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Isolation, purification and antioxidant activity of polysaccharides from the leaves of maca (*Lepidium Meyenii*)

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ABSTRACT

Two fractions of polysaccharides (MLP-1 and MLP-2) were extracted from the leaves of maca (*Lepidium Meyenii* Walp.) by water, and purified using DEAE-52 ion exchange resin and sephadex G-200 clumns chromatography. An investigation was carried out for their structural characterization and antioxidant activity *in vitro*. The results indicated that MLP-1 was mainly composed of ribose, rhamnose, arabinose, xylose, mannose, glucose and galactose, with the molar ratio of 0.12:0.32:1.50:0.32:1.03:1.00:0.93; the MLP-2 was a homopolysaccharide composed of glucose. The molecular weight (Mw) of MLP-1 was 42756 Da, and the Mw of MLP-2 was 93541 Da. The FT-IR spectra showed the general characteristic absorption peak of maca leaf polysaccharides (MLPs). The evaluation of antioxidant activity revealed that MLP-1 had strong scavenging effects *in vitro* on hydroxyl, superoxide anion and DPPH radicals, whose EC_{50} (mg/mL) was 0.44, 0.21, and 0.82, respectively. Both MLP-1 and MLP-2 presented dose-dependently positive effects on the antioxidant-related parameters. The results suggested that the purified MLP-1 displayed better antioxidant capacities than that of MLP-1, which could be explored as potential antioxidant agents for the complementary medicine or functional foods.

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1. Introduction

Maca (*Lepidium meyenii*), an annual or biennial plant of family of Brassicaceae, mainly grows in the Andes region of Peru where the altitude is between 3500 and 4500 m above sea level. This area is characterized by freezing temperature, intense sunlight, fierce winds, barren, and rocky terrain [1]. The maca hypocotyls have been used as both foodstuff and folk medicine in the Andes for over 2000 years [2]. It has been recommended as a safe edible food by the FAO in 1992, and has been promoted for global cultivation. Up to now, a variety of active components have been found in maca, including macaene and macamides [3], glucosinolates [4], alkaloid [5] sterols and flavonolignans [6], and polysaccharides [7].

Plant polysaccharides gained the attention in medicine due to their biological activities such as antioxidant [8,9], immunomodulation [10], antidiabetic [11], anti-tumor [12], antibiotic [13], and anti-inflammatory [14]. Recently, an increasing number of studies have focused on the structural characterization and activities of maca polysaccharides obtained from maca hypocotyls. For example, the polysaccharides of *Lepidium meyenii* (LMPs) were extracted by hot water, and four LMPs were obtained through increasing the concentration of ethanol in the process of polysaccharide precipitation [7]. Wang et al. [15] reported that a polysaccharide (MP21) isolated and purified from maca hypocotyls had an average Mw of 3.68×10^5 Da, and the MP21 was mainly composed of rhamnose, arabinose and galactose in a molar ratio of 1:4.84:5.34. Zhang, et al. [16] had been revealed the structural features of MC-1 (a novel polysaccharide) from maca hypocotyls. It was reported that the polysaccharides from maca hypocotyls showed antioxidant [7], anti-fatigue [17], and immunomodulatory activities [15,16]. Recently, it is proved that maca polysaccharide (MP-1) possesses a protective effect on Hep-G2 cells and alcoholic liver oxidative injury in mice [18]. However, few studies have focused on the purified polysaccharides of maca leaves and their biological activities. The maca leaves accounting for 20–40% (m/m, dry weight) of total biomass of maca were ignored and wasted, which led to the waste of maca resources.

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In this study, maca leaf polysaccharides (MLPs) were isolated by DEAE cellulose-52 chromatography, which were further purified by Sephadex G-200 column chromatography, and two polysaccharides (MLP-1 and MLP-2) were obtained. Therefore, the aims of this study were to preliminarily characterize the structure of the polysaccharide fractions isolated from the leaves of *Lepidium meyenii* and to evaluate antioxidant activities of these fractions *in vitro*.

2. Materials and methods

2.1. Materials

Fresh maca leaves were collected from Yunnan Province, in the southwest of China during in November–December, 2014. The sample was confirmed by Prof. Hou Wenbin (Tianjin Institute of Pharmaceutical Research, Tianjin, China). The leaves were shade dried, powdered, sieved through 830 μm and stored in a tightly closed container for future use.

Butylated hydroxyl toluene (BHT) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-phenyl-3-methyl-4-benzene formyl pyrazolone (PMP) was from Solarbio Co. Ltd (Beijing, China). DEAE-cellulose 52 was obtained from Beijing RuiDaHengHui Science & Technology Development Co. Ltd, and Sephadex G-200 filtration media were purchased from Beijing Hua Yuan Science and Technology Co Ltd. All of the other chemicals were analytical grade.

2.2. Extraction of crude MLPs

The powder of maca leaves was extracted with petroleum ether (b. p. 60–90 °C) using Soxhlet apparatus to remove lipids. The defatted sample was extracted with boiling water at a water/raw material ratio of 20:1 (mL/g) for 150 min, and the supernatant was collected by centrifugation (4000g, 20 min). This operation was repeated twice, and all obtained supernatants were combined. When the resulting solution was concentrated to about 1/4 of original volume, ethanol (95%, v/v) was added slowly with constant agitating, until the final concentration of ethanol reached to 70% (v/v). The supernatant and precipitate were left at 4 °C for overnight, and then separated by centrifugation at 4000*g* for 20 min. The precipitates were collected and dissolved in distilled water before lyophilization to gain crude MLPs.

2.3. Purification of MLPs

The protein in crude MLPs was removed by the Sevag method (the ratio of chloroform to butanol was 4:1, v/v), and the deproteinated process was performed until no protein precipitation exist. Then, the MLPs were further depigmented by using D101 type macroporous adsorption resin according to the method reported by Li et al. [19]. Finally, the decolored solution was collected, concentrated and lyophilized, and the polysaccharide fractions were obtained. The polysaccharide fractions obtained (100 mg) were dissolved in 10 mL of distilled water, and the precipitate and supernatant were separated by centrifugation (4000g, 15 min). The supernatant was loaded on a DEAE-52 cellulose chromatography column (2.6 cm × 60 cm) equilibrated with deionized water. The column was eluted with sodium chloride solution (linear gradient from 0 to 0.6 M) at a flow rate of 1 mL/min. Each eluent fraction was collected and determined by the phenol-sulfuric acid method [20]. The eluent was concentrated, and dialyzed (MW cut off 5.0 kDa) for 48 h and freeze-dried.

The fractions obtained by DEAE-52 cellulose column were further purified by using Sephadex G-200 column (1.6 cm × 100 cm) eluting with deionized water at 1 mL/min. The main fraction was gathered, desalted and lyophilized, respectively. According to this process, two pure MLPs were obtained and named as MLP-1 and MPL-2.

2.4. General analysis

The content of total carbohydrate was measured by the phenol-sulfuric acid method using *D*-glucose as standard [20]. Protein content was evaluated according to Commassie Brilliant Blue G-250 method using BSA (bovine serum albumin) as standard [21]. The uronic acid (UA) was measured according to *m*-hydroxybiphenyl method using D-galacturonic acid as standard [22]. All the data were detected by using a TU-1810PC UV–vis spectrophotometer (Persee, Beijing, China).

2.5. Analysis of monosaccharide compositions

The monosaccharide composition of MLP-1 and MLP-2 was determined according to the method of Olatunji et al. [23] with a minor modification. In brief, the sample (10 mg) was hydrolyzed in 6 mL trifluoroacetic acid solution (2 mol/L) at 110 °C for 4 h. The excess acid of trifluoroacetic acid was neutralized by adding $BaCO_3$, and the supernatant were gathered and lyophilized. The freeze-dried product was treated with hydroxyl-amine hydrochloride (20 mg) and pyridine (2.0 mL) for 1 h at 90 °C. The reference standards including ribose, xylose, rhamnose, mannose, arabinose, glucose and galactose were treated in the same way. The resulting products were filtered through a 0.45 μm nylon membrane (Westborough, MA, USA). Finally, the obtained samples were analyzed by gas chromatography-mass spectrometry (GC–MS) with a DB-fused-silica capillary column (60 m × 0.25 mm × 0.25 μm) and a flameionization detector.

2.6. Molecular weight determination

The average molecule weight of MLP-1 and MLP-2 was determined as described by Wang et al. [15] with slight modifications. The high-performance gel-permeation chromatography (HPGPC) with a HPLC system (Agilent 1200) was applied. This instrument was equipped with L-aquagel-OH 40 pre-column (300 × 7.5 mm, inner diameter 8 μm), PL-aquagel-OH 30 separation column (300 × 7.5 mm, inner diameter 8 μm), and a refractive index detector (RID). The loaded column was eluted with NaAc solution (0.003 mol/L) at a flow rate of 1.0 mL/min and the injection volume was 50 μL. Calculation was calibrated with standard dextrans (including T670, T410, T270, T150, T80, T50, T25, T12, T5, and T1, Sigma, USA).

2.7. FT-IR and UV spectroscopy analysis

The FT-IR spectra of MLP-1 and MLP-2 were determined by using a FT-IR spectrophotometer (Bruker, Germany) in the vibration region of 4000–400 cm^{-1}. The samples were ground into fine powder and mixed with potassium bromide, then pressed into 1 mm pellets. Their UV spectra were recorded with a TU-1810PC spectrophotometer (Persee, Beijing, China) in the wavelength range of 400–190 nm.

2.8. Assay of antioxidant activities in vitro

2.8.1. Hydroxyl radical ($OH^{\bullet}$) scavenging activity

The hydroxyl radical scavenging activity of MLP-1 and MLP-2 was determined according to the previous method [24] with a minor modification. 2.0 mL of different concentrations (0.2–1.2 mg/mL) samples were mixed with 2.0 mL of 0.6 mM

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ferrous sulphate and 2.0 mL of 0.6 mM H_2O_2, respectively. The mixtures were shaken and kept at 25 °C for 10 min. Then 2.0 mL of salicylic acid (0.6 mM) was added into the solution, shaken and kept at 25 °C for 30 min. Ascorbic acid (Vc) and BHT were used as the positive control. The resulting solution was measured at 510 nm with a spectrophotometer. Hydroxyl radical scavenging activity was calculated by the following equation: scavenging effect (%) = $[1-(A_1-A_2)/A_0]\times 100$, where A_0 is the absorbance of the control, A_1 is the absorbance of the sample, and A_2 was the background absorbance of the sample.

2.8.2. Superoxide anion radical ($O^{2-\bullet}$) scavenging activity

Superoxide anion radical scavenging activity was measured using pyrogallic acid method [25] with some modifications. Briefly, 4.5 mL of Tris–HCl (pH 8.2, 0.1 M) was preheated at 25 °C for 20 min, then 0.1 mL of different concentrations (0.2–1.2 mg/mL) samples and 0.3 mL of pyrogallic acid (5 mM) were added. Vc and BHT were used as the positive control. The obtained solution was shaken and kept at 25 °C for 5 min, then 1 mL of HCl (10 M) was added into the mixture to terminate the reaction. The absorbance of the obtained solution was measured at 320 nm with a spectrophotometer. Superoxide anion radical scavenging activity was calculated by the following equation: scavenging effect (%) = $(1-K_1/K_0)\times 100$, where K_0 is the absorbance of the control, K_1 is the absorbance of the sample.

2.8.3. DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to the reported method [26] with minor modifications. Briefly, 2.0 mL of different concentrations (0.2-1.2 mg/mL) samples were added to 2.0 mL of methanol solution (0.2 mM) of DPPH. Vc and BHT were used as the positive control. The obtained solution was shaken and kept at 25 °C in the dark for 30 min, and the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. DPPH radical scavenging activity was calculated by the following equation: scavenging effect (%) = $[1-(A_1-A_2)/A_0]\times 100$, where A_0 is the absorbance of the control, A_1 is the absorbance of the sample, and A_2 is the background absorbance of the sample.

2.9. Statistical analysis

Values from analysis are expressed as mean ± standard deviation (S.D.). Data sets with multiple comparisons were evaluated by one-way analysis of variance (ANOVA) with Duncan's test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Isolation and purification of crude MLPs

Crude MLPs were isolated from the maca leaves, and the yield was 4.6% based on the dried leaves of *L. meyenii*. After being deproteinated and decolored, the MLPs were fractionated on a DEAE-52 cellulose anion-exchange column, and the profile eluted by deionized water and NaCl solution was presented in Fig. 1A. After fractionation, two fractions (MLP-1, MLP-2) were obtained from an aqueous NaCl gradient (0, 0.2 M). These fractions were further separated by Sephadex G-200 column chromatography and eluted with deionized water. Both MLP-1 and MLP-2 showed only one symmetrical peak in Fig. 1B, C. Two fractions were collected separately, enriched and frozen. The polysaccharide content was detected using the phenol-sulfuric acid method. Thus two purified polysaccharides (MLP-1 and MLP-2) were obtained with the yield of 35.7% and 25.3% based on the total amount of deproteinatinng and decoloring MLPs, respectively. The content of total carbohydrate in MLP-1 was 94.10%, and the content of UA was 1.51%. The carbohydrate content of MLP-2 was 90.15%, and the content of UA was 20.62%. Both of MLP-1 and MLP-2 showed negative response to the Commassie Brilliant Blue method. These values were similar to those of two polysaccharide fraction (MPS-1, MPS-2) isolated from maca hypocotyls [17]. Compared with maca hypocotyl polysaccharides (MHP), the contents of UA in MLP-2 were much lower than that of MPS-2 [17] and MP21 [15].

Table 1
Monosaccharide components, molar proportion, and molecular weight of fractions (MLP-1 and MLP-2).

Sample	Molecular weight (Da)	Molar ratios						
		Rib[a]	Rha[b]	Ara[c]	Xyl[d]	Man[e]	Glc[f]	Gal[g]
MLP-1	42756	0.12	0.32	1.50	0.32	1.03	1.00	0.93
MLP-2	93541	0.00	0.00	0.00	0.00	0.00	1.00	0.00

[a] ribose.
[b] rhamnose.
[c] arabinose.
[d] xylose.
[e] mannose.
[f] glucose.
[g] galactose.

3.2. Monosaccharides composition and average molecular weight

The monosaccharide compositions of MLP-1 and MLP-2 were analyzed by GC–MS and the results were shown in Fig. 2 and Table 1. It was found that the MLP-1 was made of ribose (Rib), rhamnose (Rha), arabinose (Ara), xylose (Xyl), mannose (Man), glucose (Glc) and galactose (Gal) with the molar ratio of 0.12:0.32:1.50:0.32:1.03:1.00:0.93. Seven kinds of monosaccharide were included in MLP-1. Therefore, it was a kind of heteropolysaccharide. The MLP-2 was composed of Glc, e.g. the glucosan. According to the study of Zha et al. [7], the LMPs consisted of Rha, Ara, Glc, and Gal in a molar ratio of 2.18:9.47:1:5.21 (LMP-70). Zhang et al. [16] reported that the pure polysaccharide (MC-1) included Ara, Man, Glc and Gal with molar of 0.49:0.22:1:0.16, respectively. It was also reported that maca root polysaccharide (MP-21) was mainly composed of Rha, Ara, and Gal in molar ratio of 1:4.84:5.34 [15]. Previous study [17] showed that the MPS-1 consisted of Xyl, Ara, Gal, and Glc (the mole ratio 1:1.7:3.3:30.5), and the MPS-2 was composed of Ara, Gal and Glc (the mole ratio 1:1.3:36.8). The MP-1(isolated from maca hypocotyls) reported by Zhang et al. [18] contained five monosaccharides with different proportion, including Rha, GalUA (galacturonic acid), Glc, Gal, Xyl and Ara. This result indicated that the major monosaccharide components of MLP fractions were very similar to the polysaccharides isolated from maca hypocotyls.

The molecular weight distribution of MLP-1 and MLP-2 were determined using HPGPC and the results were shown in Fig. 3 and Table 1. The curve fitting is made by SPSS 19.0 software, and the curve equation is obtained according to the curve shape. The calibration equation of the standards was Log $M = 60.4 - 6.05t_R + 0.218t_R^2 - 0.0027t_R^3$ (where M is the molecular weight, and t_R is the retention time) with a correlation coefficient of 0.999,6. According to this formula, the average molecular weight of MLP-1 and MLP-2 were 42756 Da and 93541 Da, respectively. Zhang et al. [16] revealed that the molecular weight (Mw) of maca root polysaccharide (MC-1) was 11.3 kDa. Li et al. [17] reported that the Mw of two purified polysaccharide from maca hypocotyls was 7.6 kDa (MPS-1), and 6.7 kDa (MPS-2). Previous researches showed that the Mw of MP21 (polysaccharide from maca hypocotyls) was 3.68×10^5 Da [15], and the average Mw of MP-1 was 1067.3 kDa [18]. These results were different from our result. It implies that the difference of Mw of polysaccharides exists in maca hypocotyls or leaves in different quantity.

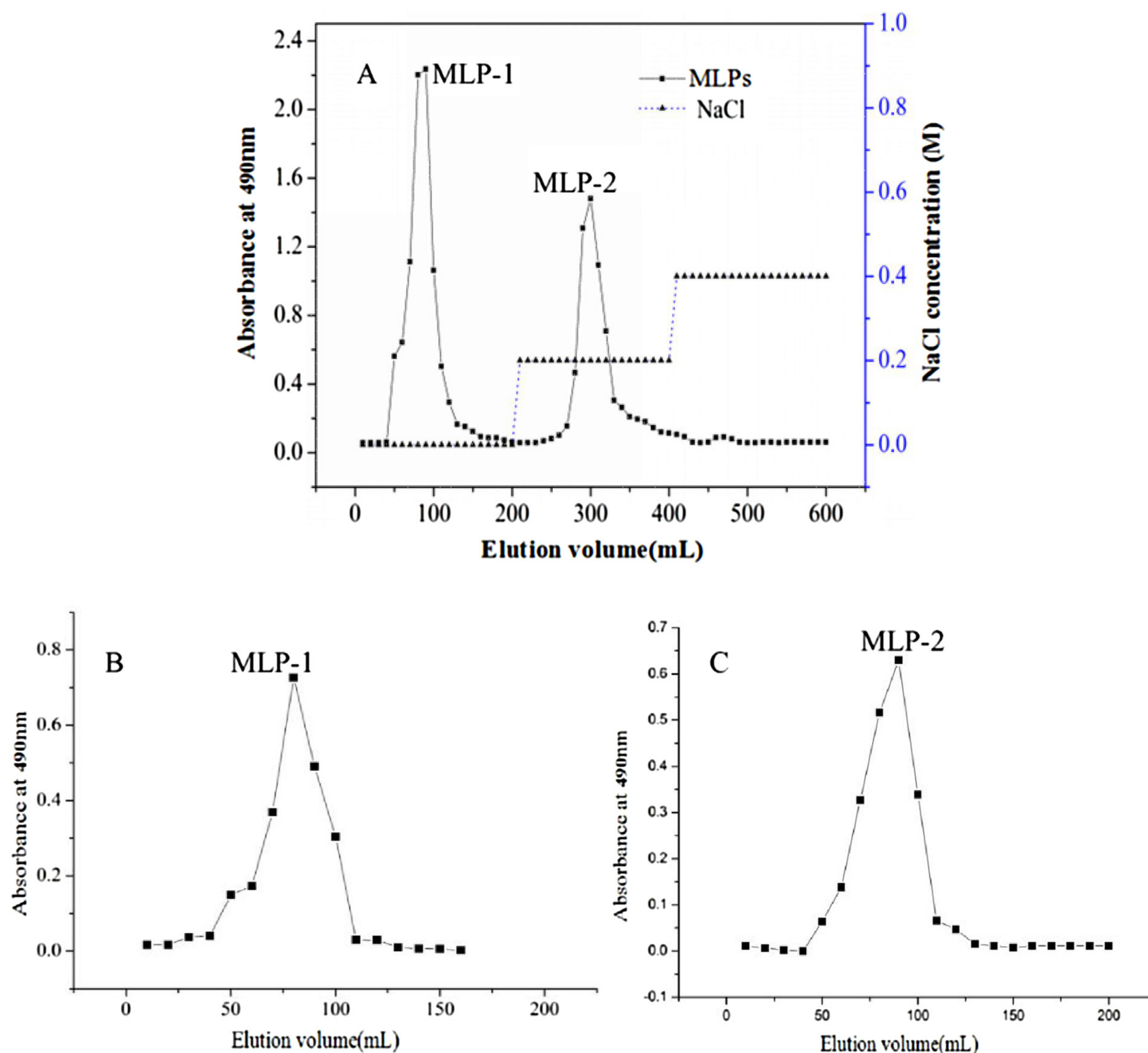

Fig. 1. Elution profiles of crude MLPs purified with DEAE-52 cellulose column (A) and gel filtration chromatography column by Sephadex G-200 (B, C).

3.3. FT-IR and UV spectra analysis of MLP-1 and MLP-2

The FT-IR spectra of the pure MLP-1 and MLP-2 fractions were shown in Fig. 4A, B, and the assignment of the most important IR bands was summarized in Table 2. It can be seen that a broad and strong band at 3445 cm^{-1} represented the O—H stretching vibration and the presence of hydrogen bonds [7,27]. The weak band at 2928 cm^{-1} in MLP-1 and 2926 cm^{-1} in MLP-2 attributed to the C—H stretching vibration of —CH and —CH_2 [7,15,16]. The absorption peak at 1629 cm^{-1} (MLP-1) and 1619 cm^{-1} (MLP-2) were due to the C=O stretching of the carbonyl group [16]. The peaks at 1395 cm^{-1} in MLP-1 and 1420 cm^{-1} in MLP-2 were distinctive of the C—O stretching vibration peak [28]. The absorption peaked at 1200–1010 cm^{-1} are attributed to the stretching vibrations of C—O—C and C—OH in the pyranose form [15,29]. A weak absorption bands at 868 and 573 cm^{-1} for MLP-1, as well as 863 and 570 cm^{-1} for MLP-2 indicated the presence of α-configuration in these two purified polysaccharides [30].

The UV spectra of MLP-1 and MLP-2 were shown in Fig. 4C, D. It was reported that the absorption peak within the 260–280 nm range is due to π–π^* electron transitions in aromatic and polyaromatic compounds present in most conjugated molecules such as proteins [31]. It can be seen that two kinds of pure polysaccharides had no absorption peaks between 260 and 280 nm, which indicated that the purified MLP-1 and MLP-2 contained no nucleic acid, protein, or polypeptide.

3.4. In vitro antioxidant activities

3.4.1. Scavenging ability on hydroxyl radicals

Hydroxyl radical is one of most harmful reactive oxygen species, which could cause cell death and tissue damage by reacting with lipids, proteins, and DNA [32]. The scavenging hydroxyl radical activities of MPL-1, MLP-2, Vitamin C and BHT were shown in Fig. 5A. The results indicated that all samples exhibited a dose dependent manner. At the concentration of 1.2 mg/mL, the scavenging effects of hydroxyl radical of MPL-1, MLP-2, Vitamin C and BHT were 82.71%, 79.07%, 99.68%, and 44.35%, respectively. The scavenging ability of MLP-1 was stronger than that of MLP-2 and BHT, but lower than Vitamin C at the range of 0.6–1.2 mg/mL. The EC_{50} (mg/mL) of MLP-1 and MLP-2 were 0.44 and 0.66, respectively. The result is in agreement with previous works that polysaccharides with low molecular weights have more reductive hydroxyl group terminals (on per unit mass basis) to accept and eliminate the free radicals [33,34]. It was reported that the polysaccharide with low molecular weight may have low viscosity of sample solu-

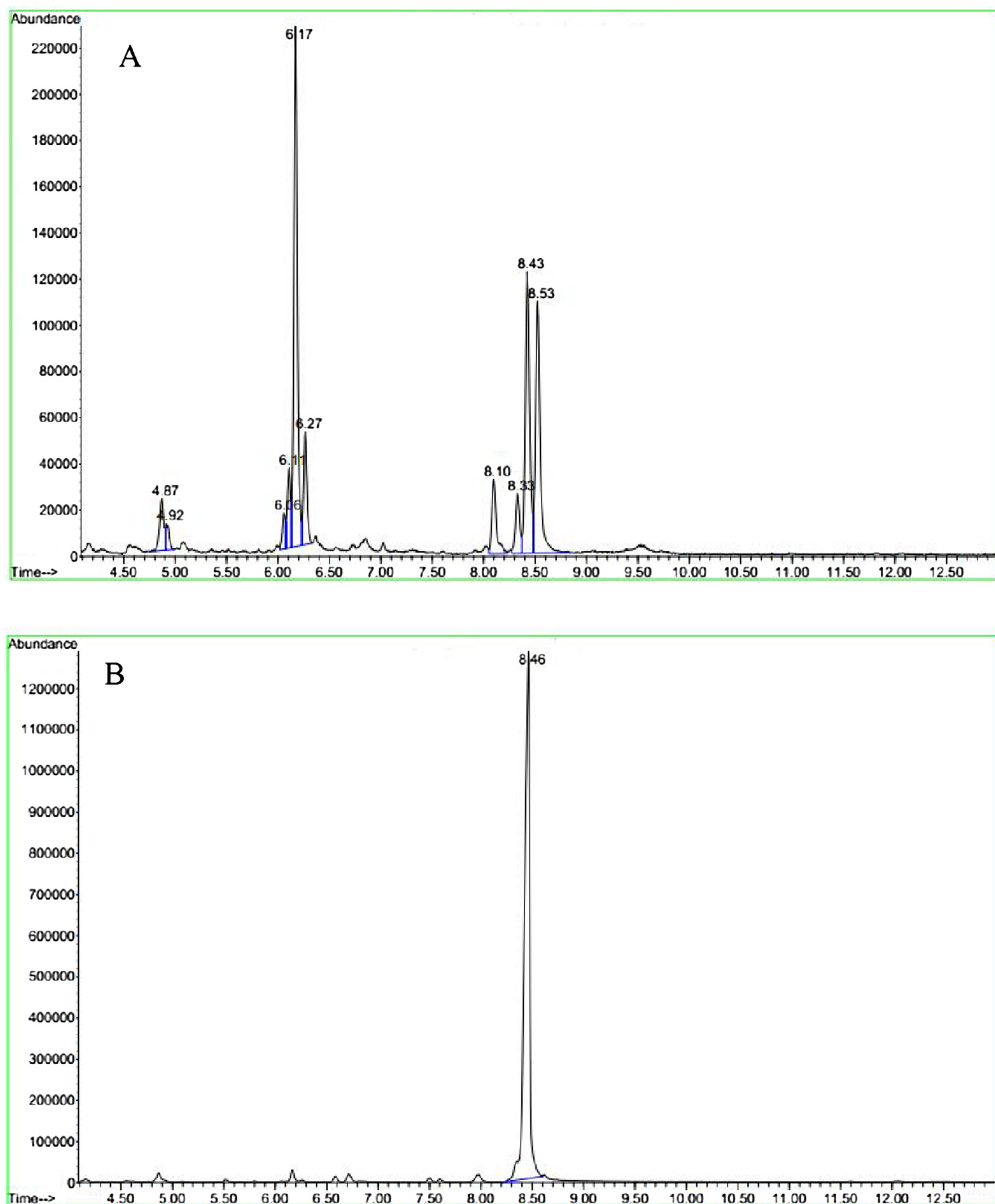

Fig. 2. GC chromatograms of hydrolysis and derivatives products of MLP-1(A) and MLP-2(B). Ribose (6.06), Rhamnose (6.11), Arabinose (6.17), Xylose (6.27), Mannose (8.36), Glucose (8.43(A); 8.46(B)) and Galactose (8.53).

tion, which could improve the quenching ability, thus increase the antioxidant activities [35,36]. Besides the molecular weight, the MLP-1 consists of relatively complex monosaccharide components (Rib, Rha, Ara, Xyl, Man, Glc, and Gal), which could promote the hydroxyl radical scavenging activity [37]. These results demonstrated that the molecular weights and chemical components of polysaccharides have great influence on their biological activities. Previous study [7] showed that the MLP-60 (polysac-

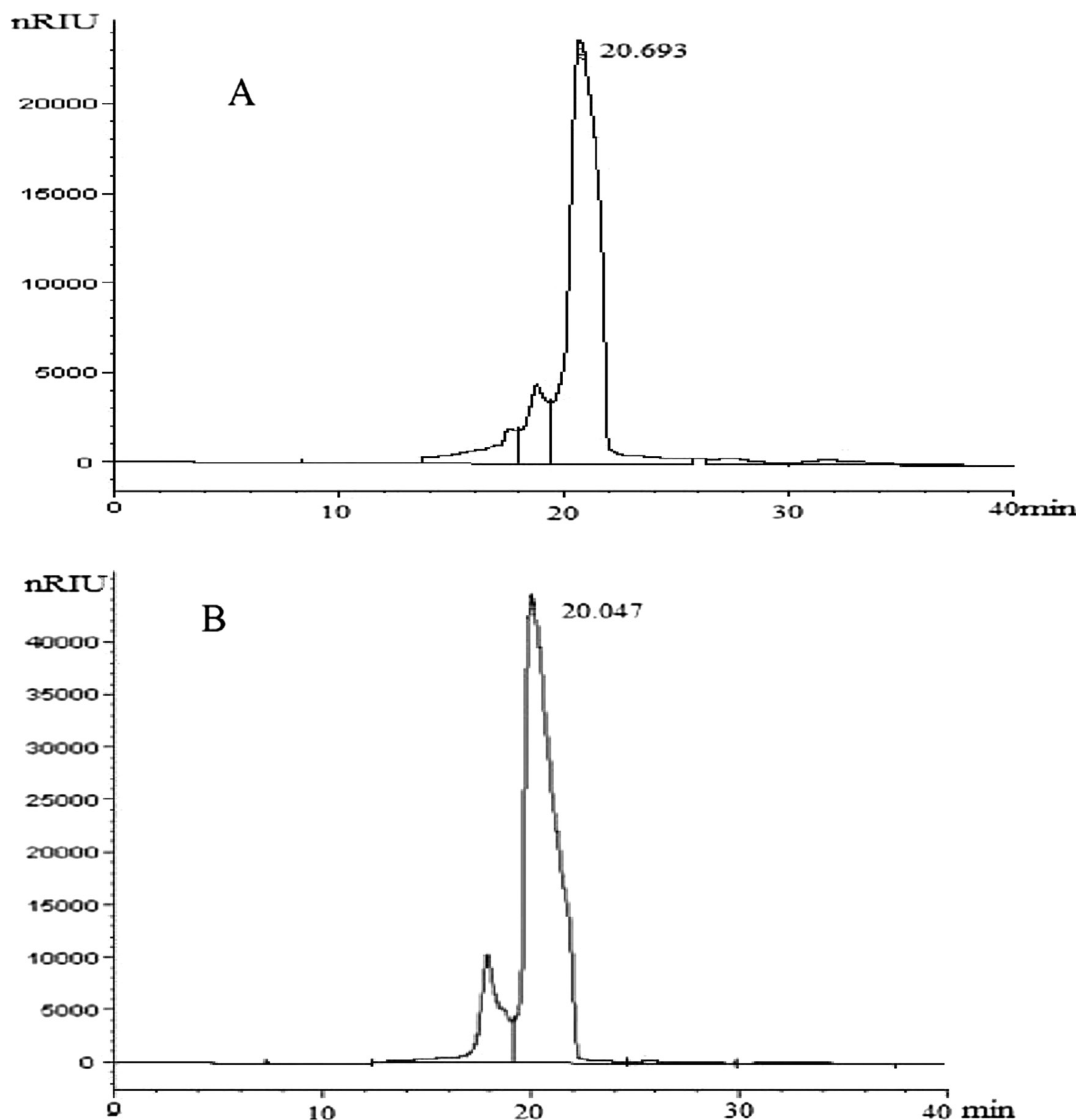

Fig. 3. HPLC chromatograms of MLP-1(A) and MLP-2(B).

Table 2
Assignment of the most important IR bands in MLP-1 and MLP-2.

Wavenumber (cm^{-1})	Assignment	References
3500-3200 (MLP-1, MLP-2)	The broad band assigned to the hydroxyl groups strestching vibration.	[19,28]
2928 (MLP-1), 2926 (MLP-2)	The C—H stretching vibration of —CH and —CH_2	[19,20,21]
1629 (MLP-1), 1619 (MLP-2)	The C=O stretching of the carbonyl group	[21]
1395 (MLP-1), 1420 (MLP-2)	The C—O stretching vibration peak	[29]
1200-1010 (MLP-1, MLP-2)	The stretching vibrations of C—O—C and C—OH in the pyranose form	[20,30]
868,573(MLP-1), 863, 570 (MLP-2)	The presence of α-configuration	[21,31]

charide obtained from maca hypocotyls) exhibited the highest hydroxyl radical scavenging ability (52.9%) at the concentration of 2.0 mg/mL, which was much less than that MLP-1 and MLP-2. It implies that the MLPs had a noticeable scavenging effect on hydroxyl radicals.

3.4.2. Scavenging ability on superoxide radicals

Superoxide radical, a highly harmful species, plays important roles in the formation of secondary radicals such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which would result in tissue damages [38]. The inhibitory effects of MPL-1, MLP-2, Vitamin C and BHT on superoxide radicals were shown in Fig. 5B. Two polysaccharide fractions also showed a dose-response relationship. The scavenging activity of MLP-1 and MLP-2 was 76.67% and 52.62% at the concentration of 1.2 mg/mL. The EC_{50} (mg/mL) of MLP-1 and MLP-2 were 0.21 and 1.01, respectively. Compared MLP-1 and MLP-2, MLP-1 had a higher scavenging effect, which is also related to its relatively low molecular weight and complex monosaccharide components [36,37]. It is known that bioactivities of polysaccharides are dependent on the monosaccharide components, molecular weight, glycoside bond types, microstructure, and configuration, etc. [37].

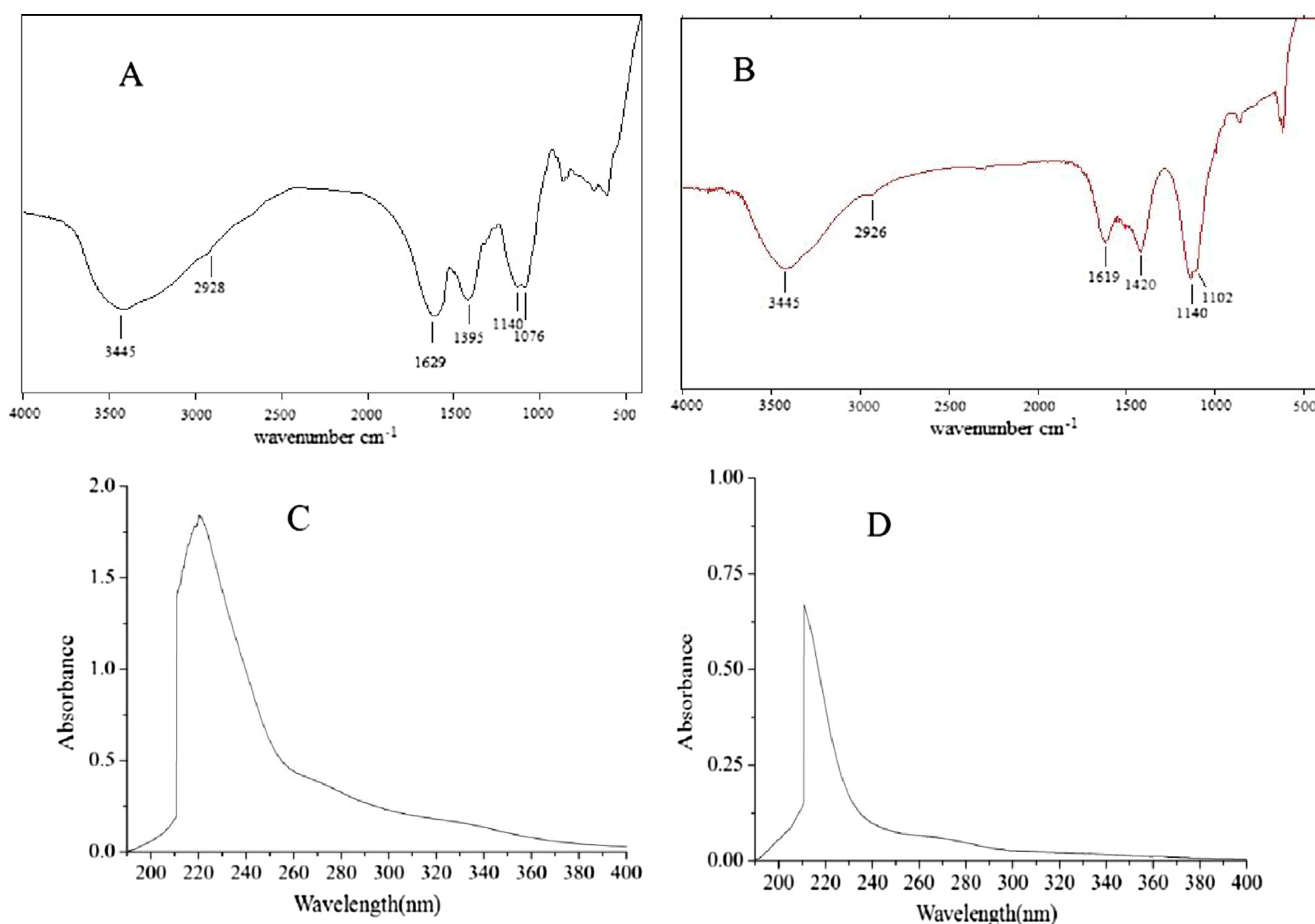

Fig. 4. The FT-IR (A, B) and UV (C, D) spectra of MLP-1 (A, C) and MLP-2 (B, D).

Previous studies on antioxidant activities revealed that the highest superoxide radical scavenging ability of maca root polysaccharides (LMP-60) was 85.8% when the concentration was 2.0 mg/mL[7], which was different from our result. The difference may be related to the fact that the LMP-60 was obtained by changing the concentration of ethanol in the process of polysaccharide precipitation. However, the MLP-1 exhibited higher superoxide anion radical scavenging activity than that of MP-1 (polysaccharide isolated from maca hypocotyls) [18]. Superoxide anion is considered as an initial free radical, which could produce other free radicals [38]. Our results suggest that the MLP-1 had a noticeable scavenging effect on superoxide anion radicals.

3.4.3. Scavenging ability on DPPH radicals

The DPPH radical scavenging activities of MPL-1, MLP-2, Vitamin C and BHT were shown in Fig. 5C. The DPPH radicals scavenging activities of MPL-1 and MLP-2 increased significantly ($P < 0.05$) with the increasing concentration. When the concentration approached to 1.2 mg/mL, the DPPH radical scavenging rate of MLP-1 and MLP-2 was 62.01% and 59.67%, which were lower than that of Vitamin C and BHT. The EC_{50} (mg/mL) of MLP-1 and MLP-2 were 0.82 and 1.11, respectively. The scavenging effect of MLP-1 was stronger than that of MLP-2. It might be because that the MLP-1 possesses lower molecular weight and more complex monosaccharide components than those of MLP-2. These results were in accordance with Luo et al. [39] saying that the low Mw polysaccharides were more effective in the antioxidant assay than high Mw polysaccharides. According to monosaccharides composition analysis, the MLP-1 mainly consists of Rib, Rha, Ara, Xyl, Man, Glc and Gal. These monosaccharides were supposed to relate to the strong antioxidant effects of polysaccharide [34,37].

Zha et al. [7] reported that the highest DPPH radical scavenging effect of maca root polysaccharides (LMP-80) is 29.5% at the concentration of 2.0 mg/mL, which was lower than that of MLP-1 (62.01%) and MLP-2 (59.67%). Compared with maca hypocotyl polysaccharides, the MLP-1 and MLP-2 extracted from maca leaves showed an obvious effect against DPPH radicals.

4. Conclusions

In this study, two polysaccharides (MLP-1 and MLP-2) were firstly isolated from leaves of maca (*Lepidium meyenii* Walp.) using DEAE-52 ion exchange resin and sephadex G-200 column. The MLP-1 was a kind of heteropolysaccharide composed of Rib, Rha, Ara, Xyl, Man, Glc and Gal, with the molar ratio of 0.12:0.32:1.50:0.32:1.03:1.00:0.93; the MLP-1 was a homopolysaccharide composed of glucose. The molecular weight (Mw) was 42756 Da, and the Mw of MLP-2 was 93541 Da. The FT-IR analysis revealed the general characteristic absorption peaks of polysaccharides. The antioxidant activity assay indicated that two polysaccharides possessed significant inhibitory effects on hydroxyl, superoxide anion, and DPPH radicals. The MLP-1 exhibited an excellent antioxidant activity, which was superior to MLP-2. The results of these assays will have a significant impact on future applications for exploring novel natural antioxidants or healthcare food. However, further investigation should be focused on

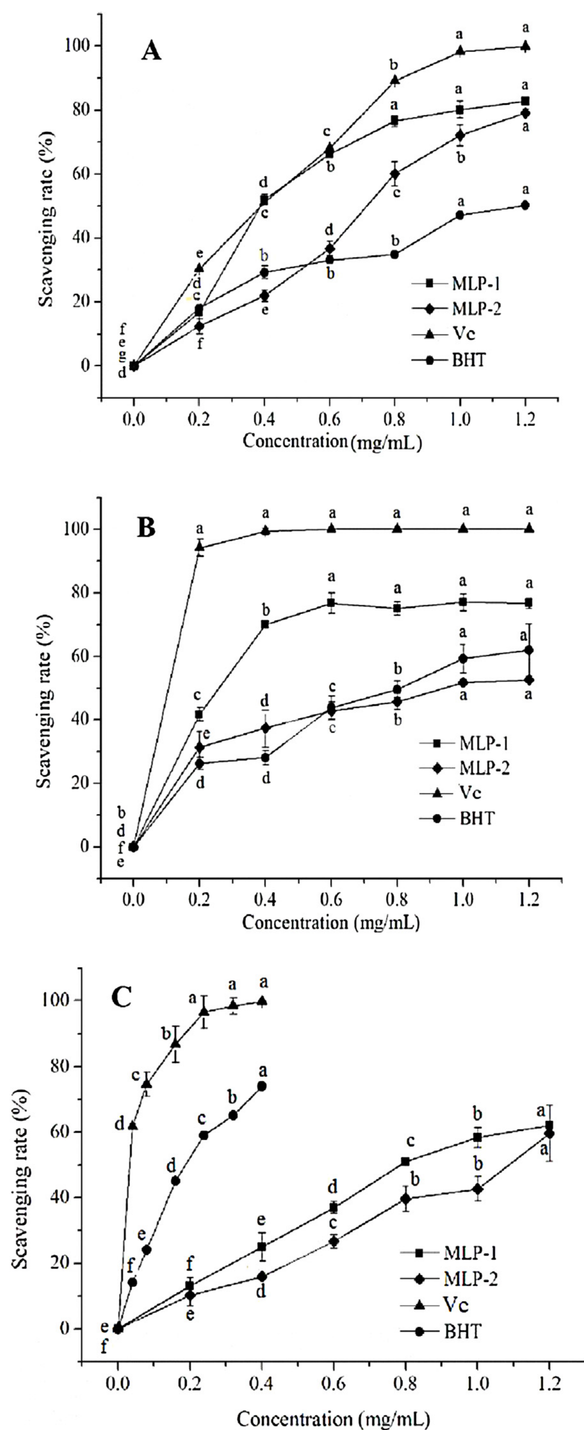

Fig. 5. The scavenging effects of MLP-1, MLP-2, Vc, and BHT on hydroxyl free radical (A); superoxide anion radical (O^{2-}•) (B); DPPH free radical (C). Different lowercase letters in the same curve indicate significant differences ($p < 0.05$). Date shown in mean ± standard deviation ($n = 3$).

more details on the structure of MLP-1 and MLP-2, the relationship between the structure and antioxidant activity, and antioxidant activity *in vivo*.

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