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HPLC-MS/MS targeting analysis of phenolics metabolism and antioxidant activity of extractions from *Lycium barbarum* and its meal using different methods

Ya-Fang SHANG[1,2], Tian-Hua ZHANG[1], Kiran THAKUR[1,2], Jian-Guo ZHANG[1,2], Carlos Leonardo Armando CESPEDES-ACUÑA[3], Zhao-Jun WEI[1,2]*

Abstract

The phenolic compounds in dried *Lycium barbarum* (LB) and *L. barbarum* meal (LBM) were extracted using ultrasonic method, reflux method and enzymatic method, and the composition, content of polyphenols and antioxidant abilities of the extracts were analyzed. 40 phenolic compounds were identified with HPLC-MS/MS, with 35 compounds detected from LB and 33 from LBM. 40 compounds were classified into phenolic acids and their derivatives, flavonoids, and their derivatives, lignanamides and other phenolic substances. Differences existed in samples extracted from different materials or under different extraction methods. It was explicitly observed that LB was higher than LBM in phenolic content. The phenolics in LB and LBM had significant antioxidant effects, with LBM extracts showing the higher antioxidant effect. This study compared the differences in polyphenols of *L. barbarum* and its meal, meanwhile explored the effects of different extraction methods, which could contribute to the exploitation of *L. barbarum* and its meal.

Keywords: *Lycium barbarum*; meal; HPLC-MS/MS; phenolic compounds; antioxidant.

Practical Application: Identification and analysis of the antioxidant activity of the polyphenol extracts from *L. barbarum* and its meal were carried out. The results provide a reference for the comprehensive exploitation of *L. barbarum* and its meal.

1 Introduction

Lycium barbarum, a plant from Solanaceae family and *Lycium* genus, grows mainly in northwestern China and other Asian regions (Zhang et al., 2016). Its fruits have long been utilized as a traditional Chinese medicine. An accumulating body of research indicates that *L. barbarum* possesses multiple physiological activities including immune modulating, tumor inhibition, hepatoprotection, and antioxidant properties due to the presence of biologically active components like polysaccharides, terpenoids, and polyphenols (Jiang et al., 2021).

Phenolic compounds as important active ingredients in natural plants, have various physiological activities such as antioxidative activity, anti-inflammatory, antibacterial effects (Singh et al., 2017; Higbee et al., 2022; Brandão et al., 2021). Polyphenols have been reported to utilize multiple pathways to inhibit the oxidation. Polyphenols contain hydroxyl groups that release H^+, neutralize free radicals such as •DPPH, $ABTS^+$, O^{2-}, •OH, and $ONOO^-$ in the body and reduce free radicals to stable substances, thus effectively prevent the free radical chain reactions. For example, chlorogenic acid chelates with ferrous ions to block the Fenton reaction to produce •OH. Phenolic acid inhibits ROS production by bursting free radicals and eliminating the activity of enzymes associated with ROS production (Lv et al., 2021). The utilization of synthetic antioxidants has shown *in vivo* toxic effects. Therefore, safe and effective natural antioxidants such as phenolics are more readily accepted by consumers than commercially synthesized antioxidants (Embuscado, 2015; Yeler & Nas, 2021). In recent years, studies have mainly focused on functional active ingredients (polysaccharides) of *L. barbarum*, while few studies have reported their phenolic constituents (Tian et al., 2019). Phenolic compounds of *L. barbarum* remain unused after the extraction of oil by pressing or solution extraction. Therefore, the extraction, identification, and quantification of active components in meals are important for the comprehensive exploitation of meals and the improvement of value-added by-products (Malešević et al., 2014; Wang et al., 2021). The embedded phenolic compounds hold promising nutritional and health benefits which can be exploited through suitable extraction methods.

Moreover, *L. barbarum* has long been known for its high nutritional value and health promoting benefits (Ma et al., 2022). However, many essential chemical constituents remain unexplored which are left inside the *L. barbarum* meal. Considering that the type and number of phenolic compounds extracted from the plant are influenced by the extraction method, it is very important to select an optimal extraction method for the application of *L. barbarum* and its by-products meal in food and pharmaceutical industry. Previous studies have shown that the selection of the suitable extraction methods also plays

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[1] *School of Food and Biological Engineering, Hefei University of Technology, Hefei, China*
[2] *Ningxia Key Laboratory for the Development and Application of Microbial Resources in Extreme Environments, School of Biological Science and Engineering, North Minzu University, Yinchuan, China*
[3] *Departamento de Ciencias Básicas, Universidad del Bío-Bío, Chillan, Chile*
*Corresponding author: zjwei@hfut.edu.cn

an immense role in deciding the optimal yield of the targeted compounds. Different kinds of methods such as ultrasonic extraction, reflux extraction, and enzymatic extraction have been shown to improve the extraction efficiency of phenolic compounds (Domínguez-Rodríguez et al., 2021). So far, there is no study reported for exploring the phenolic profile of *L. barbarum* and its meal.

The purpose of the current study was to compare the contents of total phenolics, total flavonoids, and evaluate the antioxidant activities of the extracts of *L. barbarum* and its meal obtained by ultrasonic method, reflux method, and enzymatic method. Secondly, by comparing the differences in the identity and content of polyphenols in the extracts obtained through different extraction methods and different materials, we provided a theoretical basis for the selection of extraction methods for certain polyphenols in dried *L. barbarum* (LB) and *L. barbarum* meal (LBM). This study could provide a basis for the integrated exploitation of LB and LBM.

2 Materials and methods

2.1 Polyphenols extraction of LBM and LB

The dried *L. barbarum* and meal after oil extractions were supplied by Ningxia Wolfberry Goji Industry Co. Ltd. (Yinchuan, China). LB was obtained by naturally drying. LBM was the by-product of *L. barbarum* seed oil extracted by supercritical fluid extraction. Both the samples were stored at -80 °C and lyophilized at -60 °C for 24 h. The dried samples were crushed into powder and sieved through 60 mesh sieves. The obtained LB and LBM were stored at 4 °C for further study.

The pre-experimental results showed that 70% ethanol had the best extraction effect for polyphenols, therefore, 70% ethanol was selected as extraction solution for all experiments. In brief, for ultrasonic extraction, 70% ethanol was added to 3 g material powders at a proportion of 1:20 (m/v), after that, extraction was carried out at 50 °C and 200 W for 50 min with ultrasonic cleaning machine (H1085, Zhengji Instrument Co. Ltd., Jintan, China). Further, the mixture was subjected to a centrifugation at 5600 x g for 10 min, then the supernatant was gathered and the sediments were extracted repeatedly as above. The supernatants of the two extracts were combined and concentrated by rotary evaporator under reduced pressure to a volume of less than 10 mL to obtain the extract of ultrasonic extraction. For reflux extraction, powders (3 g) were added to 60 mL of 70% ethanol and then refluxed in a closed container for 2 h at 50 °C. Following that, the mixture was centrifuged at 5600 x g for 10 min, then the supernatant was gathered and the sediment was extracted repeatedly as above. The reflux extract was obtained by the method of ultrasonic extraction solution. In terms of enzymatic extraction, powder (3 g) and 70% ethanol were blended in the same ration as above and the pH of solution was aligned to 5.5. And 3% (w/w) pectinase, 3% (w/w) celulase and 1.5% (w/w) papain were added and the solution was left at 50 °C for 60 min. After the inactivation operation, the enzyme was added again to the remaining residues after centrifugation and the extraction process was repeated, and the supernatants were concentrated to obtain the extract of enzymatic extraction (Zhang et al., 2020). All the concentrated supernatants were filtered with membrane (0.22 μm), lyophilized at -60 °C for 24 h, and then kept in -20 °C for further use.

2.2 Content determination of the major groups of polyphenols

Total phenolics

Total phenolics content of various extracts of LB and LBM were determined using previous method (Wabaidur et al., 2020) with minor modifications. In brief, 2 mL aqueous solution of lyophilized material of samples was added to Folin-Ciocalteu reagents (10%, v/v) (5 mL) followed by shaking and standing for 8 min. Then, sodium carbonate (7.5%, w/v) (4 mL) was added and the solution was kept away from light exposure and left for 1 h. The absorbance was obtained at 765 nm with a UV spectrophotometer (UV-2100, Unico Instrument Co., Ltd. Shanghai, China). Total phenolics was measured with a standard gallic acid curve y = 88.90873x + 0.01683 with a correlation coefficient (R^2) of 0.99575 and recorded as gallic acid equivalent per gram of material powders (mg GAE/g).

Total flavonoids

Total flavonoids content was measured according to previously described method (Alarcón et al., 2021). For this, 2 mL of aqueous solution of lyophilized material of each sample was added with sodium nitrite (5%, w/v) (0.15 mL) and aluminum nitrate (10%, w/v) (0.3 mL), mixed well, and was allowed to stand for 5 min at room temperature. After that, 1 mL of sodium hydroxide (4%, w/v) was added under dark conditions and the final solution was allowed to stand for 10 min at room temperature. Then the absorbance was determined at 510 nm with a UV spectrophotometer. Total flavonoids content was measured by standard catechins curve y = 32.24735x + 0.01519 with R^2 of 0.99676 and recorded as catechin equivalents per gram of material powder (mg CE/g).

2.3 HPLC-MS/MS analysis

Phenolics analysis of LB and LBM extracts by different methods was carried out using an AB Triple TOFTM 5600 + LC-MS/MS (SCIEX, Shanghai, China). Each sample was dissolved with chromatographic grade methanol. Separation of polyphenols in each sample was performed using a 100 × 4.6 mm 5-Micron C18 reverse-phase column at 25 °C. Mobile phases: 0.1% formic acid in water (phase A) and 0.1% formic acid in methanol (phase B). The applied flow rate was 0.3 mL/min, and the injection volume was 10.0 μL. The elution procedure was as follows: 95% of A for 1.5 min, 95-70% of A in 19 min, 70-10% of A in 10 min and kept at 10% of A for 5 min, 10-95% of A in 1 min and kept at 95% of A for 4 min (Liu et al., 2019). Naringin peak area (100 μg/mL) was added as an internal calibration standard for qualitative analysis. By calculating the peak areas of naringin and other components, the content of specific components were calculated. The system was fitted with an heated electrospray ionization source and was performed in negative ESI (−) ionization mode with the following key parameters: the spray voltage at +3.8 and -2.8 kV; auxiliary-gas flow rate at 10 arbitrary units (arb

unit); sheath gas flow rate at 35 arb unit; capillary temperature at 325 °C; auxiliary-gas-heater temperature at 350 °C; stepped and normalized collision energies at 20, 40, and 60 eV; scan modes in full MS with a resolution of 70,000 fwhm; scan range from m/z 10,0 to 100,000. The raw data were extracted and processed by using Mass Frontier 7.0 and PeakView 2.2 software. The freely available databases, including Pubchem and Chemspider were searched for accurate mass and molecular formula predictions of presumed molecules. The MS/MS fragments of phenolics were compared to standards reference of candidate molecules or fragments of compounds from databases and were validated with previous studies (Qu et al., 2020; Zhang et al., 2020).

2.4 Antioxidant activity analysis

•DPPH radical scavenging activity

The measurement of •DPPH free radical scavenging ability of the extracts was according to the method by Santos et al. (2021). The measurement was performed by mixing 2 mL of sample of different concentrations with 3 mL of freshly prepared •DPPH working solution of 0.1 mmol/L and the solution was left for 30 min at room temperature. The absorbance of the final solution was determined at 517 nm and recorded as A_1, the absorbance was measured as A_0 by replacing the sample solution with ethanol as a blank control, and the absorbance was measured as A_2 by replacing the •DPPH solution with ultra-pure water. The percentage of free radical scavenging ability of the analyzed extracts was calculated according to the following equation. To compare the free radical scavenging ability of extracts, half maximum inhibitory concentration (IC_{50}) values were obtained using the plotted RSA percentages against the concentrations of the extracts. All experiments were repeated three times (Equation 1).

$$DPPH \text{ scavenging activity}(\%) = \frac{(A_0 - A_1 + A_2)}{A_0} \times 100\% \quad (1)$$

$ABTS^+$ radical scavenging activity

$ABTS^+$ radical scavenging rate was determined according to the previous method with slight modifications (Shi et al., 2016). A_0, A_1 and A_2 were measured as mentioned above. The $ABTS^+$ radical scavenging capacity were measured as described below (Equation 2):

$$ABTS^+ \text{ scavenging activity}(\%) = \frac{(A_0 - A_1 + A_2)}{A_0} \times 100\% \quad (2)$$

•OH radical scavenging activity

The measurement of •OH radical scavenging capacity of LB and LBM extracts was carried out according to previously described method with minor modifications (Shi et al., 2016). A_0, A_1 and A_2 were measured as mentioned above. The OH radical scavenging capacity were measured as described below (Equation 3):

$$OH \text{ scavenging activity}(\%) = \frac{(A_0 - A_1 + A_2)}{A_0} \times 100\% \quad (3)$$

Fe^{2+} chelating activity

Fe^{2+} chelating abilities were assessed according to the method previously described by Zhu et al. (2017a). A_1. A_0 and A_2 were measured as motioned above. Additionally, the Fe^{2+} chelating activity was determined as described above (Equation 4):

$$Fe^{2+} \text{ chelating ability}(\%) = \frac{(A_0 - A_1 + A_2)}{A_0} \times 100\% \quad (4)$$

2.5 Statistical analysis

Data analysis was executed using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). One-way ANOVA and Duncan's test were used to test whether the means of the parameters were significantly different at a significance level of $p < 0.05$.

3 Results and discussion

3.1 Polyphenol content

Total phenolics and total flavonoids contents were summarized in Table 1. The contents of total phenolics and flavonoids in LB were higher than that of LBM. Among the different extraction methods, the highest extraction efficiency was obtained by enzymatic extraction and the lowest by reflux extraction. This suggests that both ultrasonic and enzymatic assistance contribute to the extraction rate, which may be related to the release of lysis of bound polyphenols and the rupture of cellular structures due to these auxiliary means.The results showed significant differences in total phenolics and total flavonoids contents between the different extraction methods.

3.2 Identification of polyphenols

The total ion flow diagram of the LBM extracted by the reflux method was shown in Figure 1 with 24 compounds showing characteristic peaks within 25 min except gallic acid

Table 1. Total phenolic contents (TP) and total flavonoids contents (TF) in *Lycium barbarum* and *L. barbarum* meal.

Sample	Extraction method	TP (mg GAE/g)	TF (mg CE/g)
LB	Reflux extraction	10.112 ± 0.336[b]	1.137 ± 0.037[c]
	Ultrasonic extraction	10.62 ± 10.347[b]	1.337 ± 0.034[b]
	Enzymatic extraction	11.857 ± 0.393[a]	1.637 ± 0.041[a]
LBM	Reflux extraction	1.857 ± 0.076[c]	0.19 ± 0.012[c]
	Ultrasonic extraction	2.236 ± 0.078[b]	0.25 ± 0.016[b]
	Enzymatic extraction	2.472 ± 0.070[a]	0.327 ± 0.012[a]

Values within the three methods in different materials are significantly different ($p < 0.05$).

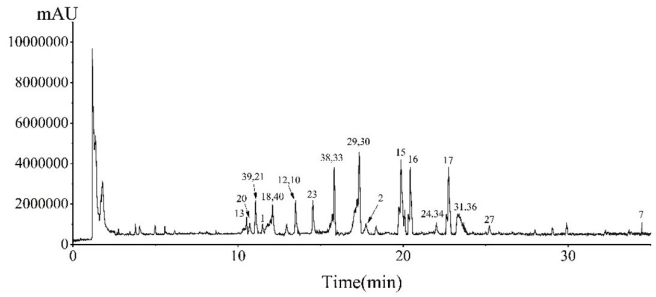

Figure 1. Taking the total ion flow diagram of liquid chromatography of LBM-reflux extraction as a sample. The numbers of peaks in the graph are consistent with that in Table 2.

showing peak within 35 min. The first detected peak responded to chlorogenic acid at 10.32 min.

A total of 40 polyphenols were characterized by HPLC-MS/MS as listed in Table 2. All the compounds were classified into phenolic acids and their derivatives, flavonoids, and their derivatives, and phenylpropanoids and their derivatives compounds according to the parent nucleus structure. 8 phenolic acids and their derivatives were reported. Phenolic acids tend to cleave CO_2 from carboxylate groups in a negative ionic mode (Liu et al., 2019). In case of vanillic acid, with a molecular ion peak m/z 167.0351 $[M-H]^-$, 152.0012 $[M-H-CH_2]^-$ and m/z 108.0208$[M-H-CH_2-CO_2]^-$, CO_2 loss was detected. Phenolic compounds in the form of glycosides were characterized by

Table 2. Information of phenolics identified by HPLC-MS/MS. substance name, extraction mass, molecular formula, MS/MS fragments; the relative content of each polyphenol (μg/g).

Number	Compound identification	Formula	Extraction Mass	Fragments (m/z)	Relative Contents (μg naringin/g material)						References
					LBM-UE	LB-UE	LBM-RE	LB-RE	LBM-EE	LB-EE	
	Phenolic acids and its derivatives										
1	Syringic acid	$C_9H_{10}O_5$	197.0457	197.0494, 182.019,166.9955, 123.0079	nd	1.71	2.79	2.14	2.77	nd	Doğu et al. (2021)
2	Salicylic acid	$C_7H_6O_3$	137.0245	93.0332, 65.0395	nd	9.99	4.38	10.55	5.95	17.29	R
3	2-hydroxybenzeneacetic acid	$C_8H_8O_3$	151.0402	108.0240, 151.0368, 136.0145, 92.0261, 79.0165	3.41	3.79	nd	nd	3.46	nd	Jia et al. (2020)
4	Protocatechuic acid	$C_7H_6O_4$	153.0198	153.0237, 109.0288,108.0203,91.0196, 80.0276,65.0024	0.69	nd	nd	nd	nd	nd	R
5	Gentisic acid	$C_7H_6O_4$	153.0198	109.0288, 108.0203 91.0196 80.0276,65.0024,153.0237	nd	nd	nd	0.24	nd	nd	R
6	Ellagic acid	$C_{14}H_6O_8$	300.9991	130.9916, 167.0160,170.9898, 190.9910,216,9889	14.54	nd	nd	11.74	5.28	25.62	R
7	Gallic acid	$C_7H_6O_5$	168.9901	168.9894,151.1132, 125.0967,97.0634	7.06	9.15	4.68	10.16	5.7	28.1	Doğu et al. (2021)
8	Vanillic acid	$C_8H_8O_4$	167.0351	152.0012, 124.0153, 108.0208	3.9	nd	nd	nd	4.2	4.49	Inbaraj et al. (2010)
	Phenylpropanoids and their derivatives										
9	Aquillochin	$C_{21}H_{20}O_9$	415.1018	415.1055, 206.0240, 192.0096,163.0039, 135.0091,369.1702	nd	4.06	nd	nd	nd	5.44	Zhang et al. (2013)
10	*P*-Coumaric acid	$C_9H_8O_3$	163.0402	119.0511, 117.0352, 93.0359	8.58	163.86	7.97	236.84	10.5	218.5	Bondia-Pons et al. (2014)
11	Caffeic acid	$C_9H_8O_4$	179.0351	135.0454, 107.0500, 89.0245, 59.0144	nd	6.35	nd	7.06	1.66	10.91	R
12	Ferulic acid	$C_{10}H_{10}O_4$	193.0506	134.0379, 89.9249, 102.9378	nd	17.58	3.07	23.14	2.54	24.06	Zhao et al. (2019)
13	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876	191.0578, 161.0249	5.99	27.54	8.04	31.9	4.95	45.95	Zhou et al. (2017)
14	Phloretic acid	$C_9H_{10}O_3$	165.0558	165.0602, 135.0456, 120.0210, 93.0345	nd	1.19	nd	2.59	nd	nd	Jiang et al. (2021)
15	*N-p-cis*-coumaroyl-tyramine	$C_{17}H_{17}NO_3$	282.1138	119.0498,282.1135,162.0555, 117.0347, 136.0764, 174.0558	32.05	9.72	36.54	nd	28.89	9.62	Jiang et al. (2021)
16	*N*-feruloyl-tyramine	$C_{18}H_{19}NO_4$	312.1242	148.0526, 177.0507, 190.0501, 297.1010, 135.0448, 147.0447, 134.0365	16.55	27.85	21.27	16.44	16.76	26.09	Jiang et al. (2021)
17	Cannabisin E	$C_{36}H_{38}N2O_9$	641.2499	623.2263, 489.1945, 431.1990, 328.1218, 312.1242, 151.0400, 136.0138	0.94	0.95	1.22	nd	nd	nd	Jiang et al. (2021)
18	Coumaric acid di-hexose	$C_{21}H_{28}O_{13}$	487.1461	145.0294,163.0395,89.0230, 235.0604, 265.0715, 308.0821	25.09	105.81	35.14	59.25	20.88	95.16	Bondia-Pons et al. (2014)
19	1-*O*-trans-feruloyl-β-*d*-glucopyranoside	$C_{16}H_{20}O_9$	355.1038	175.0385, 162.0176, 59.0156, 132.0193, 172.9779, 355.1058	nd	31.61	nd	18.46	nd	29.66	Alarcón et al. (2021)
20	*P*-coumaric acid-glycosides isomer	$C_{15}H_{18}O_8$	325.0931	145.0291, 117.0345, 89.0259, 162.0400, 325.0966	5.89	99.61	6.64	54.53	4.88	99.77	Bondia-Pons et al. (2014)
21	*P*-hydroxybenzaldehyde	$C_7H_6O_2$	121.0296	121.0278, 108.0184, 92.0257, 93.0329, 91.0165	6.43	10.32	6.27	20.63	7.77	25.85	Zhou et al. (2017)
22	Aesculetin	$C_9H_6O_4$	177.0193	123.0086, 107.0127,95.0149, 77.0433, 133.0298	5.46	nd	nd	nd	8.12	15.18	Alarcón et al. (2021)

R: comparison with standard reference substances; UE: ultrasonic extraction; RE: reflux extraction; EE: enzymatic extraction.

Table 2. Continued...

Number	Compound identification	Formula	Extraction Mass	Fragments (m/z)	Relative Contents (μg naringin/g material)						References
					LBM-UE	LB-UE	LBM-RE	LB-RE	LBM-EE	LB-EE	
23	Scopoletin	$C_{10}H_8O_4$	191.035	176.0110, 148.0165, 104.0261, 120.0209, 191.0374	17.26	100.15	12.29	261.36	16.23	264.51	Zhou et al. (2017)
	Flavonoids and their derivatives										
24	Quercetin	$C_{15}H_{10}O_7$	301.0352	150.9968	nd	4.86	5.31	10.66	5.61	11.66	Alarcón et al. (2021)
25	Morin	$C_{15}H_{10}O_7$	301.0352	285.0357, 257.0427, 227.0353, 215.0349, 212.0426, 201.0568, 150.9968	nd	1.21	nd	nd	nd	0.89	Ali et al. (2019)
26	Apigenin 7-*O*-hexoside	$C_{21}H_{20}O_{10}$	431.0982	431.0979, 269.0448, 311.0583, 225.0533, 151.0013, 162.0123	1.92	nd	nd	nd	nd	nd	Zhu et al. (2017b)
27	Kaempferol-3-*O*-β-glucoside	$C_{21}H_{20}O_{11}$	447.0932	284.0334, 191.0567, 179.0356, 162.0534	5.4	nd	5.04	nd	4.88	nd	Zhu et al. (2017b)
28	Quercitrin	$C_{21}H_{20}O_{11}$	447.0932	285.0405, 255.0321, 227.0360	nd	0.56	nd	nd	nd	nd	Jiang et al. (2021)
29	Kaempferol-*3*-*O*-rutinoside	$C_{27}H_{30}O_{15}$	593.1513	264.0729, 263.0707, 233.0590, 308.0495	101.75	17.61	95.18	19.56	90.82	26.62	Alarcón et al. (2021)
30	Isorhamnetin-3-*O*-neohespeidoside	$C_{28}H_{32}O_{16}$	623.1616	315.0494, 623.1101, 300.0264, 308.0367	1.7	14.84	1.8	15.43	nd	19.83	Inbaraj et al. (2010)
31	Kaempferol	$C_{15}H_{10}O_6$	285.0407	285.041	26.07	35.21	39.25	76.05	32.5	57.79	R
32	Isoquercetin	$C_{21}H_{20}O_{12}$	463.0881	301.0346, 300.0270, 271.0225	nd	nd	nd	1.41	nd	nd	Jiang et al. (2021)
33	Quercetin-3-*O*-galactoside (hyperoside)	$C_{21}H_{20}O_{12}$	463.0881	300.0562,255.0240,162.0254	3.79	nd	4.05	nd	3.34	nd	R
34	Isorhamnetin-3-*O*-rutinoside	$C_{28}H_{32}O_{16}$	623.1616	314.0569, 299.1270, 623.0199, 308.0491	1.76	nd	1.95	nd	nd	3.47	Alarcón et al. (2021)
35	Catechin	$C_{15}H_{14}O_6$	289.0626	243.0617, 199.9424	2.42	nd	nd	8.86	3.43	8.22	Jiang et al. (2021)
36	Taxifolin	$C_5H_{12}O_7$	303.0512	95.0124, 125.0245, 303.0499, 177.0197, 199..0421	19.19	19.76	14.34	52.18	22.01	36.59	R
37	Pinocembrin	$C_{15}H_{12}O_4$	255.0664	213.0533, 187.1356, 171.0459, 107.0150, 83.0160, 65.0031	nd	nd	nd	nd	1.9	3.6	R
38	Rutin	$C_{27}H_{30}O_{16}$	609.1465	301.0279, 179.0134, 163.0342, 125.0125, 107.0412	22.13	116.08	29.45	84.11	14.89	75.97	R
39	Rutin hexose	$C_{33}H_{40}O_{21}$	771.1991	609.1495, 462.0818, 301.0335, 162.0356	nd	37.13	3.36	22.53	nd	34.96	Bondia-Pons et al. (2014)
40	Kaempferol hexose deoxy hexose	$C_{27}H_{30}O_{15}$	593.1514	593.1514, 285.0403, 162.0342, 146.0273	12.86	nd	17.66	nd	11.52	nd	Bondia-Pons et al. (2014)

R: comparison with standard reference substances; UE: ultrasonic extraction; RE: reflux extraction; EE: enzymatic extraction.

mass spectra with characteristic ions resulting from the loss of that sugar. For example, the loss of a six-carbon sugar produces proton ions with an m/z of 162, while the loss of a disaccharide produces proton ions with an m/z of 308. Based on the above cleavage pattern and fragment ion information from the previous study (Wu et al., 2016), *P*-coumaric acid-glycosides isomer, coumaric acid di-hexose, and 1-*O* trans-feruloyl-β-*d*-glucopyranoside were identified.

Flavonoids constitute the richest group of polyphenols in LB. Total of 17 flavonoids were detected in this study. Flavonoid molecular ions can be lost by neutral fragmentation (H_2O, CO, CO_2). For example, in apigenin 7-*O*-hexose with a relative molecular mass of 432, H was prone to lost in the negative scan mode; and m/z 431.0982 $[M-H]^-$ and m/z 311.0583 $[M-H-3CO-2H_2O]^-$ were detected (Pino et al., 2020). Notably, pinocembrin was reported for the first time from *L. barbarum*.

Phenylpropanoids contain a three-carbon side chain attached to a phenol (Leonard et al., 2021). 11 phenylpropanoids were identified in the present study.

3.3 Comparison of composition and content of polyphenols

Different composition and content of phenolics in LB and LBM extracted by three methods were displayed in Table 2 and Figure 2. Overall, 40 compounds were identified in *L. barbarum*. And the specificity and superposition of the polyphenols extracted by different methods from different raw materials were shown in Figure 2. Extraction methods affected the composition of polyphenols in the extracts, with 27 polyphenols in the extracts obtained by the ultrasonic extraction, 28 polyphenols in the extracts by the enzymatic extraction and 25 polyphenols in the extracts by the reflux extraction, respectively. Differences were found in the identity and content of the substances extracted by the different methods. The results of HPLC-MS/MS showed that some compounds could only be extracted by specific method. Among the 33 phenolics identified in LBM, 27 were identified by enzymatic extraction, 27 by ultrasonic extraction, and 24 by reflux extraction, with a total of 16 overlapping compounds in the three extraction methods.

The results showed that the extraction method significantly impacted the type and content of the extracted polyphenols. Both sonication and enzymatic digestion can help to improve the extraction efficiency of polyphenols. (Singh et al., 2022).

Large differences in the types and contents of polyphenols were found between LB and LBM under the same extraction method. It indicates that the oil extraction process leads to a large difference in the types and contents of polyphenols in

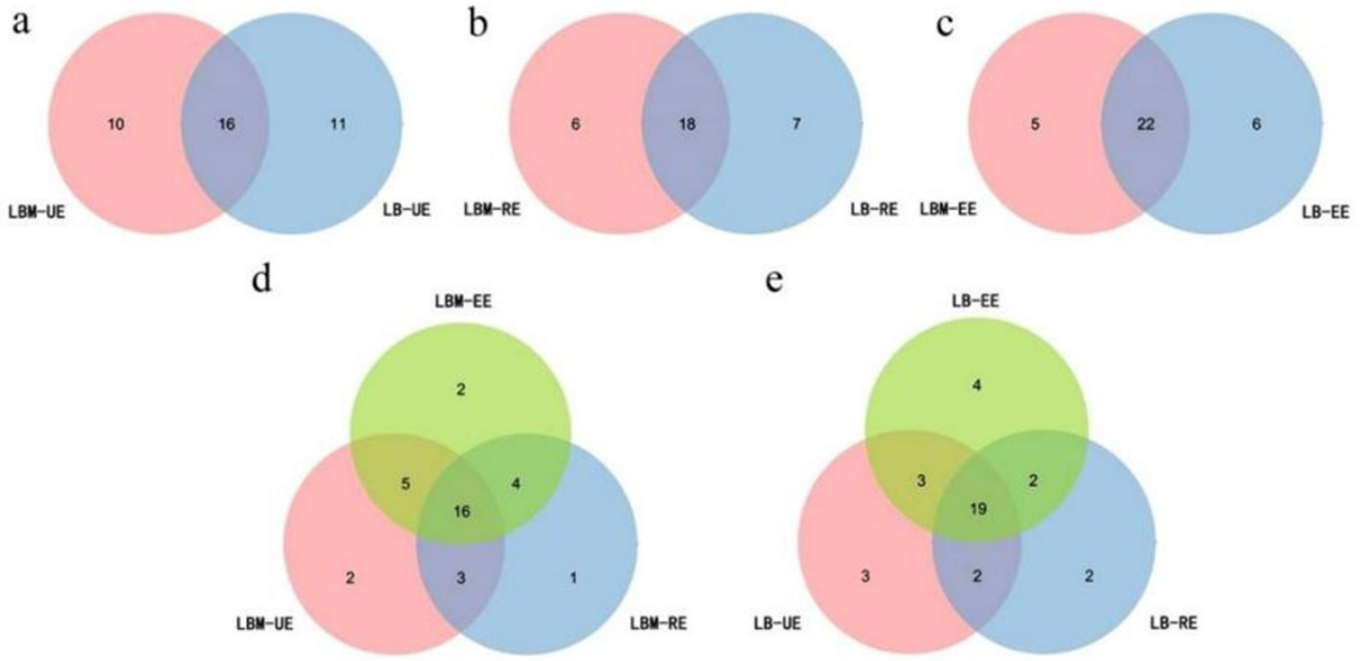

Figure 2. Venn diagrams showed the differences between the same extraction methods with different materials and differences between different extraction methods with same material. a, b, c present the variance of different materials under the same method respectively. d, and e present the variance in the amount of phenolics extracted under different extraction methods in *Lycium barbarum* meal (LBM) and *Lycium barbarum* (LB), respectively.

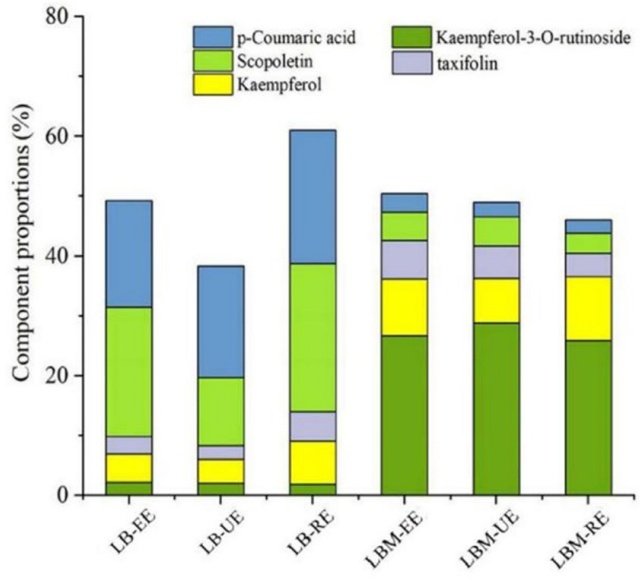

Figure 3. The proportion of the five common phenolics with the most abundant content in each sample.

the LB and LBM due to the different leaching ratios of various polyphenols; and the processing leads to the decomposition and binding of phenolic compounds.

3.4 Analysis of phenolic substances

Among the 23 common compounds found in all the six samples, the highest five substances were shown in Figure 3. The results indicated that after the process, LBM can still be used as a source of polyphenols.

The kaempferol content in LBM was lower than that in LB, while the kaempferol-3-*O*-rutinoside content in LBM much higher than that in LB. This indicates that kaempferol passes into the other phase or undergoes decomposition, while the loss of kaempferol-3-*O*-rutinoside by leaching and decomposition is smaller, or the processing may facilitate the combination of kaempferol and rutinoside. Furthermore, comparing the kaempferol content under different extraction methods revealed that enzymatic digestion was the best extraction method for kaempferol, as enzymatic digestion was more effective in breaking the glycosidic bonds (Yazdi et al., 2018).

3.5 Antioxidant activity analysis

The •DPPH, $ABTS^{+}$, •OH scavenging capacity, and Fe^{2+} chelating ability of the six extracts were compared. Table 3 presented the results. The *in vitro* antioxidant ability of LBM was stronger than LB, which contrasted with the trend in polyphenol content. The •DPPH scavenging capacity showed the same trend as the total polyphenol content, enzymatic extraction > ultrasonic extraction > reflux extraction; while, $ABTS^{+}$, •OH, and Fe^{2+} did not show this trend. In addition, statistical analysis confirmed

Table 3. Antioxidant activities in *Lycium barbarum* and *L. barbarum* meal.

Sample	Extraction method	50% inhibitory concentration [IC_{50} (μg/mL)]			
		•DPPH	$ABTS^+$	•OH	Fe^{2+}
LB	Reflux extraction	1906.87 ± 20.09^{c}	326.57 ± 2.38^{cd}	3689.07 ± 90.85^{e}	2211.81 ± 48.64^{c}
	Ultrasonic extraction	1751.1 ± 30.79^{d}	322.07 ± 10.89^{d}	3983.79 ± 141.68^{d}	2045.39 ± 75.86^{d}
	Enzymatic extraction	1729.25 ± 40.65^{d}	336.37 ± 6.31^{c}	3948.63 ± 90.67^{d}	1969.16 ± 90.20^{d}
LBM	Reflux extraction	2351.78 ± 19.86^{a}	373.65 ± 9.02^{a}	4742.99 ± 74.23^{a}	2619.94 ± 53.48^{ab}
	Ultrasonic extraction	2110.15 ± 36.28^{b}	353.95 ± 8.68^{b}	4211.44 ± 105.85^{c}	2701.50 ± 63.47^{a}
	Enzymatic extraction	2106.75 ± 28.04^{b}	360.87 ± 3.64^{ab}	4422.84 ± 143.70^{b}	2527.08 ± 54.14^{b}

Note: a, b, c, d indicate a significant difference ($P \leq 0.05$).

the differences in antioxidant activity between the different extraction methods. In subsequent studies, the results of *in vitro* antioxidant activity assays will be validated in *in vivo* antioxidant experiments.

4 Conclusion

Three extraction methods (ultrasonic extraction, reflux extraction, and enzymatic extraction) were used to extract the dried fruit and the meal of *L. barbarum,* and 40 compounds were identified and classified into phenolic acids and their derivatives, flavonoids, and their derivatives, phenylpropanoids and their derivatives. The phenolics identified in each sample showed significant differences, with 35 compounds identified in *L.barbarum* and 33 compounds identified in *L.barbarum* meal. The LBM extract showed the higher antioxidant capacity by *in vitro* method. Further studies are warranted to understand their phytochemical and biological effects.

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Conflict of interest

There is no declaim.

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