tripyridyltriazine [Fe(II)-TPTZ] (Benzie & Strain, 1996). In this study, the reducing power according to the extraction conditions for each ethanol concentration of brown rice was expressed in terms of TE/g using Trolox as a standard material (Table 2). The hot water extract showed the highest reducing power at 42.62 ± 0.19 mM TE/g, 40% ethanol extract (36.96 ± 0.19 mM TE/g), 20% ethanol extract (34.84 ± 0.51 mM TE/g), 60% ethanol extract (24.07 ± 0.00 mM TE/g), 80% ethanol extract (22.40 ± 0.00 mM TE/g), and 100% ethanol extract (14.96 ± 0.19 mM TE/g) in order, showing statistical significance. Through these results, it was confirmed that the hot water extract had the highest reducing power of Fe^{3+} to Fe^{2+}.

3.4 Free radical scavenging activity

DPPH radical scavenging activity

The DPPH radical scavenging activity according to the extraction conditions for each ethanol concentration of Puerariae Flos was converted into Trolox and shown (Table 2). The highest 40% ethanol extract was 95.74 ± 0.17 mM TE/g, followed by 60% ethanol extract (92.72 ± 0.14 mM TE/g), hot water extract (92.66 ± 0.17 mM TE/g), and 20% ethanol extract (92.33 ± 0.17 mM TE/g) did not show a significant difference. However, DPPH radical scavenging activity of the 100% ethanol extract showed the lowest scavenging activity at 80.08 ± 0.21 mM TE/g.

ABTS radical scavenging activity

The ABTS radical scavenging activity according to the extraction conditions for each ethanol concentration of Puerariae Flos was converted into Trolox and shown (Table 2). 40% ethanol extract showed the highest ABTS radical activity at 17.78 ± 0.08 mM TE/g, and 20% ethanol extract (17.34 ± 0.08 mM TE/g), hot water extract (17.04 ± 0.08 mM TE/g), 60% Ethanol extract (15.46 ± 0.09 mM TE/g) and 80% ethanol extract (14.61 ± 0.11 mM TE/g) were followed, followed by 100% ethanol extract 12.15 ± 0.13 mM TE/g to confirm statistical significance between extracts, and ABTS scavenging. There was no significant difference in activity.

Nitric oxide scavenging activity

The NO radical, one of the active nitrogens, is a highly reactive radical generated from L-arginine through the catalytic action of nitric oxide synthase in the living body (Carr et al., 2000; Sharma et al., 2007). In this experiment, the NO radical scavenging activity under the extraction conditions for each ethanol concentration of Puerariae Flos was expressed as an RC_{50} value (Table 3). As a result of measuring NO radical scavenging activity, the 40% ethanol extract showed the lowest RC_{50} value of 175.5 ± 8.7 μg/mL, and the 100% ethanol extract showed the highest RC_{50} value of 280.1 ± 19.7 μg/mL.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a non-radical reactive oxygen species that in vivo superoxide dismutase converts superoxide ($O_2\cdot$) to hydrogen peroxide, and catalase decomposes hydrogen peroxide into H_2O and O_2 (Kim et al., 2009; Patel et al., 2010). The hydrogen peroxide scavenging activity under the extraction conditions for each ethanol concentration of Puerariae Flos was expressed as an RC_{50} value (Table 4). 80% ethanol extract showed the lowest RC_{50} value of 308.39 ± 8.5 μg/mL, 40% ethanol extract (374.4 ± 12.3 μg/mL), 60% ethanol extract (399.8 ± 14.5 μg/mL), 100% ethanol extract (407.9 ± 12.7 μg/mL), 20% ethanol extract (540.3 ± 23.6 μg/mL), and hot water extract (615.3 ± 32.9 μg/mL) showed hydrogen peroxide scavenging activity in that order.

3.5 Correlation between antioxidant activity

As for the correlation value, the closer to 1 based on 0, the more positive the correlation, and the closer to -1, the more negative the correlation. The correlation between polyphenol content (TPC, TFC) and antioxidant activity (transition metal ion reducing power, free radical scavenging activity) of extracts by Puerariae Flos ethanol concentration was analyzed

(Table 4). First, as a result of confirming the correlation between polyphenol content and free radicals, the correlation between TPC and ABTS was significantly r = 0.502 ($p < 0.01$), and that of HP was r = 0.649 ($p < 0.01$), and the correlation between TFC and DPPH was r = 0.758 ($p < 0.01$), ABTS correlation r = 0.827 ($p < 0.01$), which showed a high significant correlation (Figure 2), and the correlation with HP was r = 0.408 ($p < 0.05$). indicated. Second, as a result of confirming the correlation between the polyphenol content and the reducing power of transition metal ions, the correlation between TPC and PMA was significantly r = 0.553 ($p < 0.01$) and FRAP correlation r = 0.664 ($p < 0.01$). Correlation r = 0.697 ($p < 0.01$) and FRAP correlation r = 0.866 ($p < 0.01$) showed a high significant correlation with transition metal ions (Figure 3). Third, as a result of confirming the correlation between transition metal ion reducing power and free radical scavenging activity, the correlation between PMA and ABTS was r = 0.913 ($p < 0.01$), and the HP correlation was r = 0.599 ($p < 0.01$), indicating a high significant correlation. FRAP and DPPH correlation r = 0.743 ($p < 0.01$), NO correlation r = 0.784 ($p < 0.01$), indicating a high significant correlation (Figure 4).

Table 3. Values of RC_{50} (μg/mL) for antioxidant activity of Puerariae Flos extracts.

Sample	RC50[1)]	
	NO[2)]	HP[3)]
BHA[4)]	136.8 ± 20.0[a)5)]	264.2 ± 2.9[d)]
D.W	258.2 ± 5.0[de)]	615.3 ± 32.9[c)]
20% EtOH	238.0 ± 9.3[de)]	540.3 ± 23.6[b)]
40% EtOH	175.5 ± 8.7[ab)]	374.4 ± 12.3[b)]
60% EtOH	221.4 ± 21.7[cd)]	399.8 ± 14.5[b)]
80% EtOH	186.4 ± 15.8[bc)]	308.3 ± 8.5[ab)]
100% EtOH	280.1 ± 19.7[e)]	407.9 ± 12.7[b)]

[1)]The half reduction concentration. [2)]Nitric oxide radical scavenging activity. [3)]Hydrogen peroxidescavenging activity. [4)]Positive control. [5)]Values are mean ± SD (n = 3). Means with different letters (a-e) in the same column are significantly different at $p < 0.05$ (Tukey).

Table 4. Correlation between polyphenol contents and antioxidants of Puerariae Flos extracts.

Factors	TPC[1)]	TFC[2)]	PMA[3)]	FRAP[4)]	DPPH	ABTS	NO[5)]	HP[6)]
TPC	1	0.784**[7)]	0.553**	0.664**	0.433	0.502**	0.347	0.649**
TFC		1	0.697**	0.866**	0.758**	0.827**	0.219	0.408*
PMA			1	0.678**	0.298	0.913**	0.321	0.599**
FRAP				1	0.743**	0.165	0.784**	0.458
DPPH					1	0.562*	0.349	0.051
ABTS						1	0.122	0.326
NO							1	0.140
HP								1

[1)]Total polyphenol contents. [2)]Total flavonoid contents. [3)]Phosphomolybdenum complex assay. [4)]Ferric-reducing antioxidant power. [5)]Nitric oxide radical scavenging activity. [6)]Hydrogen peroxide scavenging activity. [7)]Correlation is significantly different at *$p < 0.05$, **$p < 0.01$ (Pearson).

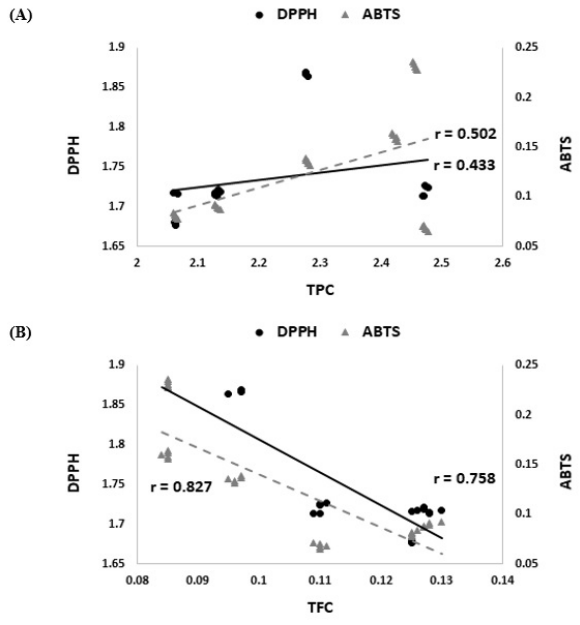

Figure 2. Correlation between polyphenol contents and free radical scavenging activity of Puerariae Flos extracts. (A) correlation between TPC and free radical scavenging activity (DPPH, ABTS), (B) correlation between TFC and free radical scavenging activity (DPPH, ABTS).

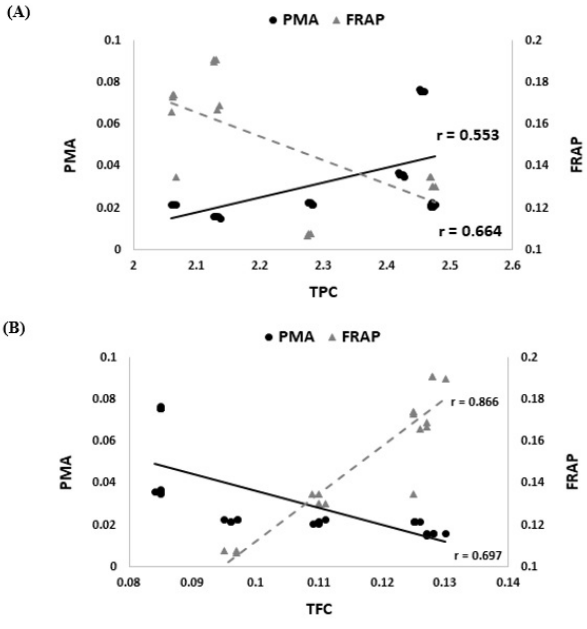

Figure 3. Correlation between polyphenol contents and transitional metal ion reducing power of Puerariae Flos extracts. (A) correlation between TPC and reducing power, (B) correlation between TFC and reducing power.

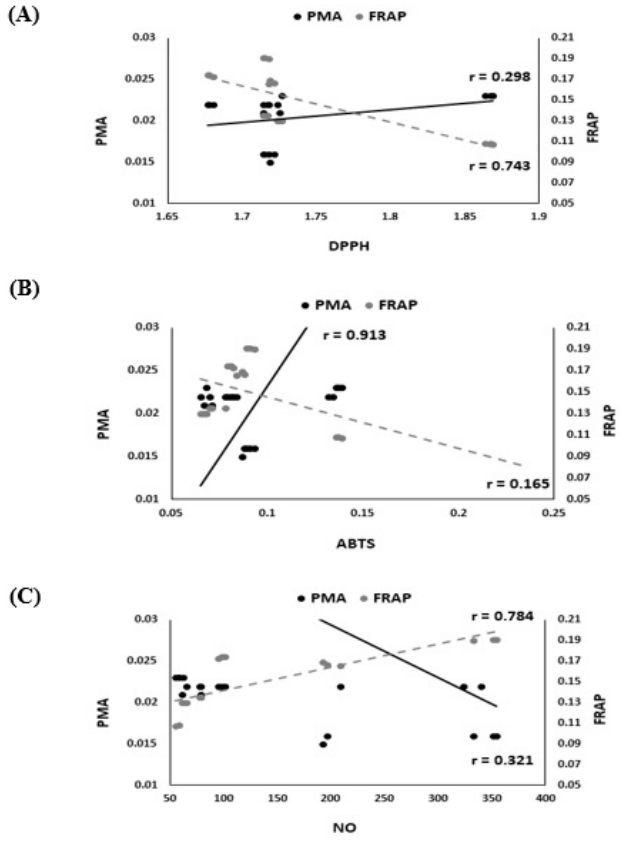

Figure 4. Correlation between free radical scavenging activity and transitional metal ion reducing power of Puerariae Flos extract. (A) correlation between DPPH radical scavenging activity and reducing power, (B) correlation between ABTS radical scavenging activity and reducing power, (C) correlation between NO radical scavenging activity and reducing power.

4 Conclusion

Our results revealed that extracts of Puerariae Flos possessed considerable amount of flavonoids and phenolic compounds. It also showed antioxidant properties and free radical scavenging activities. Although the correlation between active oxygen and antioxidant activity and polyphenol and flavonoid content in the arrowroot extract was significantly higher, it is believed that the antioxidant activity is attributed to flavonoids rather than phenolic.

The result indicates the potential of Puerariae Flos as a source of ntioxidants. However, further isolation of bioactive compounds would assist to ascertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

Conflict of interest

The authors declare that they have no conflict of interest.

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