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Physicochemical and antioxidant properties of methanol extract from Maca (*Lepidium meyenii* Walp.) leaves and roots

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Abstract

In this study, we examined the phytochemical properties and antioxidant activities of the methanol extracts of maca (*Lepidium meyenii Walp*.) leaves and roots. A total of 25 chemical compounds were identified by LC-Q-TOF and classified as saponins, phenols, flavonoids, steroids, alkybenzenes, and amines. Among all the chemical compounds identified, three saponins (tanshinone I, panaxytriol, and rotundifolioside), one phenol (gingerol), and one steroid (ergosterol peroxide) were found in the both leaf and root extracts. Levels of saponins, phenols, and flavonoids in the methanol extract from maca leaves were significantly higher than in the extract of roots. Antioxidant activities (1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and ferric reducing ability of plasma) of the methanol extracts of maca leaves were higher than those of the roots. Antioxidant activities were highly correlated with total phenol content in the methanol extracts of maca leaves. Therefore, the methanol extract from maca leaves exhibited higher antioxidant activities than those of roots, and could be used as a nutritional material with potential health benefits.

Keywords: antioxidant activity; leaves; maca; phytochemical properties; roots.

Practical Application: Maca leaves can potentially be used as a natural antioxidant for healthcare and food industry.

1 Introduction

Maca (*Lepidium meyenii* Walp.), a member of family Brassicaceae, is cultivated mainly in the central Andes of Peru at elevations of 3500-4500 m above sea level and freezing temperatures (Wang et al., 2007). These plants can be distinguished by their root colors, which are yellow, purple, white, gray, and black (Zhang et al., 2017). Yellow maca is commercially preferred, and is the most common cultivar (47.8%) (Rubio et al., 2006). Clément et al. (2010) reported that the roots of yellow maca were rich in macaene and phenolic components. It was suggested as a safe for consumption by the FAO in 1992, and has been promoted for global cultivation. In the recent years, the yellow maca is increasingly being used in Japan, Europe, China, Korea, and the US (Lee et al., 2011a; Zhang et al., 2017). According to traditional theories, it is considered an aphrodisiac also known as 'Peruvian ginseng' (Zhao et al., 2005). Maca has been used as a food health-promoting food material, stamina builder, and fertility promoter due to its various biological effects, including the regulation of metabolism and hormonal secretion, memory improvement, and antidepressant activity in humans (Zhang et al., 2017). Recently, maca has been consumed in juices, soups, extracts, and processed foods enriched with maca flour, and its availability in pills helped its commercialization in the international market, especially in Europe and Asia with functional and medicinal claims (Omran et al., 2010). It is claimed by the nutrition industry that maca has the ability to improve energy and modulate the response against oxidative stress. Previous studies reported that maca comprises various classes of bioactive compounds, including saponins, alkaloids, steroid hormones, and polyphenol compounds (Tang et al., 2017). In addition, Özdemir et al. (2015) reported that the *Lepidium* species contain triterpenoid saponins as major bioactive metabolites, but these assertions have not been scientifically substantiated. Even though Clément et al. (2010) reported the levels of major secondary metabolites in powdered maca leaves and roots of different colors, the information about biological activities of methanol extracts from maca leaves and roots is still limited and the structures of the compounds present in the extracts have yet to be established. Therefore, the main objectives of the present study were to uncover the antioxidant properties and characterize the detailed chemical compositions of the methanol extracts from maca leaves and roots using modern techniques including LC-Q-TOF analyses and to further promote its food and pharmaceutical applications.

2 Materials and methods

2.1 Materials

The dried maca leaves and roots were collected from High Dongbang Agricultural Products Cooperative (Kangwondo, Chunchen, Korea). HPLC-grade acetonitrile, water and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemical reagents were of analytical grade.

2.2 Chemical and mineral composition of maca leaves and roots

The chemical composition of dried maca leaves and roots, including moisture, ash, protein, and fat contents, was determined according to AOAC methods (AOAC, 2005)

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925.09B, 923.03, 979.09, and 920.39C, respectively. Their mineral contents were determined by the inductively coupled plasma optical emission spectrometry (ICP-OES 720 series, Agilent Technologies, Palo Alto, CA, USA).

2.3 Extraction procedure for maca leaves and roots

The extraction procedure for maca leaves and roots was based on the procedure described by Lee et al. (2017) with some modifications. Dried maca leaves and roots (100 g) were crushed into small blocks and soaked with 80% methanol (1:20, w/v). The temperature during extraction was maintained at 70 °C for 3 h using a shaking water bath (BS-11, Jeio tech Co., Ltd., Daejeon, Korea), and extracts were centrifuged (VS-5000N, Vision scientific Co., Ltd., Daejeon, Korea) at 3,500 rpm for 10 min. The supernatants were combined and subjected to vacuum evaporation. The resulting solid was collected, solubilized in distilled water, freeze-dried, and ground. The yield of crude extracts was measured as the percentage of dry weight that was calculated by using the following formula: yield (% dry weight) = (weight of extracted sample/dried sample weight) ×100.

2.4 Phytochemical properties of methanol extracts from maca leaves and roots

Liquid chromatography–quadrupole-time of flight (LC-Q-TOF)

Quantitative analysis of the compounds was performed using a LC-Q-TOF system (model 6530, Agilent Technologies, Santa Clara, CA, USA), equipped with a diode array detector (DAD), a quaternary pump and an autosampler. Chromatographic separation was performed on an Agilent Eclipse XDB C18 column (150 mm × 2.1 mm i.d., 3.5 µm) at 35 °C. The mixture of (A) 0.1% formic acid in deionized water and (B) acetonitrile was chosen as a mobile phase. The gradient elution program was as follows: 95% A and 5% B (0–5 min), 5% A and 95% B (5–10 min), 5% A and 95% B (10–10.10 min), 95% A and 5% B (10.10–15 min). The flow rate was set at 0.3 mL/min. The post-run equilibrium time of 5 min and an injection volume of 1 µm were used for all samples. The LC-Q-TOF was equipped with an ESI source in the positive mode and mass analysis conditions were set as follows: drying gas (N_2), 7 L/min; drying gas temperature, 300 °C; nebulizer, 40 psi; sheath gas temperature, 325 °C; nitrogen sheath gas flow, 10 L/min; capillary voltage, 4000 V; nozzle voltage, 0 V; fragmentor, 150 V; skimmer, 65 V. Mass spectra were acquired in the *m/z* range of 100 to 1,000. All the operations and data analysis were controlled using the Qualitative analysis software (Agilent Technologies, Santa Clara, CA, USA).

2.5 Comparison of in-vitro antioxidative activities of methanol extracts from maca leaves and roots

Total saponin content

The total saponin content of the methanol extract from maca leaves and roots was determined using the method of Nguyen et al. (2017) with some modifications. Briefly, 0.25 mL of the extract was mixed with 0.25 mL of 8% (w/v) vanillin solution and then 2.5 mL of 72% H_2SO_4 solution was added. The mixture was then incubated at 60 °C for 15 min and rapidly cooled to room temperature using an ice water bath. The absorbance of the mixture was measured at 560 nm using a Multiskan Go spectrophotometer (Thermo Scientific, Vantaa, Finland). Escin were used as a standard. The saponin content was expressed as escin equivalents (mg EE/ 100 g dry weight).

Total phenolic content

Total phenolic content of the methanol extract from maca leaves and roots was determined using the Folin–Ciocalteu method (Pontoni et al., 2017). Briefly, 0.5 mL of extract (2.0 mg/mL) was added in 0.5 mL of Folin–Ciocalteu reagent and allowed to stand at room temperature for 3 min. Next, 1.5 mL of sodium carbonate (10% w/w) solution was added to the mixture. After 60 min at room temperature, absorbance was measured at 725 nm using a Multiskan Go spectrophotometer (Thermo Scientific, Vantaa, Finland). Total phenolic content was expressed as gallic acid equivalents (mg GAE/100 g dry weight).

Total flavonoid content

The spectroscopic determination of total flavonoid in the crude extract was carried out following the method earlier described by Mottaghipisheh et al. (2018). Briefly, 150 µL of the sample was incubated at room temperature for 5 min. After mixing with 1.7 mL of 30% methanol, 750 µL of 0.5 M $NaNO_2$ solution and 75 µL of 0.3 M $AlCl_3$ were mixed. Next, 500 µL of 1 M NaOH was added and the absorbance was recorded at 415 nm using a Multiskan Go spectrophotometer (Thermo Scientific, Vantaa, Finland). Total flavonoid content was calculated and expressed as quercetin equivalents (mg QE/ 100 g dry weight).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of methanol extract from maca leaves and roots was measured by DPPH test using a previously described method (Maietta et al., 2018) with some modifications. Briefly, samples were dissolved in distilled water. 0.2 mL of extract with different concentration (1.0-5.0 mg/mL) was added to 0.8 mL of a DPPH methanol solution. The mixture was kept at room temperature for 15 min, and the absorbance was recorded at 517 nm using a Multiskan Go spectrophotometer (Thermo Scientific, Vantaa, Finland). The DPPH radical scavenging activity was calculated by the following formula Equation 1:

$$\text{Scavenging activity}(\%) = \left\{1 - \frac{(A_2 - A_1)}{A_0}\right\} \times 100 \quad (1)$$

A_0: the absorbance of DPPH without sample

A_1: the absorbance of sample without DPPH

A_2: was the absorbance of sample and DPPH

Ferric Reducing Ability of Plasma (FRAP)

The FRAP assay was performed according to the method of Wang et al. (2014) with minor modifications. Extracts were diluted prior to the FRAP assay. The FRAP working solution contained 2.5 mL of 10 mM neocuproine 2, 4, 6-tripyridyl-s-

triazine (TPTZ) solution (dissolved in 40 mM hydrochloric acid), 2.5 mL of 20 mM ferric chloride solution, and 25 mL of the acetate buffer solution (pH 3.6). Next, 10 μL samples were combined with 990 μL of the FRAP working solution, and the redox reaction performed at room temperature for 15 min without light before the absorbance at 593 nm was recorded. Total FRAP was expressed as gallic acid equivalents (mg GAE/g dry weight).

2.6 Statistical analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level. The CORR procedure was used to obtain correlation coefficients.

3 Results and discussion

3.1 Chemical and mineral composition of maca leaves and roots

The yields of the methanol extracts from maca leaves and roots were calculated as a percentage of the weight of dried powder, which were 22.3 g/ 100 g dry weight and 27.6 g/ 100 g dry weight, respectively (data not shown). The chemical composition of maca leaves was 28.95% protein, 15.98% ash, 7.41% moisture, and 0.35% fat. Maca roots were 21.40% protein, 8.83% moisture, 7.48% ash, and 0.02% fat (data not shown).

The mineral compositions of maca leaves and roots are presented in Table 1. The K^+ contents of both maca leaves and roots were the highest (28.42 and 30.37 mg/g, respectively) compared to other minerals and were higher than that of Peru powdered maca (Valentová et al., 2006), suggesting it to be a potential potassium source. An earlier study showed that dietary intake of K^+ has a beneficial effect on the coronary heart disease by decreasing blood pressure and maintaining an adequate Na^+ to K^+ ratio (Weaver, 2009). The Ca^{2+} contents of maca leaves and roots was observed to be higher (6.58 and 2.25 mg/g, respectively) than that reported by other studies of maca roots (Li et al., 2017). Ca^{2+} is an important component of the human body, and is required for vascular contraction, muscle function, nerve transmission, and intracellular signaling (Institute of Medicine, 2010). The Na^+ contents of maca leaves and roots were found to be higher compared to other studies of maca roots as well (Li et al., 2017). Maca leaves and roots are an excellent source of Na^+ which is an essential mineral for human health required for cellular homeostasis and fluid balance (Farquhar et al., 2015). Therefore, in this study it was suggested that maca leaves and roots are an excellent source of minerals and can serve as nutritional supplements.

Table 1. Mineral composition of Maca leaves and roots.

Mineral compounds (mg/g)	Maca leaves	Maca roots
Na	0.25±20.84	0.70±10.65
Ca	6.58±17.66	2.25±20.11
K	28.42±21.52	30.37±23.00
Mg	1.52±2.13	1.27±1.45
Fe	0.16±0.98	0.02±0.98
Mn	0.18±0.79	ND
Zn	0.09±0.35	ND
P	3.79±1.69	5.83±0.987

ND: Not detected.

3.2 Phytochemical properties of methanol extract from maca leaves and roots

LC-Q-TOF

Retention times and LC-Q-TOF data for the target compounds in the methanol extracts from maca leaves and roots are shown in the Table 2. By combining LC-Q-TOF data and elution order, 25 compounds were identified in the methanol extracts from maca leaves and roots. Saponins tanshinone I, panaxytriol, and rotundifolioside were found in both methanol extract from maca leaves and roots. Tanshinone I, panaxytriol, and rotundifolioside were confirmed to be the aglycons with *m/z* 276.0775, 277.1815, and 895.5049, respectively (Zhao et al., 1996). However, the *m/z* 470.1925, 357.0825, 276.0775, 277.1815, 967.4907, 935.5038, 981.5047, and 895.5049 corresponding to triterpene, esculin hydrate, tanshinone I, panaxytriol, lanatoside, marsdekoside B, colubrinoside, and rotundifolioside, respectively, were only detected in the methanol extract from maca leaves (Ueda et al., 2012; Sánchez-Rabaneda et al., 2003). The *m/z* 457.3574 of oleanoic acid was only found in the methanol extract from maca roots. Thus, it was found that a methanol extract from maca leaves had a bigger variety of saponins compared to the methanol extract from maca roots.

As for the phenols, the aglycon with *m/z* 293.1748 was confirmed to be gingerol in both methanol extracts from maca leaves and roots. The unique phenols in the methanol extract from maca leaves were *m/z* 692.2200 and 495.1482 identified as kuwanon G and oxypaenoniflorin, respectively (Ding et al., 2012). The *m/z* 256.1748 of tolmetin was only found in the methanol extract from maca roots.

Flavonoids in the methanol extract from maca leaves include aglycons of laricitrin and ligustroflavone with *m/z* 330.0379 and 723.2140, respectively (Kowalska et al., 2007); however, no flavonoids were detected in the methanol extract from maca roots. Castillo-Muñoz et al. (2008) reported a new type of flavonoid in the *Vitis vinifera Cv. Petit Verdot* red wine grapes, a 3',4'-dihydroxy-5'-dimethoxy derivative named laricitrin (*m/z* 331).

It is well known that maca improves the functions of male hormones such as sexual development at puberty, sexual function, maintenance of mass and strength of the bone and muscle, and maintenance of the body composition in males (Nique et al., 2012). In this study, analysis of steroid derivatives showed that the *m/z* 428.3276 in both the methanol extract from maca leaves and roots corresponds to ergosterol peroxide, a steroid hormone derivative. The methanol extract from maca roots, but not from the mama leaves, was found to contain the *m/z* 442.3425 corresponding to the testosterone decanoate.

Table 2. LC-Q-TOF of methanol extract from Maca leaves and roots.

t_R (min)	Experimental m/z $[M-H]^-$	Theoretical m/z $[M-H]^-$	Error (ppm)	Formula $[M-H]^-$	Identification	Occurrence	
						Leaves	Roots
Saponin group							
0.568	470.1925	470.1946	4.69	$C_{26}H_{31}O_8$	Triterpene	+	-
0.930	357.0825	357.0827	1.08	$C_{15}H_{18}O_{10}$	Esculin hydrate	+	-
5.181	276.0775	276.0792	6.02	$C_{18}H_{12}O_3$	Tanshinone I	+	+
6.477	277.1815	277.1809	-2	$C_{17}H_{26}O_3$	Panaxytriol	+	+
6.762	456.3574	457.3691	7.70	$C_{30}H_{48}O_3$	Oleanoic acid	-	+
8.052	967.4907	967.4908	-0.29	$C_{49}H_{76}O_{19}$	Lanatoside	+	-
8.733	935.5038	935.5010	-2.53	$C_{49}H_{76}O_{17}$	Marsdekoiside B	+	-
8.734	981.5047	981.5065	1.34	$C_{50}H_{78}O_{19}$	Colubrinoside	+	-
10.253	895.5049	895.5061	1.19	$C_{47}H_{76}O_{16}$	Rotundifolioside	+	+
Phenol group							
4.436	691.2200	692.2258	-2.09	$C_{40}H_{36}O_{11}$	Kuwanon G	+	-
5.646	256.0994	255.0917	-8	$C_{15}H_{14}NO_3$	Tolmetin	-	+
5.955	293.1748	294.1833	3.74	$C_{17}H_{26}O_4$	Gingerol	+	+
6.475	495.1482	495.1508	4.68	$C_{23}H_{28}O_{12}$	Oxypaeoniflorin	+	-
Flavonoid group							
0.593	330.0379	330.0381	0.68	$C_{16}H_{11}O_8$	Laricitrin	+	-
4.431	723.2140	724.2215	0.27	$C_{33}H_{40}O_{18}$	Ligustroflavone	+	-
Steroid group							
6.681	428.3276	428.3296	4.73	$C_{28}H_{44}O_3$	Ergosterol peroxide	+	+
6.755	442.3425	442.3452	-1.79	$C_{29}H_{47}O_3$	Testosterone decanoate	-	+
Alkybenzene group							
6.759	303.2190	303.2204	-2.54	$C_{19}H_{30}NO_2$	Pentapiperium Metilsulfate	-	+
Amino group							
0.583	133.0497	133.0872	2.77	$C_5H_9O_4$	Ribosyl	-	+
0.588	200.0643	200.0631	-3.37	$C_{16}H_9$	4-Pyrenyl radical	-	+
0.586	181.0765	181.0744	-11.22	$C_9H_{12}NO_3$	Tyrosinium	-	+
0.587	179.0583	179.0588	-6.64	$C_9H_{10}NO_3$	L-tyrosine	-	+
0.591	221.0688	221.0290	-4.4	$C_{11}H_{12}NO_4$	N-acetyl-L-tyrosine	-	+
5.818	255.1644	255.1629	-0.3	$C_{17}H_{22}NO$	Bephenium	-	+
10.259	367.3496	367.3459	-11.51	$C_{23}H_{46}NO_2$	Oleoylcholine	-	+

(+) presence; (-) absence.

In addition, the *m/z* 303.2190 in the methanol extract from maca roots was a pentapiperium methylsulfate, an alkybenzene derivative with antibacterial properties. However, it was not found in the maca leaves.

Seven amines were identified in the methanol extract from maca roots, but none were detected in the methanol extract from maca leaves. The aglycons of *m/z* 133.0497, 200.0643, 181.0765, 179.0583, 221.0688, 255.1644, and 367.3496 were ribosyl, 4-pyrenyl radical, tyrosinium, L-tyrosine, N-acetyl-L-tyrosinate, bephenium, and oleoylcholine, respectively (Peek et al., 1991; Shi et al., 2002; Lehmann & Kessler, 1983). According to Slany et al. (1993), the ribosyl moiety of a new function of S-adenosylmethionine was found to be the *m/z* 132. Shen et al. (2004) reported that tyrosinium is an α-amino-acid cation, and it is the conjugate acid of tyrosine, arising from protonation of the amino group. L-tyrosine was detected at the *m/z* 180. Therefore, LC-Q-TOF results showed that the chemical structure of methanol extract from maca leaves and roots can be classified as saponin, phenol, flavonoid, steroid, alkybenzene, and amino fractions. In this study, the methanol extract from maca leaves was found to rich in saponins, which the maca roots were rich in amines.

3.3 Comparison of in-vitro antioxidant activities of the methanol extracts from maca leaves and roots

Total saponin content

Saponins are bioactive compounds with various health benefits, including some anti-cancer agents and the compounds reducing the cardiovascular disease risk (Vuong et al., 2013). They are found in a number of plant-derived foods and medicinal plants (Karimi et al., 2011). Total saponin contents of the methanol extracts from maca leaves and roots are shown in the Table 3. Total saponin content was higher in the methanol extract from maca leaves (6.09 mg EE/ 100 g DW) in comparison to maca roots (5.10 mg EE/ 100 g DW). According to Hwang et al. (2014), saponin content of ginseng leaves was higher than that of the ginseng roots. In addition, total saponin content was higher in the *Labisa pumila* Benth leaves in comparison to the roots (Karimi et al., 2011). According to the LC-Q-TOF data (Table 2), the methanol extract from maca leaves had a wider variety of saponins than the maca roots. Therefore, the methanol extract from maca leaves exhibited a higher total saponin contents than the maca roots and can be a potential source of antioxidants.

Total phenolic content

Phenolic compounds have a variety of biological activities including anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic properties. These properties are likely to be related to their antioxidant activities (Talhaoui et al., 2015). Total phenolic contents of the methanol extracts from maca leaves and roots were measured using the Folin–Ciocalteu method and are shown in the Table 3. The total phenolic content of the methanol extract from maca leaves (0.37 mg GAE/ 100 g DW) was higher than that of the maca roots (0.17 mg GAE/ 100 g DW). Higher total phenolic content in the methanol extract from maca leaves was expected and could be explained by the elevated biosynthesis of polyphenols due to light exposure in these organs (Tuzcu et al., 2017). In addition, according to our findings on the types of phenols present (Table 2), the methanol extract from maca leaves exhibited a wide variety of phenols than the maca roots. Fukuoka et al., (2018) also reported that total phenolic content of leaves from sweet potato was higher than that of roots. Feng et al. (2017) observed that the extract from leaves from *Vaccinium glaucoalbum* was higher in the total phenol content than that of fruits. Thus, the methanol extract from maca leaves revealed higher total phenolic content than that of maca roots, and could be used a potential antioxidant.

Table 3. Bioactive compounds of methanol extract from Maca leaves and roots.

Parameters	Maca leaves	Maca roots
Saponin (mg EE/100 g dry weight)	6.09±0.59$^{a1)}$	5.10±0.48^{b}
Polyphenol (mg GAE/100 g dry weight)	0.37±0.42^{a}	0.17±0.26^{b}
Flavonoid (mg QE/100 g dry weight)	0.23 ±0.90^{a}	0.01±0.17^{b}

[1)]Means with different superscripts in a row (a-b) is significant at $p<0.05$ by Ducan's multiple range test.

Total flavonoid content

Total flavonoid contents of the methanol extracts from maca leaves and roots are shown in the Table 3. The methanol extracts from maca leaves and roots contained 0.23 and 0.01 mg QE/ 100 g dry weight total flavonoids, respectively. Karimi et al. (2011) reported that the total flavonoid content of the methanol extract from *Labisa pumila* Benth. leaves was higher than that of the roots. Flavonoids are an important part of the human nutrition due to the radical scavenging ability conferred by their hydroxyl groups that contributes to their antioxidant activity (Sun et al., 2011). Flavonoids are distributed widely in the plants and are especially common in the leaves (Siddhuraju & Becker, 2003).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity is often used to evaluate the antioxidant activity of natural products. Antioxidant activity of primary antioxidants is often assessed by evaluating their hydrogen donating ability. These antioxidants have the ability to donate hydrogen to free radicals, thereby converting them to non-toxic species and thus inhibiting the propagation of lipid oxidation (Lugasi et al., 1998).

In this study, the DPPH radical scavenging activity of the methanol extracts from both maca leaves and roots gradually increased with an increase in concentrations, and the DPPH radical scavenging activity of maca leaves was higher than that of roots (Figure 1A). Similarly, Sandoval et al. (2002) reported that the DPPH radical scavenging activity of the extract from maca roots increased with increasing concentration. Matkowski et al. (2008) reported that the DPPH radical scavenging activity of extract from leaves of *Salvia miltiorrhiza Bunge* and S. *Verticillata* L. was higher than that of roots. Therefore, the methanol extract from maca leaves could be a potential source of antioxidants.

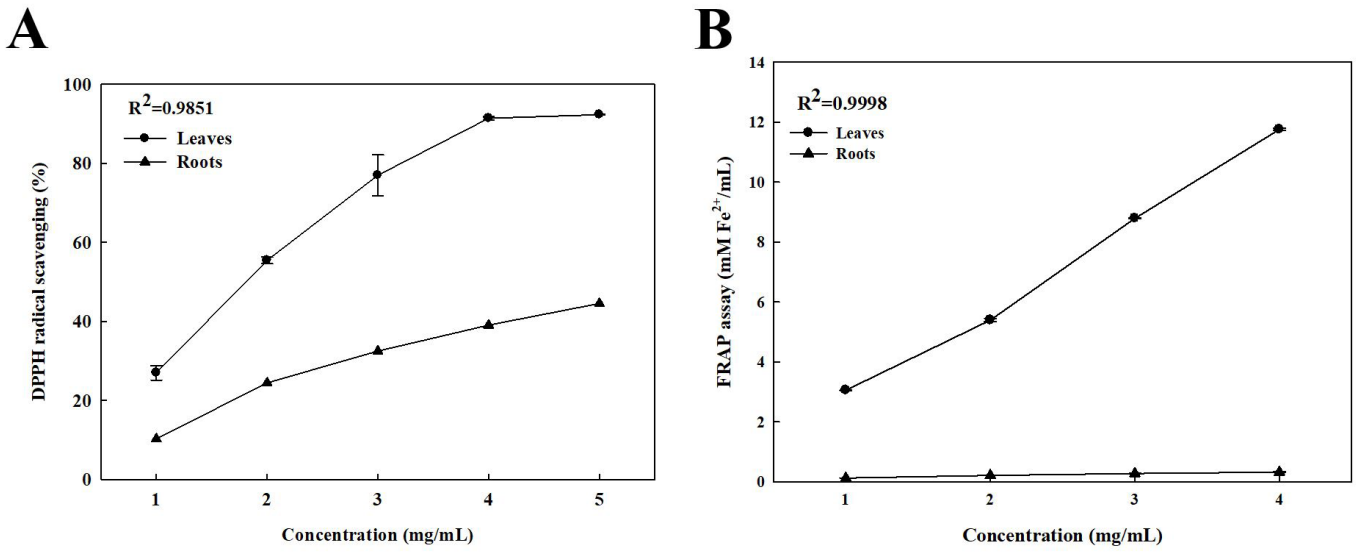

Figure 1. DPPH and FRAP assay of methanol extract from Maca leaves and roots. A: DPPH radical scavenging activity, B: FRAP assay.

Table 4. Pearson's coefficients of correlation among the total saponin content, total phenol content, total flavonoid content, and antioxidant activities (DPPH radical scavenging activity and FRAP assay) of the methanol extract from maca leaves and roots (n=6).

	Methanol extract from maca leaves					Methanol extract from maca roots				
	Saponin	Phenol	Flavonoid	DPPH	FRAP	Saponin	Phenol	Flavonoid	DPPH	FRAP
Saponin		0.899*	0.345	0.779	0.798		0.901**	0.621	0.800	0.751
Phenol			0.838*	0.991***	0.998***			0.573	0.835*	0.742
Flavonoid				0.841*	0.837*				0.735	0.823*
DPPH					0.985***					0.874*
FRAP										

Significantly different at the *p<0.05%, **p<0.01%, and ***p<0.001% levels.

Ferric reducing ability of plasma (FRAP)

FRAP provided a direct estimate of antioxidant activities or reductants present in the methanol extracts from maca leaves and roots and is based on the ability of the analytes to reduce the Fe^{3+}/Fe^{2+} pair. The methanol extract from maca leaves exhibited the higher FRAP activity, whereas maca roots had a lower FRAP activity (Figure 1B). Alam et al. (2012) reported that the FRAP activity of the methanol extracts of *Withania somnifera* leaves indicated a greater reduction of Fe^{3+}/Fe^{2+} pair and higher antioxidant activities compared with the extracts from fruits and roots.

3.4 Correlations between the antioxidant activities of the methanol extracts from maca leaves and roots

The correlation (r^2) was calculated for the total saponin content, total phenolic content, total flavonoid content, and antioxidant activities (DPPH radical scavenging activity and the FRAP assay) of the methanol extracts from maca leaves and roots (Table 4). The total phenolic content of the methanol extract from the maca leaves showed a weak positive correlation with the total saponin content (r^2 = 0.801, p < 0.05). In the methanol extract from the maca roots total phenolic content was well correlated with the total saponin content (r^2 = 0.901, p < 0.01). Lee et al. (2011b) indicated that the saponin contents of the extracts from yellow soybeans and mung beans were weakly correlated with total phenolic contents of the same extracts.

In addition, for the methanol extract from maca leaves high correlation was found between the total phenolic content and both the DPPH radical scavenging activity (r^2 = 0.991, p<0.001) and the FRAP assay results (r^2 = 0.998, p < 0.001). However, the total phenolic content of methanol extract from maca roots had a positive correlation only with the DPPH radical scavenging activity (r^2 = 0.835, p < 0.05). Correlation between the total phenol content and the DPPH radical scavenging activity suggested that an increase in the total phenol content of methanol extract from maca leaves and roots improved the hydroxyl radical scavenging capacity (Siddhuraju & Becker, 2003). In general, higher phenolic content corresponded to the higher antioxidant activity because phenols have high hydrogen atom donating abilities (Siddhuraju & Becker, 2003). According to Islam et al. (2003), phenol content of the extract from sweet potato leaves was significantly correlated with the DPPH radical scavenging activity. High correlation was obtained between the phenolic content and antioxidant activities of various plants (Stintzing et al., 2005; Rabah et al., 2004), and thus could be used in evaluating antioxidant potential of foods.

The total flavonoid content of methanol extract from maca leaves was positively correlated with total phenolic content (r^2 = 0.838, p < 0.05), DPPH radical scavenging activity (r^2 = 0.841, p < 0.05), and the FRAP assay results (r^2 = 0.837, p < 0.05). On the other hand, the methanol extract from maca roots showed that the total flavonoid content had only correlation with the FRAP assay results (r^2 = 0.823, p < 0.05). Similarly, the correlation was found between the total flavonoid content of the extract from *Salacia chinensis* L and the FRAP assay results (Ngo et al., 2017). Zhou et al. (2011) also reported that the total flavonoid content of the extract from *Eriobortrya japonica* Lindl. was positively correlated with the antioxidant activities such as the DPPH radical scavenging activity and the FRAP assay results.

The correlation was found between the DPPH radical scavenging activity and the FRAP assay results for the methanol extracts from maca leaves (r^2 = 0.985, p < 0.001) and roots (r^2 = 0.874, p < 0.05). Sulaiman et al. (2011) reported a moderate correlation between the DPPH radical scavenging activity and the FRAP assay results for the extract from *Malaysian* bananas. Du et al. (2009) also reported a correlation between the DPPH radical scavenging activity and the FRAP assay results for the extract from *Actinidia* fruits. Based on these results, the correlation between the total phenol content and antioxidant activities (DPPH radical scavenging activity and the FRAP assay) was higher for the methanol extract from maca leaves compared to the methanol extract from maca roots. Therefore, the methanol extract from maca leaves can be considered a rich natural source of phenolic compounds with good antioxidant properties.

4 Conclusion

In this study, the phytochemical properties and antioxidant activities of the methanol extracts from maca leaves and roots were evaluated. Total of 25 chemical compounds were identified by LC-Q-TOF and used for classification of chemical compositions and antioxidant activities of the methanol extracts from maca leaves and roots. In both methanol extracts from maca leaves and roots, the chemical compounds were found in three saponin groups, one phenol group, and one steroid group. Total saponin (6.09 mg EE/ 100 g dry weight), phenol (0.37 mg GAE/ 100 g dry weight), and flavonoid contents (0.23 mg QE/ 100 g dry weight) were higher in the methanol extract from maca leaves in comparison to that from the maca

roots. The antioxidant activities of maca leaves and roots were determined by two complementary tests such as the DPPH radical scavenging activity and the FRAP assay. Both assays followed a similar trend that the methanol extract from maca leaves had higher antioxidant activities compared to the methanol extract from maca roots. High correlation was found between total phenol content and antioxidant activities (DPPH radical scavenging activity and the FRAP assay) in the methanol extract from maca leaves. However, the total phenol content of the methanol extract from maca roots was correlated only with the DPPH radical scavenging activity. Therefore, the methanol extract from maca leaves has more potential antioxidants that could be processed into foods.

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