Nutritional Composition and Antioxidant Properties of the Fruit of Berberis heteropoda Schrenk

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Keywords: Berberis heteropoda Schrenk; nutritional composition; antioxidant properties; phenol; flavonoid

Abstract:
Objective
This study aimed to assess the major nutrients and antioxidant properties of Berberis heteropoda Schrenk fruits collected from the Nanshan Mountain area of Urumqi City, Xinjiang Uygur Autonomous Region, China.

Methods and Materials
Basic nutrients including amino acids, minerals, and fatty acids were assessed, and the total phenol, flavonoid, and anthocyanin contents of the extracts were determined.

Results
The analytical results showed that average water, total fat, total protein, ash, and carbohydrates contents in Berberis heteropoda Schrenk fruits were 75.22, 0.506, 2.55, 1.31, and 17.72 g 100 g fresh fruit, respectively. The total phenol, flavonoid, and anthocyanin contents of Berberis heteropoda Schrenk fruits were 68.55 mg gallic acid equivalents g, 108.42 mg quercetin equivalents g, and 19.83 mg cyanidin-3-glucoside equivalents g, respectively. The UPLC-Q-TOF-MSE analysis of phenols revealed 32 compounds.

Conclusions
The findings suggest Berberis heteropoda Schrenk fruits may have potential nutraceutical value and can be considered a potential source of nutritional components and antioxidant phytochemicals in the human diet.

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Nutritional Composition and Antioxidant Properties of the Fruit of *Berberis heteropoda* Schrenk

Short title: Nutrition and antioxide of *Berberis heteropoda* Schrenk

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ABSTRACT

Objective: This study aimed to assess the major nutrients and antioxidant properties of Berberis heteropoda Schrenk fruits collected from the Nanshan Mountain area of Urumqi City, Xinjiang Uygur Autonomous Region, China.

Methods and Materials: Basic nutrients including amino acids, minerals, and fatty acids were assessed, and the total phenol, flavonoid, and anthocyanin contents of the extracts were determined.

Results: The analytical results showed that average water, total fat, total protein, ash, and carbohydrates contents in Berberis heteropoda Schrenk fruits were 75.22, 0.506, 2.55, 1.31, and 17.72 g/100 g fresh fruit, respectively. The total phenol, flavonoid, and anthocyanin contents of Berberis heteropoda Schrenk fruits were 68.55 mg gallic acid equivalents, 108.42 mg quercetin equivalents, and 19.83 mg cyanidin-3-glucoside equivalents, respectively. The UPLC-Q-TOF-MSE analysis of phenols revealed 32 compounds.

Conclusions: The findings suggest Berberis heteropoda Schrenk fruits may have potential nutraceutical value and can be considered a potential source of nutritional components and antioxidant phytochemicals in the human diet.

Keywords: Berberis heteropoda Schrenk; nutritional composition; antioxidant properties; phenol; flavonoid

Introduction

Berberis heteropoda Schrenk is a shrub of the family Berberidaceae and is distributed in the Altai, Tianshan, and Baluke mountains of the Xinjiang Uygur Autonomous Region, China, as
well as in Mongolia and Kazakhstan [1]. The roots, bark, stems, and fruits of *Berberis heteropoda* Schrenk are traditionally used as herbal medicine, and the fruits in particular have historically been consumed as tea [2,3]. Studies have confirmed traditional beliefs, suggesting that this fruit can be used to treat dysentery, enteritis, pharyngitis, stomatitis, eczema, and hypertension [4,5]. The nutritional and antioxidant properties of *Berberis heteropoda* Schrenk fruit are significantly related to the distribution of molecular and secondary metabolites, which should be understood to obtain benefits from its consumption.

In addition to protein, fat, dietary fiber, minerals, and other nutrients, plants also contain numerous phenolic components that play an important role in human health [6]. Polyphenols are secondary metabolites produced by plants during growth and are widely found in vegetables, fruits, forages, and medicinal plants [7]. Phenolic compounds are effective in preventing oxidation at the cellular and physiological levels, and their antioxidant capacity is determined based on the arrangement of hydroxyl and carbonyl groups in their structures, as well as the gain and loss of electrons from hydrogen atoms to reduce free radicals and form stable phenoxy groups [8,9]. Flavonoids are the major components of plant polyphenols, which with high redox plays an important role in antioxidant effects, including reduction, hydrogen donor, singlet oxygen quenching, and metal chelation. Thus, evaluating the content of polyphenols and flavonoids and antioxidant capacity of *Berberis heteropoda* Schrenk fruit and developing its medicinal and nutritional value are important.

While recent studies have focused on the anthocyanin composition of *Berberis heteropoda* Schrenk fruit [3], the nutritional composition and phenolic components remain less understood. Therefore, we conducted this study to assess the major nutrients and their
antioxidant properties of *Berberis heteropoda* Schrenk, providing additional information regarding the nutritional roles and health benefits of consuming these fruits.

### Materials and Methods

#### Plant Material

Mature *Berberis heteropoda* Schrenk fruits were collected from shrubs in the Nanshan Mountain area of Urumqi City, Xinjiang Uygur Autonomous Region, China (latitude 89°29′36″E, longitude 43°27′32″N), TH in September 2019. Fig. 1 shows its distribution in Xinjiang. The specimens were identified by expert Lude Xin from Xinjiang Medical University. A voucher specimen was deposited in the Institute of Clinical Nutrition, People's Hospital of Xinjiang Autonomous Region. High-quality *Berberis heteropoda* Schrenk fruit were selected, and stem and seeds were removed. The fruits were transported back to the laboratory for a pre-cooling treatment 2 h after harvest. Fruits with uniform size and maturity without mechanical damage were selected for dark storage at -20°C until further analysis.

![Fig. 1. Berberis heteropoda Schrenk shrub plant and distribution in Xinjiang.](image)

#### Standards and Reagents

The reagents 1,1-diphenyl-2-picryl-hydrazl (DPPH) and 2,2-azinobis-(3-ethylbenzthiazoline-6- sulfonic acid) (ABTS) were purchased from Shanghai Macklin Biochemical Co., Ltd. Gallic acid and rutin standards were purchased from Chengdu Munster Biotechnology Co., Ltd. Folin–Ciocalteu’s phenol reagent was purchased from Tianjin Kaitong Chemical Reagent Co., Ltd. Anhydrous methanol, anhydrous ethanol,
concentrated hydrochloric acid, sodium nitrite, sodium hydroxide, sodium carbonate, and ferrous sulfate were obtained from Sinopharm Chemical Reagent Co., Ltd.

**Nutritional Composition**

**Determination of general nutrients**

The crude protein content was determined using the Kjeldahl method according to Chinese National Standard (CNS) GB/T5009.5-2016 “Determination of protein in food.” The ash content was measured using muffle furnace burning method according to CNS GB 5009.4-2016 “Determination of ash in food.” The crude fat was determined using the Soxhlet extraction method according to CNS GB 5009.6-20163 “Determination of fat in food.” The moisture was measured using the direct drying method according to CNS GB5009.3-2016 “Determination of moisture in food.” The carbohydrate content was determined based on CNS NY/T 2332-2013. The total energy of each sample was calculated as follows: Total Energy (kJ) = 17 × (g crude protein + g total carbohydrate) + 37 (g crude fat) [10].

**Mineral composition**

The mineral and element contents were determined according to CNS GB5009.268-2016 “Determination of multi-elements in food,” using inductively-coupled plasma-mass spectrometry (Agilent 5110 ICP-OES). Briefly, 1.0 g slurry sample was digested in 2 mL concentrated HNO₃ in a microwave oven and then diluted with distilled water to 25 mL. The solution was filtered before storage, and a blank digest was performed in a similar manner. The blank solution and the test solution were each injected into the ICP-OES, and the contents of K, Ca, Na, Mg, Fe, Cu, Zn, and P were determined.

**Amino acid analysis**
The amino acid contents were measured by an automatic amino acid analyzer (L-8900, Hitachi, Japan) according to the Chinese National Standard CNS GB 5009.124-2016 “Determination of amino acid in food.” Continuous flash evaporation at reduced pressure was used to remove excess acid, and the sample was dissolved in citrate buffer (pH 2.2) [11].

**Fatty acids**

The composition and content of fatty acids were determined by gas-chromatography mass spectroscopy (7890B/7000D Gas Chromatography-Mass Spectrometer, Agilent, USA) according to CNS GB 5009.168-2016. The triglyceride undecarbonate was applied as internal standard, and 37 different fatty acid methylester standard solutions were used for the external standard. The fatty acid content was quantitatively measured using the chromatographic peak.

**Extraction and Quantification of Total Phenol Content (TPC), Total Flavonoid Content (TFC) and Total Anthocyanin Content (TAC) Content**

**Extraction**

The samples were extracted using a modification method [12]. Briefly, 1.0 g stirred Berberis heteropoda Schrenk fruit was obtained, 30 mL 70% acidified ethanol (0.1% v/v HCl) was added, and the solution was extracted three times under ultrasonic (40 kHz, 100 W) treatment for 30 min at 25 °C. Then, the mixture was centrifuged at 1000 r/min for 15 min, and the supernatant was collected. The residue was extracted twice, and all the collected supernatant was mixed, and then concentrated under vacuum. The extraction was preserved at -20°C until further analysis. This final extract was used for the quantification of TPC, TFC, TAC, and antioxidant activity.

**Determination of TPC**
The TPC was measured using Folin–Ciocalteu’s phenol reagent with the colorimetric method [13]. Briefly, 0.5 mL of reagent and 1.5 mL of sodium carbonate solution (10% w/v) were added to 1 mL of *Berberis heteropoda* Schrenk fruit extract. Then, 8 mL pure water was immediately added, and the mixture was left for 10 min in a water bath at 75 °C. Afterward, an ultraviolet–visible (UV–vis) spectrophotometer (PERSEE New century T6, China) was used to detect the absorbance at 760 nm. The standard curve of the absorbance value of gallic acid concentration solution was measured. TPC was denoted as milligram of gallic acid equivalent per gram of plant mass.

**Determination of TFC**

The TFC was measured using rutin as a reference standard with the aluminum nitrate method [14]. Briefly, 0.5 mL of *Berberis heteropoda* Schrenk fruit extract was added to 1 mL of sodium nitrite, left to stand for 6 min, mixed with 1 mL of 10% aluminum nitrate, and then left to stand again for 6 min. Afterward, 10 mL of 1.0 mol/L sodium hydroxide was added, the volume of water was fixed to 20 mL, and the solution was placed for 15 min. UV–vis spectrometry was used to detect the absorbance at 510 nm, and the standard curve of absorbance was determined. TFC was denoted as milligram of rutin equivalent per gram of weight of plant.

**Determination of TAC**

The TAC was determined by the pH differential method [15]. Briefly, 2 mL of fruit extract was added to the centrifuge tube and centrifuged at 1000 r/min for 5 min. Then, 0.5 mL supernatant was added into two equal 10 mL volumetric flasks: one with a pH 1.0 buffer, and the other with a pH 4.5 buffer. The absorbance at 517 nm and 700 nm was measured after 15
min balance. The data were expressed as milligram of cyanidin-3-glycoside equivalents per gram of fresh fruit weight. The TAC was calculated according to the following formula:

\[
A = \left[ (A_{517} - A_{700})_{pH1.0} - (A_{517} - A_{700})_{pH4.5} \right], \quad \text{BHSTAC (mg/g)} = A \times \text{MW} \times \text{DF} \times \frac{1}{\varepsilon \times L \times \frac{V}{M}},
\]

where MW represents the molecular weight of centrinin-3-glycoside (449.2 g/mol), centrothrin-3-glycoside molar extinction coefficient (26900 L·cm⁻¹·mol⁻¹), DF represents diluted multiples, L denotes absorption pool thickness (1 cm), V represents extraction volume (mL), and M denotes the weight of peel powder.

**Measurements of Antioxidant Capacity**

**DPPH free radical assay**

The DPPH free radical scavenging assay was carried out according to the method described by Vlase et al [16]. Briefly, *Berberis heteropoda* Schrenk fruit extract was dissolved in 70% ethanol at different concentrations and mixed with 2 mL of a freshly prepared ethanol solution of DPPH free radicals (100 μmol/L). The solution was mixed vigorously and stored in darkness at room temperature for 30 min, and UV–vis spectrometry was used to detect the absorbance at 517 nm. The positive control group was measured with Vitamin C (V₃). The results were expressed as half maximal inhibitory concentration (IC₅₀), which was used to indicate the corresponding concentration of the extract when the antioxygenation free radical scavenging capacity was 50%.

\[
\text{DPPH free radical scavenging rate} = \left( 1 - \frac{A_s - A_0}{A_c} \right) \times 100\%.
\]

where \(A_c\) denotes absorbance value of the control, \(A_0\) represents absorbance value of the blank, and \(A_s\) is the absorbance value of the sample.

**ABTS free radical assay**
The ABTS free radical scavenging assay was carried in accordance with the method described by Lyu et al [17]. Briefly, 2 mL of 10 mmol/L potassium persulfate solution and 2 mL of 10 mmol/L ABTS free radical solution were mixed and then stored in the dark for 12 h. Ethanol was added to the mixed solution until its UV–vis absorbance value reached 0.700 ± 0.020 at 736 nm. Subsequently, 2 mL of *Berberis heteropoda* Schrenk fruit extract or ascorbic acid solution was mixed vigorously with 2 mL of ABTS working solution and stored in the dark at room temperature for 10 min. The IC₅₀ values of the sample extract were calculated based on the concentration and capacity of free radical scavenging curves.

\[
\text{ABTS free radical scavenging rate} = \frac{A_c - A_s}{A_c} \times 100\%
\]

where \(A_c\) represents absorbance value of the control and \(A_s\) denotes absorbance value of the sample.

**Hydroxyl free radical assay**

The hydroxyl free radical assay was carried out in accordance with the method described by Liang et al [18]. Briefly, 0.5 mL of 7.5 mmol/L ferrous sulfate heptahydrate, 0.5 mL of 7.5 mmol/L salicylic acid, 1 mL of *Berberis heteropoda* Schrenk fruit extract, and 0.2 mL of 30% hydrogen peroxide were mixed and left for 30 min in a water bath at 37 °C. After cooling, the absorbances of the sample, blank, and control groups were determined at 510 nm on the UV–vis spectrometer.

\[
\text{HRSA(\%) = } \left( \frac{A_s - A_c}{A_0 - A_c} \right) \times 100\%;
\]

where \(A_c\) denotes absorbance value of the control, \(A_0\) represents absorbance value of the blank, and \(A_s\) represents absorbance value of the sample.

**Superoxide anion free radical assay**
The superoxide anion free radical assay was carried out in accordance with the method described by Liu et al [15]. Briefly, 4.5 mL of 50 mmol/L Tris-Hydrochloric acid and 1 mL of *Berberis heteropoda* Schrenk fruit extract were mixed and left for 15 min in a water bath at 25 °C. Afterward, 0.4 mL of 5 mmol/L pyrogallic acid was added and left for 5 min in a water bath at 25 °C. Subsequently, 0.1 mL of 8 mol/L hydrochloric acid was added to terminate the reaction, and the absorbance values of the sample, blank, and control were measured at 325 nm on the UV–vis spectrometer.

\[
\text{Superoxide anion scavenging rate} = \frac{A_c - A_s}{A_s} \times 100\% 
\]

where Ac denotes absorbance value of the control, and As represents absorbance value of the sample.

**Chromatography and Mass Spectrometry**

**Chromatographic conditions**

Chromatographic separations were performed on an Agilent ultra-high performance liquid chromatography 1290 UPLC system with a Waters UPLC BEH C18 column (1.7 μm 2.1 × 100 mm). The flow rate was set to 0.4 mL/min, and the sample injection volume was set to 5 μL. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The multi-step linear elution gradient program was as follows: 0–3.5 min, 95–85% A; 3.5–6 min, 85–70% A; 6–6.5 min, 70–70% A; 6.5–12 min, 70–30% A; 12–12.5 min, 30–30% A; 12.5–18 min, 30–0% A; 18–25 min, 0–0% A; 25–26 min, 0–95% A; 26–30 min, 95–95% A.

**Mass spectrometry (MS) conditions**
An Q Exactive Focus mass spectrometer coupled with Xcalibur software was employed to obtain the MS and MS/MS data based on the IDA acquisition mode. During each acquisition cycle, the mass range was set from 100 to 1500, the top three of every cycle were screened, and the corresponding MS/MS data were further acquired. Sheath gas flow rate: 45 Arb; Aux gas flow rate: 15 Arb; Capillary temperature: 400 °C, Full MS resolution: 70000; MS/MS resolution: 17500; Collision energy: 15/30/45 in NCE mode; Spray Voltage: 4.0 kV (positive) or -3.6 kV (negative).

**Statistical Analysis**

All experimental data were collected in triplicate, and the data were expressed as mean with standard deviation. Statistical analysis of the data was carried out in Graphpad Prism v7.0 (Graphpad Software, Inc.) and SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Nutritional Composition of the Berberis heteropoda Schrenk Fruit**

Proximate compositions of *Berberis heteropoda Schrenk* fruit

The major nutrients of *Berberis heteropoda Schrenk* fruit are summarized in Table 1. The major components were identified as water, crude fiber, and total protein. Water content was the highest (75.22±1.75 g/100 g), followed by crude fiber (17.72±0.52 g/100 g) and protein (2.55±0.03 g/100 g) contents. The ash content was 1.31±0.04 g/100 g, which indicates that the fruit is rich in minerals. The contents of total sugar and total fat were 0.05±0.00 and 0.51±0.02 g/100 g, respectively. The energy content per 100 g fruit was 363.52 kJ.

**Table 1.** Proximate compositions of *Berberis Heteropoda* Shrenk fruit
| Compositions (Unit)                           | Data        |
|---------------------------------------------|-------------|
| Water (g/100 g fresh fruit)                 | 75.22±1.75  |
| Total fat (g/100 g fresh fruit)             | 0.506±0.02  |
| Total Protein (g/100 g fresh fruit)         | 2.55±0.03   |
| Ash (g/100 g fresh fruit)                   | 1.31±0.04   |
| Total sugars (g/100 g fresh fruit)          | 0.05±0.00   |
| Carbohydrates (g/100 g fresh weight)        | 17.72±0.52  |
| Total Energy (kJ)                           | 363.52±7.51 |

**Minerals**

A total of eight mineral elements were detected in *Berberis heteropoda* Schrenk fruit and are summarized in Table 2. Potassium (582.67±8.02 mg/100 g) was the most abundant element [19]. Calcium (78.5±1.62 mg/100 g), phosphorus (73.24±1.72 mg/100 g), and magnesium (30.61±0.56 mg/100 g) were also abundant.

**Table 2.** Nutritional composition (minerals, amino acids) of *Berberis Heteropoda* Shrenk fruit

| Minerals | mg Per 100 g of FW | Percentage of Total Minerals (%) |
|----------|--------------------|---------------------------------|
| Na       | 1.38±0.03          | 0.18                            |
| K        | 582.67±8.02        | 75.73                           |
| Ca       | 78.5±1.62          | 10.20                           |
| Cu       | 0.27±0.01          | 0.04                            |
| Zn       | 0.59±0.01          | 0.08                            |
| Fe       | 2.31±0.05          | 0.30                            |
| Amino Acids    | g Per 100 g of FW | Percentage of Total Amino Acids (%) |
|---------------|------------------|-----------------------------------|
| Phenylalanine | 0.12±0.01        | 4.24                              |
| Alanine       | 0.17±0.01        | 6.01                              |
| Methionine    | 0.015±0.00       | 0.53                              |
| proline       | 0.2±0.02         | 7.07                              |
| glycine       | 0.21±0.01        | 7.43                              |
| Glutamic acid | 0.53±0.01        | 18.74                             |
| arginine      | 0.22±0.01        | 7.78                              |
| lysine        | 0.21±0.00        | 7.43                              |
| tyrosine      | 0.11±0.00        | 3.89                              |
| Leucine       | 0.18±0.01        | 6.36                              |
| Serine        | 0.13±0.01        | 4.60                              |
| threonine     | 0.13±0.01        | 4.60                              |
| Aspartic acid | 0.27±0.01        | 9.55                              |
| valine        | 0.15±0.01        | 5.30                              |
| histidine     | 0.073±0.00       | 2.58                              |
| isoleucine    | 0.11±0.01        | 3.89                              |
| Total amino acids | 2.828          |                                   |
The details of 16 identified amino acids in *Berberis heteropoda* Schrenk fruit are shown in Table 2. Glutamic acid was the most abundant, and the contents of aspartic acid, arginine, lysine, and glycine were also abundant. It contained 6 types of essential amino acids (EAAs), and the content of essential amino acids was 0.9 g/100 g, accounting for 31.8% of the total amino acids. The content of remaining ten non-EAAs (NEAAs) was 1.93 g/100 g.

**Fatty acids**

The content of fatty acid in *Berberis heteropoda* Schrenk fruit is presented in Table 3. A total of 10 different fatty acids were determined, including saturated and unsaturated varieties. Tetrahexanoic acid (C24:0) was found to be the dominant fatty acid, followed by octadecatrienoic acid (C18:3) and octadecadienoic acid (C18:2). Unsaturated fatty acid content (UFA, 51.52%) was slightly higher than that of saturated fatty acids (SFA, 48.48%).

**Table 3. Fatty Acid Content in Breberis Heteropoda Shrenk fruit**

| Fatty acids                      | Formula   | g/100 g fatty acid | Proportion (%) |
|----------------------------------|-----------|--------------------|----------------|
| Myristic acid (C14:0)            | C14H28O2  | 0.0039             | 1.41           |
| 2-methyl-heptanoic acid (C8:0)   | C8H16O2   | 0.0019             | 0.71           |
| Hexadecanoic acid (C16:0)        | C16H32O2  | 0.0285             | 10.35          |
| Stearyl acid (C18:0)             | C18H36O2  | 0.0041             | 1.50           |
| Octadecenoic acid (C18:1)*       | C18H32O2  | 0.0263             | 9.55           |
| Octadecadienoic acid (C18:2)*    | C18H32O2  | 0.0526             | 19.11          |
| Octadecatrienoic acid (C18:3)*   | C18H30O2  | 0.0630             | 22.86          |
| Arachidic acid (C20:0)           | C20H40O2  | 0.0038             | 1.38           |
| Fatty Acid                  | Chemical Structure          | Concentration (mg/g) |
|----------------------------|----------------------------|----------------------|
| Docosanoic acid (C22:0)    | CH₃(CH2)₂₀-COOH             | 0.0112               |
| Tetrahexanoic acid (C24:0) | CH₃(CH2)₂₂-COOH             | 0.0801               |
| **Subtotal**               |                            | 0.2754               |

Note: *unsaturated fatty acids

**TPC, TFC, TAC, and Antioxidant Activity of Berberis heteropoda Schrenk Fruit Extract**

The TPC, TFC, and TAC values in *Berberis heteropoda* Schrenk fruit are shown in Fig. 2. The determination methods of flavonoids and polyphenols exhibited a good linear relationship within the measurement range \((r^2 = 0.995; r^2 = 0.999)\). The regression equations used were \(y = 0.0109x + 0.0157\) and \(y = 0.067x - 0.0173\). Compared with the extraction effect of different solvents, the extraction effect of solvent from high to low is methanol > acetone > ethanol for total phenol, total flavonoids, and total anthocyanins. Using methanol as extraction solvent, the TFC, TPC, and TAC values were 108.42, 68.55, and 19.83 mg/g fruit, respectively. Evidently, with methanol as the extraction solvent, total flavonoids and total phenol can obtain a higher extraction rate.

**Fig. 2.** Quantification of TPC (A), TFC (B), TAC (C) of *Berberis heteropoda* Schrenk shrub extract.

The antioxidant activity of the *Berberis heteropoda* Schrenk fruit extracts was evaluated using Vc as the control, and the IC₅₀ value of DPPH free radical, ABTS, •OH, O₂•- scavenging ability were 20.27±0.26 µg/mL, 13.89±0.13 mg/mL, 5.81±0.13 mg/mL, and 0.57±0.02 mg/mL, respectively (Fig. 3). We observed that methanol extract had the best antioxidant activity, and the IC₅₀ of DPPH radical, hydroxyl radical, ABTS radical, and superoxide anion radical were 20.13 µg/mL, 5.44 mg/mL, 8.79 µg/mL, and 1.35 mg/mL, respectively. The IC₅₀ values of methanol extraction were higher than those of vitamin C but lower than those of
ethanol and acetone extraction. Methanol, acetone, and ethanol extracts were ranked from high to low in the order of free radical scavenging ability, and *Berberis heteropoda* Schrenk fruit extracts had good antioxidant activity and could scavenge free radicals effectively.

Fig. 3. IC<sub>50</sub> value (mg/mL) of different extracts on radicals (A: DPPH radical; B: ABTS radical; C: hydroxyl radical; D: superoxide anion radical).

**Identification of Phenols in Berberis heteropoda Schrenk Fruit Extract**

The UPLC-QTOF-MS spectra indicated that the compounds in the extract of *Berberis heteropoda* Schrenk fruit were primarily concentrated within 2–10 min, when the mobile phase was 15%–70% ethyl alcohol solution, indicating that the polyphenols of *Berberis heteropoda* Schrenk fruit belonged to polar compounds (Table 4; Fig. 4). The details of 32 kinds of compounds are listed as follows: Compounds 1 (t<sub>R</sub>=1.52 min), 2 (t<sub>R</sub>=2.96 min), and 3 (t<sub>R</sub>=3.67 min) with fragment [M-H]<sup>-</sup> ions at m/z 633.0787 [12], 477.1030 [20], and 289.0719 [21]. Compounds 4 (t<sub>R</sub>=4.53 min), 5 (t<sub>R</sub>=5.03 min), and 6 (t<sub>R</sub>=5.50 min) with fragment [M-H]<sup>-</sup> ions at m/z 479.0835 [22], 507.1144 [23], and 479.0834 [24]. Compounds 7 (t<sub>R</sub>=5.80 min), 8 (t<sub>R</sub>=5.84 min), and 9 (t<sub>R</sub>=5.85 min) with fragment [M-H]<sup>-</sup> ions at m/z 447.0926, 507.1143, and 449.1095 [24,25]. Compounds 10 (t<sub>R</sub>=5.89 min), 11 (t<sub>R</sub>=5.91 min), and 12 (t<sub>R</sub>=6.02 min) with fragment [M-H]<sup>-</sup> ions at m/z 285.0393, 463.0892, and 317.0300 [10,24]. Compounds 13 (t<sub>R</sub>=6.14 min), 14 (t<sub>R</sub>=6.20 min), and 15 (t<sub>R</sub>=6.73 min) with fragment [M-H]<sup>-</sup> ions at m/z 347.0927 [24], 319.0458 [26], and 301.0357 [27]. Compounds 16 (t<sub>R</sub>=6.97 min), 17 (t<sub>R</sub>=7.97 min), and 18 (t<sub>R</sub>=8.04 min) with fragment [M-H]<sup>-</sup> ions at m/z 301.0358, 285.0408, and 299.0556 [28,29]. Compounds 19 (t<sub>R</sub>=8.19 min), 20 (t<sub>R</sub>=9.56 min), and 21 (t<sub>R</sub>=4.52 min) with fragment [M-H]<sup>-</sup> ions at m/z 315.0507 [21], 269.0455 [29] and fragment [M+H]<sup>+</sup> ions at m/z 271.0588 [30]. Compounds 22 (t<sub>R</sub>=4.75 min), 23 (t<sub>R</sub>=4.85 min), and 24 (t<sub>R</sub>=5.05 min) with fragment [M+H]<sup>+</sup> ions at m/z 285.1122 [31], 291.0858 [32], and 303.0478 [33]. Compounds 25 (t<sub>R</sub>=5.08 min), 26
(t_R=5.55 min), and 27 (t_R = 7.25 min) with fragment [M+H]^+ ions at m/z 305.0650, 333.0602, and 435.127884 [24,34]. Compounds 28 (t_R=7.29 min), 29 (t_R=7.75 min), and 30 (t_R=8.22 min) with fragment [M+H]^+ ions at m/z 437.1445 [35], 437.1445 [36], and 437.1445 [36]. Finally, compounds 31 (t_R=9.92 min) and 32 (t_R=14.88 min) with fragment [M+H]^+ ions at m/z 437.1445 and 595.1658 [10,38].

Fig. 4. Representative total ion chromatogram of extract sample obtained from Berberis heteropoda Schrenk fruit in positive mode (A) and in negative mode (B) by UHPLC-ESI-Q-TOF/MS.

Table 4. Characterisation of phenolic compounds of Berberis Heteropoda Shrenk fruit by UPLC-Q-TOF-MSE.

| Compound | t_R/min | Ionisation mode | Identification | Molecular formula | MS(m/z) | MS^2(m/z) |
|----------|---------|-----------------|----------------|-------------------|---------|-----------|
| 1        | 1.52    | [M-H]^−        | Corilagin      | C_{27}H_{22}O_{18} | 633.0787 | 261.667; 181.051 |
| 2        | 2.96    | [M-H]^−        | Petunidin-3-O-beta-glucopyranoside | C_{22}H_{23}O_{12} | 477.1030 | 299.013; 314.043 |
| 3        | 3.67    | [M-H]^−        | Cianidanol     | C_{15}H_{14}O_{6} | 289.0719 | 245.0827 |
| 4        | 4.53    | [M-H]^−        | Gossypetin-8-C-glucoside | C_{21}H_{20}O_{13} | 479.0835 | 316.0244; 271.216 |
| 5        | 5.03    | [M-H]^−        | Syringetin-3-O-glucoside | C_{23}H_{24}O_{13} | 507.1144 | 301.067; 345.0604 |
| 6        | 5.50    | [M-H]^−        | Myricetin-3-O-galactoside | C_{21}H_{20}O_{13} | 479.0834 | 115.0551; 133.014 |
| 7        | 5.80    | [M-H]^−        | kaempferol 7-O-glucoside | C_{21}H_{20}O_{11} | 447.0926 | 285.0365 |
| 8        | 5.84    | [M-H]^−        | Syringetin-3-O-galactoside | C_{23}H_{24}O_{13} | 507.1143 | 344.053; 273.032 |
| 9        | 5.85    | [M-H]^−        | Flavanomarein  | C_{21}H_{22}O_{11} | 449.1095 | 287.057; 150.0037 |
| 10       | 5.89    | [M-H]^−        | Luteolin       | C_{15}H_{10}O_{6} | 285.0393 | 151.0022; 133.0302 |
| 11       | 5.91    | [M-H]^−        | Spiraeoside    | C_{21}H_{20}O_{12} | 463.089 | 301.034; 179.0188 |
| 12       | 6.02    | [M-H]^−        | Myricetin      | C_{15}H_{10}O_{8} | 317.0300 | 137.0248; 151.00568 |
| No. | MW   | Compound                      | Formula       | Exact Mass 1 | Exact Mass 2 | Exact Mass 3 | Exact Mass 4 |
|-----|------|-------------------------------|---------------|-------------|-------------|-------------|-------------|
| 13  | 6.14 | [M-H] Luteolin-4’-O-glucoside | C21H20O11     | 447.0927    | 285.0388    |             |             |
| 14  | 6.20 | [M-H] Dihydromyricetin        | C15H12O8      | 319.0458    | 150.999; 107.0111 |           |             |
| 15  | 6.73 | [M-H] Morin                   | C15H10O7      | 301.0356    | 165.02      |             |             |
| 16  | 6.97 | [M-H] Quercetin               | C15H10O7      | 301.0357    | 121.0272; 151.0038; 178.9974 |           |             |
| 17  | 7.97 | [M-H] Kaempferol              | C15H10O6      | 285.0408    |             |             |             |
| 18  | 8.04 | [M-H] Kaempferide             | C16H12O6      | 299.0556    | 284.0329; 256.036 |           |             |
| 19  | 8.19 | [M-H] Isorhamnetin            | C16H12O7      | 315.0507    | 300.029     |             |             |
| 20  | 9.56 | [M-H] Galangin                | C15H10O5      | 269.0455    | 225.0558    |             |             |
| 21  | 4.52 | [M+H]+ Genistein              | C15H10O5      | 271.0588    | 121.028     |             |             |
| 22  | 4.75 | [M+H]+ Flavokawain B          | C17H16O4      | 285.1122    | 249.1829; 267.141 |           |             |
| 23  | 4.85 | [M+H]+ Epicatechin            | C15H14O6      | 291.0858    | 123.0446; 139.039 |           |             |
| 24  | 5.05 | [M+H]+ Herbacetin             | C15H10O7      | 303.0478    | 257.042     |             |             |
| 25  | 5.08 | [M+H]+ Dihydro-Quer           | C15H12O7      | 305.0650    | 289.631; 290.365 |           |             |
| 26  | 5.55 | [M+H]+ Flavonol base + 4O, 1MeO | C16H12O8    | 333.0602    | 58.065; 318.036 |           |             |
| 27  | 7.25 | [M+H]+ Naringenin-7-O-glucoside | C21H22O10  | 435.1279    | 153.0835; 273.0744 |           |             |
| 28  | 7.29 | [M+H]+ Phlorizin              | C21H24O10     | 437.1445    | 107.045; 275.0905 |           |             |
| 29  | 7.75 | [M+H]+ Hyperoside             | C21H20O12     | 465.1028    | 61.0285; 85.0285 |           |             |
| 30  | 8.22 | [M+H]+ Aurantio-obtusin beta-D-glucoside | C23H24O12 | 493.1329    | 331.0826    |             |             |
| 31  | 9.92 | [M+H]+ Kaempferol 3-glucorhamnoside | C27H30O15  | 595.1650    | 85.0305; 287.0686 |           |             |
| 32  | 9.92 | [M+H]+ Vicenin 2              | C27H30O15     | 595.1658    | 325.071; 317.0645 |           |             |

### Discussion

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Berberis heteropoda Schrenk is an important local plant resource in the Nanshan Mountain region, and understanding the active components of the plant can guide its nutritional utilization. A prior study on anthocyanin composition in Berberis heteropoda Schrenk fruit considered it as a potential anthocyanin pigment source [3]. This study focused on the chemical information of Berberis heteropoda Schrenk fruit; however, there has been a lack of comprehensive scientific research on the overall nutritional composition of the fruit. This study systematically evaluated the major nutrients and antioxidant properties of Berberis heteropoda Schrenk fruits and found them to be rich in various nutrients, providing evidence for potential healthcare or nutritional use. Moreover, a total of 32 kinds of polyphenols were detected from Berberis heteropoda Schrenk fruit extract.

This study revealed that Berberis heteropoda Schrenk fruit exhibited nutritional properties suggesting potential nutraceutical value. Moreover, the major nutrients of Berberis heteropoda Schrenk fruit was comparable to that of wolfberry (Lycium ruthenicum Murr), which as a wild plant and widely observed in Xinjiang [39]. Moreover, the protein content in Berberis heteropoda Schrenk fruit was higher than black mulberry (1.17±0.06%) [19]. Furthermore, the content of fat and sugar in Berberis heteropoda Schrenk fruit was lower, which did not cause excess risk of obesity, and it could be considered a functional food or medicine rather than edible fresh fruits due to poor taste.

Our study revealed that Berberis heteropoda Schrenk fruit contained a large amount of minerals, including sodium, potassium, calcium, copper, zinc, iron, magnesium, phosphorus, and other elements. Studies have demonstrated that such minerals play an important role in the physiological function of human tissues, maintaining the osmotic pressure of cells, supporting the pH balance of the body, and regulating special physiological functions of the body as cofactors [40,41]. Moreover, we observed that sodium/potassium ratio in Berberis heteropoda Schrenk fruit was 0.002, which could prevent hypertension risk [42]. Based on
these findings, *Berberis heteropoda* Schrenk fruit may be considered helpful for controlling blood pressure.

The ratio of EAAs to NEAAs was 0.47, which does not meet the ideal protein condition proposed by FAO/WHO [43], and therefore, this fruit is not recommended as a high-quality protein food. The percentage of glutamic acid, glycine, and aspartic acid were 18.72, 7.42, and 9.54 %, respectively, accounting for more than one-third of the total amino acids in *Berberis heteropoda* Schrenk fruit. Moreover, the UFA: SFA ratio in *Berberis heteropoda* Schrenk fruit was 1.06, suggesting that *Berberis heteropoda* Schrenk fruit should not be recommended as a rich fatty acid food.

Phenolic and flavonoid compounds have been demonstrated as important phytonutrients in plants [44,45]. Flavonoids are the secondary metabolites with activity in plants, which are widely existed and abundant [46]. Phenols are important plant compounds that mimic the biological effects of vitamin E [47]. Moreover, a previous study revealed that anthocyanins are rich in many plants, and the fruits presented red, yellow, purple, black, and other colorful colors induced by anthocyanins [48]. The basic structural unit of anthocyanins is 2-phenylbenzopyran, comprising C6-C3-C6 skeleton [49]. Because of its unique structure, it has the functions of anti-oxidation, anti-inflammation, anti-tumor [50], prevention of cardiovascular disease, and enhancement of vision [51]. Our study found that the content of *Berberis heteropoda* Schrenk fruit was higher than that of wolfberry [39] but lower than that of *Passiflora foetida* [10]. This result suggested that TPC is significantly related to geographical, climate, and soil conditions. The anthocyanin content of *Berberis heteropoda* Schrenk fruit found in this study was slightly inconsistent with a prior study, possibly because the previous samples were obtained from Yili, Xinjiang [3].

The free radical scavenging activity of flavonoids and polyphenols has been demonstrated in previous study [52]. The IC$_{50}$ value is typically used to evaluate the antioxidant activity, and
smaller IC$_{50}$ values indicate stronger antioxidant capacity. The present results indicated that *Berberis heteropoda* Schrenk fruit extract had strong scavenging effects on DPPH$\bullet$, •OH, O$_2$$\bullet$- and ABTS$^{\bullet}$. These results suggested that *Berberis heteropoda* Schrenk fruit could be considered an excellent source of natural antioxidants.

This study provides an examination of the major nutrients and antioxidant properties of *Berberis heteropoda* Schrenk fruits. However, several shortcomings should be mentioned: (1) functional monomers of the *Berberis heteropoda* Schrenk fruits were not addressed; (2) structures of specific phenolic compounds and their antioxidant effects were not assessed, and the mechanism of action needs further evaluation; and (3) potential effect of *Berberis heteropoda* Schrenk fruits on health needs further assessment.

**Conclusions**

The present study analyzed the major nutrients, mineral elements, fatty acids, and amino acids of *Berberis heteropoda* Schrenk fruits and found a wide array of important nutrient components. Moreover, the TPC, TFC, and TAC values were high, with excellent antioxidant properties. Therefore, *Berberis heteropoda* Schrenk fruit may be used as a potential health-promoting food for resisting oxidation damage.

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**Competing interests**
The authors declare no conflict of interest.

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Author Contributions. Conceptualization: JGL; Methodology: JXS and QL; Writing-original draft preparation: JXS and FX; Writing-review and editing: JXS; Data curation: FX and JL; Project administration: JGL.

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annotation:
1. Altai mountains
2. Tianshan mountains
3. Baluke mountains
